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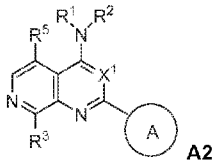
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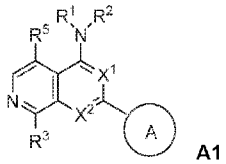
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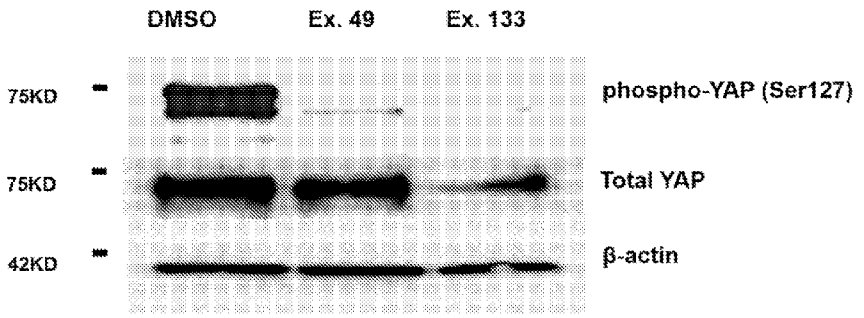


A2



A1

Fig. 1



(57) Abstract: The present invention is related to 6-6 Fused Bicyclic Heteroaryl Compounds of the Formula A2 or A1 and their Use as LATS Inhibitors, or a salt, stereoisomer or pharmaceutical composition thereof; wherein the variables are as defined herein (A1 & A2). The present invention further relates to a method of LATS inhibition in a cell population using a compound of Formula A1, or a salt, stereoisomer or pharmaceutical composition thereof. The present invention further provides a method for manufacturing compounds of the invention, and its therapeutic uses. The invention further provides methods to their preparation, to their medical use, their use in the treatment and management of diseases or disorders.

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6-6 Fused Bicyclic Heteroaryl Compounds and their Use as LATS Inhibitors

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 23, 2018, is named PAT057699-WO-PCT_SL.txt and is 35,778 bytes in size.

INTRODUCTION

The present invention relates to LATS (large tumor suppressor kinase) inhibitors. The present invention further relates to 6-6 fused bicyclic heteroaryl compounds and compositions comprising such compounds.

The present invention also relates to ex-vivo use of such compounds to produce cellular material for cell therapy/transplantation. The present invention further relates to methods of generating an expanded population of cells, such as an expanded population of ocular cells for example comprising limbal stem cells (LSCs) or corneal endothelial cells (CECs) involving the use of a LATS inhibitor, as well as the population of cells such as ocular cells comprising for example limbal stem cells (LSCs) or corneal endothelial cells (CECs) and preparations, uses and methods of therapy comprising said cells.

The present invention also relates to 6-6 fused bicyclic heteroaryl compounds, compositions comprising such compounds, and their use in in promoting wound healing, particularly for treatment of burns, acute and chronic skin ulcers, including vascular, diabetic and pressure ulcers, such as venous leg ulcers, diabetic foot ulcers, pressure ulcers.

The present invention additionally relates to 6-6 fused bicyclic heteroaryl compounds, compositions comprising such compounds, and their use in liver regeneration and liver regrowth as well as in the prevention of damage and in the maintenance or improvement of function of organs ex-vivo, with or without perfusion devices.

BACKGROUND OF THE INVENTION

Organ regeneration and/or healing is an issue crucial to treat many serious health issues.

For example in the eye, it is known that corneal blindness is the third leading cause of blindness worldwide. Approximately half of all the cornea transplants worldwide are performed for treatment of corneal endothelial dysfunction.

The cornea is a transparent tissue comprising different layers: corneal epithelium, Bowman's membrane, stroma, Descemet's Membrane and endothelium. The corneal endothelium also comprises a monolayer of human corneal endothelial cells and helps maintain corneal transparency via its barrier and ionic pump functions. It plays a crucial role in maintaining the balance of fluid, nutrients and salts between the corneal stroma and the aqueous humor. To maintain transparency, endothelial cell density must be maintained, however endothelial cell density can be significantly decreased as a result of trauma, disease or endothelial dystrophies. The density of the cells also decreases with aging. Human corneal endothelium has a limited propensity to proliferate in vivo. If the density of cells falls too low, the barrier function may be compromised. Loss of endothelial barrier function results in corneal edema and loss of visual acuity. The clinical condition of bullous keratopathy may be one resulting complication.

Currently the only treatment for blindness caused by corneal endothelial dysfunction is corneal transplantation. Although corneal transplantation is one of the most common forms of organ transplantation, the availability of donor corneas required is extremely limited. A 2012-2013 global survey quantified the considerable shortage of corneal graft tissue, finding that only one cornea is available for every 70 needed (Gain at el., (2016) Global Survey of Corneal Transplantation and Eye Banking. JAMA Ophthalmol. 134:167-173).

New therapeutic approaches to supply corneal endothelial cells for the treatment of corneal endothelial dysfunction are thus greatly needed.

The corneal epithelium also needs to be maintained in the eye. The corneal epithelium is composed of a layer of basal cells and multiple layers of a non-keratinized, stratified, squamous epithelium. It is essential in maintaining the clarity and the regular refractive surface of the cornea. It acts as a transparent, renewable protective layer over the corneal stroma and is replenished by a stem cell population located in the limbus. In limbal stem cell deficiency, a condition in which limbal stem cells are diseased or absent, a decrease in the

number of healthy limbal stem cells results in a decreased capacity for corneal epithelium renewal.

Limbal stem cell deficiency may arise as a result of injuries from chemical or thermal burns, ultraviolet and ionizing radiation, or even as a result of contact lens wear; genetic disorders like aniridia, and immune disorders such as Stevens Johnson syndrome and ocular cicatricial pemphigoid. Loss of limbal stem cells can be partial or total; and may be unilateral or bilateral. Symptoms of limbal stem cell deficiency include pain, photophobia, non healing painful corneal epithelial defects, corneal neovascularization, replacement of the corneal epithelium by conjunctival epithelium, loss of corneal transparency and decreased vision that can eventually lead to blindness.

A product for use in treating limbal stem cell deficiency was granted a conditional marketing authorisation in the European Union in 2015 (under the name Holoclar®), making it the first Advanced Therapy Medicinal Product (ATMP) containing stem cells in Europe. Holoclar is an ex vivo expanded preparation of autologous human corneal epithelial cells containing stem cells. A biopsy of healthy limbal tissue is taken from the patient, expanded ex vivo and frozen until surgery. For administration to the patient the thawed cells are grown on a membrane comprising fibrin, and then surgically implanted onto the eye of the patient. The therapy is intended for use in adults with moderate to severe limbal stem cell deficiency due to physical or chemical ocular burns. (Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G. (2010) Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med. 363:147-155). However the method is limited in that it is for autologous use only and there must be enough surviving limbus in one eye to allow a minimum of 1- 2 square millimeters of undamaged tissue to be extracted from the patient. There is also the risk that for each specific patient the culture of his/her cells may not be successful and the patient cannot receive this treatment. Furthermore also feeder cells of murine origin are used to prepare the Holoclar cell preparation, which introduces potential safety concerns, due to the risk of disease transmission and potential immunogenicity into the preparation for use in humans. Moreover the Holoclar cell preparation only contains approximately 5% of limbal stem cells, as identified by p63alpha staining.

New therapeutic approaches to supply limbal stem cells for the treatment of limbal stem cell deficiency are thus greatly needed.

Functional liver regeneration during homeostasis and in disease conditions is critical to maintaining essential physiological processes. Despite the liver's marked potential to regenerate, this process can be impaired following severe acute or chronic liver injury. Liver damage and impaired liver regeneration often result in serious morbidity and mortality and therefore require life-saving liver transplantation. Unfortunately, the need for liver transplants currently far eclipses the supply of available donor organs. As a result, many patients continue to die while awaiting a life-saving transplant. The use of split-liver transplants from deceased donors or partial-liver transplants from living donors is limited by graft size constraints. Transplantation of a partial liver that has an inadequate graft-to-recipient weight ratio (GRWR) increases the incidence of graft dysfunction and failure. Therapies that increase liver regrowth may allow transplantation of partial livers that otherwise would be deemed inadequate for transplantation based on size. Alternatively, regenerating livers by inhibiting liver cell death, improving liver function and repairing the aberrant liver architecture could normalize liver function, preventing the need for transplantation (Forbes SJ and Newsome PN (2016) Liver regeneration — mechanisms and models to clinical application. Nature Reviews Gastroenterology & Hepatology, 13(8):473-485; Dutkowski P, Linecker M, DeOliveira ML, Müllhaupt B, Clavien PA (2015) Challenges to Liver Transplantation and Strategies to Improve Outcomes. Gastroenterology, 148(2):307-323).

Hence, there remains an urgent need for more efficacious therapeutics to promote liver regrowth.

Chronic skin ulcers, including vascular, diabetic and pressure ulcers, constitute a major public health issue. The increased demand for wound care is reflected in the association of wounds with comorbidities, increased mortality and patient's quality of life (Demidova-Rice TN, Hamblin MR, & Herman IM (2012) Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. Advances in skin & wound care 25(7):304-314; Demidova-Rice TN, Hamblin MR, & Herman IM (2012) Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 2: role of growth factors in normal and pathological wound healing: therapeutic potential and methods of delivery. Advances in skin & wound care 25(8):349-370). The health care cost of patients with chronic wounds is around 25 billion dollars per year in the US alone. No new chemical entities have been approved by the

FDA since the approval of Regranex (PDGF) in 1997, which has limited efficacy (Eaglstein WH, Kirsner RS, & Robson MC (2012) Food and Drug Administration (FDA) drug approval end points for chronic cutaneous ulcer studies. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 20(6):793-796). Intrinsically, growth factors are not stable in the proteolytic environment of wound bed. Growth factor therapy could also suffer from low expression of its corresponding receptors in the wounds, as demonstrated in the clinic patient samples (Demidova-Rice TN, Hamblin MR, & Herman IM (2012) Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 2: role of growth factors in normal and pathological wound healing: therapeutic potential and methods of delivery. Advances in skin & wound care 25(8):349-370).

Hence, there remains an urgent need for more efficacious therapeutics to promote wound healing in patients with chronic wounds.

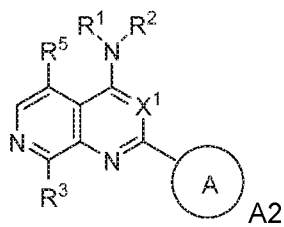
New therapeutic approaches to promote cell proliferation are thus greatly needed for conditions affecting a range of organs throughout the body, such as the eye, liver and skin.

SUMMARY OF THE INVENTION

The present invention relates to compounds, salts thereof, and compositions thereof, wherein the compounds are LATS (large tumor suppressor kinase) inhibitors. These compounds have use in therapies for the conditions and purposes detailed above.

Various aspects of the invention are described herein.

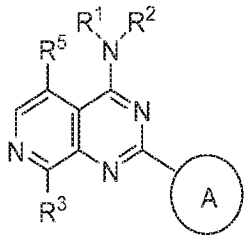
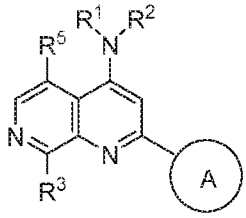
The present invention relates to a compound of Formula A2 or a subformulae thereof, or a salt, or stereoisomer thereof,



A2

wherein X^1, ring A, R^1, R^2, R^3, and R^5 are as defined in the detailed description *infra*.

In a preferred embodiment, the compound is according to Formula I or Formula II, or subformulae thereof, or a salt thereof:


I or

II.

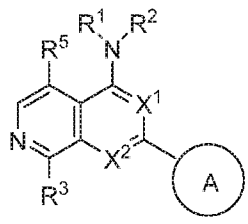
wherein ring A, R^1, R^2, R^3, and R^5 are as defined in the detailed description *infra*.

In another aspect, the invention relates to a pharmaceutical composition comprising a therapeutically effective amount of a compound according to the definition of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or subformulae thereof and one or more pharmaceutically acceptable carriers.

In another aspect, the invention relates to a combination, in particular a pharmaceutical combination, comprising a therapeutically effective amount of the compound according to the definition of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, and one or more therapeutically active agent.

In another aspect, the invention relates to compounds and compositions that may be used in therapy.

In an embodiment the present invention relates to a method of LATS inhibition in a cell or cell population using a compound of Formula A1 or subformulae thereof or a salt thereof, or a stereoisomer thereof:


A1

wherein X^1, X^2, ring A, R^1, R^2, R^3, and R^5 are as defined in the detailed description *infra*. Preferably the salt is a pharmaceutically acceptable salt. In a specific embodiment of the method of LATS inhibition in a cell population according to the invention, the compound, or a

salt thereof, is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine. In another specific embodiment of the method of LATS inhibition in a cell population according to the invention, the compound, or a salt thereof, is selected from N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

In another embodiment the present invention relates to a method of LATS inhibition in an ocular cell population using a compound of Formula A1 or subformulae thereof or a salt thereof, or a stereoisomer thereof. In yet another embodiment the present invention relates to a method of LATS inhibition in a cell population comprising limbal stem cells using a compound of Formula A1 or subformulae thereof or a salt thereof, or a stereoisomer thereof. In yet a further embodiment the present invention relates to a method of LATS inhibition in a cell population comprising corneal endothelial cells using a compound of Formula A1 or subformulae thereof or a salt thereof, or a stereoisomer thereof.

Also preferably the method of LATS inhibition in a cell population is performed *ex vivo*. In yet another preferred embodiment said compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar. In one preferred embodiment of the method of LATS inhibition in a cell population comprising limbal stem cells the compound is present for 12 to 16 days, particularly preferably the compound is present for 14 days. In another embodiment of the method of LATS inhibition in a cell population comprising corneal endothelial cells the compound is present for one to two weeks and subsequently the cells are cultured for a period in growth medium without supplementation with said compound, preferably wherein the period is one to two weeks. In an embodiment of the invention of the method of LATS inhibition in a cell population the LATS inhibitor inhibits LATS1 or LATS2, or LATS1 and LATS2. In a more preferred embodiment the LATS inhibitor inhibits LATS1 and LATS2. In another preferred embodiment of the method of LATS inhibition in a cell population comprising limbal stem cells said method further comprises genetically modifying said limbal stem cells. In another preferred embodiment of the method of LATS inhibition in a cell population comprising corneal endothelial cells said method further comprises genetically modifying said corneal endothelial cells. Preferably said genetically modifying comprises

introducing into said cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. In yet another preferred embodiment the method of LATS inhibition in a cell population, the method comprises the further step after generation of an expanded population of cells of rinsing those cells to substantially remove the compound according to the invention. In one aspect the invention relates to an expanded cell population comprising limbal stem cells obtainable by the method of LATS inhibition in a cell population comprising limbal stem cells according to the invention. In another aspect the invention relates to a an expanded cell population comprising limbal stem cells obtained by the method of LATS inhibition in a cell population comprising limbal stem cells according to the invention. In one aspect the invention relates to a population of corneal endothelial cells obtainable by the method of LATS inhibition in a cell population comprising corneal endothelial cells according to the invention. In another aspect the invention relates to a population of corneal endothelial cells obtained by the method of LATS inhibition according to the invention. In yet another aspect the invention relates to an ocular cell delivery preparation, comprising a cell population obtainable or obtained by the method of LATS inhibition according to the invention and a composition suitable for ocular delivery which is a localising agent. In a specific embodiment the localising agent is GelMa (which is methacrylamide modified gelatin, and is also known as gelatin methacrylate). In another specific embodiment the localising agent is fibrin or fibrin glue. Preferably the cell delivery preparation of limbal stem cells has greater than 20% limbal stem cells. Also preferably the cell delivery preparation of limbal stem cells has greater than 20% p63alpha positive cells. In certain preferred aspects the cell population obtainable or obtained by the method of LATS inhibition according to the invention or cell delivery preparation according to the invention has only trace levels of the compound according to the invention. Preferably, in the cell delivery preparation of corneal endothelial cells, corneal endothelial cells are present in the cell delivery preparation at a density greater than 500 cells per mm^2 (area). In certain preferred aspects the cell population obtainable or obtained by the method of LATS inhibition according to the invention or cell delivery preparation according to the invention has only trace levels of the compound according to the invention.

In another aspect the invention relates to a method of culturing cells comprising culturing a population of cells in the presence of a LATS inhibitor. The cells can be a cell population as described and/or as provided herein. Preferably the cells are ocular cells or liver cells. In a preferred embodiment the cells are ocular cells. In a further aspect the invention relates to a

method of culturing cells comprising culturing a population of cells comprising limbal stem cells in the presence of a LATS inhibitor. In another aspect the invention relates to a method of culturing cells comprising culturing a population of cells comprising corneal endothelial cells in the presence of a LATS inhibitor. In a preferred embodiment the invention relates to a method of culturing cells comprising culturing a cell population comprising limbal stem cells, wherein the LATS inhibitor is a compound of Formula A1 or subformulae thereof or salt thereof according to the invention. In another preferred embodiment the invention relates to a method of culturing cells comprising culturing a population comprising corneal endothelial cells, wherein the LATS inhibitor is a compound of Formula A1 or subformulae thereof or salt thereof according to the invention. Preferably the salt is a pharmaceutically acceptable salt. In a preferred embodiment said compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar. In one preferred embodiment of the method of culturing cells comprising culturing a cell population comprising limbal stem cells, the compound is present for 12 to 16 days, particularly preferably the compound is present for 14 days. In another preferred embodiment of the method of culturing cells comprising culturing a cell population comprising corneal endothelial cells, the compound is present for one to two weeks and subsequently the cells are cultured for a period in growth medium without supplementation with said compound, preferably wherein the period is one to two weeks. In an embodiment of the invention the LATS inhibitor inhibits LATS1 or LATS2, or LATS1 and LATS2. In a more a preferred embodiment the LATS inhibitor inhibits LATS1 and LATS2. In one embodiment said method further comprises genetically modifying cells. Preferably said genetically modifying comprises introducing into said cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. Preferably the cells are ocular cells. In one embodiment said method further comprises genetically modifying limbal stem cells. In another preferred embodiment said method further comprises genetically modifying corneal endothelial cells. In yet another preferred embodiment the method of culturing cells comprises the further step after generation of an expanded population of cells of rinsing those cells to substantially remove the compound according to the invention. In one aspect the invention relates to an expanded cell population obtainable by the method of culturing cells according to the invention. In another aspect the invention relates to an expanded cell population obtained by the method of culturing cells according to the invention. Preferably the cells are ocular cells.

In one aspect the invention relates to an expanded cell population comprising limbal stem cells obtainable by the method of culturing cells comprising limbal stem cells according to the invention. In another aspect the invention relates to an expanded cell population comprising limbal stem cells obtained by the method of culturing cells comprising limbal stem cells according to the invention. In one aspect the invention relates to a population of corneal endothelial cells obtainable by the method of culturing cells comprising corneal endothelial cells according to the invention. In another aspect the invention relates to a population of corneal endothelial cells obtained by the method of culturing cells comprising corneal endothelial cells according to the invention. In another aspect the invention relates to an ocular cell delivery preparation, comprising a cell population obtainable or obtained by the method of culturing cells according to the invention and a composition suitable for ocular delivery which is a localising agent. In a specific embodiment the localising agent is GelMa. In another specific embodiment the localising agent is fibrin or fibrin glue. Preferably the cell delivery preparation of limbal stem cells has greater than 20% limbal stem cells. Also preferably the cell delivery preparation of limbal stem cells has greater than 20% p63alpha positive cells. Preferably corneal endothelial cells are present in the cell delivery preparation of corneal endothelial cells, at a density greater than 500 cells per mm^2 (area). In certain preferred aspects the cell population obtainable or obtained by the method of culturing cells according to the invention or cell delivery preparation according to the invention has only trace levels of the compound according to the invention.

In another aspect the invention relates to a method of cell population expansion comprising the step of a) culturing a seeding population of cells in the presence of a LATS inhibitor to generate an expanded population of cells. In a preferred embodiment the method of cell population expansion is performed ex vivo. Preferably the cells are ocular cells or liver cells. In a preferred embodiment the cells are ocular cells. In yet another aspect the invention relates to a method of cell population expansion comprising the step of a) culturing a seeding population of cells comprising limbal stem cells in the presence of a LATS inhibitor to generate an expanded population of cells comprising limbal stem cells. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof or salt thereof, according to the invention. In a futher aspect the invention relates to a method of cell population expansion comprising the step of a) culturing a seeding population of cells comprising limbal stem cells in the presence of a compound of Formula A1 or subformulae thereof, or a salt thereof to generate an expanded population of cells comprising limbal stem

cells. In another embodiment of the invention said compound is selected from Formula A2 or subformulae thereof or a salt thereof. In another aspect the invention relates to a method of cell population expansion comprising the step of a) culturing a seeding population of cells comprising corneal endothelial cells in the presence of a LATS inhibitor to generate an expanded population of cells comprising corneal endothelial cells. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof or salt thereof, according to the invention. In a further aspect the invention relates to a method of cell population expansion comprising the step of a) culturing a seeding population of cells comprising corneal endothelial cells in the presence of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof to generate an expanded population of cells comprising corneal endothelial cells. Preferably said compound is selected from Formula A2 or subformulae thereof. Also preferably the salt is a pharmaceutically acceptable salt. Preferably said compound is selected from the group of compounds consisting of N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-(2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propoxy)propan-2-ol; 2,4-dimethyl-4-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)pentan-2-ol; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclobutyl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-isopropyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propan-1-ol; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
N-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propoxy)ethan-1-ol; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; and 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine.

Also preferably said compound or a salt thereof, is selected from N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine and (S)-N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine.

In a preferred embodiment of the method of cell population expansion said compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar. In one preferred embodiment of the method of cell population expansion relating to limbal stem cells in step a) the compound is present for 12 to 16 days, particularly preferably the compound is present for 14 days. In another preferred embodiment of the method of cell population expansion relating to corneal endothelial cells in step a) the compound is present for one to two weeks and subsequently step b) is performed wherein the cells are cultured for a period in growth medium without supplementation with said compound, preferably wherein the period is one to two weeks. In a specific embodiment of the method of cell population expansion relating to limbal stem cells the compounds according to Formula A1 or subformulae thereof produce greater than 30 fold expansion of the seeded amount of cells. In another specific embodiment of the method of cell population expansion relating to limbal stem cells the compounds according to Formula A1 and subformulae thereof produce 100 fold to 2200 fold, preferably 600 fold to 2200 fold expansion of the seeded amount of cells. In an embodiment of the method of cell population expansion relating to limbal stem cells, the method according to the invention produces a cell population with greater than 20% limbal stem cells. In another embodiment of the method of cell population expansion relating to limbal stem cells, the method according to the invention produces a cell population with greater than 50% limbal stem cells. In another aspect the method of cell population expansion relating to limbal stem cells according to the invention produces a cell population with greater than 20% expressing p63alpha. In yet another aspect the method of cell population expansion relating to limbal stem cells according to the invention produces a cell population with greater than 50% expressing p63alpha. In a specific embodiment of the method of cell population expansion relating to corneal endothelial cells the compounds according to Formula A1 or subformulae thereof produce greater than 10 fold expansion of the seeded amount of cells. In another specific embodiment of the method of cell population expansion relating to corneal endothelial cells the compounds according to Formula A1 or subformulae thereof produce 15 fold to 600 fold, preferably 20 fold to 550 fold expansion of the seeded amount of cells. In an embodiment of the invention the LATS inhibitor inhibits LATS1 or LATS2, or LATS1 and LATS2. In a more preferred embodiment the LATS inhibitor inhibits LATS1 and LATS2. In another preferred embodiment said method of cell population expansion further comprises use of a gene editing system. Preferably said method comprises use of a gene editing system which specifically targets a gene associated with

facilitating a host versus graft immune response. Also preferably the cells are ocular cells or liver cells. In a preferred embodiment the cells are ocular cells. In another preferred embodiment said method of cell population expansion further comprises genetically modifying limbal stem cells, preferably wherein said genetically modifying comprises introducing into said cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. In another preferred embodiment said method further comprises genetically modifying corneal endothelial cells, preferably wherein said genetically modifying comprises introducing into said cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. In yet another preferred embodiment, the method of cell population expansion further comprises step c) rinsing the expanded population of cells to substantially remove the compound according to the invention.

In one aspect the invention relates to a kit comprising a LATS inhibitor, growth medium and instructions for cell population expansion. In another aspect the invention relates to a cell population obtainable by the method of cell population expansion according to the invention. In yet another aspect the invention relates to a cell population obtained by the method of cell population expansion according to the invention. In another aspect the invention relates to an ocular cell population obtainable by the method of cell population expansion according to the invention. In another aspect the invention relates to an ocular cell population obtained by the method of cell population expansion according to the invention. In one aspect the invention relates to a cell population comprising limbal stem cells obtainable by the method of cell population expansion relating to limbal stem cells according to the invention. In another aspect the invention relates to a cell population comprising limbal stem cells obtained by the method of cell population expansion relating to limbal stem cells according to the invention. In one aspect the invention relates to a cell population comprising corneal endothelial cells obtainable by the method of cell population expansion relating to corneal endothelial cells according to the invention. In another aspect the invention relates to a cell population comprising corneal endothelial cells obtained by the method of cell population expansion relating to corneal endothelial cells according to the invention. In yet another aspect the invention relates to an ocular cell delivery preparation, comprising a cell population obtainable or obtained by the method of cell population expansion according to the invention and a composition suitable for ocular delivery which is a localising agent. In a specific embodiment the localising agent is GelMa. In another specific embodiment the

localising agent is fibrin or fibrin glue. Preferably the cell delivery preparation of limbal stem cells has greater than 20% limbal stem cells. Also preferably the cell delivery preparation of limbal stem cells has greater than 20% p63alpha positive cells. Preferably corneal endothelial cells are present in the cell delivery preparation of corneal endothelial cells, at a density greater than 500 cells per mm^2 (area). In certain preferred aspects the cell population obtainable or obtained by the method of cell population expansion or cell delivery preparation according to the invention has only trace levels of the compound according to the invention.

In some aspects of the method of cell population expansion, the method further comprises use of a gene editing system. Preferably the gene editing system is used for genetically modifying cells. In embodiments of methods according to the invention genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response. Also preferably the gene editing system specifically targets a gene associated with facilitating a host versus graft immune response. Preferably said gene editing system is selected from the group consisting of a CRISPR gene editing system, a TALEN gene editing system, a zinc finger nuclease gene editing system, a meganuclease gene editing system, AAV vector driven homologous recombination and lentiviral vectors-based genome editing technologies.

In one aspect the invention relates to an isolated cell population, wherein greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of cells are limbal stem cells. Preferably greater than 20% are limbal stem cells. More preferably greater than 50% are limbal stem cells. In another preferred embodiment greater than 70% are limbal stem cells. Particularly preferably greater than 90% are limbal stem cells. In one embodiment the cells have been gene edited.

In another aspect the invention relates to an isolated cell population, wherein greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of cells are p63alpha expressing cells. Preferably greater than 20% are p63alpha positive. More preferably greater than 50% are p63alpha positive. In another preferred embodiment greater than 70% are p63alpha positive. Particularly preferably greater than 90% are p63alpha positive. In one embodiment the cells have been gene edited.

In a further aspect the invention relates to a cell population comprising limbal stem cells or the cell population according to the invention, wherein one or more of said cells comprises a non-naturally occurring insertion or deletion of one or more nucleic acid residues of a gene associated with facilitating a host vs graft immune response, wherein insertion and/or deletion results in reduced or eliminated expression or function of said gene. In a preferred embodiment said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C. In a specific embodiment the cells have genetically modified levels of B2M expression.

In a further aspect the invention relates to a cell population comprising corneal endothelial cells or the cell population according to the invention, wherein one or more of said cells comprises a non-naturally occurring insertion or deletion of one or more nucleic acid residues of a gene associated with facilitating a host vs graft immune response, wherein insertion and/or deletion results in reduced or eliminated expression or function of said gene. In a preferred embodiment said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C. In a specific embodiment the cells have genetically modified levels of B2M expression.

In a further aspect the invention relates to a cell population comprising limbal stem cells or corneal endothelial cells which have been gene edited. In a further aspect the invention relates to a cell population comprising limbal stem cells which have been gene edited. Preferably the gene editing was performed by CRISPR. Preferably also the B2M gene was edited.

In one aspect the invention relates to a growth promoting agent of cells comprising a LATS inhibitor. In one embodiment the invention relates to a growth promoting agent of ocular cells comprising a LATS inhibitor. In one aspect the invention relates to a growth promoting agent of limbal stem cells comprising a LATS inhibitor. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof or salt thereof. In another aspect the invention relates to a growth promoting agent of limbal stem cells comprising a compound of Formula A1 or subformulae thereof or salt theroef. In one aspect the invention relates to a growth promoting agent of corneal endothelial cells comprising a LATS inhibitor. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof or a salt thereof. In

another aspect the invention relates to a growth promoting agent of corneal endothelial cells comprising a compound of Formula A1 or subformulae thereof or a salt thereof.

In one aspect the invention relates to a pharmaceutical composition comprising a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt, or stereoisomer thereof, according to the invention and at least one pharmaceutically acceptable excipient. Preferably the composition further comprises a preservation or cryopreservation solution.

In another aspect the invention relates to a cell proliferation medium comprising a LATS inhibitor and a growth medium. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof according to the invention. In one aspect the invention relates to a cell proliferation medium comprising a compound of Formula A1 or subformulae thereof according to the invention and a growth medium. In one embodiment the cell proliferation medium additionally comprises cells as provided herein. Preferably the cell proliferation medium additionally comprises ocular cells. In another embodiment the cell proliferation medium additionally comprises limbal stem cells. Preferably the limbal stem cells are in suspension. In yet another embodiment the cell proliferation medium comprises corneal endothelial cells. Preferably the corneal endothelial cells are in suspension.

In one aspect the invention relates to a cell preparation comprising a LATS inhibitor and cells of a cell population as described and/or provided herein. In another aspect the invention relates to a cell preparation comprising a LATS inhibitor and ocular cells. In another aspect the invention relates to a cell preparation comprising a LATS inhibitor and limbal stem cells. In yet another aspect the invention relates to a cell preparation comprising a LATS inhibitor and corneal endothelial cells. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof according to the invention. In another aspect the invention relates to a cell preparation comprising a compound of Formula A1 according to the invention and limbal stem cells. In an alternative aspect the invention relates to a cell preparation comprising a compound of Formula A1 according to the invention and corneal endothelial cells. Preferably the cell preparation further comprises a growth medium. Particularly preferably the cell preparation further comprises a preservation or cryopreservation solution.

In another aspect the invention relates to an ocular cell delivery preparation, comprising a cell preparation according to the invention and a composition suitable for ocular delivery which is a localising agent. In a specific embodiment the localising agent is GelMa. In another specific embodiment the localising agent is fibrin or fibrin glue. In another aspect the invention relates to an ocular cell delivery preparation, comprising a cell preparation according to the invention and a composition suitable for ocular delivery which is a localising agent. In a specific embodiment the localising agent is GelMa. In another specific embodiment the localising agent is fibrin or fibrin glue. In certain preferred aspects the cell preparation according to the invention has only trace levels of the compound according to the invention. In yet another specific embodiment greater than 20% of the cells in the cell delivery preparation are limbal stem cells. In a further specific embodiment greater than 20% of the cells in the cell delivery preparation are p63alpha expressing cells.

In yet another aspect the invention relates to an ocular cell delivery preparation, comprising a cell preparation according to the invention and a composition suitable for ocular delivery which is a localising agent. In a specific embodiment the localising agent is GelMa. Preferably the corneal endothelial cells are in suspension. In an alternative embodiment the corneal endothelial cells are present in the cell delivery preparation at a density greater than 500 cells per mm^2 (area). Particularly preferably the corneal endothelial cells are present at a density of 1000 to 3500 cells/mm^2 (area), more preferably 2000 to about 3000 cells/mm^2 (area). In certain preferred aspects the cell preparation according to the invention has only trace levels of the compound according to the invention.

Preferably the growth medium in the methods or cell preparation according to the invention is selected from the group consisting of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with Fetal Bovine Serum (FBS), human endothelial Serum Free (SF) Medium with human serum, X-VIVO15 medium and DMEM/F12 which is optionally supplemented with calcium chloride; preferably X-VIVO15 medium.

Preferably the preservation or cryopreservation solution according to the invention comprises a solution which is Optisol or PBS (phosphate buffered saline) and the cryopreservation solution additionally comprises glycerol, dimethyl sulfoxide, propylene glycol or acetamide.

In another aspect the invention relates to a kit comprising a composition suitable for ocular delivery and a LATS inhibitor. Preferably the LATS inhibitor is a compound of Formula A2 or subformulae thereof according to the invention. In another aspect the invention relates to a kit comprising a composition suitable for ocular delivery and compound of Formula A2 or subformulae thereof according to the invention. Preferably the kit has instructions for use. In an embodiment the composition suitable for ocular delivery is a localising agent or topical eye drops. Preferably the composition suitable for ocular delivery is a localising agent. In a specific embodiment the kit further comprises limbal stem cells. In another specific embodiment of the kit the composition suitable for ocular delivery is a localising agent which is GelMa. In an alternative specific embodiment of the kit the composition suitable for ocular delivery is a localising agent which is fibrin or fibrin glue. In yet another specific embodiment, greater than 20% of the cells in the kit are limbal stem cells. In a further specific embodiment, greater than 20% of the cells in the kit are p63alpha expressing cells. In an alternative specific embodiment the kit comprises corneal endothelial cells. In another specific embodiment the composition suitable for ocular delivery of corneal endothelial cells is a localising agent which is GelMa. In yet another specific embodiment corneal endothelial cells are present in a monolayer. Preferably the corneal endothelial cells are present at a density greater than 500 cells per mm^2 (area). Particularly preferably the corneal endothelial cells are present at a density of 1000 to 3500 cells/mm^2 (area), more preferably 2000 to about 3000 cells/mm^2 (area).

In preferred embodiments according to the invention the composition suitable for ocular delivery is a localising agent which is a biomatrix. Preferably the composition suitable for ocular delivery according to the invention is a localising agent selected from the group consisting of fibrin, collagen, gelatin, cellulose, amniotic membrane, fibrin glue, polyethylene (glycol) diacrylate (PEGDA), GelMA, localising agents comprising a polymer, cross-linked polymer, or hydrogel comprising one or more of hyaluronic acid, polyethylene glycol, polypropylene glycol, polyethylene oxide, polypropylene oxide, poloxamer, polyvinyl alcohol, polyacrylic acid, polymethacrylic acid, polyvinyl pyrrolidone, poly(lactide-co-glycolide), alginate, gelatin, collagen, fibrinogen, cellulose, methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropylmethylcellulose, hydroxypropyl-guar, gellan gum, guar gum, xanthan gum and carboxymethylcellulose, as well as derivatives thereof, co-polymers thereof, and combinations thereof. In preferred embodiments according to the invention the composition suitable for ocular delivery is a localising agent which is GelMa,

fibrin or fibrin glue. In specific embodiments according to the invention the composition suitable for ocular delivery is a localising agent which is GelMa. In other specific embodiments according to the invention the composition suitable for ocular delivery is a localising agent which is fibrin or fibrin glue. Preferably the localising agent is fibrin glue. Fibrin glues are known in the art, including, for example, TISSEEL VH Fibrin sealant (Baxter AG, Vienna, Austria) (Panda et al., 2009, Indian J Ophthalmol. Sep-Oct; 57(5): 371–379). In one embodiment fibrin glue is used for the delivery of limbal stem cells. In another embodiment GelMa is used for the delivery of corneal endothelial cells.

In more preferred embodiments according to the invention wherein limbal stem cells are present in combination with the localising agent, greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of cells are limbal stem cells. Preferably greater than 20% are limbal stem cells. More preferably greater than 50% are limbal stem cells. In another preferred embodiment greater than 70% are limbal stem cells. Particularly preferably greater than 90% are limbal stem cells.

In further preferred embodiments according to the invention wherein cells are present in combination with the localising agent, greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of cells are p63alpha expressing cells. Preferably greater than 20% are p63alpha positive cells. More preferably greater than 50% are p63alpha positive cells. In another preferred embodiment greater than 70% are p63alpha positive cells. Particularly preferably greater than 90% are p63alpha positive cells.

In more preferred embodiments according to the invention corneal endothelial cells are present in combination with the localising agent. Preferably the corneal endothelial cells are in a monolayer. More preferably the corneal endothelial cells are present at a density greater than 500 cells per mm^2 (area). Particularly preferably the corneal endothelial cells are present at a density of 1000 to 3500 cells/mm^2 (area), more particularly preferably 2000 to about 3000 cells/mm^2 (area).

In further particularly preferred embodiments of the invention the LATS inhibitor inhibits LATS1 or LATS2, or LATS1 and LATS2. In a more particularly preferred embodiments according to the invention the LATS inhibitor inhibits LATS1 and LATS2.

In preferred embodiments according to the invention the compound is selected from the group consisting of 2-methyl-1-(2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propoxy)propan-2-ol; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; (S)-N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)pentan-2-ol; N-isopropyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclobutyl)pyrido[3,4-d]pyrimidin-4-amine; N-isopropyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propan-1-ol; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propoxy)ethan-1-ol; and (R)-N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine.

Also preferably said compound or a salt thereof, is selected from N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine and (S)-N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine. Preferably said compound or a salt thereof, is N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine.

In particularly preferred embodiments according to the invention the compound according to the invention is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

The invention relates in one aspect to a method of transplanting a population of cells to a subject, said method comprising administering the population of cells obtainable or obtained by the method of cell population expansion or method of culturing cells or method of LATS inhibition according to the invention.

The invention further relates to a method of transplanting a population of ocular cells onto the eye of a subject, said method comprising administering the population of cells obtainable or obtained by the method of cell population expansion or method of culturing cells or method of LATS inhibition according to the invention, wherein the cells are ocular cells. Preferably ocular cells are limbal stem cells or corneal endothelial cells. The invention relates in another aspect to a method of transplanting a population of ocular cells onto the cornea of a subject, said method comprising administering the cell delivery preparation according to the invention.

The invention relates in another aspect to a method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject, said method comprising administering the population of cells comprising limbal stem cells obtainable or obtained by the method of cell population expansion or method of culturing cells or method of LATS inhibition according to the invention. The invention relates in another aspect to a method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject, said method comprising administering the cell delivery preparation according to the invention.

The invention relates in another aspect to a method of transplanting a cell population comprising limbal stem cells onto the cornea of a subject, said method comprising expanding a cell population comprising limbal stem cells by culturing said population with cell proliferation medium comprising a LATS inhibitor according to the invention, preferably rinsing the expanded cell population to substantially remove the LATS inhibitor, and administering said cells onto the cornea of said subject. Preferably said cell population is combined with a biomatrix prior to said administration. In a specific embodiment said cell population is combined with a biomatrix which is GelMA prior to said administration. In a another specific embodiment said cell population is combined with fibrin glue prior to said administration. In an embodiment said cell population is combined with a carrier which is a contact lens. In a specific embodiment the cell population comprising limbal stem cells is combined with a biomatrix which is GelMA and the GelMA is polymerized on a carrier which is a contact lens. In another specific embodiment the cell population comprising limbal stem cells is combined with fibrin glue and a contact lens.

The invention relates in one aspect to a method of transplanting a population of corneal endothelial cells onto the cornea of a subject, said method comprising administering the population of corneal endothelial cells obtainable or obtained by the method of cell population expansion or method of culturing cells or method of LATS inhibition according to the invention. The invention relates in another aspect to a method of transplanting a population of corneal endothelial cells onto the cornea of a subject, said method comprising administering the cell delivery preparation according to the invention.

The invention relates in another aspect to a method of transplanting a population of cells comprising corneal endothelial cells onto the cornea of a subject, said method comprising expanding a population of cells comprising corneal endothelial cells by culturing said population with cell proliferation medium comprising a LATS inhibitor according to the invention, rinsing the expanded population of cells to substantially remove the LATS inhibitor, and administering said cells onto the cornea of said subject. Preferably said cells are combined with a biomatrix prior to said administration. In a specific embodiment said cells are combined with a biomatrix which is GelMA prior to said administration. In a more specific embodiment said corneal endothelial cells are combined with a biomatrix which is bioprinted onto the ocular surface. Particularly preferably said corneal endothelial cells are combined with a biomatrix which is GelMA and bioprinted onto the ocular surface by polymerising the GelMA by a light triggered reaction.

The invention relates in another aspect to a method of transplanting a population of cells to the eye of a subject, comprising combining the cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject or applying the mixture onto the surface of the eye of the subject, and bioprinting the cells in or on the eye by guiding and fixing the cells, such as on the cornea, using a light source, such as an Ultraviolet A or white light source. In certain embodiments, the light source produces light of a wavelength that is at least 350 nm. In certain embodiments, the light source produces light in the 350 nm to 420 nm range. For example, an LED light source can be used to produce a light having a wavelength of 365 nm or 405 nm, or any other wavelength above 350 nm, or a mercury lamp with a bandpass filter can be used to produce a light having a wavelength of 350 nm to 700 nm, for example a wavelength of 365 nm or 405 nm. In another embodiment, the light source produces visible, white light having a wavelength, for example, in the 400 nm to 700

nm range. In certain embodiments, the cells are ocular cells, such as corneal cells, for example corneal endothelial cells.

The invention relates in another aspect to a method of transplanting a population of corneal endothelial cells to the eye of a subject, comprising culturing a population of corneal endothelial cells in a cell proliferation medium that comprises a LATS inhibitor, combining the corneal endothelial cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject, and bioprinting the cells in the eye by guiding and fixing the cells on the cornea using a light source, such a UVA or LED or visible light source.

The invention relates in a further aspect to a method of prophylaxis or treatment of an ocular disease or disorder using a LATS inhibitor. Preferably the LATS inhibitor is a compound of Formula A1 or subformulae thereof according to the invention. The invention relates in yet a further aspect to a method of prophylaxis or treatment of an ocular disease or disorder using a compound according to Formula A2 or subformulae thereof according to the invention. In preferred specific embodiments the method of prophylaxis or treatment of an ocular disease or disorder further comprises the method of LATS inhibition in a cell population or the method of cell population expansion according to the invention, wherein said cells are ocular cells. Preferably the method of prophylaxis or treatment of an ocular disease or disorder comprises administering to a subject in need thereof of a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population expansion according to the invention, wherein said cells are ocular cells. In another preferred embodiment the method of prophylaxis or treatment of an ocular disease or disorder comprises administering to a subject in need thereof of a therapeutically effective amount of the cell delivery preparation according to the invention, wherein said cells are ocular cells. In yet another preferred embodiment the method of prophylaxis or treatment of an ocular disease or disorder the method comprises the steps of the method of transplanting a population of cells comprising ocular cells to the eye of a subject according to the invention. Preferably the ocular cells are limbal stem cells or corneal endothelial cells. In yet another preferred embodiment the method of prophylaxis or treatment of an ocular disease or disorder the method comprises the steps of the method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject according to the invention. In an alternatively preferred embodiment the method of prophylaxis or treatment of an ocular

disease or disorder the method comprises the steps of the method of transplanting a population of corneal endothelial cells onto the cornea of a subject according to the invention. In a specific embodiment of the method of prophylaxis or treatment of an ocular disease or disorder according to the invention the cell population obtainable or obtained by the method of cell population expansion according to the invention or cell delivery preparation according to the invention is administered simultaneously or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin, and cefazolin.

In one aspect the invention relates to a YAP (yes associated protein) modulator for use in a method of transplanting a population of cells to a subject, which comprises administering to a subject in need thereof of a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population expansion according to the invention or cell delivery preparation according to the invention. Preferably said YAP modulator is a LATS inhibitor. Preferably the cells are ocular cells.

In one aspect the invention relates to a YAP (yes associated protein) modulator for use in a method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject, which comprises administering to a subject in need thereof of a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population expansion according to the invention or cell delivery preparation according to the invention. Preferably said YAP modulator is a LATS inhibitor.

In another aspect the invention relates to a YAP modulator for use in a method of treating limbal stem cell deficiency, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population expansion according to the invention or cell delivery preparation according to the invention. Preferably said YAP modulator is a LATS inhibitor.

In one aspect the invention relates to a YAP (yes associated protein) modulator for use in a method of transplanting a population of corneal endothelial cells onto the cornea of a subject, which comprises administering to a subject in need thereof of a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population

expansion according to the invention or cell delivery preparation according to the invention. Preferably said YAP modulator is a LATS inhibitor.

In another aspect the invention relates to a YAP modulator for use in a method of treating corneal endothelial dysfunction, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population expansion according to the invention or cell delivery preparation according to the invention. Preferably said YAP modulator is a LATS inhibitor.

In yet another embodiment, the present invention relates to a method of treatment of a disease or disorder comprising administering to a subject in need thereof a cell population, wherein the population has been grown in the presence of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof. Preferably the cells are ocular cells.

In one aspect the invention relates to a compound according to Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof according to the invention for use in therapy or as a medicament. Preferably the compound is for use in an ocular disease or disorder.

In another aspect the invention relates to a LATS inhibitor for use in an ocular disease or disorder, preferably wherein the LATS inhibitor is a compound. Preferably the compound is a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof according to the invention.

In yet another aspect the invention relates to the use of a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof according to the invention in the manufacture of a medicament. In a further aspect the invention relates to the use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof according to the invention in the manufacture of a medicament to treat an ocular disease or disorder.

In preferred specific embodiments of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the use further comprises the method of LATS inhibition in a cell population or the method of cell population expansion according to the invention.

In further preferred specific embodiments of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the use comprises administering to a subject in need thereof a therapeutically effective amount of a cell population obtainable or obtained by the method of cell population expansion according to the invention. In yet further preferred embodiments of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the use comprises administering to a subject in need thereof of a therapeutically effective amount of the cell delivery preparation according to the invention. In one preferred embodiment of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the use comprises the steps of the method of transplanting a population of cells comprising ocular cells onto the cornea of a subject according to the invention. In yet more preferred embodiments of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the use comprises the steps of the method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject according to the invention. In yet more preferred embodiments of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the use comprises the steps of the method of transplanting a population of cells comprising corneal endothelial cells onto the cornea of a subject according to the invention. In specific embodiments of the compound for use according to the invention or LATS inhibitor for use according to the invention or use of the compound in a manufacture of a medicament according to the invention, the cell population obtainable or obtained by the method of cell population expansion according to the invention or cell delivery preparation according to the invention is administered simultaneously or sequentially with an agent or

agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin, and cefazolin.

In preferred embodiments according to the invention, the ocular disease or disorder is associated with limbal stem cell deficiency. In more preferred embodiments the ocular disease or disorder is limbal stem cell deficiency. More preferably the ocular disease or disorder is limbal stem cell deficiency which arises due to an injury or disorder selected from the group consisting of chemical burns, thermal burns, radiation injury, aniridia, sclerocornea, multiple endocrine neoplasia, Stevens Johnson syndrome, ocular cicatricial pemphigoid, collagen vascular diseases; chronic non-auto-immune inflammatory disorders arising from contact lens use, dry eye disease, rosacea, staph marginal, keratitis (including bacterial, fungal & viral keratitis), pterygia or neoplasm, limbal stem cell deficiency arising after multiple eye surgeries, excision of pterygia or neoplasm or cryotherapy; and limbal stem cell deficiency arising as a result of medication toxicity from a medication selected from the group consisting of preservatives (thimerosal, benzalkonium), topical anesthetics, pilocarpine, beta blockers, mitomycin, 5-fluorouracil, silver nitrate, and oral medications causing Stevens Johnson syndrome. Particularly preferably the ocular disease or disorder is limbal stem cell deficiency which arises due to an injury or disorder selected from the group consisting of chemical burns, aniridia, Stevens Johnson Syndrome and contact lens use.

In preferred embodiments according to the invention, the ocular disease or disorder is associated with decreased corneal endothelial cell density. In more preferred embodiments the ocular disease or disorder is corneal endothelial dysfunction. More preferably the ocular disease or disorder is corneal endothelial dysfunction which is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy), corneal transplant failure, posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, X-linked endothelial corneal dystrophy, aniridia, and corneal endothelitis. In a specific embodiment the ocular disease or disorder is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy) and corneal transplant failure.

The invention further relates to methods of promoting wound healing, particularly for treating or ameliorating the symptoms of burns, acute and chronic skin ulcers, comprising administering to a subject in need thereof an effective amount of a LATS inhibitor.

Within certain other aspects, the invention relates to a method of promoting wound healing comprising administering a therapeutically effective amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt, or a stereoisomer thereof.

In another aspect, the invention relates to compounds and compositions that may be used in promoting wound healing. In another aspect, the invention relates to compounds and compositions that may be used for the manufacture of a medicament for promoting wound healing.

The present invention also relates to a method of promoting wound healing, particularly for treating or ameliorating the symptoms of burns, acute skin ulcers, and chronic skin ulcers, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula A1 or subformulae thereof, and optionally with a second therapeutic agent that is another compound of the invention or one other type of therapeutic agent.

The present invention also relates to a method of promoting ocular wound healing comprising administering to an eye of a subject a therapeutically effective amount of a compound of the invention. In one embodiment, the ocular wound is a corneal wound. In other embodiments, the ocular wound is an injury or surgical wound.

In another aspect, the invention relates to compounds and compositions that may be used in liver regeneration and liver regrowth. Within certain other aspects, the invention relates to a method of promoting liver regeneration and liver regrowth comprising administering a therapeutically effective amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof. In another aspect, the invention relates to compounds and compositions that may be used for the manufacture of a medicament for liver regeneration and liver regrowth.

The present invention also relates to a method for liver regeneration and liver regrowth, particularly for treatment of insufficient liver regrowth following transplantation of marginal

grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of the present invention and optionally with a second therapeutic agent that is another compound of the invention or one other type of therapeutic agent.

In certain embodiments, the invention relates to a method of generating cellular material for cell therapy and/or transplantation comprising the ex-vivo use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof. The cellular material may comprise ocular, liver or skin cells.

Furthermore, in certain embodiments, the invention relates to a method of promoting liver regeneration and liver regrowth comprising the ex-vivo use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof.

The present invention also relates to an ex-vivo method for liver cell population expansion, comprising use of a compound of the present invention or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof.

Other features and advantages of the present invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: LATS inhibitors (compound ex. 49 and ex. 133) induce YAP dephosphorylation in LSCs within one hour of treatment as shown by Western blot.

Figure 2: Immunolabelling of p63-alpha in limbal stem cell cultures indicates that the LSC population can be expanded when it is maintained in medium comprising the LATS inhibitors (compound ex. 49 and ex. 133). **Figure 2A**: In the presence of growth medium and DMSO, only a few isolated cells attach to the culture dish and survive up to 6 days. Most cells expressed the human nuclear marker, but few expressed p63alpha. **Figures 2B** and **2C:** In contrast, in the presence of LATS inhibitors: compound example no. 49 and example no. 133, the cells formed colonies and expressed p63alpha. This result indicated that the LATS inhibitors promote the expansion of the population of cells with the p63alpha-positive phenotype. **Figure 2D:** Passaging cells and culturing them in the presence of LATS inhibitor compound example no. 49 for two weeks enabled cell population expansion and the formation of confluent cultures expressing p63alpha.

Figure 3: LATS1 and LATS2 knockdown by siRNA activates LSC proliferation in culture as shown by percentage of EdU positive cells.

Figure 4: Immunolabelling of LSC markers DeltaN-p63 alpha/beta/gamma, ABCG2 and C/EBP delta indicates that LSCs maintained in a culture medium containing the LATS inhibitors (compound ex. 49 and ex. 133) express typical markers of LSCs. Results indicate that cells cultured in DMSO do not typically express markers such as DeltaN-p63 alpha/beta/gamma, ABCG2 and C/EBP delta, normally expressed by LSCs. In contrast, cells cultured in the presence of LATS inhibitors express markers such as DeltaN-p63 alpha/beta/gamma, ABCG2 and C/EBP delta, normally expressed by LSCs.

Figure 5: Immunolabelling of undifferentiated LSC marker p63alpha and corneal epithelium cell marker keratin 12 shows that LSC populations expanded using a culture medium comprising the LATS inhibitors (**Fig 5A**: compound ex. 49, **Fig 5B:** compound ex. 47, **Fig 5C:** compound ex. 12, **Fig 5D:** compound ex. 261) can differentiate into corneal epithelium cells when transferred to conditions that enable differentiation. Shown are views where the transition from the p63alpha-positive cell identity to the keratin-12 identity are occurring.

Figure 6: LSCs were labeled using a fluorescent protein in order to confirm that LSCs attached to a contact lens using GelMA polymerization can be delivered to the surface of the rabbit eye ex vivo. Arrows show site of attachment of LSCs.

Figure 7: **Fig 7A**: Transplanted eye, keratin-12 staining; **Fig. 7B:** Transplanted eye, keratin-19 staining; **Fig. 7C**: non-transplanted control eye, keratin-12 staining; **Fig 7D:** non-transplanted control eye, keratin- 19 staining. These figures show that in a rabbit model of limbal stem cell deficiency, a population of LSCs expanded in medium comprising compound example no. 12, combined with GelMA and delivered via a contact lens in vivo to the rabbit's corneal surface, lead to regeneration of a keratin-12-positive corneal epithelium (**Fig. 7A**) and prevented conjunctivalization by keratin-19-positive conjunctival cells in the transplanted eye. **Fig. 7B:** Arrow is pointing to absence of keratin 19 staining. In contrast, non-transplanted rabbit eyes showed absence of keratin-12-positive corneal epithelium restoration. **Fig. 7C:** Arrow is pointing to absence of keratin-12 staining. Instead, signs of conjunctivalization were observed, as showed by the presence of keratin-19 staining. **Fig. 7D**: Arrow is pointing to areas of positive keratin-19 staining.

Figure 8: Fig. 8A: Rabbit eye transplanted with human LSCs; **Fig. 8B:** Control rabbit eye, non-transplanted with human LSCs. These Figures show that the cells that restored the corneal epithelium of transplanted eyes were human (**Fig. 8A**), as demonstrated by the presence of human mitochondrial protein (arrow shows the human mitochondrial marker is present). In contrast, the ocular surface of non-transplanted eyes did not exhibit human mitochondrial protein staining (**Fig. 8B:** human mitochondrial marker is absent).

Figure 9: Microscopic images of LSCs delivered via TISSEEL to collagen coated 24 well plate show repopulation of the cells to cover the culture surface within 2 weeks.

Figure 10: CellTracker Green CMFDA labeled LSCs were delivered to human cornea ex vivo and covered with protective contact lens.

Figure 11: Red and green dye labeled HEK-293 cells were bioprinted into a Yin-Yang pattern on top of a rabbit cornea ex vivo (shown as dark and light gray pattern).

Figure 12: Reducing immune rejection by CRISPR/Cas9-mediated deletion of the beta-2-microglobulin (B2M) gene in LSCs: FACS analyses show that CRISPR-mediated deletion of B2M and subsequent elimination of HLA A, B and C occurred in 21 percent of the LSCs.

Figure 13: The population of B2M-negative/HLA A,B,C-negative LSCs was expanded using compound example no. 48a to produce a cell preparation where 97 percent of the cells do not express HLA A, B, C.

Figure 14: LATS inhibitors (compounds ex. 133 and ex. 49) induce translocation of YAP into the nucleus in Corneal Endothelial Cells (CECs).

Figure 15: LATS inhibitors (compound ex. 133 and ex. 49) induce YAP dephosphorylation in CECs within one hour of treatment. As shown in Fig. 15a by Western blot; Fig. 15b: graph showing phosphorylated YAP levels normalized to beta-actin; and Fig.15c: graph showing phosphorylated YAP levels normalized to total YAP.

Figure 16: CECs grown in presence or absence of LATS inhibitors. An Incucyte system (Essen Biosciences) was used to measure CEC confluence by real-time quantitative live-cell analysis over a time course. Compound ex. 49 (black squares) and ex. 133 (light grey squares) induced strong CEC proliferation; whereas CEC proliferation was minimal in the vehicle (DMSO, dark grey squares).

Figure 17: LATS1 and LATS2 knockdown by siRNA activates corneal endothelial cell proliferation in culture as shown by percentage of EdU positive cells.

Figure 18: Zonula Occludens-1 (ZO-1) immunolabelling indicates that CECs proliferated in the presence of the LATS inhibitor, compound ex. 49, (Fig. 18b) form tight junctions, an endothelial structure and retain a normal cell size and morphology characteristic of functional CECs. CECs proliferated in the presence of the vehicle alone (DMSO) show signs of polymegatism characteristic of dysfunctional CECs (Fig. 18a).

Figure 19: Quantitative RT-PCR analysis indicates that a corneal endothelial cell population expanded with the LATS inhibitor, compound ex. 49, express genes normally expressed by corneal endothelial cells in vivo, including Collagen 8a2, AQP1, SLc4A11. The cells do not express markers of other epithelia present in the eye, including RPE65 (a marker of retinal pigmented epithelium) and CD31 (a marker of vascular epithelium).

Figure 20: Immunohistochemical analysis indicates that a corneal endothelial cell population expanded with the LATS inhibitor, compound ex. 49, express genes normally expressed by corneal endothelial cells in vivo, including Na/K ATPase (Fig. 20a) and Collagen 8a2 (Fig. 20b).

Figure 21: FACS analysis of the corneal endothlelium cell population expanded in the presence of the LATS inhibitor, compound ex. 47, and CECs cultured in the absence of a LATS inhibitor. The cell population expanded in the presence of the LATS inhibitor expresses low levels of CD73 (Fig. 21a, grey line), CD44 (Fig. 21b, grey line), CD166 (Fig. 21c, grey line) and CD105 (Fig. 21d, grey line); while the cell population cultured without the LATS inhibitor expresses high levels of CD44, CD73, CD105 and CD166 (black lines).

Figure 22: Bubble depression method depicting method 1 as described further under the "bio-printing section", in which a biomatrix is applied to an eye to deliver a cell preparation according to the invention. **Fig. 22a**. Inject bolus of biomatrix; **Fig. 22b** Inject bubble beneath biomatrix to spread over cornea; **Fig. 22c** Cure biomatrix with UV or blue light source; **Fig. 22d** Remove bubble and replace with balanced salt solution.

Figure 23: Subtractive method using femtosecond (FS) laser, depicting method 2 as described further under the "bio-printing section", in which a biomatrix is applied to an eye to deliver a cell preparation according to the invention. **Fig. 23a** Remove dysfunctional endothelium with FS; **Fig. 23b** Inject biomatrix in anterior chamber; **Fig. 23c** Cure biomatrix with UV or blue light source**; Fig. 23d** Detach unwanted biomatrix with FS; **Fig. 23e** Remove detached biomatrix with forceps.

Figure 24: Dye mask method, depicting method 3 as described further under the "bio-printing section", in which a biomatrix is applied to an eye to deliver a cell preparation according to the invention. **Fig. 24a** Stain the endothelium with dye (e.g. Typan Blue); **Fig. 24b** Peel the dysfunctional endothelium; **Fig. 24c** Inject dyed biomatrix; **Fig. 24d** Cure biomatrix with UV or blue light source; **Fig. 24e** Flush uncured biomatrix.

Figure 25: Dry dispense method, depicting method 4 as described further under the "bio-printing section", in which a biomatrix is applied to an eye to deliver a cell preparation according to the invention. **Fig. 25a** Drain anterior chamber; **Fig. 25b** Dispense biomatrix to

posterior cornea with soft tip / brush cannula; **Fig. 25c** Cure biomatrix with UV or blue light source; **Fig. 25d** Refill eye with balanced salt solution.

Figure 26 Schematic showing bioprinting device as described further in Example C11.

Figure 27 Results showing cells can be bioprinted on the posterior side of the cornea by using a handheld device that projects 365 nm UVA light through the cornea. After unpolymerized and unattached material was rinsed, a circular pattern of fluorescent protein-labelled cells was retained on the posterior side of the cornea.

Figure 28 CECs bioprinted on the posterior side of the cornea can rebuild a corneal endothelium in a rabbit model of corneal endothelium dystrophy. Results indicated that in experimental rabbits, the corneal endothelium structure can be detected using ZO-1 immunohistochemistry (Fig. 28A). In the right eye of a rabbit where the corneal endothelium was surgically removed and no CEC was bioprinted, the ZO-1 staining is absent, indicating an absence of normal corneal endothelium structure (Fig. 28B). In the right eye of a rabbit where the corneal endothelium was surgically removed and CEC were bioprinted, the ZO-1 staining is present, indicating that a corneal endothelium structure has been rebuilt (Fig. 28C). Fig. 28D and Fig. 28E show that human nuclear antigen immunostaining is absent in eyes that did not receive any human CECs. In contrast, human nuclear antigen-positive cells cover the imaged field in eyes where human CECs were bioprinted (Fig.28F), indicating that the ZO-1-labeled corneal endothelium structure shown in Fig. 28C is composed of the human CECs bioprinted on the posterior side of the rabbit cornea.

Figure 29: Red-fluorescent-protein labeled HEK-293 cells were bioprinted into constructs of different letters on the posterior side of human cornea ex vivo.

Figure 30: Vector map shows the design of the AAV2 vector used to express the CRISPR system in LSCs and CECs. (Figure 30 discloses SEQ ID NO: 28).

Figure 31: FACS analysis of AAV-mediated expression of the CRISPR system enabled deletion of B2M and subsequent elimination of HLA A, B and C in LSCs.

Figure 32: FACS analysis of AAV-mediated expression of the CRISPR system enabled deletion of B2M and subsequent elimination of HLA A, B and C in CECs.

Figure 33A: Fig. 33A is a western blot of pYAP in lysate of human HaCaT cells that were untreated or treated by 40 pM each of siRNA against MST1/2 or LATS1/2; actin was used as control.

Figure 33B: Fig. 33B is a western blot of pYAP in lysate of human HaCaT cells that were untreated or treated by 9 μM of Example 133; actin was used as control.

Figure 33C: Fig. 33C is a graph of the relative inhibitory activity against LATS1 versus concentration of Example 133 ranging from ~10^{-4} to 1 μm. The calculated IC_{50} of Example 133 against LATS1 was 1.3 nM.

Figure 34: is a bar graph of relative *Cyr61*/*Gapdh* expression levels versus concentration of Example 133 at 0, 0.2 and 2 mg/mL.

Figure 35A: Fig. 35A shows micrographs of mouse skin treated topically with vehicle or Example 133.

Figure 35B: Fig. 35B is a scatter plot comparing the percentage of Ki67+ cells in mouse skin treated with vehicle or Example 133.

DETAILED DESCRIPTION OF THE INVENTION

LATS

LATS is the abbreviated name of the large tumor suppressor kinase. LATS as used herein refers to LATS1 and/or LATS2. LATS1 as used herein refers to the large tumor suppressor kinase 1 and LATS2 refers to the large tumor suppressor kinase 2. LATS1 and LATS2 both have serine/threonine protein kinase activity. LATS1 and LATS2 have been given the Human Genome Organisation (HUGO) Gene Nomenclature Committee identifiers: HGNC ID 6514 and HGNC ID 6515 respectively. LATS1 is sometimes also referred to in the art as WARTS or wts, and LATS2 is sometimes referred to in the art as KPM. Representative LATS sequences, include, but are not limited to, the protein sequences available from the

National Center for Biotechnology Information protein database with the accession numbers NP_004681.1 (LATS1) and NP_001257448.1 (LATS1) and NP_055387.2 (LATS 2), as shown below.

LATS1 : NP_004681.1 (Serine/threonine–protein kinase LATS1 isoform 1, homo sapiens)(SEQ ID NO: 1:)

1 mkrsekpegy rqmrpktfpa snytvssrqm lqeireslrn lskpsdaaka ehnmskmste
61 dprqvrnppk fgthhkalqe irnsllpfan etnssrstse vnpqmlqdlq aagfdedmvi
121 qalqktnnrs ieaaiefisk msyqdprreq maaaaarpin asmkpgnvqq svnrkqswkg
181 skeslvpqrh gpplgesvay hsespnsqtd vgrplsgsgi safvqahpsn gqrvnppppp
241 qvrsvtpppp prgqtpprg ttppppswep nsqtkrysgn meyvisrisp vppgawqegy
301 pppplntspm nppnqgqrgi ssvpvgrqpi imqssskfnf psgrpgmqng tgqtdfmihq
361 nvvpagtvnr qppppyplta angqspsalq tggsaapssy tngsipqsmm vpnrnshnme
421 lynisvpglq tnwpqsssap aqsspssghe iptwqpnipv rsnsfnnplg nrashsansq
481 psattvtait papiqqpvks mrvlkpelqt alapthpswi pqpiqtvqps pfpegtasnv
541 tvmppvaeap nyqgpppppyp khllhqnpsv ppyesiskps kedqpslpke deseksyenv
601 dsgdkekkqi ttspitvrkn kkdeerresr iqsyspqafk ffmeqhvenv lkshqqrlhr
661 kkqlenemmr vglsqdaqdq mrkmlcqkes nyirlkrakm dksmfvkikt lgigafgevc
721 larkvdtkal yatktlrkkd vllrnqvahv kaerdilaea dnewvvrlyy sfqdkdnlyf
781 vmdyipggdm msllirmgif peslarfyia eltcavesvh kmgfihrdik pdnilidrdg
841 hikltdfglc tgfrwthdsk yyqsgdhprq dsmdfsnewg dpsscrcgdr lkplerraar
901 qhqrclahsl vgtpnyiape vllrtgytql cdwwsvgvil femlvgqppf laqtpletqm
961 kvinwqtslh ippqaklspe asdliiklcr gpedrlgkng adeikahpff ktidfssdlr
1021 qqsasyipki thptdtsnfd pvdpdklwsd dneeenvndt lngwykngkh pehafyeftf
1081 rrffddngyp ynypkpieye yinsqgseqq sdeddqntgs eiknrdlvyv

LATS1 : serine/threonine-protein kinase LATS1 isoform 2 [Homo sapiens]
NCBI Reference Sequence: NP_001257448.1 (SEQ ID NO: 2:)

1 mkrsekpegy rqmrpktfpa snytvssrqm lqeireslrn lskpsdaaka ehnmskmste
61 dprqvrnppk fgthhkalqe irnsllpfan etnssrstse vnpqmlqdlq aagfdedmvi
121 qalqktnnrs ieaaiefisk msyqdprreq maaaaarpin asmkpgnvqq svnrkqswkg
181 skeslvpqrh gpplgesvay hsespnsqtd vgrplsgsgi safvqahpsn gqrvnppppp
241 qvrsvtpppp prgqtpprg ttppppswep nsqtkrysgn meyvisrisp vppgawqegy
301 pppplntspm nppnqgqrgi ssvpvgrqpi imqssskfnf psgrpgmqng tgqtdfmihq

361 nvvpagtvnr qppppyplta angqspsalq tggsaapssy tngsipqsmm vpnrnshnme
421 lynisvpglq tnwpqsssap aqsspssghe iptwqpnipv rsnsfnnplg nrashsansq
481 psattvtait papiqqpvks mrvlkpelqt alapthpswi pqpiqtvqps pfpegtasnv
541 tvmppvaeap nyqgpppppyp khllhqnpsv ppyesiskps kedqpslpke deseksyenv
601 dsgdkekkqi ttspitvrkn kkdeerresr iqsyspqafk ffmeqhvenv lkshqqrlhr
661 kkqlenemmr vkpfkmsifi lnhlfawclf

LATS 2: NP_055387.2 serine/threonine-protein kinase LATS2 [Homo sapiens]. ((SEQ ID NO: 3:)

1 mrpktfpatt ysgnsrqrlq eireglkqps kssvqglpag pnsdtsldak vlgskdatrq
61 qqqmratpkf gpyqkalrei rysllpfane sgtsaaaevn rqmlqelvna gcdqemagra
121 lkqtgsrsie aaleyiskmg yldprneqiv rvikqtspgk glmptpvtrr psfegtgdsf
181 asyhqlsgtp yegpsfgadg ptaleemprp yvdylfpgvg phgpghqhqh ppkgygasve
241 aagahfplqg ahygrphllv pgeplgygvq rspsfqsktp petggyaslp tkgqgggppga
301 glafpppaag lyvphphhkq agpaahqlhv lgsrsqvfas dsppqslltp srnslnvdly
361 elgstsvqqw paatlarrds lqkpgleapp rahvafrpdc pvpsrtnsfn shqprpgppg
421 kaepslpapn tvtavtaahi lhpvksvrvl rpepqtavgp shpawvpapa papapapapa
481 aegldakeeh alalggagaf pldveyggpd rrcppppypk hlllrskseq ydldslcagm
541 eqslragpne peggdksrks akgdkggkdk kqiqtspvpv rknsrdeekr esriksyspy
601 afkffmeqhv enviktyqqk vnrrlqleqe makaglceae qeqmrkilyq kesnynrlkr
661 akmdksmfvk iktlgigafg evclackvdt halyamktlr kkdvlnrnqv ahvkaerdil
721 aeadnewvvk lyysfqdkds lyfvmdyipg gdmmsllirm evfpehlarf yiaeltlaie
781 svhkmgfihr dikpdnilid ldghikltdf glctgfrwth nskyyqkgsh vrqdsmepsd
841 lwddvsncrc gdrlktleqr arkqhqrcla hslvgtpnyi apevllrkgy tqlcdwwsvg
901 vilfemlvgq ppflaptpte tqlkvinwen tlhipaqvkl speardlitk lccsadhrlg
961 rngaddlkah pffsaidfss dirkqpapyv ptishpmdts nfdpvdeesp wndasegstk
1021 awdtltspnn khpehafyef tfrrffddng ypfrcpkpsg aeasqaessd lessdlvdqt
1081 egcqpvyv

LATS is thought to negatively regulate YAP1 activity. “YAP1” refers to the yes-associated protein 1, also known as YAP or YAP65, which is a protein that acts as a transcriptional regulator of genes involved in cell proliferation. LATS kinases are serine/threonine protein kinases that have been shown to directly phosphorylate YAP which results in its cytoplasmic retention and inactivation. Without phosphorylation by LATS, YAP translocates into the

nucleus, forming a complex with a DNA binding protein, TEAD, and results in downstream gene expression. (Barry ER & Camargo FD (2013) The Hippo superhighway: signaling crossroads converging on the Hippo/Yap pathway in stem cells and development. Current opinion in cell biology 25(2):247-253.; Mo JS, Park HW, & Guan KL (2014) The Hippo signaling pathway in stem cell biology and cancer. EMBO reports 15(6):642-656; Pan D (2010) The hippo signaling pathway in development and cancer. Developmental cell 19(4):491-505.)

The Hippo/YAP pathway is involved in numerous cell types and tissues in mammalian systems, including various cancers. In particular, the Hippo pathway is evidently involved in the intestine, stomach and esophagus, pancreas, salivary gland, skin, mammary gland, ovary, prostate, brain and nervous system, bone, chrondrocytes, adipose cells, myocytes, T lymphocytes, B lymphocytes, myeloid cells, kidney, and lung. See Nishio et al., 2017, *Genes to Cells* 22:6-31.

LATS1 and LATS2 Inhibition

Compounds of Formula A1 or subformulae thereof, in free form or in salt form are potent inhibitors of LATS1 and/or LATS2.

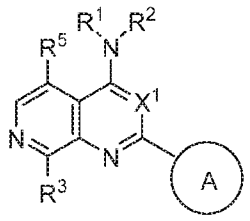
In a preferred embodiment the compounds of Formula A2 or subformulae thereof, in free form or in salt form are potent inhibitors of LATS1 and LATS2.

The inhibition efficacy of the compounds against LATS1 were assayed by the LATS1 Biochemical HTRF Assay as described in Example A1 below. The inhibition efficacy of the compounds of the invention against LATS1 (LATS1 IC_{50} in micromolar) in this assay are reported in Table 1A. It should be noted that compounds with IC_{50} greater than 1 micromolar are considered inactive in this assay.

The inhibition efficacy of selected compounds against LATS2 were assayed by the LATS2 Biochemical Caliper Assay as described in Example A3 below. The inhibition efficacy of the compounds of the invention against LATS2 (LATS2 IC_{50} in micromolar) in this assay are also reported in Table 1A. It should be noted that compounds with IC_{50} greater than 1 micromolar are considered inactive in this assay.

LATS Inhibitors

The invention therefore relates to a compound of Formula A2:



A2

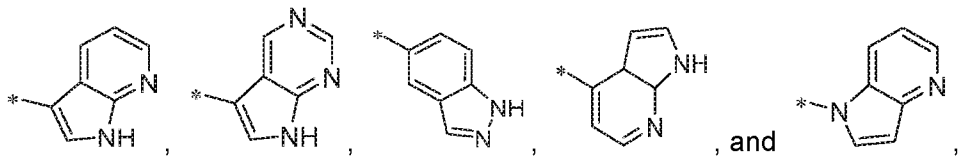
or a salt, or stereoisomer thereof, wherein

X^1 is CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

a 9-membered fused bicyclic heteroaryl that is selected from



, and ,

wherein "*" represents the point of attachment of ring A to the remainder of the molecule; and

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$haloalkyl, $-NH_2$, $C_{1\text{-}6}$alkylamino, di-($C_{1\text{-}6}$alkyl)amino, $C_{3\text{-}6}$cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or $C_{1\text{-}6}$alkoxy;

R^1 is hydrogen or $C_{1\text{-}6}$alkyl;

R^2 is selected from

(a) $C_{1\text{-}8}$alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) -OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -$C(O)R^0$;

(viii) -$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;

(ix) -$C(O)R^8$, wherein R^8 is R^0 or -NH-C_{1-6}alkyl-$C(O)R^0$;

(x) -$S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

or, provided that when X^1 is CH, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as

ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0;

with the proviso that:

(1) when X^1 is N, ring A is 4-primidinyl or 3-fluoro-4-primidinyl, R^1 is H or methyl, R^3 is H or Cl and R^5 is H; then R^2 is not C_{2-4}alkyl that is substituted with a substituent selected from $–NH_2$, C_{1-6}alkylamino or *t*-butyl-carbamoyl-amino and that is optionally further substituted with unsubstituted phenyl; and

(2) when X^1 is N, ring A is indazol-5-yl, R^1, R^3 and R^5 are H; then R^2 is not C_4alkyl that is substituted with $–NH_2$.

Unless specified otherwise, the term “compounds of the present invention” refers to compounds of Formula A2 or subformulae thereof, or salts thereof, as well as all stereoisomers (including diastereoisomers and enantiomers), rotamers, tautomers and isotopically labeled compounds (including deuterium substitutions), as well as inherently formed moieties.

Various (enumerated) embodiments of the invention are described herein. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention. When an embodiment is described as being “according to” a previous embodiment, the previous embodiment includes sub-embodiments thereof, for example such that when Embodiment 20 is described as being “according to” embodiments 1 to 19, embodiments 1 to 19 includes embodiments 19 and 19A.

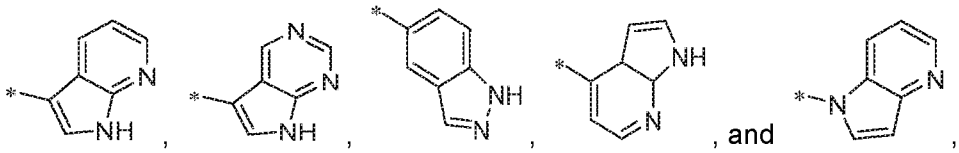
Embodiment 1. A compound of Formula A2 or a salt thereof, as described above.

Embodiment 2. A compound of Formula A2 according to embodiment 1, or a salt thereof, wherein

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 2 heteroatoms that are selected from N, provided that at least one of the nitrogen atom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

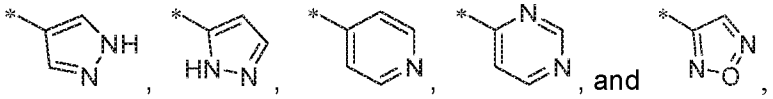
(b) a 9-membered fused bicyclic heteroaryl that is selected from

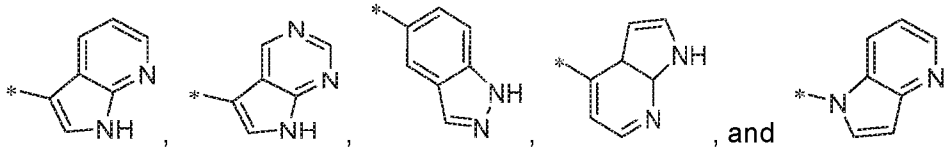


wherein "*" represents the point of attachment of ring A to the remainder of the molecule; and

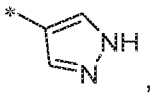
wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, -NH_2 and C_{3-6}cycloalkyl.

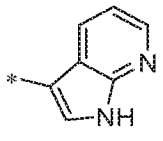
Embodiment 3. A compound of Formula A2, or a salt thereof, according to embodiment 1, wherein ring A is selected

from  , which are each unsubstituted or substituted by 1 to 2 substituents independently selected from cyano, halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, NH_2, and C_{3-6}cycloalkyl;

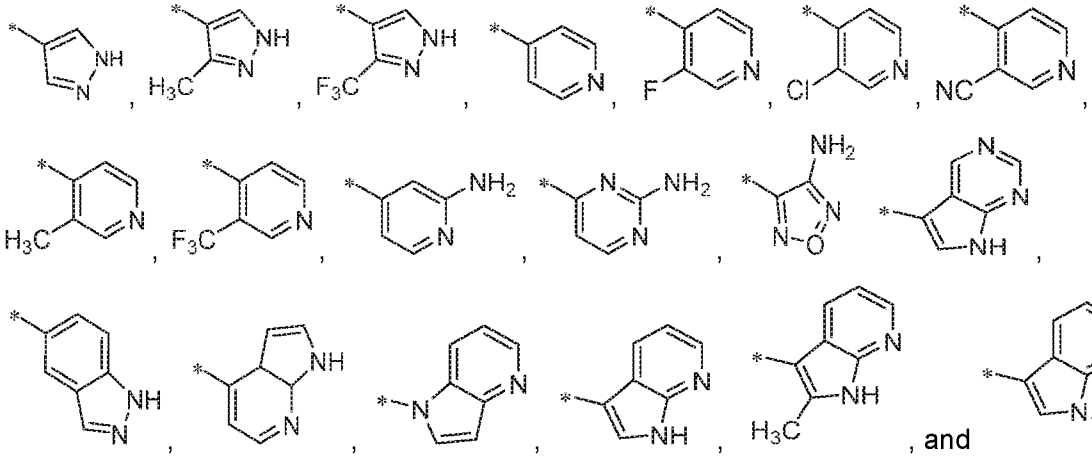
or from  , which are each unsubstituted or substituted by C_{1-6}alkyl.

Embodiment 4. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 3, wherein ring A is selected

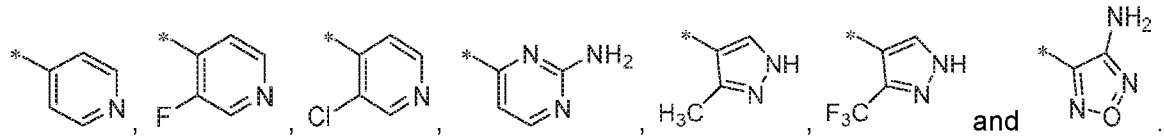
from  , which are each unsubstituted or substituted by a substituent selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, and -NH_2;

or is  , which is unsubstituted or substituted by C_{1-6}alkyl.

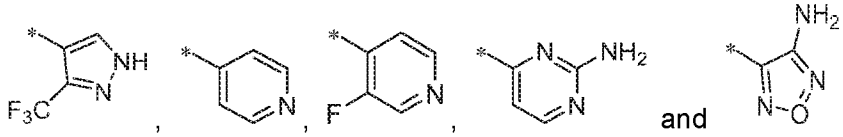
Embodiment 5. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 3, wherein ring A is selected from

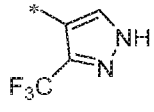


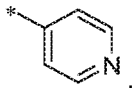
Embodiment 6. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 5, wherein ring A is selected from

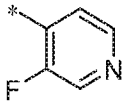


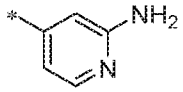
Embodiment 7. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 6, wherein ring A is selected from

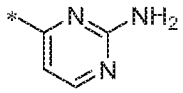


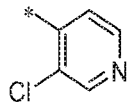
Embodiment 8. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 7, wherein ring A is  .

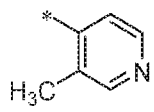
Embodiment 9. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 7, wherein ring A is  .

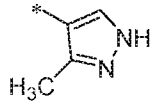
Embodiment 10. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 7, wherein ring A is  .

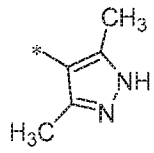
Embodiment 11. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 7, wherein ring A is  .

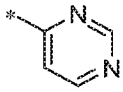
Embodiment 12. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 7, wherein ring A is  .

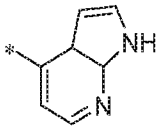
Embodiment 13. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 5, wherein ring A is  .

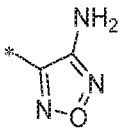
Embodiment 14. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 5, wherein ring A is  .

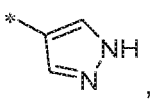
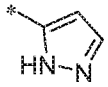
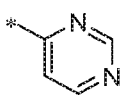
Embodiment 15. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 6, wherein ring A is  .

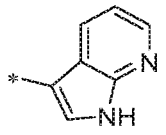
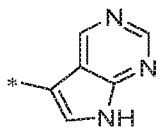
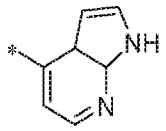
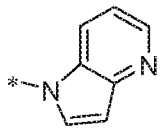
Embodiment 16. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 3, wherein ring A is  .

Embodiment 17. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 4, wherein ring A is  .

Embodiment 18. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 5, wherein ring A is  .

Embodiment 18A. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 5, wherein ring A is  .

Embodiment 19. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 3, wherein ring A is selected from  ,  , and  , which are each unsubstituted or substituted by 1 to 2 substituents independently selected from cyano, halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, NH_2, and C_{3-6}cycloalkyl;

or from  ,  ,  , and  , which are each unsubstituted or substituted by C_{1-6}alkyl.

Embodiment 20. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 19, wherein R^1 is selected from hydrogen, methyl and ethyl.

Embodiment 21. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 20, wherein R^1 is methyl.

Embodiment 22. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 20, wherein R^1 is hydrogen.

Embodiment 23. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 22, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(vi) $-C(O)R^8$, wherein R^8 is R^0;

(vii) $-S(O)_2C_{1-6}$alkyl;

(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}alkyl, hydroxyC_{1-6}alkyl and R^0;

(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O and that is unsubstituted or substituted by C_{1-6}alkyl; and

(x) phenyl that is unsubstituted or substituted by halogen;

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, -C(O)R^0, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –C(O)R^0; and

(c) 4-membered heterocycloalkyl comprising, as ring member, a heteroatom selected from N and O and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, -C(O)R^0, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –C(O)R^0.

Embodiment 24. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 22, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) -OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -C(O)R^0;

(v) -N$R^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -C(O)R^0, C_{1-6}alkyl that is unsubstituted or substituted by –C(O)R^0;

(vi) -C(O)R^8, wherein R^8 is R^0;

(vii) -$S(O)_2C_{1-6}$alkyl;

(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}alkyl, hydroxyC_{1-6}alkyl and R^0;

(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O and that is unsubstituted or substituted by C_{1-6}alkyl; and

(x) phenyl that is unsubstituted or substituted by halogen;

and wherein the C atom of the C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents (i) to (x) that is the point of attachment of R^2 to the remainder of the molecule is not a –CH_2- group;

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, -C(O)R^0, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –C(O)R^0; and

(c) 4-membered heterocycloalkyl comprising, as ring member, a heteroatom selected from N and O and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, -C(O)R^0, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –C(O)R^0.

Embodiment 25. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 23, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) -OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl or -C(O)H;

(v) -$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -C(O)-C_{1-6}alkoxy, and C_{1-6}alkyl that is unsubstituted or substituted by –C(O)OH; and

(vi) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by hydroxyl; and

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl or –C(O)-C_{1-6}alkoxy.

Embodiment 26. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 25, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from

(i) C_{1-6}haloalkyl;

(ii) -OR^6, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl; and

(iii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by hydroxyl; and

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 27. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26, wherein R^2 is C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituent independently selected from C_{1-6}haloalkyl and -OR^6, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 28. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, wherein R^2 is C_{1-8}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 29. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by C_{1-6}haloalkyl.

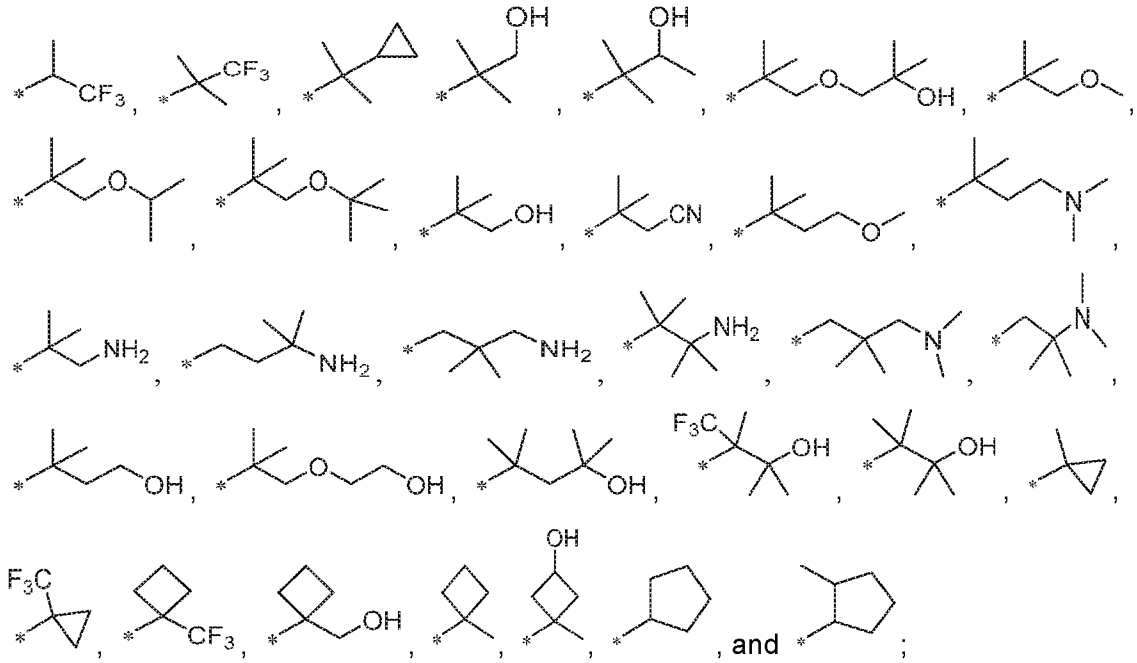
Embodiment 30. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by –O-C_{1-6}alkyl-OH.

Embodiment 31. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26, wherein R^2 is C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}haloalkyl, C_{1-6}alkylamino, R^0, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 32. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26 and 31, wherein R^2 is unsubstituted C_{3-6}cycloalkyl.

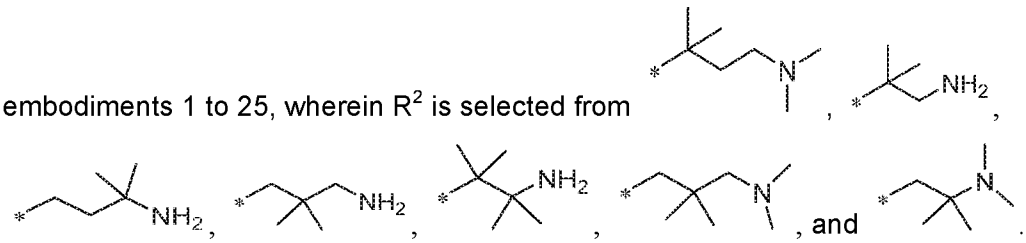
Embodiment 33. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26 and 31, wherein R^2 is C_{3-6}cycloalkyl that is substituted by C_{1-6}alkyl or C_{1-6}haloalkyl.

Embodiment 34. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 25, wherein R^2 is selected from isopropyl, *s*-butyl, *t*-butyl, 2-methyl-but-2-yl, 2,4,4-trimethylpentan-2-yl,

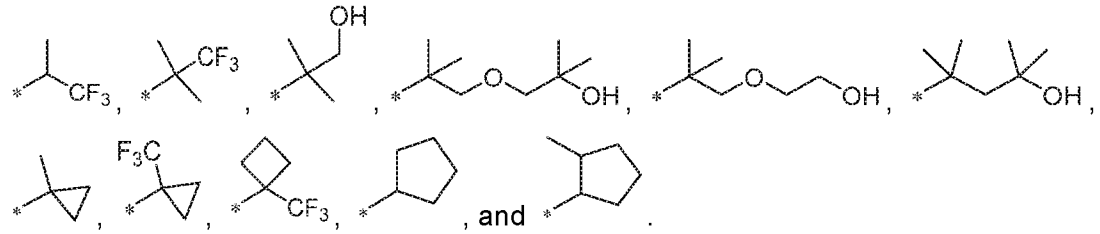


wherein "*" represents the point of attachment of R^2 to the remainder of the molecule.

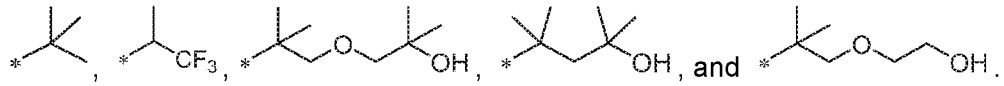
Embodiment 34A. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 25, wherein R^2 is selected from



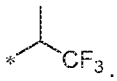
Embodiment 35. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 23 and 25 to 27, wherein R^2 is selected from n-propyl, isopropyl, *t*-butyl,



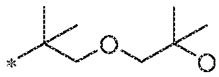
Embodiment 36. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, 34 and 35, wherein R^2 is selected from



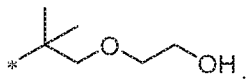
Embodiment 37. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, 29, and 34 to 36, wherein R^2 is



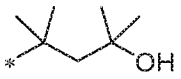
Embodiment 38. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, 30, and 34 to 36 wherein R^2 is



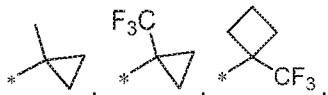
or

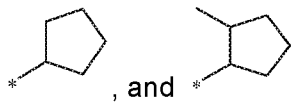


Embodiment 39. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 27, 31, and 34 to 36 wherein R^2 is

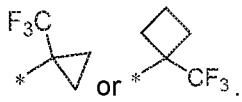


Embodiment 40. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26, 31, 34 and 35 wherein R^2 is selected from

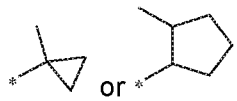


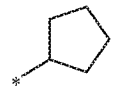


Embodiment 41. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26, 31, 33 to 35 and 40, wherein R^2 is selected from



Embodiment 42. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26, 31, 33 to 35 and 40, wherein R^2 is



Embodiment 43. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 26, 31, 32, 34, 35 and 40 wherein R^2 is  .

Embodiment 44. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 23, 25 to 30, and 35 wherein R^2 is selected from n-propyl, isopropyl and *t*-butyl.

Embodiment 45. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 23, 25 to 30, 35, and 44 wherein R^2 is n-propyl.

Embodiment 46. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 30, 35, and 44, wherein R^2 is isopropyl.

Embodiment 47. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 30, 35, and 44, wherein R^2 is *t*-butyl.

Embodiment 48. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 19, wherein

X^1 is CH; and

R^1 and R^2 taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$haloalkyl, and R^0.

Embodiment 49. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 19, and 48 wherein

X^1 is CH; and

R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 5- or 6-membered heterocycloalkyl that can include, as ring member, 1 to 2 additional heteroatom selected from N, O and S, wherein the 5- or 6-membered heterocycloalkyl

formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl, C_{1-4}alkyl and C_{1-4}haloalkyl.

Embodiment 50. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 19, 48 and 49 wherein

X^1 is CH; and

R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 6-membered heterocycloalkyl that can include, as ring member, an additional heteroatom selected from N and O, wherein the 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl, C_{1-6}alkyl and C_{1-6}haloalkyl.

Embodiment 51. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 19, and 48 to 50 wherein

X^1 is CH; and

R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 6-membered heterocycloalkyl selected from piperidinyl, piperazinyl and morpholinyl, wherein the piperidinyl, piperazinyl or morpholinyl is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl and C_{1-6}alkyl.

Embodiment 52. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 51, wherein R^3 is selected from hydrogen, chloro and methyl.

Embodiment 53. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 52, wherein R^3 is hydrogen.

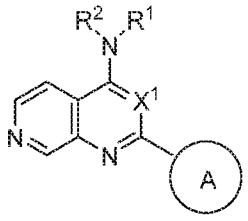
Embodiment 53A. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 52, wherein R^3 is chloro.

Embodiment 53B. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 52, wherein R^3 is methyl.

Embodiment 54. A compound of the Formula A2, or a salt thereof, according to any one of embodiments 1 to 53, wherein R^5 is selected from hydrogen and chloro.

Embodiment 55. A compound of Formula A2, or a salt thereof, according to any one of embodiments 1 to 54, wherein R^5 is hydrogen.

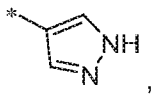
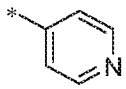
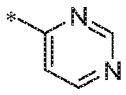
Embodiment 56. A compound of Formula A2, or a salt thereof, according to embodiment 1, wherein the compound is of Formula A3:



A3

wherein

X^1 is CH or N;

Ring A is  ,  or  , each of which is unsubstituted or substituted by a substituent selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and $-NH_2$;

R^1 is hydrogen or unsubstituted C_{1-6}alkyl; and

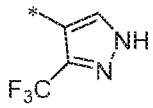
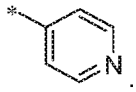
R^2 is

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 substituents selected from

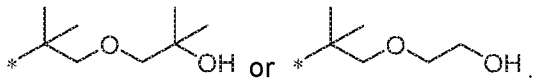
(i) C_{1-4}haloalkyl and

(ii) $-OR^6$, wherein R^6 is selected from hydrogen and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl; or

(b) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by C_{1-6}alkyl or C_{1-6}haloalkyl.

Embodiment 57. A compound of Formula A2, or a salt thereof, according to embodiment 56, wherein ring A is  or  .

Embodiment 58. A compound of Formula A2, or a salt thereof, according to embodiment 56 or embodiment 57, wherein R^2 is



Embodiment 59. A compound of Formula A2, or a salt thereof, according to embodiment 56 or embodiment 57, wherein R^2 is n-propyl or tert-butyl that is unsubstituted or substituted by trifluromethyl.

Embodiment 60. A compound of the Formula A2, or a salt thereof, according to embodiment 1, selected from: N-(2-cyclopropylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N,N-diethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-butyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; N-ethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-cyclohexylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(3-methyloxetan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; N-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methyl-4-phenylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-cyclopropyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methanesulfonyl-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol; 3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)acetic acid; (1R,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-(1-methanesulfonyl-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (2S)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-[(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)amino]acetic acid; (2R)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-

d]pyrimidin-4-yl]amino}propanoic acid; methyl 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoate; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-(2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethoxy)ethan-1-ol; 2-(hydroxymethyl)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol; 3-methyl-3-(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanamido)butanoic acid; 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-3-phenylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-{[4-(dimethylamino)oxan-4-yl]methyl}-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-(2-methanesulfonylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-(adamantan-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propanamide; 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propane-2-sulfonamide; 2-(pyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 4,4,4-trifluoro-2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentyl)methanol; N-(3-methoxycyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (1R,2R)-1-N,2-N-dimethyl-1-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]cyclohexane-1,2-diamine; methyl (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate; ethyl 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate; 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid; (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid; 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-

yl]amino}propan-1-ol; 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; N-(butan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylbut-3-yn-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (1r,3s)-3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutan-1-ol; 2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; 2-(pyridin-4-yl)-N-(2,4,4-trimethylpentan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(pentan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[1-(tert-butoxy)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4,4,4-trifluoro-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-pentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-[1-(1H-indol-3-yl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[1-(4-fluorophenyl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-phenylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-(4-fluorophenyl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethan-1-ol; N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 1-({[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}methyl)cyclopentan-1-ol; N,N-dimethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methoxyphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-phenyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(3-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 6-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}hexanoic acid; N-(3-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-(1-phenylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; tert-butyl N-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)carbamate; (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; methyl 2-(1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)acetate; N-(2-methylpropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanenitrile; N-(6-aminohexyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-aminobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-

yl]amino}propanenitrile; N-[2-methyl-1-(2-methylpiperidin-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine; N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-cyclopentyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-[4-(tert-butylamino)pyrido[3,4-d]pyrimidin-2-yl]pyridin-2-amine; 2-[1-(benzenesulfonyl)-2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl]-N-tert-butylpyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-methylpyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)pentan-2-ol; N-ethyl-2-(3-fluoropyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-[2-methyl-2-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)propoxy]propan-2-ol; 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1-methoxy-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol; 2-(3-chloropyridin-4-yl)-N-cyclopentylpyrido[3,4-d]pyrimidin-4-amine; 1-(2-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2-methylpropoxy)-2-methylpropan-2-ol; N-methyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(4-methanesulfonyl-2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[3-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[2-chloro-5-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridin-2-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile; 2-{2-methyl-

1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(1H-indazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3,5-dimethyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1,1,1-trifluoro-2-methylpropan-2-yl)-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(4-hydroxy-2,4-dimethylpentan-2-yl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile; 2-(3,5-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2,3-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1,3-thiazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-[2-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-2-carbonitrile; N-(1-methylcyclopropyl)-2-(1,2-oxazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(dimethyl-1,2-oxazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-{1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methylpyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclobutyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyrimidin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol; N-propyl-2-{7H-pyrrolo[2,3-d]pyrimidin-5-yl}pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(3-cyclopropyl-1H-pyrazol-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(3-methylpyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-{1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-[(1R)-1-phenylethyl]-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(5-methyl-1H-pyrazol-4-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-ethyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyridazin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1,3-oxazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-

methyl-1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-1,2,3-triazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1,2-oxazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(2H-1,2,3,4-tetrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclobutyl)pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; 2-(1H-pyrazol-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)methanol; 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-ethyl-1H-pyrazol-4-yl)-N-(2-methylpropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-amino-2-methylpropan-2-yl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-{[4-(tert-butylamino)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-5-yl]amino}ethoxy)ethan-1-ol; N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[2-methyl-1-(propan-2-yloxy)propan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[(2S)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[(2R)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butan-1-ol; N-tert-butyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2,2-dimethyl-1-[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]piperidin-4-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}pentan-2-ol; N-cyclopentyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; dimethyl(3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butyl)amine; N,N-diethyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2-methyl-1-(2-

methyl-2-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}propoxy)propan-2-ol; N-propyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine; N-tert-butyl-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyridazin-4-yl)-1,7-naphthyridin-4-amine; 2-(2-aminopyridin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine; N,N-diethyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine; (3-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-3-methylbutyl)dimethylamine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)-1,7-naphthyridin-4-amine; 2-(3-fluoropyridin-4-yl)-4-(piperidin-1-yl)-1,7-naphthyridine; 2-(3-fluoropyridin-4-yl)-4-(morpholin-4-yl)-1,7-naphthyridine; N-tert-butyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine; 2-(3-fluoropyridin-4-yl)-N-(2-methylbutan-2-yl)-1,7-naphthyridin-4-amine; 2-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-2-methylpropan-1-ol; 1-[2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-yl]-2,2-dimethylpiperidin-4-ol; 2-(3-fluoropyridin-4-yl)-N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-1,7-naphthyridin-4-amine; 4-(4-methylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 4-(piperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 4-(2-methylpiperidin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 2-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclobutyl)-1,7-naphthyridin-4-amine; 2-methyl-N1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,2-diamine; N-(oxetan-3-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-(1-methylcyclopropyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 4-(3,3-dimethylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 2,2-dimethyl-4-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)morpholine; N-(1-methylcyclobutyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2,2-dimethyl-N1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,3-diamine; N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,2-diamine; 4-(2-methylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 2-methyl-N^1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,3-diamine; (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)-1,7-naphthyridine; N-(tert-butyl)-N-methyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; *N*-(1-methylcyclobutyl)-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; N^1,N^1,3-trimethyl-N^3-(2-(pyrimidin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; *tert*-butyl (2-methyl-1-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propan-2-yl)carbamate; *tert*-butyl (2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)ethyl)carbamate; 2-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine; N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)ethane-1,2-diamine; *N,N*,2-trimethyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide; N^1,3-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-

1,3-diamine; *tert*-butyl (2,2-dimethyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propyl)carbamate; 2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; 3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butanamide; (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine; 2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine; (*S*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine; ethyl 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanoate; N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; 4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine; 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide; (*S*)-1,1,1-trifluoro-2-methyl-3-((2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)amino)propan-2-ol; N-tert-butyl-2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-amine; 2-(3-chloropyridin-4-yl)-N,N-diethyl-1,7-naphthyridin-4-amine; N-((1R,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (S)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1S,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (R)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1S,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1R,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *tert*-butyl (3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butyl)carbamate; N^1,N^1,N^3,2,2-pentamethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; N^1,N^1-diethyl-3-methyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^3-(2-(2-fluoropyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; N^3-(2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; N^1,N^1,3-trimethyl-N^3-(2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^3-(2-(2-aminopyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; and 3-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine.

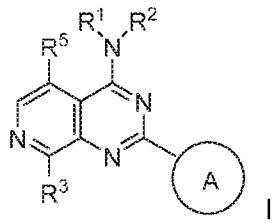
Embodiment 60A. A compound of the Formula A2, or a salt thereof, according to embodiment 1 , selected from: N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;

2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *N*-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 60B. A compound of the Formula A2, or a salt thereof, according to embodiment 1 , selected from: N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 60C. A compound of the Formula A2, or a salt thereof, according to embodiment 1 , wherein the compound is N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine.

Embodiment 61. A compound of Formula I, or a salt thereof



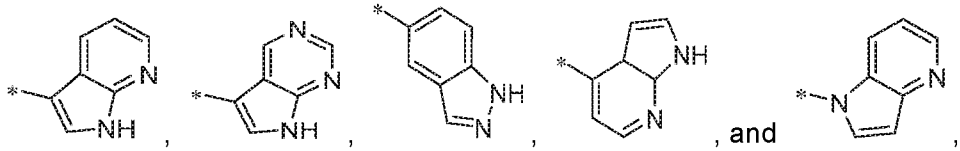
I

wherein

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-}

$_6$alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0;

with the proviso that:

(1) when ring A is 4-primidinyl or 3-fluoro-4-primidinyl, R^1 is H or methyl, R^3 is H or Cl and R^5 is H; then R^2 is not C_{2-4}alkyl that is substituted with a substituent selected from –NH_2, C_{1-6}alkylamino or *t*-butyl-carbamoyl-amino and that and that is optionally further substituted with unsubstituted phenyl; and

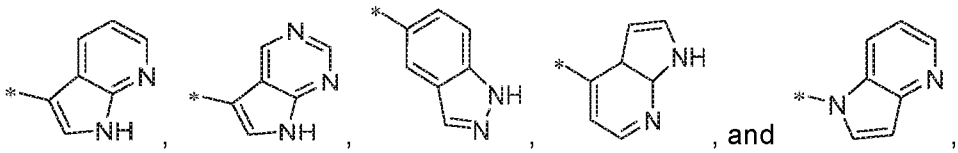
(2) when ring A is indazol-5-yl, R^1, R^3 and R^5 are H; then R^2 is not C_4alkyl that is substituted with –NH_2.

Embodiment 62. A compound of Formula I according to embodiment 61, or a salt thereof, wherein

Ring A is

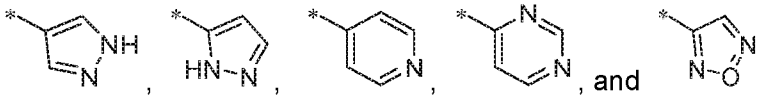
(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 2 heteroatoms that are selected from N, provided that at least one of the nitrogen atom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 5-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

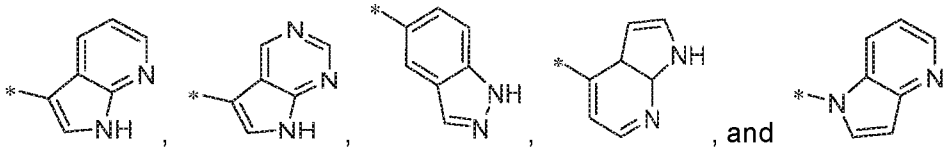
(b) a 9-membered fused bicyclic heteroaryl that is selected from



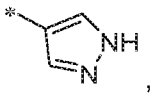
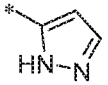
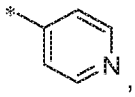
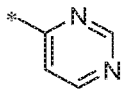
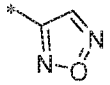
wherein "*" represents the point of attachment of ring A to the remainder of the molecule and ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$ and C_{3-6}cycloalkyl.

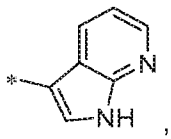
Embodiment 63. A compound of Formula I, or a salt thereof, according to embodiment 61 or embodiment 62, wherein ring A is selected

from  which are each unsubstituted or substituted by 1 to 2 substituents independently selected from cyano, halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, NH_2, and C_{3-6}cycloalkyl;

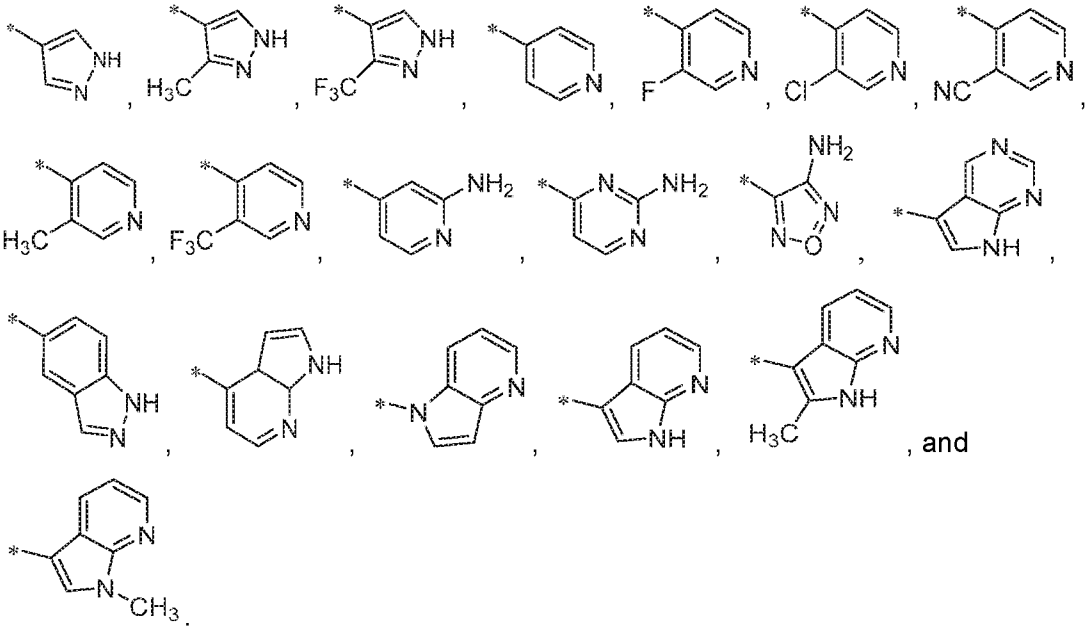
or from  which are each unsubstituted or substituted by C_{1-6}alkyl.

Embodiment 64. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 63, wherein ring A is selected

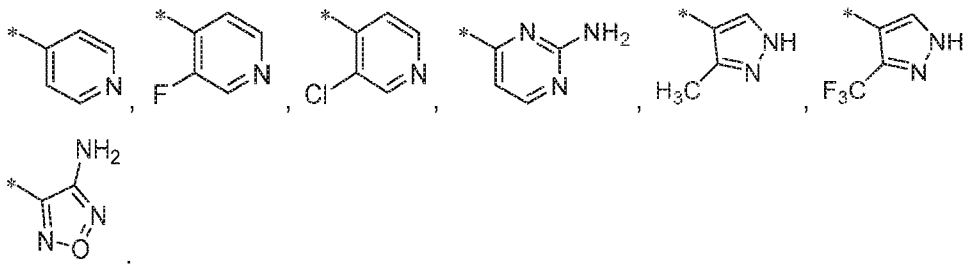
from  ,  ,  ,  , and  that are each unsubstituted or substituted by a substituent selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, and -NH_2;

or is  , that is unsubstituted or substituted by C_{1-6}alkyl.

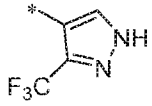
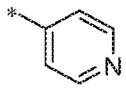
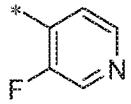
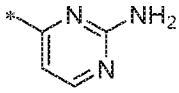
Embodiment 65. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 63, wherein ring A is selected from

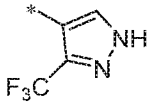


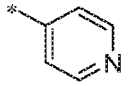
Embodiment 66. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 65, wherein ring A is selected from

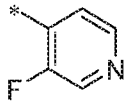


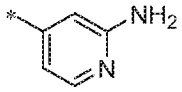
Embodiment 67. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 66, wherein ring A is selected from

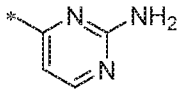
 ,  ,  , and  .

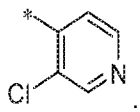
Embodiment 68. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 67, wherein ring A is  .

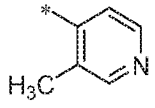
Embodiment 69. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 67, wherein ring A is  .

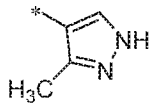
Embodiment 70. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 67, wherein ring A is  .

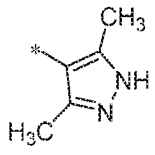
Embodiment 71. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 67, wherein ring A is  .

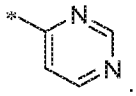
Embodiment 72. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 67, wherein ring A is  .

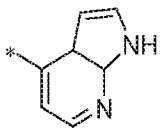
Embodiment 73. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 65, wherein ring A is  .

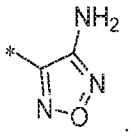
Embodiment 74. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 65, wherein ring A is  .

Embodiment 75. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 66, wherein ring A is  .

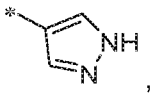
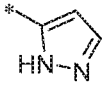
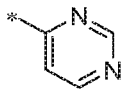
Embodiment 76. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 63, wherein ring A is  .

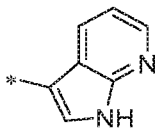
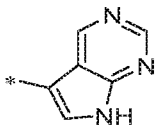
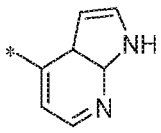
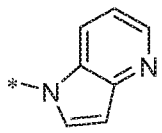
Embodiment 77. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 64, wherein ring A is  .

Embodiment 78. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 65, wherein ring A is  .

Embodiment 78A. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 65, wherein ring A is  .

Embodiment 79. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 63, wherein ring A is selected

from  ,  , and  , which are each unsubstituted or substituted by 1 to 2 substituents independently selected from cyano, halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, NH_2, and C_{3-6}cycloalkyl;

or from  ,  ,  , and  , which are each unsubstituted or substituted by C_{1-6}alkyl.

Embodiment 80. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 79, wherein R^1 is selected from hydrogen, methyl and ethyl.

Embodiment 81. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 80, wherein R^1 is methyl.

Embodiment 82. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 80, wherein R^1 is hydrogen.

Embodiment 83. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 82, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(vi) $-C(O)R^8$, wherein R^8 is R^0;

(vii) $-S(O)_2C_{1-6}$alkyl;

(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}alkyl, hydroxyC_{1-6}alkyl and R^0;

(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O and that and that is unsubstituted or substituted by C_{1-6}alkyl; and

(x) phenyl that is unsubstituted or substituted by halogen;

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(c) 4-membered heterocycloalkyl comprising, as ring member, a heteroatom selected from N and O and that and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$.

Embodiment 84. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 82, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(vi) $-C(O)R^8$, wherein R^8 is R^0;

(vii) $-S(O)_2C_{1-6}$alkyl;

(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}alkyl, hydroxyC_{1-6}alkyl and R^0;

(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O and that and that is unsubstituted or substituted by C_{1-6}alkyl; and

(x) phenyl that is unsubstituted or substituted by halogen;

and wherein the C atom of the C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents (i) to (x) that is the point of attachment of R^2 to the remainder of the molecule is not a $–CH_2-$ group.

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $–C(O)R^0$; and

(c) 4-membered heterocycloalkyl comprising, as ring member, a heteroatom selected from N and O and that and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $–C(O)R^0$.

Embodiment 85. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 83, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl or -C(O)H;

(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)-C_{1-6}$alkoxy, and C_{1-6}alkyl that is unsubstituted or substituted by –C(O)OH; and

(vi) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by one hydroxyl; and

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl or $–C(O)-C_{1-6}$alkoxy.

Embodiment 86. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 85, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from
  (i) C_{1-6}haloalkyl;
  (ii) $-OR^6$, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl; and
  (iii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by hydroxyl; and
(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 87. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by 1 to 2 substituent independently selected from C_{1-6}haloalkyl and $-OR^6$, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 88. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 89. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by C_{1-6}haloalkyl.

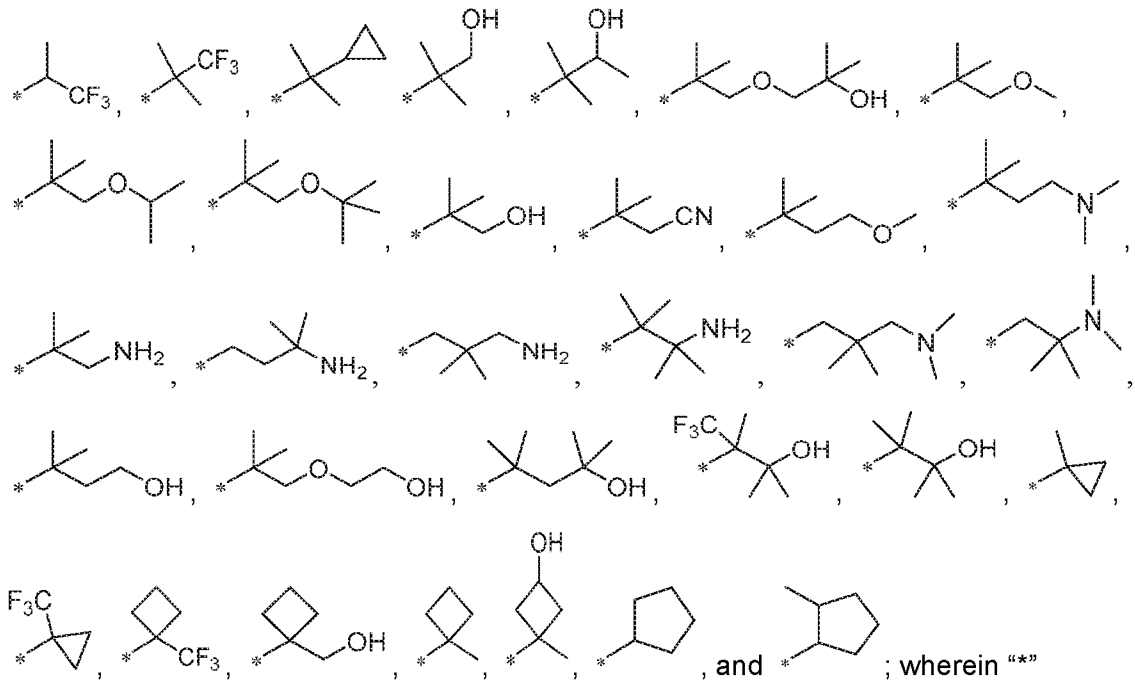
Embodiment 90. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by $-O-C_{1-6}$alkyl-OH.

Embodiment 91. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86, wherein R^2 is C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}haloalkyl, C_{1-6}alkylamino, R^0, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 92. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86 and 91, wherein R^2 is unsubstituted C_{3-6}cycloalkyl.

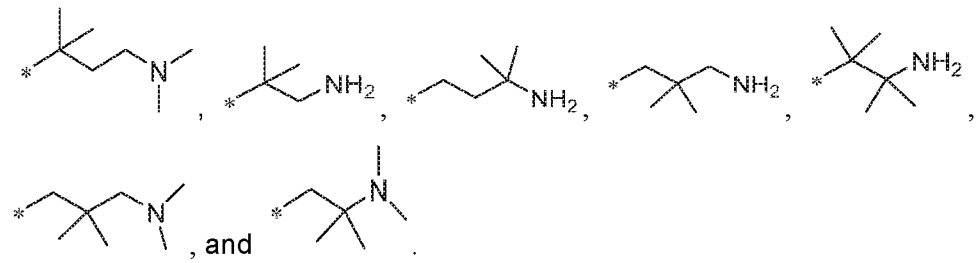
Embodiment 93. A compound of the Formula I, or a salt thereof, according to any one of embodiments 61 to 86 and 91, wherein R^2 is C_{3-6}cycloalkyl that is substituted by C_{1-6}alkyl or C_{1-6}haloalkyl.

Embodiment 94. A compound of the Formula I, or a salt thereof, according to any one of embodiments 61 to 85, wherein R^2 is selected from n-propyl, isopropyl, *s*-butyl, *t*-butyl, 2-methyl-but-2-yl, 2,4,4-trimethylpentan-2-yl,

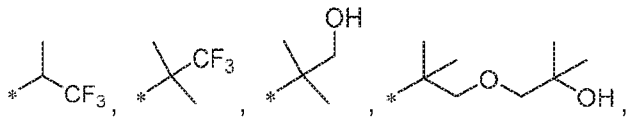
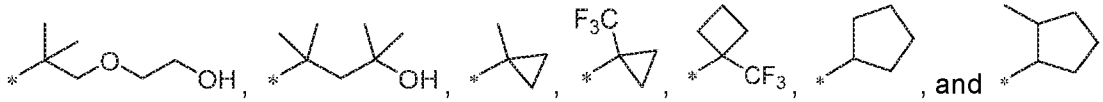


represents the point of attachment of R^2 to the remainder of the molecule.

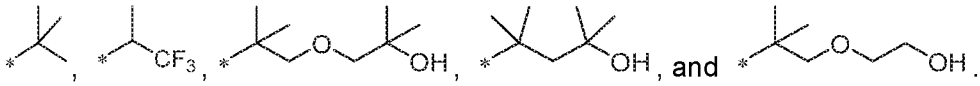
Embodiment 94A. A compound of the Formula I, or a salt thereof, any one of according to embodiments 61 to 85, wherein R^2 is selected from

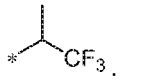


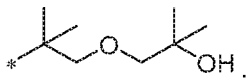
Embodiment 95. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 83 and 85 to 87, wherein R^2 is selected from

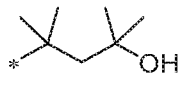
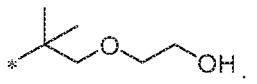
n-propyl, isopropyl, *t*-butyl,  ,  , and .

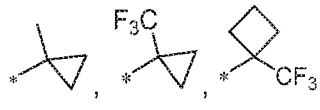
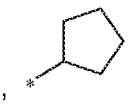
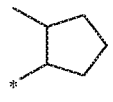
Embodiment 96. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, 94 and 95, wherein R^2 is selected from

 , and .

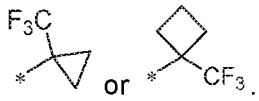
Embodiment 97. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, 89, and 94 to 96, wherein R^2 is  .

Embodiment 98. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, 90, and 94 to 96 wherein R^2 is  .

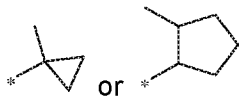
Embodiment 99. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 87, 91, and 94 to 96, wherein R^2 is  or  .

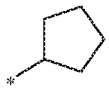
Embodiment 100. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86, 91, 94 and 95 wherein R^2 is selected from  ,  , and  .

Embodiment 101. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86, 91, 93 to 95 and 100, wherein R^2 is



Embodiment 102. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86, 91, 93 to 95 and 100, wherein R^2 is



Embodiment 103. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 86, 91 to 95, 100 and 102, wherein R^2 is  .

Embodiment 104. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 83, 85 to 90, and 95, wherein R^2 is selected from n-propyl, isopropyl and *t*-butyl.

Embodiment 105. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 83, 85 to 90, 95, and 104, wherein R^2 is n-propyl.

Embodiment 106. A compound of the Formula I, or a salt thereof, according to any one of embodiments 61 to 90, 95, and 104, wherein R^2 is isopropyl.

Embodiment 107. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 90, 95, and 104, wherein R^2 is *t*-butyl.

Embodiment 108. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 107, wherein R^3 is selected from hydrogen, chloro and methyl.

Embodiment 109. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 108, wherein R^3 is hydrogen.

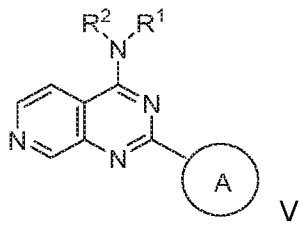
Embodiment 109A. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 108, wherein R^3 is chloro.

Embodiment 109B. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 108, wherein R^3 is methyl.

Embodiment 110. A compound of the Formula I, or a salt thereof, according to any one of embodiments 61 to 109, wherein R^5 is selected from hydrogen, and chloro.

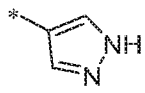
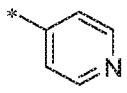
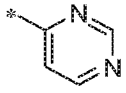
Embodiment 111. A compound of Formula I, or a salt thereof, according to any one of embodiments 61 to 110, wherein R^5 is hydrogen.

Embodiment 112. A compound of Formula I, or a salt thereof, according to embodiment 61, wherein the compound is of Formula V:



V

wherein

Ring A is  ,  or  , each of which is unsubstituted or substituted by a substituent selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and $-NH_2$;

R^1 is hydrogen or unsubstituted C_{1-6}alkyl; and

R^2 is

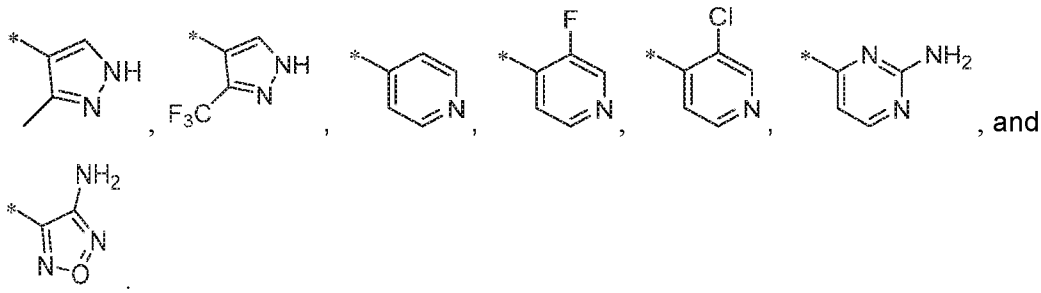
(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 substituents selected from

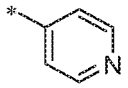
(i) C_{1-4}haloalkyl or

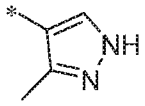
(ii) $-OR^6$, wherein R^6 is selected from hydrogen and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl; or

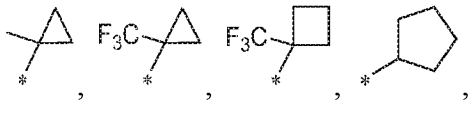
(b) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by C_{1-6}alkyl or C_{1-6}haloalkyl.

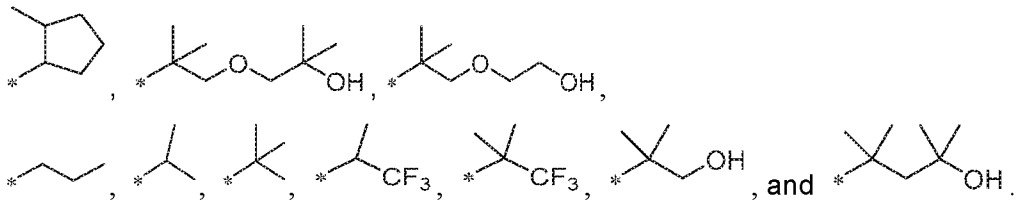
Embodiment 113. A compound of Formula I, or a salt thereof, according to embodiment 112, wherein ring A is selected from



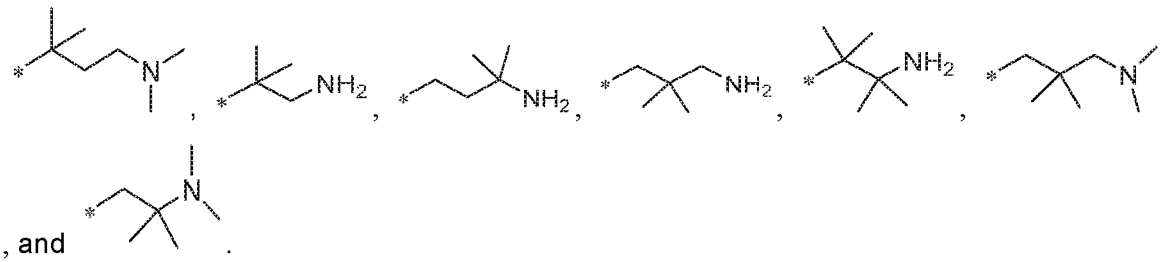
Embodiment 114. A compound of Formula I, or a salt thereof, according to embodiment 112 or embodiment 113, wherein ring A is  .

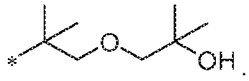
Embodiment 115. A compound of Formula I, or a salt thereof, according to embodiment 112 or embodiment 113, wherein ring A is  .

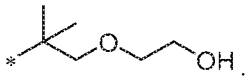
Embodiment 116. A compound of Formula I, or a salt thereof, according to any one of embodiments 112 to 115, wherein R^2 is selected from 

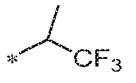


Embodiment 116A. A compound of Formula I, or a salt thereof, according to any one of embodiments 112 to 115, wherein R^2 is selected from



Embodiment 117. A compound of Formula I, or a salt thereof, according to any one of embodiments 112 to 116, wherein R^2 is  .

Embodiment 118. A compound of Formula I, or a salt thereof, according to any one of embodiments 112 to 116, wherein R^2 is  .

Embodiment 119. A compound of Formula I, or a salt thereof, according to any one of embodiments 112 to 116, wherein R^2 is  .

Embodiment 120. A compound of the Formula I , or a salt thereof, according to embodiment 61 , selected from: N-(2-cyclopropylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N,N-diethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-butyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; N-ethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-cyclohexylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(3-methyloxetan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; N-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methyl-4-phenylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-cyclopropyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methanesulfonyl-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol; 3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-

d]pyrimidin-4-yl]amino}propoxy)acetic acid; (1R,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-(1-methanesulfonyl-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (2S)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-[(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)amino]acetic acid; (2R)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; methyl 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoate; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-(2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethoxy)ethan-1-ol; 2-(hydroxymethyl)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol; 3-methyl-3-(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanamido)butanoic acid; 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-3-phenylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-{[4-(dimethylamino)oxan-4-yl]methyl}-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-(2-methanesulfonylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-(adamantan-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propanamide; 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propane-2-sulfonamide; 2-(pyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 4,4,4-trifluoro-2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentyl)methanol; N-(3-methoxycyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (1R,2R)-1-N,2-N-dimethyl-1-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]cyclohexane-1,2-diamine; methyl (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate; ethyl 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate; 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid; (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid; 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-(1-methylcyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; N-(butan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylbut-3-yn-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (1r,3s)-3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutan-1-ol; 2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; 2-(pyridin-4-yl)-N-(2,4,4-trimethylpentan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(pentan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[1-(tert-butoxy)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4,4,4-trifluoro-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-pentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-[1-(1H-indol-3-yl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[1-(4-fluorophenyl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-phenylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-(4-fluorophenyl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethan-1-ol; N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 1-({[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}methyl)cyclopentan-1-ol; N,N-dimethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methoxyphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-phenyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(3-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 6-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}hexanoic acid; N-(3-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-(1-phenylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; tert-butyl N-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-

d]pyrimidin-4-yl]amino}propyl)carbamate; (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; methyl 2-(1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)acetate; N-(2-methylpropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanenitrile; N-(6-aminohexyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-aminobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanenitrile; N-[2-methyl-1-(2-methylpiperidin-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine; N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-cyclopentyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-[4-(tert-butylamino)pyrido[3,4-d]pyrimidin-2-yl]pyridin-2-amine; 2-[1-(benzenesulfonyl)-2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl]-N-tert-butylpyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-methylpyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)pentan-2-ol; N-ethyl-2-(3-fluoropyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-[2-methyl-2-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)propoxy]propan-2-ol; 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1-methoxy-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol; 2-(3-chloropyridin-4-yl)-N-cyclopentylpyrido[3,4-d]pyrimidin-4-amine; 1-(2-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2-methylpropoxy)-2-methylpropan-2-ol; N-methyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(4-methanesulfonyl-2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[3-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[2-chloro-5-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-

N-methyl-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridin-2-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile; 2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(1H-indazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3,5-dimethyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1,1,1-trifluoro-2-methylpropan-2-yl)-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(4-hydroxy-2,4-dimethylpentan-2-yl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile; 2-(3,5-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2,3-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1,3-thiazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-[2-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-2-carbonitrile; N-(1-methylcyclopropyl)-2-(1,2-oxazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(dimethyl-1,2-oxazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-{1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methylpyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclobutyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyrimidin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol; N-propyl-2-{7H-pyrrolo[2,3-d]pyrimidin-5-yl}pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(3-cyclopropyl-1H-pyrazol-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(3-methylpyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-{1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-[(1R)-1-phenylethyl]-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(5-methyl-1H-pyrazol-4-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(1-methylcyclopropyl)-2-(1H-

pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-ethyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyridazin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1,3-oxazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-1,2,3-triazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1,2-oxazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(2H-1,2,3,4-tetrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclobutyl)pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; 2-(1H-pyrazol-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)methanol; 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-ethyl-1H-pyrazol-4-yl)-N-(2-methylpropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-amino-2-methylpropan-2-yl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N^1,N^1,3-trimethyl-N^3-(2-(pyrimidin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; *tert*-butyl (2-methyl-1-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propan-2-yl)carbamate; *tert*-butyl (2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)ethyl)carbamate; 2-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine; N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)ethane-1,2-diamine; *N,N*,2-trimethyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-

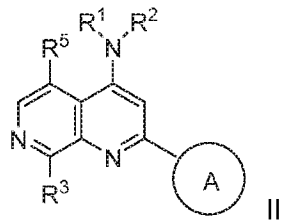
yl)amino)propanamide; N^1,3-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; *tert*-butyl (2,2-dimethyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propyl)carbamate; 2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; 3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butanamide; (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine; 2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine; (*S*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine; ethyl 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanoate; N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; 4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine; 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide; (*S*)-1,1,1-trifluoro-2-methyl-3-((2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)amino)propan-2-ol; 5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-{[4-(tert-butylamino)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-5-yl]amino}ethoxy)ethan-1-ol; N-((1R,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (S)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1S,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (R)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1S,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1R,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *tert*-butyl (3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butyl)carbamate; N^1,N^1,N^3,2,2-pentamethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; N^1,N^1-diethyl-3-methyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^3-(2-(2-fluoropyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; N^3-(2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; N^1,N^1,3-trimethyl-N^3-(2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^3-(2-(2-aminopyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; and 3-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine.

Embodiment 120A. A compound of the Formula I , or a salt thereof, according to embodiment 61, selected from: N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-

d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *N*-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 120B. A compound of the Formula I , or a salt thereof, according to embodiment 61 , wherein the compound is N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 121. A compound of Formula II, or a salt thereof,



II

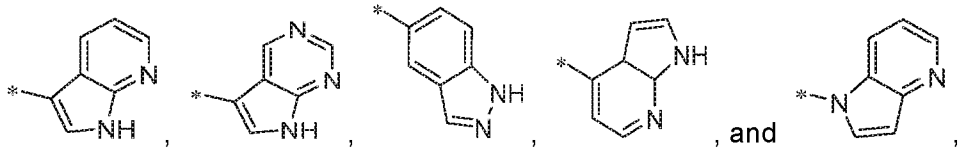
wherein

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the

3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted

by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; or

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

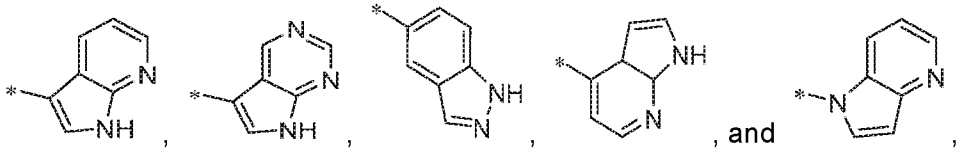
R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 122. A compound of formula II according to embodiment 121, or a salt thereof, wherein

Ring A is

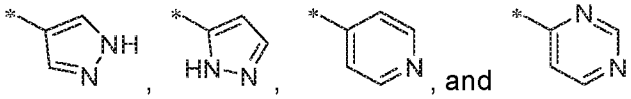
(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 2 heteroatoms that are selected from N, provided that at least one of the nitrogen atom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

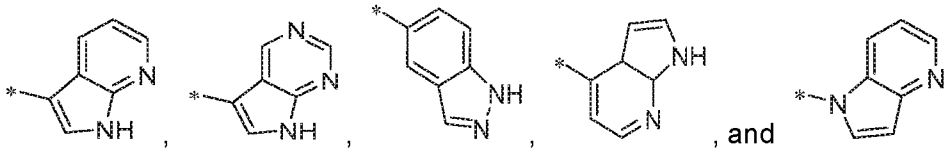
(b) a 9-membered fused bicyclic heteroaryl that is selected from



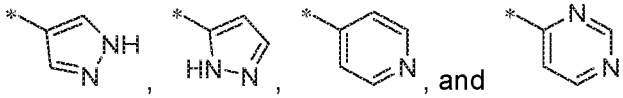
wherein "*" represents the point of attachment of ring A to the remainder of the molecule and that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$ and C_{3-6}cycloalkyl.

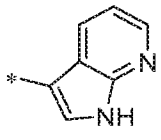
Embodiment 123. A compound of Formula II, or a salt thereof, according to embodiment 121 or embodiment 122, wherein ring A is selected

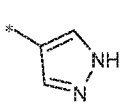
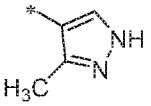
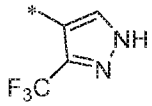
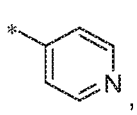
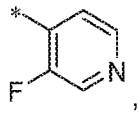
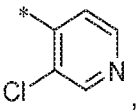
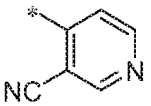
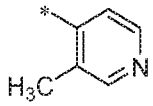
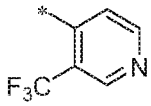
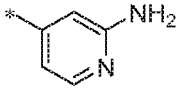
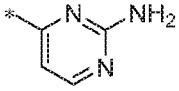
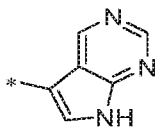
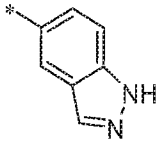
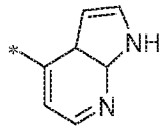
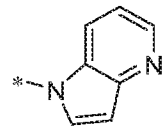
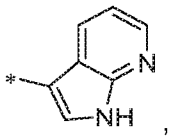
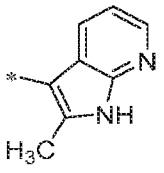
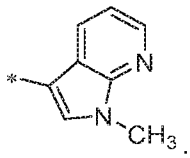
from  , which are each unsubstituted or substituted by 1 to 2 substituents independently selected from cyano, halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, NH_2, and C_{3-6}cycloalkyl;

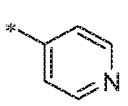
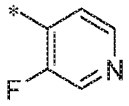
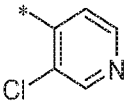
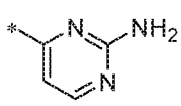
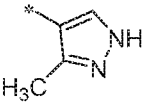
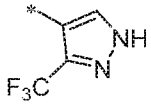
or from  , which are each unsubstituted or substituted by C_{1-6}alkyl.

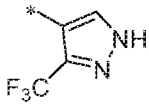
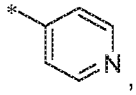
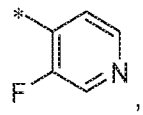
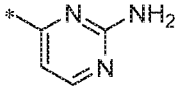
Embodiment 124. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 123, wherein ring A is selected

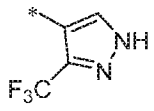
from  , which are each unsubstituted or substituted by a substituent selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, and $-NH_2$;

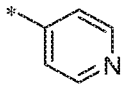
or is  , which is unsubstituted or substituted by C_{1-6}alkyl.

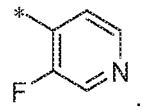
Embodiment 125. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 123, wherein ring A is selected from  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  .

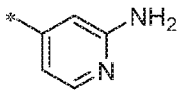
Embodiment 126. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 125, wherein ring A is selected from  ,  ,  ,  ,  , and  .

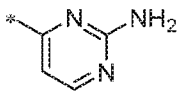
Embodiment 127. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 126, wherein ring A is selected from  ,  ,  , and  .

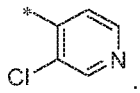
Embodiment 128. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 127, wherein ring A is  .

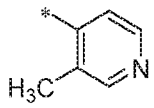
Embodiment 129. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 127, wherein ring A is  .

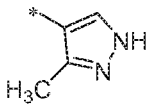
Embodiment 130. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 127, wherein ring A is  .

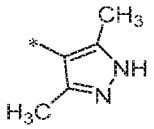
Embodiment 131. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 127, wherein ring A is  .

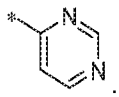
Embodiment 132. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 127, wherein ring A is  .

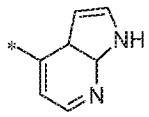
Embodiment 133. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 125, wherein ring A is  .

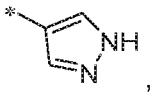
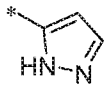
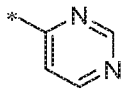
Embodiment 134. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 125, wherein ring A is  .

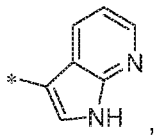
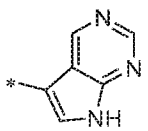
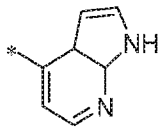
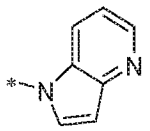
Embodiment 135. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 126, wherein ring A is  .

Embodiment 136. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 123, wherein ring A is  .

Embodiment 137. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 124, wherein ring A is  .

Embodiment 138. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 125, wherein ring A is  .

Embodiment 139. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 123, wherein ring A is selected from  ,  , and  , which are each unsubstituted or substituted by 1 to 2 substituents independently selected from cyano, halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, NH_2, and C_{3-6}cycloalkyl;

or from  ,  ,  , and  , which are each unsubstituted or substituted by C_{1-6}alkyl.

Embodiment 140. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 139, wherein R^1 is selected from hydrogen, methyl and ethyl.

Embodiment 141. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 140, wherein R^1 is methyl.

Embodiment 142. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 140, wherein R^1 is hydrogen.

Embodiment 143. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 142, wherein
R^2 is selected from
(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from
(i) cyano;
(ii) C_2alkynyl;
(iii) C_{1-6}haloalkyl;
(iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;
(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;
(vi) $-C(O)R^8$, wherein R^8 is R^0;
(vii) $-S(O)_2C_{1-6}$alkyl;
(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}alkyl, hydroxyC_{1-6}alkyl and R^0;
(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O and that is unsubstituted or substituted by C_{1-6}alkyl; and
(x) phenyl that is unsubstituted or substituted by halogen;
(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and
(c) 4-membered heterocycloalkyl comprising, as ring member, a heteroatom selected from N and O and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$.

Embodiment 144. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 142, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(vi) $-C(O)R^8$, wherein R^8 is R^0;

(vii) $-S(O)_2C_{1-6}$alkyl;

(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}alkyl, hydroxyC_{1-6}alkyl and R^0;

(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O and that is unsubstituted or substituted by C_{1-6}alkyl; and

(x) phenyl that is unsubstituted or substituted by halogen;

wherein the C atom of the C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents (i) to (x) that is the point of attachment of R^2 to the remainder of the molecule is not a $-CH_2-$ group;

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(c) 4-membered heterocycloalkyl comprising, as ring member, a heteroatom selected from N and O and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$.

Embodiment 145. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 143, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from
  (i) cyano;
  (ii) C_2alkynyl;
  (iii) C_{1-6}haloalkyl;
  (iv) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl or -C(O)H;
  (v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -C(O)-C_{1-6}alkoxy, and C_{1-6}alkyl that is unsubstituted or substituted by –C(O)OH; and
  (vi) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a hydroxyl; and
(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl or –C(O)-C_{1-6}alkoxy.

Embodiment 146. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 145, wherein
R^2 is selected from
(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from
  (i) C_{1-6}haloalkyl;
  (ii) $-OR^6$, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl; and
  (iii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by hydroxyl; and
(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 147. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 146, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by 1 to 2 substituent independently selected from C_{1-6}haloalkyl and $-OR^6$, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 148. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 149. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by C_{1-6}haloalkyl.

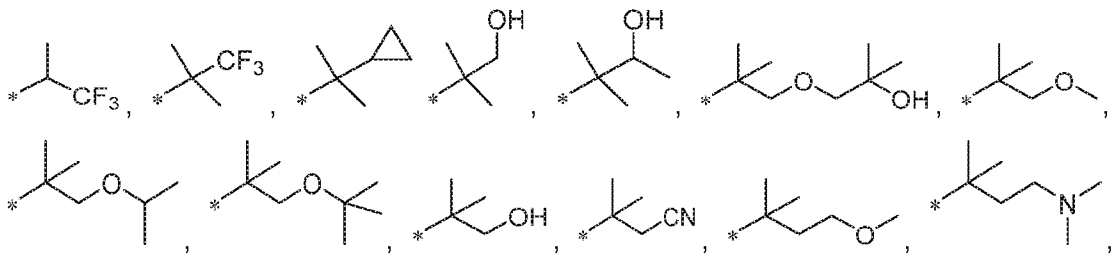
Embodiment 150. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, wherein R^2 is C_{1-6}alkyl that is unsubstituted or substituted by –O-C_{1-6}alkyl-OH.

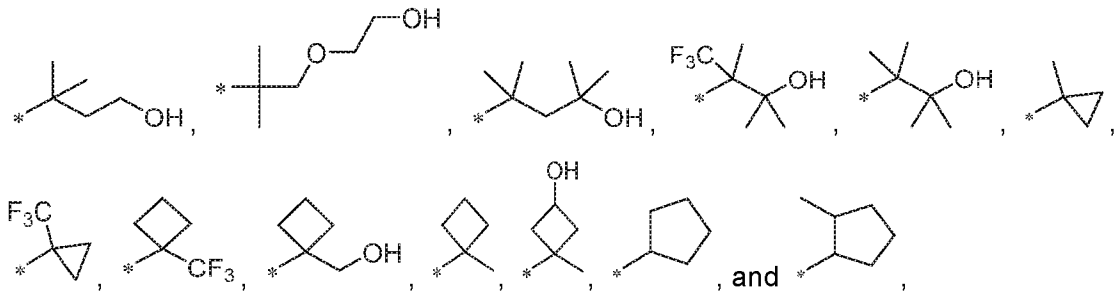
Embodiment 151. A compound of the Formula II, or a salt thereof, according to any one of embodiments 121 to 146, wherein R^2 is C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C_{1-6}haloalkyl, C_{1-6}alkylamino, R^0, and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl.

Embodiment 152. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 146 and 151, wherein R^2 is unsubstituted C_{3-6}cycloalkyl.

Embodiment 153. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 146 and 151, wherein R^2 is C_{3-6}cycloalkyl that is substituted by C_{1-6}alkyl or C_{1-6}haloalkyl.

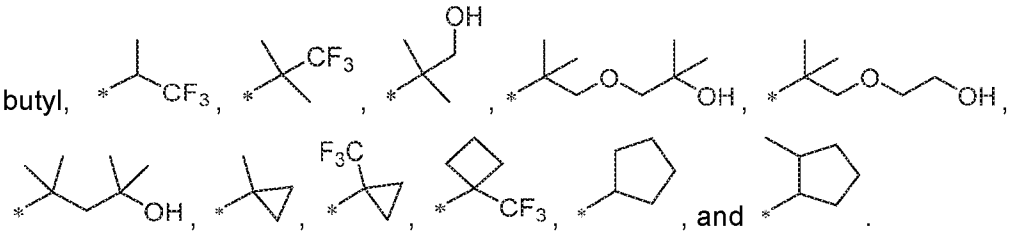
Embodiment 154. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 145, wherein R^2 is selected from n-propyl, isopropyl, *s*-butyl, *t*-butyl, 2-methyl-but-2-yl, 2,4,4-trimethylpentan-2-yl,



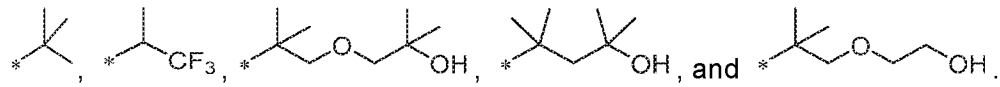


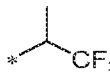
wherein "*" represents the point of attachment of R^2 to the remainder of the molecule.

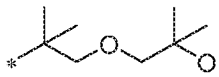
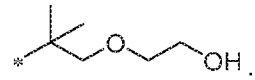
Embodiment 155. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 143 and 145 to 147, wherein R^2 is selected from n-propyl, isopropyl, *t*-butyl,



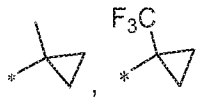
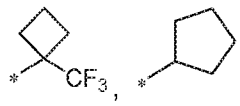
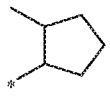
Embodiment 156. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, 154 and 155, wherein R^2 is selected from

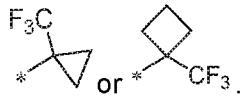


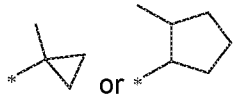
Embodiment 157. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, 149, and 154 to 156, wherein R^2 is  .

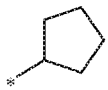
Embodiment 158. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, 150, and 154 to 156, wherein R^2 is  or  .

Embodiment 159. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 147, 151, and 154 to 156, wherein R^2 is  .

Embodiment 160. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 146, 151, 154 and 155, wherein R^2 is selected from  ,  , and  .

Embodiment 161. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 146, 151, 153 to 155 and 160, wherein R^2 is  .

Embodiment 162. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 136, 151, 153 to 155 and 160, wherein R^2 is  .

Embodiment 163. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 136, 151, 152, 154, 155, and 160, wherein R^2 is  .

Embodiment 164. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 133, 135 to 150, and 155, wherein R^2 is selected from n-propyl, isopropyl and *t*-butyl.

Embodiment 165. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 143, 145 to 150, 155, and 164, wherein R^2 is n-propyl.

Embodiment 166. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 150, 155, and 164, wherein R^2 is isopropyl.

Embodiment 167. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 150, 155, and 164, wherein R^2 is *t*-butyl.

Embodiment 168. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 139, wherein R^1 and R^2 taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0.

Embodiment 169. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 139, and 168, wherein R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 5- or 6-membered heterocycloalkyl that can include, as ring member, 1 to 2 additional heteroatom selected from N, O and S, wherein the 5- or 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl, C_{1-4}alkyl and C_{1-4}haloalkyl.

Embodiment 170. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 139, 168, and 169, wherein R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 6-membered heterocycloalkyl that can include, as ring member, an additional heteroatom selected from N and O, wherein the 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl, C_{1-6}alkyl and C_{1-6}haloalkyl.

Embodiment 171. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 139, and 168 to 170, wherein R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 6-membered heterocycloalkyl selected from piperidinyl, piperazinyl and morpholinyl, wherein the piperidinyl, piperazinyl or morpholinyl is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl and C_{1-6}alkyl.

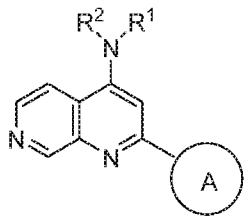
Embodiment 172. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 171, wherein R^3 is selected from hydrogen, chloro and methyl.

Embodiment 173. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 172, wherein R^3 is hydrogen.

Embodiment 174. A compound of the Formula II, or a salt thereof, according to any one of embodiments 121 to 173, wherein R^5 is selected from hydrogen, and chloro.

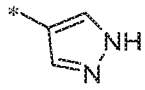
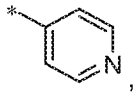
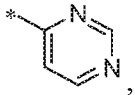
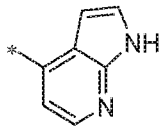
Embodiment 175. A compound of Formula II, or a salt thereof, according to any one of embodiments 121 to 174, wherein R^5 is hydrogen.

Embodiment 176. A compound of the Formula II, or a salt thereof, according to embodiment 121, wherein the compound is of Formula VI:



VI

wherein

Ring A is  ,  ,  , and  , each unsubstituted or substituted by a substituent selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and $-NH_2$;

R^1 is hydrogen or unsubstituted C_{1-6}alkyl; and

R^2 is

(a) C_{1-8}alkyl that is unsubstituted or substituted by a substituent selected from

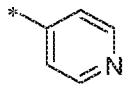
(i) di-C_{1-6}alkylamino;

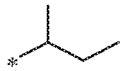
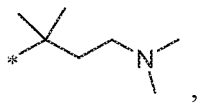
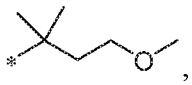
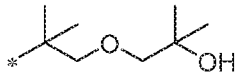
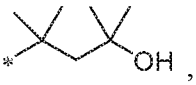
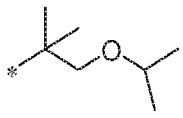
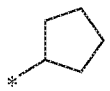
(ii) C_{1-6}haloalkyl;

(iii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0; or

(b) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by C_{1-6}alkyl or C_{1-6}haloalkyl;

or R^1 and R^2 may be taken together with the nitrogen to which they are bound to form a 6-membered heterocycloalkyl, which is unsubstituted or substituted by 1 to 3 substituted selected from C_{1-6}alkyl and hydroxyl.

Embodiment 177. A compound of the Formula II, or a salt thereof, according to embodiment 176, wherein ring A is  .

Embodiment 178. A compound of the Formula II, or a salt thereof, according to embodiment 176 or embodiment 177, wherein R^2 is selected from ethyl,  ,  ,  ,  ,  ,  ,  , and  .

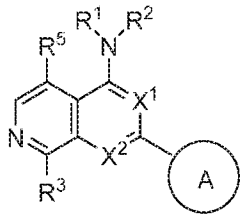
Embodiment 179. A compound of the Formula II, or a salt thereof, according to any one of embodiments 176 to 178, wherein R^2 is tert-butyl.

Embodiment 180. A compound of the Formula II, or a salt thereof, according to embodiment 121, selected from: N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[2-methyl-1-(propan-2-yloxy)propan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[(2S)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[(2R)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butan-1-ol; N-tert-butyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2,2-dimethyl-1-[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]piperidin-4-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}pentan-2-ol; N-cyclopentyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; dimethyl(3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butyl)amine; N,N-diethyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}propoxy)propan-2-ol; N-propyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine; N-tert-butyl-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyridazin-4-yl)-1,7-naphthyridin-4-amine; 2-(2-aminopyridin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine; N,N-diethyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine; (3-{[2-

(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-3-methylbutyl)dimethylamine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)-1,7-naphthyridin-4-amine; 2-(3-fluoropyridin-4-yl)-4-(piperidin-1-yl)-1,7-naphthyridine; 2-(3-fluoropyridin-4-yl)-4-(morpholin-4-yl)-1,7-naphthyridine; N-tert-butyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine; 2-(3-fluoropyridin-4-yl)-N-(2-methylbutan-2-yl)-1,7-naphthyridin-4-amine; 2-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-2-methylpropan-1-ol; 1-[2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-yl]-2,2-dimethylpiperidin-4-ol; 2-(3-fluoropyridin-4-yl)-N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-amine; and 2-(3-chloropyridin-4-yl)-N,N-diethyl-1,7-naphthyridin-4-amine.

Embodiment 180a. A compound of the Formula II, or a salt thereof, according to embodiment 121 , wherein the compound is N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine.

Embodiment 181. A compound of Formula A1, or a salt thereof,



A1

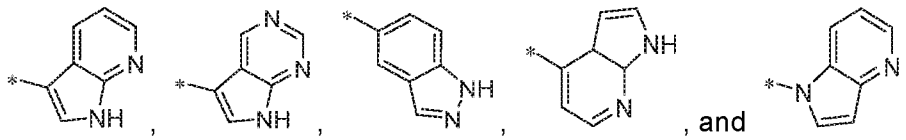
for use in ocular diseases or disorders, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

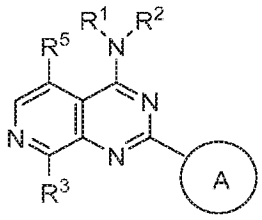
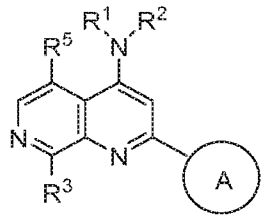
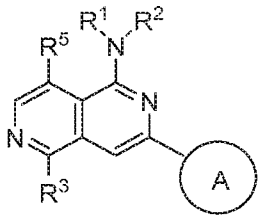
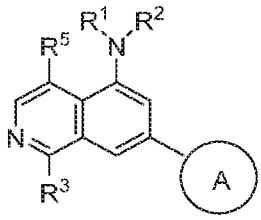
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

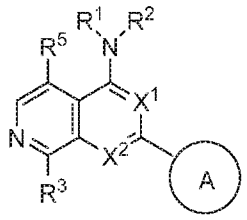
Embodiment 182. A compound of Formula A1 or a salt thereof, for use in ocular diseases or disorders according to embodiment 181, wherein the compound is of the formula selected from Formulae I to IV:


I,

II,

III, and

IV.

Embodiment 183. A compound of Formula A1 or a salt thereof, for use in ocular diseases or disorders according to embodiment 181, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 184. A compound of the Formula A1 or a salt thereof, for use in ocular diseases or disorders according to embodiment 181, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 185. Use of a compound of the Formula A1, or a salt thereof,


A1

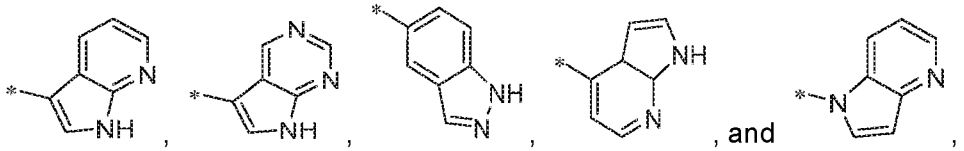
In a method of generating an expanded population of limbal stem cells, preferably ex vivo, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-}

$_6$alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

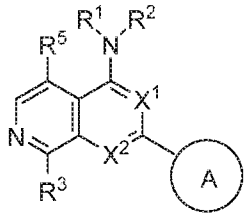
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 186. Use of a compound of the Formula A1, or a salt thereof,



A1

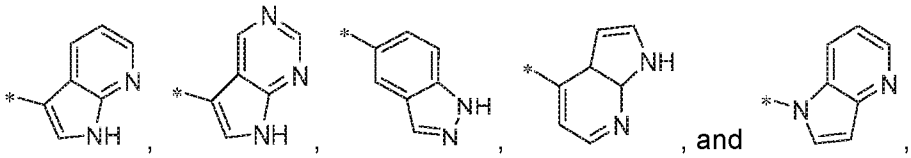
in a method of generating an expanded corneal endothelial cell population, preferably ex vivo, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

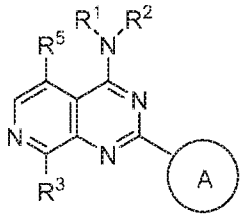
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered

heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

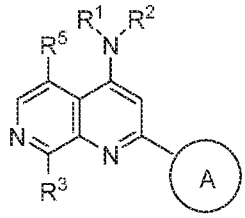
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

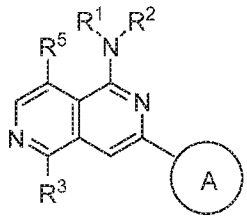
Embodiment 187. Use of a compound of the Formula A1 or a salt thereof, according to embodiment 185 or 186, wherein the compound is of the formula selected from Formulae I to IV:



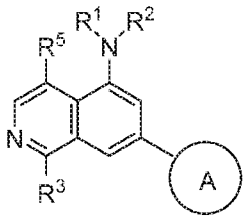
I,



II,



III, and



IV.

Embodiment 188. Use of a compound of Formula A1 or a salt thereof, according to embodiment 185 or 186, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; and 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine.

Embodiment 188A. Use of a compound of the Formula AI, or a salt thereof, according to embodiment 185 or 186, wherein the compound is selected from: N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; 2,4-dimethyl-4-{[2-

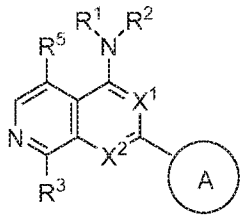
(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *N*-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine and N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 188B. Use of a compound of the Formula AI, or a salt thereof, according to embodiment 185 or 186, wherein the compound is selected from: N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 188C. Use of a compound of the Formula AI, or a salt thereof, according to embodiment 185 or 186, wherein the compound is compound is N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine.

Embodiment 189. Use of a compound of Formula A1 or a salt thereof, according to embodiment 185 or 186, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 190. A method of treatment of an ocular disease or disorder comprising administering to a subject in need thereof a cell population, wherein the cell population has been grown in the presence of a compound of Formula A1, or a salt thereof,


A1

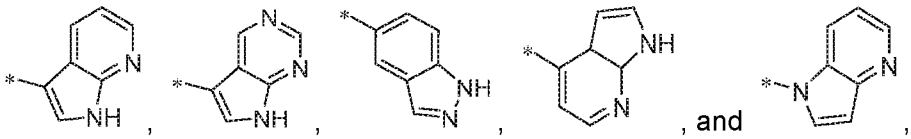
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

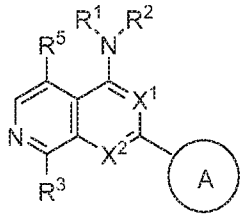
or, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-

membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 190A. A method of treatment of an ocular disease or disorder comprising administering to a subject in need thereof a limbal stem cell population, wherein said population has been grown in the presence of a compound of Formula A1, or a salt thereof,


A1

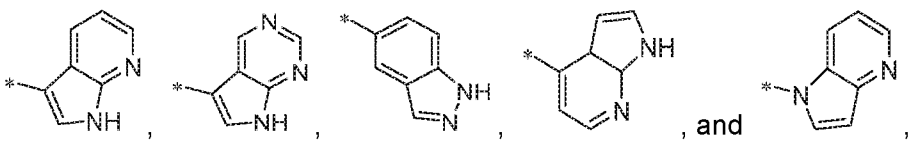
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from


, and ,

wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, -NH_2, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

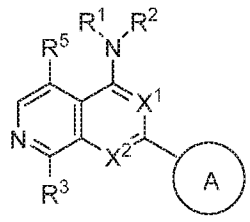
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

or, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 191. A method of treatment of an ocular disease or disorder comprising administering to a subject in need thereof a corneal endothelial cell population, wherein the population has been grown in the presence of a compound of Formula A1, or a salt thereof,



A1

wherein

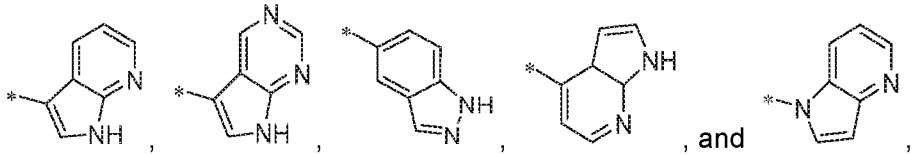
X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least

one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to

2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

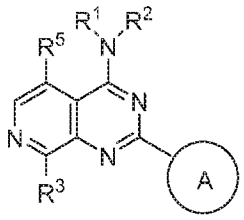
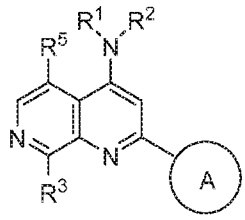
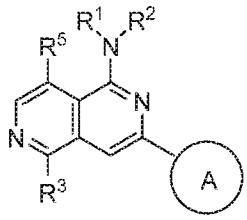
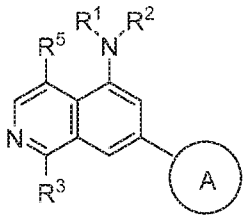
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 192. A method of treatment of an ocular disease or disorder according to embodiment 190 or 191, wherein the compound is of the formula selected from Formulae I to IV:


I,

II,

III, and

IV.

Embodiment 193. A method of treatment of an ocular disease or disorder according to embodiment 190 or 191, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 193A. A method of treatment of an ocular disease or disorder according to embodiment 190 or 191, wherein the compound is selected from: N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *N*-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-

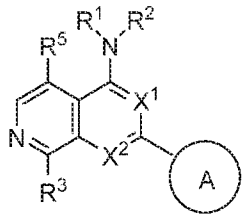
yl]amino}propoxy)ethan-1-ol; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine and N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 193B. A method of treatment of an ocular disease or disorder according to embodiment 190 or 191, wherein the compound is selected from: N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 193C. A method of treatment of an ocular disease or disorder according to embodiment 190 or 191, wherein the compound is N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine.

Embodiment 194. A method of promoting cell proliferation according to embodiment 190 or 191, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 195. Use of a compound of Formula A1, or a salt thereof,



A1

in the manufacture of a medicament for an ocular disease or disorder, wherein

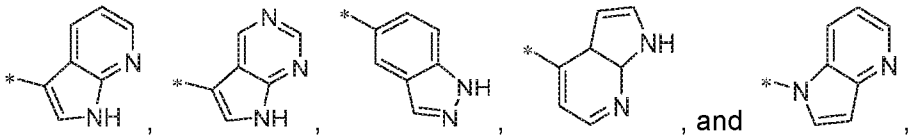
X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the

3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted

by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

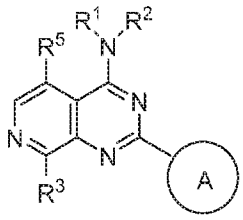
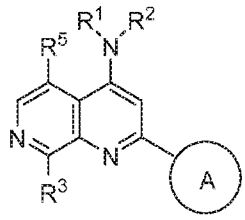
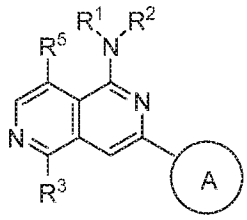
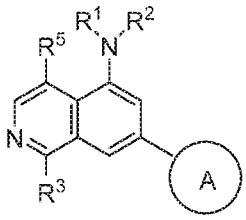
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 196. Use of a compound of Formula A1 or a salt thereof, in the manufacture of a medicament for an ocular disease or disorder according to embodiment 195, wherein the compound is of the formula selected from Formulae I to IV:


I,

II,

III, and

IV.

Embodiment 197. Use of a compound of Formula A1 or a salt thereof, in the manufacture of a medicament for an ocular disease or disorder according to embodiment 195, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; and 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine.

Embodiment 197A. Use of a compound of the Formula AI, or a salt thereof, in the manufacture of a medicament for an ocular disease or disorder according to embodiment 195, wherein the compound is selected from: N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *N*-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-

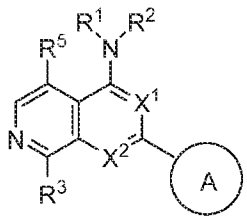
yl]amino}propoxy)ethan-1-ol; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine and N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 197B. Use of a compound of the Formula AI, or a salt thereof, in the manufacture of a medicament for an ocular disease or disorder according to embodiment 195, wherein the compound is selected from: N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 197C. Use of a compound of the Formula AI, or a salt thereof, in the manufacture of a medicament for an ocular disease or disorder according to embodiment 195, wherein the compound is compound is N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine.

Embodiment 198. Use of a compound of Formula A1 or a salt thereof, in the manufacture of a medicament for an ocular disease or disorder according to embodiment 195, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 199. A compound of Formula A1, or a pharmaceutically acceptable salt thereof,



A1

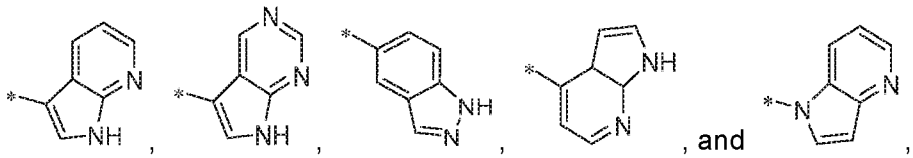
for use in promoting liver regeneration and liver regrowth, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

halogen;

cyano;

oxo;

C_2alkenyl;

C_2alkynyl;

C_{1-6}haloalkyl;

$-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

$-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

$-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

$-S(O)_2C_{1-6}$alkyl;

monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

phenyl that is unsubstituted or substituted by halogen;

5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

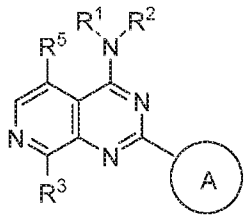
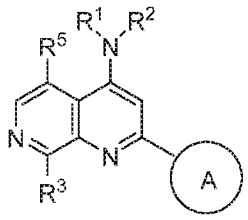
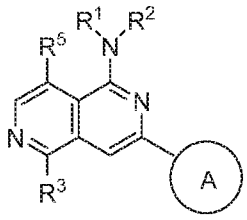
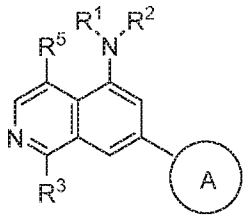
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 200. A compound of Formula A1 or a pharmaceutically acceptable salt thereof, for use in promoting liver regeneration and liver regrowth according to embodiment 199, wherein the compound is of the formula selected from Formulae I to IV:


I,

II,

III, and

IV.

Embodiment 201. A compound of Formula A1 or a pharmaceutically acceptable salt thereof, for use in promoting liver regeneration and liver regrowth according to embodiment 199, wherein the compound is selected from:

2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol;

dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine;

N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;

N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine;

N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;

2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine;

N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;

N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;

2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;

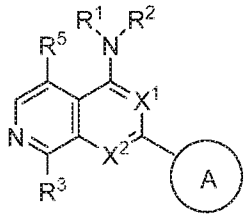
2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine;

N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;

4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; and
N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine.

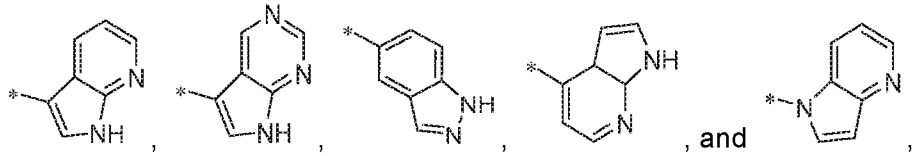
Embodiment 202. A compound of the Formula A1 or a pharmaceutically acceptable salt thereof, for use in promoting liver regeneration and liver regrowth according to embodiment 199, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 203. Use of a compound of the Formula A1, or a pharmaceutically acceptable salt thereof,



A1

for promoting liver regeneration and liver regrowth, wherein
X^1 and X^2 are each independently CH or N;
Ring A is
(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or
(b) a 9-membered fused bicyclic heteroaryl that is selected from



, and

,

wherein "*" represents the point of attachment of ring A to the remainder of the molecule;
wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;
R^0 is hydroxyl or C_{1-6}alkoxy;
R^1 is hydrogen or C_{1-6}alkyl;
R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from
halogen;
cyano;
oxo;
C_2alkenyl;
C_2alkynyl;
C_{1-6}haloalkyl;
-OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -$C(O)R^0$;
-$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;
-$C(O)R^8$, wherein R^8 is R^0 or -NH-C_{1-6}alkyl-$C(O)R^0$;
-$S(O)_2C_{1-6}$alkyl;
monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;
6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;
phenyl that is unsubstituted or substituted by halogen;
5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and
9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents

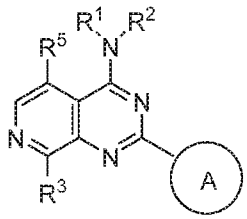
independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -C(O)R^0, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –C(O)R^0;

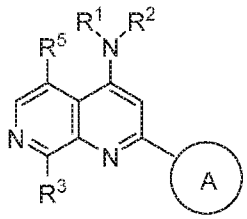
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

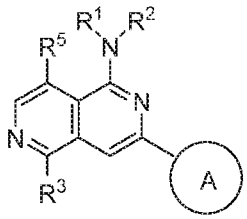
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

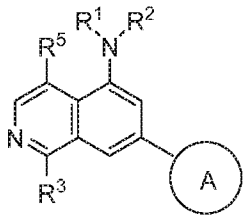
R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 204. Use of a compound of the Formula A1 or a pharmaceutically acceptable salt thereof, for promoting liver regeneration and liver regrowth according to embodiment 203, wherein the compound is of the formula selected from Formulae I to IV:


I,


II,


III, and


IV.

Embodiment 205. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, for promoting liver regeneration and liver regrowth according to embodiment 203, wherein the compound is selected from:

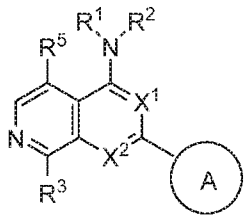
2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol;

dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine;

N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;
N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine;
N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;
2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine;
N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;
N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;
2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;
2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine;
N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;
4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; and
N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine.

Embodiment 206. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, for promoting liver regeneration and liver regrowth according to embodiment 203, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 207. A method of promoting liver regeneration and liver regrowth, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula A1, or a pharmaceutically acceptable salt thereof,

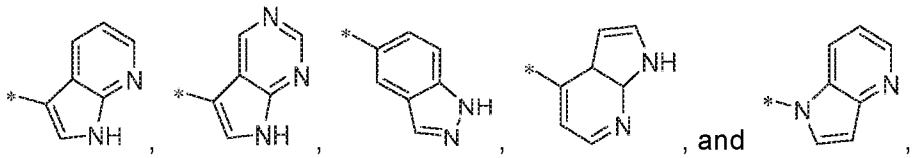


A1

wherein
X^1 and X^2 are each independently CH or N;
Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

halogen;

cyano;

oxo;

C_2alkenyl;

C_2alkynyl;

C_{1-6}haloalkyl;

$-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

$-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

$-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

$-S(O)_2C_{1-6}$alkyl;

monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

phenyl that is unsubstituted or substituted by halogen;

5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

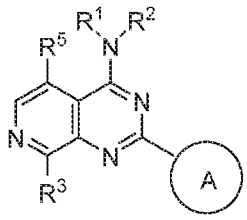
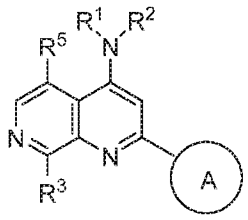
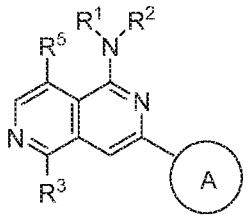
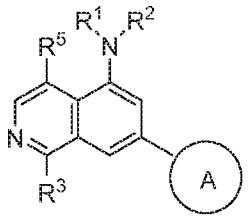
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 208. A method of promoting liver regeneration and liver regrowth, according to embodiment 207, wherein the compound is of the formula selected from Formulae I to IV:


I,

II,

III, and

IV.

Embodiment 209. A method of promoting liver regeneration and liver regrowth according to embodiment 207, wherein the compound is selected from:

2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol;

dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine;

N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;

N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine;

N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;

2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine;

N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;

N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;

2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;

2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine;

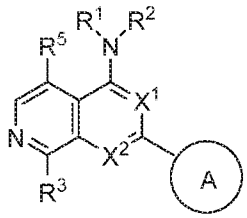
N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;

4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; and

N^2,*N*^{2},2-trimethyl-*N*^{1}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine.

Embodiment 210. A method of promoting liver regeneration and liver regrowth according to embodiment 207, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 211. Use of a compound of Formula A1, or a pharmaceutically acceptable salt thereof,



A1

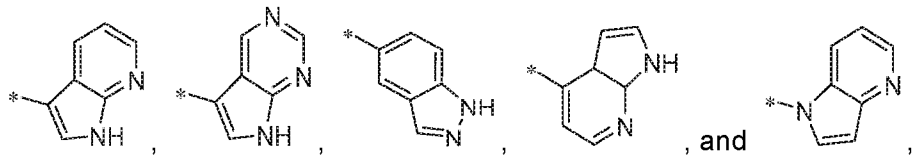
in the manufacture of a medicament for promoting liver regeneration and liver regrowth, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



, and

wherein "*" represents the point of attachment of ring A to the remainder of the molecule; wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, -NH_2, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from
halogen;
cyano;
oxo;
C_2alkenyl;
C_2alkynyl;
C_{1-6}haloalkyl;
-OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -$C(O)R^0$;
-$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;
-$C(O)R^8$, wherein R^8 is R^0 or -NH-C_{1-6}alkyl-$C(O)R^0$;
-$S(O)_2C_{1-6}$alkyl;
monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;
6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;
phenyl that is unsubstituted or substituted by halogen;
5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and
9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents

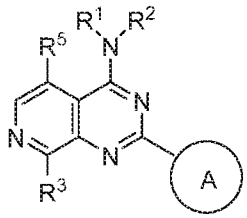
independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -C(O)R^0, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –C(O)R^0;

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

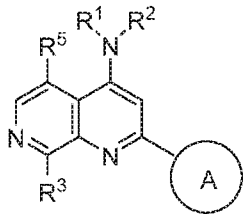
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

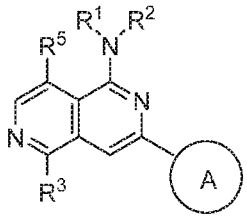
Embodiment 212. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for promoting liver regeneration and liver regrowth according to embodiment 211, wherein the compound is of the formula selected from Formulae I to IV:



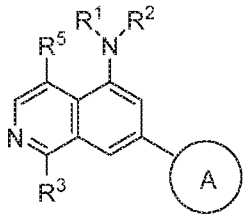
I,



II,



III, and



IV.

Embodiment 213. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for promoting liver regeneration and liver regrowth according to embodiment 211, wherein the compound is selected from:

2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol;

dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine;
N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;
N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;
N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine;
N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;
2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine;
N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;
N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;
2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;
2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine;
N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;
4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; and
N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine.

Embodiment 214. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for promoting liver regeneration and liver regrowth according to embodiment 211, wherein the compound is according to any one of embodiments 1 to 180.

In another embodiment, the present invention relates to a pharmaceutical composition comprising at least one of the compounds of the present invention or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates a pharmaceutical composition comprising at least one of the compounds of the present invention or a pharmaceutically acceptable salt thereof and at least one pharmaceutically acceptable carrier, diluent or excipient.

In another embodiment, the present invention relates to a compound of the present invention, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition for use as a medicament.

In another embodiment, the present invention relates to a pharmaceutical composition for use in promoting liver regeneration and liver regrowth, comprising a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention also relates the use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for promoting liver regeneration and liver regrowth alone, or optionally in combination with another compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof and/or at least one other type of therapeutic agent.

In another embodiment, the present invention relates to a method for promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method for promoting liver regeneration and liver regrowth, particularly for treatment of insufficient liver regrowth following transplantation of marginal grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution.

In another embodiment, the present invention relates to a method for promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutically effective amount of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene

expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method for promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutically effective amount of an agent capable of inhibiting the activity of LATS kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a preferred embodiment, the agent is a compound according to any one of embodiments 1 to 180.

In another embodiment, the present invention relates to a method for expanding a population of liver cells *ex vivo* which comprises contacting the liver cells with a compound according to any one of embodiments 1 to 180. In a preferred embodiment, the method further comprises gene editing said liver cells. Preferably said gene editing targets a gene involved in the host versus graft immune response.

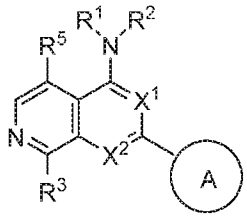
In another embodiment, the present invention relates to a method for expanding a population of liver progenitor cells *ex vivo* which comprises contacting the liver progenitor cells with a compound according to any one of embodiments 1 to 180. In a preferred embodiment, the method further comprises gene editing said liver progenitor cells. Preferably said gene editing targets a gene involved in the host versus graft immune response.

In another embodiment, the present invention relates to a population of liver cells obtained by contacting liver cells with a compound according to any one of embodiments 1 to 180. In a preferred embodiment, the liver cells obtained by contacting liver cells with a compound according to any one of embodiments 1 to 180 have been gene edited. Preferably said gene editing targets a gene involved in the host versus graft immune response.

In another embodiment, the present invention relates to a population of liver progenitor cells obtained by contacting liver progenitor cells with a compound according to any one of embodiments 1 to 180. In a preferred embodiment, the liver progenitor cells obtained by contacting liver progenitor cells with a compound according to any one of embodiments 1 to

180 have been gene edited. Preferably said gene editing targets a gene involved in the host versus graft immune response.

Embodiment 215. A compound of Formula A1, or a pharmaceutically acceptable salt thereof,


A1

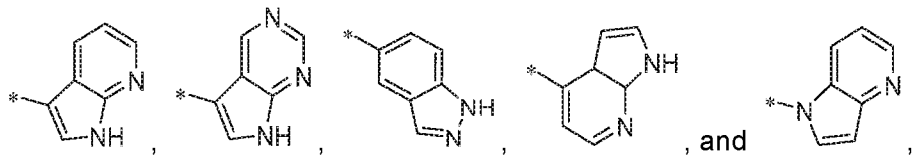
for use in promoting wound healing, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

halogen;

cyano;

oxo;

C_2alkenyl;

C_2alkynyl;

C_{1-6}haloalkyl;

-OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -$C(O)R^0$;

-$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;

-$C(O)R^8$, wherein R^8 is R^0 or -NH-C_{1-6}alkyl-$C(O)R^0$;

-$S(O)_2C_{1-6}$alkyl;

monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

phenyl that is unsubstituted or substituted by halogen;

5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

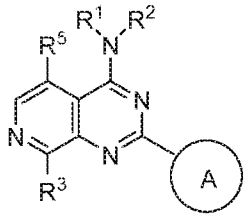
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional

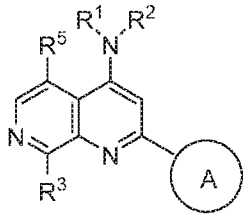
heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

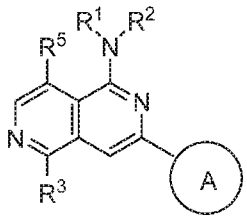
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

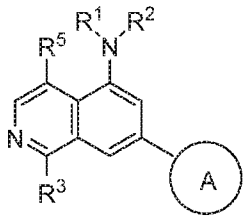
R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 216. A compound of Formula A1 or a pharmaceutically acceptable salt thereof, for use in promoting wound healing according to embodiment 215, wherein the compound is of the formula selected from Formulae I to IV:


I,


II,

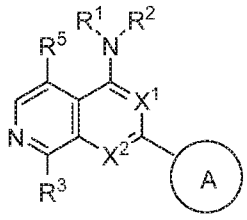

III, and


IV.

Embodiment 217. A compound of Formula A1 or a pharmaceutically acceptable salt thereof, for use in promoting wound healing according to embodiment 215, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 218. A compound of the Formula A1 or a pharmaceutically acceptable salt thereof, for use in promoting wound healing according to embodiment 215, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 219. Use of a compound of the Formula A1, or a pharmaceutically acceptable salt thereof,


A1

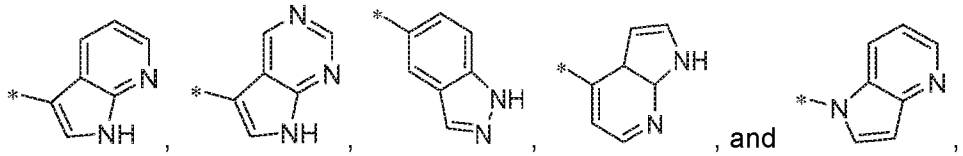
for promoting wound healing, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from


, and
,

wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

halogen;

cyano;

oxo;

C_2alkenyl;

C_2alkynyl;

C_{1-6}haloalkyl;

$-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

$-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

$-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl$-C(O)R^0$;

$-S(O)_2C_{1-6}$alkyl;

monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

phenyl that is unsubstituted or substituted by halogen;

5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

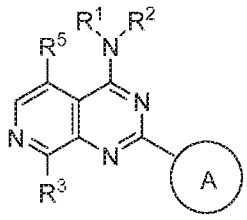
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

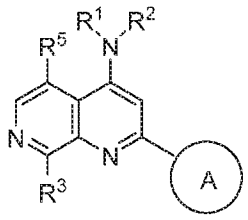
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

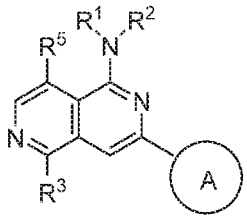
Embodiment 220. Use of a compound of the Formula A1 or a pharmaceutically acceptable salt thereof, for promoting wound healing according to embodiment 219, wherein the compound is of the formula selected from Formulae I to IV:



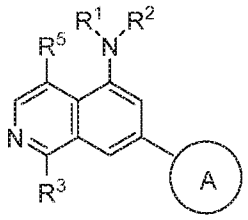
I,



II,



III, and

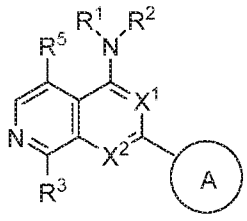


IV.

Embodiment 221. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, for promoting wound healing according to embodiment 219, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; and 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine.

Embodiment 222. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, for promoting wound healing according to embodiment 219, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 223. A method of promoting wound healing, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula A1, or a pharmaceutically acceptable salt thereof,


A1

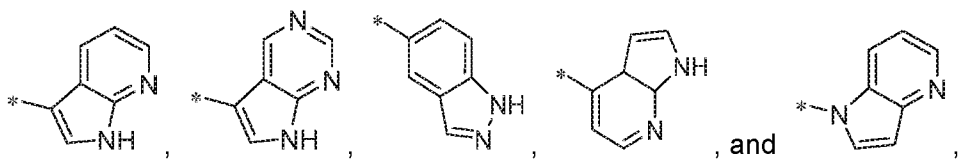
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from


, and ,

wherein "*" represents the point of attachment of ring A to the remainder of the molecule; wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

halogen;
cyano;
oxo;
C_2alkenyl;
C_2alkynyl;
C_{1-6}haloalkyl;
-OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -$C(O)R^0$;
-$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;
-$C(O)R^8$, wherein R^8 is R^0 or -NH-C_{1-6}alkyl-$C(O)R^0$;
-$S(O)_2C_{1-6}$alkyl;
monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;
6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;
phenyl that is unsubstituted or substituted by halogen;
5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and
9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

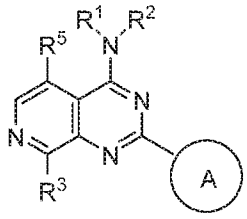
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

or, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$haloalkyl, and R^0;

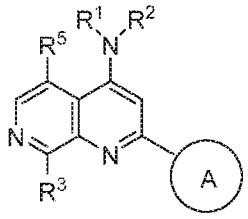
R^3 is selected from hydrogen, halogen and $C_{1\text{-}6}$alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered hetero$C_{3\text{-}8}$alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

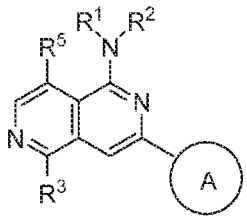
Embodiment 224. A method of promoting wound healing, according to embodiment 223, wherein the compound is of the formula selected from Formulae I to IV:



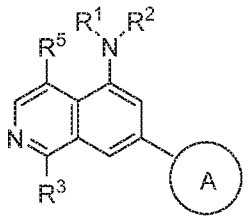
I,



II,



III, and

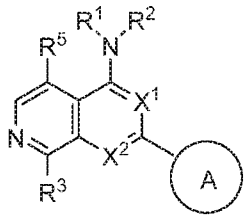


IV.

Embodiment 225. A method of promoting wound healing according to embodiment 223, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

Embodiment 226. A method of promoting wound healing according to embodiment 223, wherein the compound is according to any one of embodiments 1 to 180.

Embodiment 227. Use of a compound of Formula A1, or a pharmaceutically acceptable salt thereof,


A1

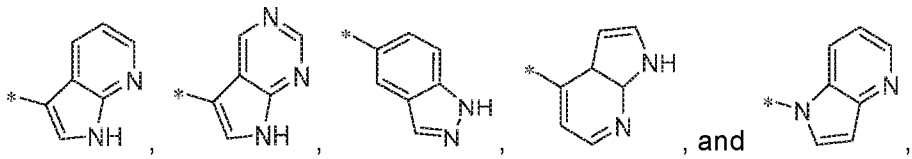
in the manufacture of a medicament for promoting wound healing, wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

halogen;

cyano;

oxo;

C_2alkenyl;

C_2alkynyl;

C_{1-6}haloalkyl;

-OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or -$C(O)R^0$;

-$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;

-$C(O)R^8$, wherein R^8 is R^0 or -NH-C_{1-6}alkyl-$C(O)R^0$;

-$S(O)_2C_{1-6}$alkyl;

monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

phenyl that is unsubstituted or substituted by halogen;

5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

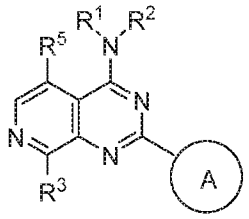
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both

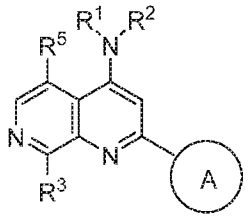
are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

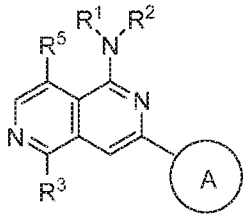
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

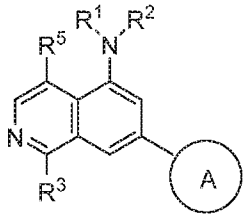
R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

Embodiment 228. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for promoting wound healing according to embodiment 227, wherein the compound is of the formula selected from Formulae I to IV:


I,


II,


III, and


IV.

Embodiment 229. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for promoting wound healing according to embodiment 227, wherein the compound is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; and 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine.

Embodiment 230. Use of a compound of Formula A1 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for promoting wound healing according to embodiment 227, wherein the compound is according to any one of embodiments 1 to 180.

In another embodiment, the present invention relates to a composition comprising at least one of the compounds of Formula A2 or subformulae thereof or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates a pharmaceutical composition comprising at least one of the compounds of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof and at least one pharmaceutically acceptable carrier, diluent or excipient.

In another embodiment, the present invention relates to a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition for use as a medicament.

In another embodiment, the present invention relates to a pharmaceutical composition for use in promoting liver regeneration and liver regrowth, comprising a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention also relates the use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for promoting liver regeneration and liver regrowth alone, or optionally in combination with another compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof and/or at least one other type of therapeutic agent.

In another embodiment, the present invention relates to a method for promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method for promoting liver regeneration and liver regrowth, particularly for treatment of insufficient liver regrowth following transplantation of marginal grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic

liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution.

In another embodiment, the present invention related to a method for promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutically effective amount of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a pharmaceutical composition for use in promoting wound healing, comprising a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention also relates to the use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for promoting wound healing alone, or optionally in combination with another compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof and/or at least one other type of therapeutic agent.

In another embodiment, the present invention relates to a method of promoting wound healing comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method of promoting wound healing comprising treating or ameliorating the symptomology of burns, acute and chronic skin ulcers, wherein the skin ulcers include, but are not limited to vascular ulcers, diabetic ulcers, and pressure ulcers.

In another embodiment, the present invention related to a method of promoting wound healing comprising administering to a subject in need thereof a therapeutically effective

amount of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method of treatment of an ocular disease or disorder comprising administering to a subject in need thereof a cell population, wherein the population has been grown in the presence of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a salt thereof.

In another embodiment, the present invention relates to a method of treatment of an ocular disease or disorder comprising administering to a subject in need thereof a limbal stem cell population, wherein the population has been grown in the presence of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a salt thereof.

In another embodiment, the present invention relates to a method of treatment of an ocular disease or disorder comprising administering to a subject in need thereof a corneal endothelial cell population, wherein the population has been grown in the presence of an agent capable of inhibiting the activity of LATS1 and LATS2 kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation. In a further embodiment, the agent is a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof.

In another embodiment, the present invention relates to a method of promoting ocular wound healing comprising administering to an eye of a subject a therapeutically effective amount of a compound of the invention. In one embodiment, the ocular wound is a corneal wound. In other embodiments, the ocular wound is an injury or surgical wound.

DEFINITIONS

The general terms used hereinbefore and hereinafter preferably have within the context of this invention the following meanings, unless otherwise indicated, where more general terms wherever used may, independently of each other, be replaced by more specific definitions or remain, thus defining more detailed embodiments of the invention.

All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.* "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed.

The term "a," "an," "the" and similar terms used in the context of the present invention (especially in the context of the claims) are to be construed to cover both the singular and plural unless otherwise indicated herein or clearly contradicted by the context.

As used herein, the term "C_{1-8}alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to eight carbon atoms, and which is attached to the rest of the molecule by a single bond. The term "C_{1-4}alkyl" is to be construed accordingly. As used herein, the term "*n*-alkyl" refers to straight chain (un-branced) alkyl radical as defined herein. Examples of C_{1-8}alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), $-C(CH_3)_2CH_2CH(CH_3)_2$ and $-C(CH_3)_2CH_3$.

As used herein, the term "C_{2-6}alkenyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, having from two to six carbon atoms, which is attached to the rest of the molecule by a single bond. The term "C_{2-4}alkenyl" is to be construed accordingly. Examples of C_{2-6}alkenyl include, but are not limited to, ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, pent-4-enyl and penta-1,4-dienyl.

The term "alkylene" refers to a divalent alkyl group. For example, the term "C_{1-6}alkylene" or "C_1 to C_6 alkylene" refers to a divalent, straight, or branched aliphatic group containing 1 to 6 carbon atoms. Examples of alkylene include, but are not limited to methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), n-propylene ($-CH_2CH_2CH_2-$), iso-propylene ($-CH(CH_3)CH_2-$), n-

butylene, sec-butylene, iso-butylene, tert-butylene, n-pentylene, isopentylene, neopentylene and n-hexylene.

As used herein, the term "C_{2-6}alkynyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one triple bond, having from two to six carbon atoms, and which is attached to the rest of the molecule by a single bond. The term "C_{2-4}alkynyl" is to be construed accordingly. Examples of C_{2-6}alkynyl include, but are not limited to, ethynyl, prop-1-ynyl, but-1-ynyl, pent-1-ynyl, pent-4-ynyl and penta-1,4-diynyl.

As used herein, the term "C_{1-6}alkoxy" refers to a radical of the formula -OR_a, where R_a is a C_{1-6}alkyl radical as generally defined above. "C_{1-6} alkoxy" or "C_1 to C_6 alkoxy" is intended to include C_1, C_2, C_3, C_4, C_5, and C_6 alkoxy groups (that is 1 to 6 carbons in the alkyl chain). Examples of C_{1-6}alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, pentoxy, and hexoxy.

As used herein, the term "C_{1-6}alkylamino" refers to a radical of the formula -NH-R_a, where R_a is a C_{1-4}alkyl radical as defined above.

As used herein, the term "di-(C_{1-6}alkyl)amino" refers to a radical of the formula -N(R_a)-R_a, where each R_a is a C_{1-4}alkyl radical, which may be the same or different, as defined above.

As used herein, the term "cyano" means the radical *—C≡N.

The term "cycloalkyl" refers to nonaromatic carbocyclic ring that is a fully hydrogenated ring, including mono-, bi- or poly-cyclic ring systems. "C_{3-10}cycloalkyl" or "C_3 to C_{10} cycloalkyl" is intended to include C_3, C_4, C_5, C_6, C_7, C_8, C_9 and C_{10} cycloalkyl groups that is 3 to 10 carbon ring members). Example cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and norbornyl.

"Fused ring", as used herein, refers to a multi-ring assembly wherein the rings comprising the ring assembly are so linked that the ring atoms that are common to two rings are directly bound to each other. The fused ring assemblies may be saturated, partially saturated, aromatics, carbocyclics, heterocyclics, and the like. Non-exclusive examples of common

fused rings include decalin, naphthalene, anthracene, phenanthrene, indole, benzofuran, purine, quinoline, and the like.

"Halogen" refers to bromo, chloro, fluoro or iodo; preferably fluoro, chloro or bromo.

As used herein, the term "haloalkyl" is intended to include both branched and straight-chain saturated alkyl groups as defined above having the specified number of carbon atoms, substituted with one or more halogens. For example, "C_1-$_6$haloalkyl" or "C_1 to C_6 haloalkyl" is intended to include C_1, C_2, C_3, C_4, C_5, and C_6 alkyl chain. Examples of haloalkyl include, but are not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, trichloromethyl, pentafluoroethyl, pentachloroethyl, 2,2,2-trifluoroethyl, 1,3-dibromopropan-2-yl, 3-bromo-2-fluoropropyl and 1,4,4-trifluorobutan-2-yl, heptafluoropropyl, and heptachloropropyl.

"Heteroalkyl", as used herein, refers to an alkyl, as defined herein, where one or more of the carbon atoms within the alkyl chain are replaced by heteroatoms independently selected from N, O and S. In C_{X-Y}hetereoalkyl or x- to y-membered heteroalkyl, as used herein, x-y describe the number of chain atoms (carbon and heteroatoms) on the heteroalkyl. For example C_{3-8}heteroalkyl refers to an alkyl chain with 3 to 8 chain atoms. Further, heteroalkyl as defined herein the atom linking the radical to the remainder of the molecule must be a carbon. Representative example of 3- to 8-membered heteroalkyl include, but are not limited to -$(CH_2)OCH_3$, -$(CH_2)_2OCH(CH_3)_2$, -$(CH_2)_2$-O-$(CH_2)_2$-OH and -$(CH_2)_2$-(O-$(CH_2)_2)_2$-OH.

The term "heteroaryl" refers to aromatic moieties containing at least one heteroatom (e.g., oxygen, sulfur, nitrogen or combinations thereof) within a 5- to 10-membered aromatic ring system Examples of heteroaryl include, but are not limited to pyrrolyl, pyridyl, pyrazolyl, indolyl, indazolyl, thienyl, furanyl, benzofuranyl, oxazolyl, isoxazolyl, imidazolyl, triazolyl, tetrazolyl, triazinyl, pyrimidinyl, pyrazinyl, thiazolyl, purinyl, benzimidazolyl, quinolinyl, isoquinolinyl, quinoxalinyl, benzopyranyl, benzothiophenyl, benzoimidazolyl, benzoxazolyl and 1H-benzo[d][1,2,3]triazolyl. The heteroaromatic moiety may consist of a single or fused ring system. A typical single heteroaryl ring is a 5- to 6-membered ring containing one to four heteroatoms independently selected from N, O and S and a typical fused heteroaryl ring system is a 9- to 10-membered ring system containing one to four heteroatoms

independently selected from N, O and S. The fused heteroaryl ring system may consist of two heteroaryl rings fused together or a heteroaryl fused to an aryl (e.g., phenyl).

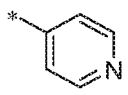
As used herein, the term “heteroatoms” refers to nitrogen (N), oxygen (O) or sulfur (S) atoms. Unless otherwise indicated, any heteroatom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences, and when the heteroatom is sulfur, it can be unoxidized (S) or oxidized to S(O) or $S(O)_2$.

The term “hydroxyl” or “hydroxy”, as used herein, refers to the radical –OH.

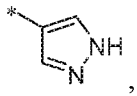
“Heterocycloalkyl” means cycloalkyl, as defined in this application, provided that one or more of the ring carbons indicated, are replaced by a moiety selected from -O-, -N=, -NH-, -S-, -S(O)- and $-S(O)_2-$, Examples of 3 to 8 membered heterocycloalkyl include, but are not limited to oxiranyl, aziridinyl, azetidinyl, imidazolidinyl, pyrazolidinyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydrothienyl 1,1-dioxide, oxazolidinyl, thiazolidinyl, pyrrolidinyl, pyrrolidinyl-2-one, morpholinyl, piperazinyl, piperidinyl, pyrazolidinyl, hexahydropyrimidinyl, 1,4-dioxa-8-aza-spiro[4.5]dec-8-yl, thiomorpholinyl, sulfanomorpholinyl, sulfonomorpholinyl and octahydropyrrolo[3,2-b]pyrrolyl.

The term “oxo", as used herein, refers to the divalent radical =O.

As referred to herein, the term "substituted" means that at least one hydrogen atom is replaced with a non-hydrogen group, provided that normal valencies are maintained and that the substitution results in a stable compound. When a substituent is oxo (*i.e.*, =O), then 2 hydrogens on the atom are replaced. In cases wherein there are nitrogen atoms (*e.g.*, amines) present in compounds of the present invention, these may be converted to N-oxides by treatment with an oxidizing agent (*e.g.*, mCPBA and/or hydrogen peroxides) to afford other compounds of the invention.

As used herein, the term “unsubstituted nitrogen” refers to a nitrogen ring atom that has no capacity for substitution due to its linkage to its adjacent ring atoms by a double bond and a single bond (-N=). For example, the nitrogen at the para position of the 4-pyridyl 

is an “unsubstituted” nitrogen, andthe nitrogen at the 4-position, in reference to the linking

C-ring atom, of 1H-pyrazol-4-yl,  , is an “unsubstituted” nitrogen.

As a person of ordinary skill in the art would be able to understand, for example, a ketone (-CH-C(=O)-) group in a molecule may tautomerize to its enol form(-C=C(OH)-). Thus, this invention is intended to cover all possible tautomers even when a structure depicts only one of them.

As used herein, “  ” and “” are symbols denoting the point of attachment of X, to other part of the molecule.

When any variable occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-3 R groups, then said group may be unsubstituted or substituted with up to three R groups, and at each occurrence R is selected independently from the definition of R.

Unless specified otherwise, the term “compound of the present invention” or “compounds of the present invention” refers to compounds of Formula A2 and subformulae thereof, as well as isomers, such as stereoisomers (including diastereoisomers, enantiomers and racemates), geometrical isomers, conformational isomers (including rotamers and astropisomers), tautomers, isotopically labeled compounds (including deuterium substitutions), and inherently formed moieties (e.g., polymorphs, solvates and/or hydrates). When a moiety is present that is capable of forming a salt, then salts are included as well, in particular pharmaceutically acceptable salts.

It will be recognized by those skilled in the art that the compounds of the present invention may contain chiral centers and as such may exist in different isomeric forms. As used herein, the term “isomers” refers to different compounds that have the same molecular formula but differ in arrangement and configuration of the atoms.

“Enantiomers” are a pair of stereoisomers that are non-superimposable mirror images of each other. A 1:1 mixture of a pair of enantiomers is a "racemic” mixture. The term is used to designate a racemic mixture where appropriate. When designating the stereochemistry

for the compounds of the present invention, a single stereoisomer with known relative and absolute configuration of the two chiral centers is designated using the conventional RS system (e.g., (1S,2S)); a single stereoisomer with known relative configuration but unknown absolute configuration is designated with stars (e.g., (1R*,2R*)); and a racemate with two letters (e.g, (1RS,2RS) as a racemic mixture of (1R,2R) and (1S,2S); (1RS,2SR) as a racemic mixture of (1R,2S) and (1S,2R)). "Diastereoisomers" are stereoisomers that have at least two asymmetric atoms, but which are not mirror-images of each other. The absolute stereochemistry is specified according to the Cahn- Ingold- Prelog R-S system. When a compound is a pure enantiomer the stereochemistry at each chiral carbon may be specified by either *R* or *S*. Resolved compounds whose absolute configuration is unknown can be designated (+) or (–) depending on the direction (dextro- or levorotatory) which they rotate plane polarized light at the wavelength of the sodium D line. Alternatively, the resolved compounds can be defined by the respective retention times for the corresponding enantiomers/diastereomers *via* chiral HPLC.

Certain of the compounds described herein contain one or more asymmetric centers or axes and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)-.

Geometric isomers may occur when a compound contains a double bond or some other feature that gives the molecule a certain amount of structural rigidity. If the compound contains a double bond, the substituent may be E or Z configuration. If the compound contains a disubstituted cycloalkyl, the cycloalkyl substituent may have a cis- or trans-configuration.

Conformational isomers (or conformers) are isomers that can differ by rotations about one or more a bonds. Rotamers are conformers that differ by rotation about only a single a bond.

The term "atropisomer" refers to a structural isomer based on axial or planar chirality resulting from restricted rotation in the molecule.

Unless specified otherwise, the compounds of the present invention are meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate

mixtures. Optically active (*R*)- and (*S*)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques (e.g., separated on chiral SFC or HPLC chromatography columns, such as CHIRALPAK® and CHIRALCEL® available from DAICEL Corp. using the appropriate solvent or mixture of solvents to achieve good separation**).**

The present compounds can be isolated in optically active or racemic forms. Optically active forms may be prepared by resolution of racemic forms or by synthesis from optically active starting materials. All processes used to prepare compounds of the present invention and intermediates made therein are considered to be part of the present invention. When enantiomeric or diastereomeric products are prepared, they may be separated by conventional methods, for example, by chromatography or fractional crystallization.

As used herein, the term “LATS” is the abbreviated name of the large tumor suppressor protein kinase. LATS as used herein refers to LATS1 and/or LATS2. LATS1 as used herein refers to the large tumor suppressor kinase 1 and LATS2 refers to the large tumor suppressor kinase 2. LATS1 and LATS2 both have serine/threonine protein kinase activity.

As used herein, the term “YAP1” refers to the yes-associated protein 1, also known as YAP or YAP65, which is a protein that acts as a transcriptional regulator of genes involved in cell proliferation.

As used herein, the term “MST1/2” refers to mammalian sterile 20–like kinase -1 and -2.

As used herein, the term “pharmaceutical composition” refers to a compound of the invention, or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutically acceptable carrier. In one embodiment pharmaceutical composition is in a form suitable for topical, parenteral or injectable administration.

The term "a therapeutically effective amount" of a compound of the present invention refers to an amount of the compound of the present invention that will elicit the biological or medical response of a subject, for example, reduction or inhibition of an enzyme or a protein activity, or ameliorate symptoms, alleviate conditions, slow or delay disease progression, or prevent a disease, etc. In one non-limiting embodiment, the term “a therapeutically effective

amount" refers to the amount of the compound of the present invention that, when administered to a subject, is effective to (1) at least partially alleviate, inhibit, prevent and/or ameliorate a condition, or a disorder or a disease (i) mediated by LATS activity, or (ii) characterized by activity (normal or abnormal) of LATS; or (2) reduce or inhibit the activity of LATS; or (3) reduce or inhibit the expression of LATS. In another non-limiting embodiment, the term "a therapeutically effective amount" refers to the amount of the compound of the present invention that, when administered to a cell, or a tissue, or a non-cellular biological material, or a medium, is effective to at least partially reducing or inhibiting the activity of LATS; or at least partially reducing or inhibiting the expression of LATS.

The term "subject" includes human and non-human animals. Non-human animals include vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, cats, horses, cows, chickens, dog, mouse, rat, goat, rabbit, and pig. Preferably, the subject is human. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

The term "IC_{50}", as used herein, refers to the molar concentration of an inhibitor that produces 50% of the inhibition effect.

As used herein, the term "treat", "treating" or "treatment" of any disease or disorder refers to alleviating or ameliorating the disease or disorder (i.e., slowing or arresting the development of the disease or at least one of the clinical symptoms thereof); or alleviating or ameliorating at least one physical parameter or biomarker associated with the disease or disorder, including those which may not be discernible to the patient.
As used herein, the term "prevent", "preventing" or "prevention" of any disease or disorder refers to the prophylactic treatment of the disease or disorder; or delaying the onset or progression of the disease or disorder.

As used herein, a subject is "in need of" a treatment if such subject would benefit biologically, medically or in quality of life from such treatment.

Depending on the process conditions the compounds of the present invention are obtained either in free (neutral) or salt form. Both the free form and salt form, and particularly

"pharmaceutically acceptable salts" of these compounds are within the scope of the invention.

As used herein, the terms "salt" or "salts" refers to an acid addition or base addition salt of a compound of the invention. "Salts" include in particular "pharmaceutical acceptable salts". The term "pharmaceutically acceptable salts" refers to salts that retain the biological effectiveness and properties of the compounds of the invention and, which typically are not biologically or otherwise undesirable. In many cases, the compounds of the present invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.
Pharmaceutically acceptable acid addition salts can be formed with inorganic acids and organic acids.

Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like.

Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, toluenesulfonic acid, sulfosalicylic acid, and the like.

Pharmaceutically acceptable base addition salts can be formed with inorganic and organic bases.

Inorganic bases from which salts can be derived include, for example, ammonium salts and metals from columns I to XII of the periodic table. In certain embodiments, the salts are derived from sodium, potassium, ammonium, calcium, magnesium, iron, silver, zinc, and copper; particularly suitable salts include ammonium, potassium, sodium, calcium and magnesium salts.

Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like. Certain organic amines include

isopropylamine, benzathine, cholinate, diethanolamine, diethylamine, lysine, meglumine, piperazine and tromethamine.

In another aspect, the present invention provides compounds of Formula A2 in acetate, ascorbate, adipate, aspartate, benzoate, besylate, bromide/hydrobromide, bicarbonate/carbonate, bisulfate/sulfate, camphorsulfonate, caprate, chloride/hydrochloride, chlortheophyllonate, citrate, ethandisulfonate, fumarate, gluceptate, gluconate, glucuronate, glutamate, glutarate, glycolate, hippurate, hydroiodide/iodide, isethionate, lactate, lactobionate, laurylsulfate, malate, maleate, malonate, mandelate, mesylate, methylsulphate, mucate, naphthoate, napsylate, nicotinate, nitrate, octadecanoate, oleate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, polygalacturonate, propionate, sebacate, stearate, succinate, sulfosalicylate, sulfate, tartrate, tosylate trifenatate, trifluoroacetate or xinafoate salt form.

Any formula given herein is also intended to represent unlabeled forms as well as isotopically labeled forms of the compounds. Isotopically labeled compounds have structures depicted by the formulas given herein except that one or more atoms are replaced by an atom having a selected atomic mass or mass number. Isotopes that can be incorporated into compounds of the invention include, for example, isotopes of hydrogen.

Further, incorporation of certain isotopes, particularly deuterium (i.e., ^{2}H or D) may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements or an improvement in therapeutic index or tolerability. It is understood that deuterium in this context is regarded as a substituent of a compound of the Formula A1 or sub-formulae thereof. The concentration of deuterium, may be defined by the isotopic enrichment factor. The term "isotopic enrichment factor" as used herein means the ratio between the isotopic abundance and the natural abundance of a specified isotope. If a substituent in a compound of this invention is denoted as being deuterium, such compound has an isotopic enrichment factor for each designated deuterium atom of at least 3500 (52.5% deuterium incorporation at each designated deuterium atom), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), at least 5500 (82.5% deuterium incorporation), at least 6000 (90% deuterium incorporation), at least 6333.3 (95% deuterium incorporation), at least 6466.7 (97% deuterium incorporation), at least 6600 (99%

deuterium incorporation), or at least 6633.3 (99.5% deuterium incorporation). It should be understood that the term "isotopic enrichment factor" can be applied to any isotope in the same manner as described for deuterium.

Other examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, and chlorine, such as ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{18}F ^{31}P, ^{32}P, ^{35}S, ^{36}Cl, ^{123}I, ^{124}I, ^{125}I respectively. Accordingly it should be understood that the invention includes compounds that incorporate one or more of any of the aforementioned isotopes, including for example, radioactive isotopes, such as ^{3}H and ^{14}C, or those into which non-radioactive isotopes, such as ^{2}H and ^{13}C are present. Such isotopically labelled compounds are useful in metabolic studies (with ^{14}C), reaction kinetic studies (with, for example ^{2}H or ^{3}H), detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. In particular, an ^{18}F or labeled compound may be particularly desirable for PET or SPECT studies. Isotopically-labeled compounds of Formula A2 can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples and Preparations using an appropriate isotopically-labeled reagents in place of the non-labeled reagent previously employed.

Any asymmetric atom (e.g., carbon or the like) of the compound(s) of the present invention can be present in racemic or enantiomerically enriched, for example the (*R*)-, (*S*)- or (*R,S*)-configuration. In certain embodiments, each asymmetric atom has at least 50 % enantiomeric excess, at least 60 % enantiomeric excess, at least 70 % enantiomeric excess, at least 80 % enantiomeric excess, at least 90 % enantiomeric excess, at least 95 % enantiomeric excess, or at least 99 % enantiomeric excess in the (*R*)- or (*S*)-configuration. Substituents at atoms with unsaturated double bonds may, if possible, be present in *cis*- (*Z*)- or *trans*- (*E*)- form.

Accordingly, as used herein a compound of the present invention can be in the form of one of the possible stereoisomers, rotamers, atropisomers, tautomers or mixtures thereof, for example, as substantially pure geometric (*cis* or *trans*) stereoisomers, diastereomers, optical isomers (antipodes), racemates or mixtures thereof.

Any resulting mixtures of stereoisomers can be separated on the basis of the physicochemical differences of the constituents, into the pure or substantially pure geometric or optical isomers, diastereomers, racemates, for example, by chromatography and/or fractional crystallization.

Any resulting racemates of final compounds or intermediates can be resolved into the optical antipodes by known methods, *e.g.*, by separation of the diastereomeric salts thereof, obtained with an optically active acid or base, and liberating the optically active acidic or basic compound. In particular, a basic moiety may thus be employed to resolve the compounds of the present invention into their optical antipodes, *e.g.*, by fractional crystallization of a salt formed with an optically active acid, *e.g.*, tartaric acid, dibenzoyl tartaric acid, diacetyl tartaric acid, di-*O,O'*-*p*-toluoyl tartaric acid, mandelic acid, malic acid or camphor-10-sulfonic acid. Racemic products can also be resolved by chiral chromatography, *e.g.*, high pressure liquid chromatography (HPLC) using a chiral adsorbent.

"Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (e.g., a polypeptide of the invention), which does not comprise additions or deletions, for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same sequences. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (i.e., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity over a specified region, or, when not specified, over the entire sequence of a reference sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual

alignment and visual inspection. The invention provides polypeptides or polynucleotides that are substantially identical to the polypeptides or polynucleotides, respectively, exemplified herein.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide or cell naturally present in a living animal is not "isolated," but the same nucleic acid or peptide or cell partially or completely separated from the coexisting materials of its natural state is "isolated."

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

A "cell population" or "population of cells" as used herein comprises cells that proliferate in the presence of a LATS1 and/or LATS2 inhibitor in vivo or ex vivo. In such cells, Hippo signaling typically suppresses cell growth, but will proliferate when the pathway is disrupted by LATS inhibition. In certain embodiments, a cell population useful in a method, preparation, medium, agent, or kit of the invention comprises cells from tissues described above or cells described or provided herein. Such cells include, but are not limited to ocular cells (e.g., limbal stem cells, corneal endothelial cells), epithelial cells (e.g., from skin), neural stem cells, mesenchymal stem cells, basal stem cells of the lungs, embryonic stem cells, adult stem cells, induced pluripotent stem cells and liver progenitor cells.

Pharmacology and Utility

In one embodiment the present invention relates to small molecule LATS kinase inhibitors for all indications where cell proliferation would be favorable in vivo and/or ex vivo.

Ex vivo cell therapies generally involve expansion of a cell population isolated from a patient or healthy donor to be transplanted to a patient to establish a transient or stable graft of the expanded cells. Ex vivo cell therapies can be used to deliver a gene or biotherapeutic molecule to a patient, wherein gene transfer or expression of the biotherapeutic molecule is achieved in the isolated cells. Non-limiting examples of ex vivo cell therapies include, but are not limited to, stem cell transplantation (e.g., hematopoietic stem cell transplantation, autologous stem cell transplantation, or cord blood stem cell transplantation), tissue regeneration, cellular immunotherapy, and gene therapy. See for example, Naldini, 2011, Nature Reviews Genetics volume 12, pages 301–315.

In one specific aspect the invention relates to small molecule LATS inhibitors, which are capable of activating the YAP pathway to promote skin cell proliferation, and thereby are useful in promoting wound healing.

Further examples of the uses of the compounds of the present invention are *inter alia*

1) in the treatment of insufficient liver regrowth following transplantation of marginal grafts (organs that are felt inadequate and thus frequently discarded for reasons that include but not limited to excessive fatty content, small for body size and older age of donor);

2) to support enhanced regrowth of the remnant liver mass following extensive hepatectomy, for example in the setting of primary and metastatic tumors of the liver, traumatic liver injury, and resection of other space occupying lesions of the liver such as vascular malformations and liver abscesses;

3) to promote growth of hepatic cells (e.g. hepatic progenitor cells (HPCs)) in culture (ex vivo expansion) and possible subsequent transplantation;

4) to regenerate livers of patients with acute liver failure from viral hepatitis, drug-induced liver injury, autoimmune hepatitis, ischemic and congestive liver disease;

5) to support treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution; or

6) to prevent damage and maintain or improve function of organs ex vivo, with or without perfusion devices.

The YAP/Hippo pathway regulates tissue growth and regeneration in skin and other tissues. The Hippo (MST) kinase cascade, with LATS being the terminal kinases, negatively regulates YAP activity. LATS kinases are serine/threonine protein kinases that have been shown to directly phosphorylate YAP which results in its cytoplasmic retention and inactivation. Without phosphorylation by LATS, YAP translocates to the nucleus, forming a complex with TEAD and driving the downstream gene expression for cell proliferation and survival.

The activity of a compound according to the present invention can be assessed by the following *in vitro* & *in vivo* methods.

LATS1 and LATS2 Inhibition

Compounds of Formula A1 or subformulae thereof, in free form or in pharmaceutically acceptable salt form are potent inhibitors of LATS1 and LATS2.

The inhibition efficacy of the compounds against LATS1 were assayed by the LATS1 Biochemical HTRF Assay as described in the Examples Section A. The inhibition efficacy of the compounds of the invention against LATS1 (LATS1 IC_{50} in μM) are reported in Table 1A.

The LATS1 IC_{50} of the compounds ranged from >10 μM to less than 1 nM, with the majority of the compounds having IC_{50} below 1 μM. It should be noted that compounds with IC_{50} greater than 1 μM are considered inactive in this assy.

The inhibition efficacy of selected compounds against LATS2 were assayed by the LATS2 Biochemical Caliper Assay as described in the Examples Section A. The inhibition efficacy of the compounds of the invention against LATS2 (LATS2 IC_{50} in μM) are also reported in Table 1A.

pYAP Suppression (HaCaT cells)

By the inhibition of LATS1 and LATS2 kinases, compounds of Formula A1 suppress phosphorylation of YAP. The ability of the compounds to reduce phosphorylation of YAP in human HaCaT cells (a keratinocytes cell line) was measured by the pYAP HTRF Assay as described in the Examples. The assay results are reported in Table 1C. The data demonstrated that treatment by compounds of Formula A1 reduced phosphorylation of YAP.

The ability of the compounds of Formula A1 in reducing phosphorylation of YAP can be demonstrated in similar fashion in JHH-5 cells (Fujise et al., Hepatogastroenterology. 1990 Oct;37(5):457-60).

YAP Nuclear Translocation (HaCaT cells)

Without phosphorylation by LATS, YAP translocates to the nucleus. The effect of the compounds of Formula A1, or subformulae thereof, on YAP translocation in human HaCaT cells were assessed by the YAP Nuclear Translocation Assay as described in the Examples Section A. The resulted EC_{50} values are reported in Table 1C. The nuclear translocation EC_{50} ranged from >20 μM to 0.3 μM, and selected compounds having EC_{50} values about 1 μM or below. The data supports that treatment by selected compounds of Formula A1 activate the translocation of YAP from the cytoplasma into the nucleus.

The effect of the compounds of Formula A1, or subformulae thereof, on YAP translocation can be demonstrated in similar fashion in JHH-5 cells (Fujise et al., Hepatogastroenterology. 1990 Oct;37(5):457-60).

Target Identification

Compounds of Formula A1 , or subformulae thereof, selectively target LATS1/2 versus MST1/2 kinases. To identify the target of the hits from the YAP translocation assay screen,

the Target Identification Assay as described in the Examples Section A was performed. The results of the assay are described in Figures 33A to 33C.

Figure 33A shows the western blot analyses for pYAP (Ser127) in lysates of human HaCaT cell, treated with vehicle, or transfected with 40 pM of siRNA against MST1/2 or LATS1/2. siRNA against LATS1/2 eliminated pYAP signal whereas siRNA against MST1/2 had minimal impact. It is consistent with the consensus in the field that LATS is responsible for YAP phosphorylation.

Figure 33B shows the western blot analysis of cell lysates of human HaCaT cells that were untreated or treated with 9 μM of Example 133 for 1 hour in the presence of 0.5 μM Okadaic acid. pYAP was dramatically inhibited. The result is similar to the study of siRNA against LATS and MSTS (Figure 33A), which suggests Example 133 targets the LATS kinases.

Figure 33C shows the inhibition of LATS1 activity (relative to DMSO control) in response to concentration of Example 133 ranging from about 10^{-4} to 1 μM. The data show that Example 133 strongly inhibited LATS1 in this assay with an IC_{50} of 1.3 nM. The result further confirmed that the compounds of the invention are potent inhibitors of the LATS kinases.

Mouse *in vivo* Pharmacodynamics (PD): use in wound healing

The compounds of the invention also activate the YAP pathway *in vivo*. A study of YAP pathway target gene expression was conducted in an *in vivo* mouse model as described in the *In Vivo* PD Assay in the Examples Section A. Two full-thickness excisional wounds on the dorsum of an anesthetized mouse were treated topically with vehicle, or 0.2 and 2 mg/mL of Example 133. After two daily doses, the skin samples around the wound edge were collected and Taqman analyses were performed using *Cyr61* and *Gapdh* probes. mRNA expression levels for the target genes were normalized to Gapdh mRNA levels and plotted against the concentration of Example 133 in Figure 34. The data shows that Example 133 significantly up-regulated the YAP target gene Cyr61 when compared to vehicle, and in a dose-dependent manner. The result is consistent with known LATS biology for activation of the YAP pathway.

In Vivo Skin Cell Proliferation

Further, the compounds of the invention increase skin cell proliferation *in vivo* when applied topically. The study was conducted as described the "*In Vivo* Histology and Ki67 Staining Assay" in the Examples Section A. Example 133 or vehicle were applied to intact mouse dorsal skin. After three days of twice-a-day dosing, the skin samples were collected and subjected to immunohistology staining for Ki67. The sections were evaluated visually for cell proliferation and for the abundance of Ki67 positive cells. The study results are documented in Figure 35A and 35B.

Figure 35A shows representative micrographs of the Ki67 stained mouse skin treated by either vehicle or Example 133. Mouse skin treated by Example 133 shows significant increase in basal cell proliferation and epidermal thickness as compared to vehicle. Figure 35B compares the abundance of Ki67 positive cell in untreated and treated mouse skin. The data shows a 10% higher abundance of Ki67 positive cells in skin treated by Example 133, which is indicative of a statistically significant induction of Ki67 positive cells. Accordingly, Example 133 significantly increased skin cell proliferation in mice *in vivo*.
In summary, from high-content imaging-based phenotypic HTS, compounds of the invention have been shown to be potent LATS inhibitors, which was confirmed by both cellular and biochemical LATS assays. Also, by using siRNA to knockdown both LATS1 and LATS2, LATS as a negative regulator of the YAP pathway has also been validated.

Further, it has been shown, *in vitro*, that Example 133 activated the YAP pathway, promoted cell proliferation in human keratinocytes (HaCaT). LATS inhibitors induced YAP target gene expression in wounded mouse PD models. Topical treatment of Example 133 in mice induced YAP target gene expression and increased basal layer skin cell growth as evidenced by Ki67 staining. Accordingly, Example 133 is useful in promoting skin regeneration.

Compounds of Formula A1 or subformulae thereof, in free form or in pharmaceutically acceptable salt form, therefore may be useful as a therapy for a disease or condition which can benefit from proliferation of cells as described herein. For example, where regeneration of injured or diseased tissue and organs would benefit a subject, including but not limited to

skin, liver and the cornea. Additionally, the compounds of Formula A2, or subformulae thereof, may also be used as research chemicals, e.g. as tool compounds.

Thus, as a further aspect, the present invention provides the use of a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, in therapy.

In a one embodiment, the present invention provides the use of a compound of Formula A1, or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for treating and ameliorating the symptomology of burns, acute skin ulcers and chronic skin ulcers. In one embodiment, the chronic skin ulcer is selected from vascular, diabetic and pressure ulcers. In a further embodiment, the chronic skin ulcer is selected from venous leg ulcers, diabetic foot ulcers, and bed sores.

In another aspect, the invention provides a method of treating a disease or condition, which is treated by inhibition of LATS kinases, comprising administration of a therapeutically acceptable amount of a compound of Formula A2, or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof.

In one embodiment, the disease or condition is selected from burns, acute skin ulcer or chronic skin ulcer. In a further embodiment, the chronic skin ulcer is selected from vascular, diabetic, and pressure ulcers. In a further embodiment, the chronic skin ulcer is selected from venous leg ulcers, diabetic foot ulcers, and bed sores.

Thus, as a further aspect, the present invention provides the use of a compound of Formula A2, or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for the manufacture of a medicament.

In a further embodiment, the present invention provides the use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for the manufacture of a medicament for promoting wound healing, or for the treatment of a disease which may be treated by inhibition of LATS kinases. In another embodiment, the disease is burns, acute or chronic skin ulcer. In another embodiment, the chronic skin ulcer is selected from diabetic ulcers, pressure ulcers and vascular ulcers. In

another embodiment, the chronic skin ulcer is selected from venous leg ulcers, diabetic foot ulcers and bed sores.

Thus, as a further aspect, the present invention provides a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for use as a medicament.

In a further embodiment, the present invention provides a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for use as a medicament for promoting wound healing, or for the treatment of a disease which may be treated by inhibition of LATS kinases. In another embodiment, the disease is burns, acute skin ulcer or chronic skin ulcer. In another embodiment, the chronic skin ulcer is selected from diabetic ulcers, pressure ulcers and vascular ulcers. In another embodiment, the chronic skin ulcer is selected from venous leg ulcers, diabetic foot ulcers and bed sores.

In vivo treatment of mice: use in liver

The ability of the compounds of Formula A1 to induce liver regeneration and liver regrowth was measured by treating mice with the compounds as described in the in vivo Assay as described in the Biologic Assay section infra. The assay results are reported in Table 1C. The results show that the tested compounds reduce pYAP levels in mouse livers when compared to vehicle control treated mice, indicating LATS kinase inhibition in vivo in the liver 2 hours post dosing. Increased mRNA expression of YAP target genes Cyr61 and Ctgf indicates activation of YAP signaling 2 hours post dosing. Immunohistochemistry staining for the proliferation marker Ki67 further indicates increased liver cell proliferation 24 hours post dosing.

Thus, as a further aspect, the present invention provides the use of a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, in therapy.

In a one embodiment, the present invention provides the use of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer

thereof, for promoting liver regeneration and liver regrowth, particularly for the treatment of insufficient liver regrowth following transplantation of marginal grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution.

In another aspect, the invention provides a method of treating a disease which is treated by inhibition of LATS kinases comprising administration of a therapeutically acceptable amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof.

In a further embodiment, the invention provides a method of promoting liver regeneration and liver regrowth, particularly for treatment of insufficient liver regrowth following transplantation of marginal grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution, comprising administration of a therapeutically acceptable amount of a compound of Formula A1 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof.

Thus, as a further aspect, the present invention provides the use of a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for the manufacture of a medicament.

In a further embodiment, the medicament is for promoting liver regeneration and liver regrowth, particularly for treatment of insufficient liver regrowth following transplantation of

marginal grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution

Thus, as a further aspect, the present invention provides a compound of Formula A2 or subformulae thereof, or a pharmaceutically acceptable salt thereof, or a stereoisomer thereof, for use as a medicament. In a further embodiment, the medicament is for promoting liver regeneration and liver regrowth, particularly for treatment of insufficient liver regrowth following transplantation of marginal grafts; for supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; for regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and for treatment of patients with chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis to enhance both regenerative capacity and accelerate fibrosis resolution.

In another aspect, the present invention provides the use of a compound of Formula A1 or subformulae thereof, or a salt thereof, or a stereoisomer thereof, for promoting growth of hepatic cells in culture outside the donor (ex-vivo expansion) or for induction of liver regeneration ex-vivo.

In another aspect, the present invention provides the method for promoting growth of hepatic cells in culture outside the donor (ex-vivo expansion) or for induction of liver regeneration ex-vivo using a compound of Formula A1 or subformulae thereof, or a salt thereof, or a stereoisomer thereof.

Pharmaceutical Composition and Administration

In another aspect, in embodiments of the invention relating to in vivo use, the present invention provides a pharmaceutical composition comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. In a further embodiment, the composition comprises at least two pharmaceutically acceptable carriers, such as those described herein. In embodiments of the invention relating to topical uses of the compounds of the invention, the pharmaceutical composition is formulated in a way that is suitable for topical administration such as aqueous solutions, suspensions, ointments, creams, gels or sprayable formulations, *e.g.*, for delivery by aerosol or the like, comprising the active ingredient together with one or more of solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives that are known to those skilled in the art.

In embodiments of the invention relating to in vivo use, the compound of the present invention is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to give the patient an elegant and easily handleable product. The dosage regimen for the compounds of the present invention will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the species, age, sex, health, medical condition, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; the route of administration, the renal and hepatic function of the patient, and the effect desired. In embodiments of the invention relating to in vivo use, the compounds of the invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three, or four times daily.

In embodiments of the invention relating to in vivo use, the pharmaceutical composition or combination of the present invention can be in unit dosage of about 1-1000 mg of active ingredient(s) for a subject of about 50-70 kg. The therapeutically effective dosage of a compound, the pharmaceutical composition, or the combinations thereof, is dependent on the species of the subject, the body weight, age and individual condition, the disorder or disease or the severity thereof being treated. A physician, clinician or veterinarian of ordinary skill can readily determine the effective amount of each of the active ingredients necessary to prevent, treat or inhibit the progress of the disorder or disease.

The compounds of the present invention can be applied topically in the form of aqueous solutions, suspensions, ointments, creams, lotion, gels or sprayable formulations, *e.g.*, for delivery by aerosol or the like. The dosage may range between about 10^{-3} molar and 10^{-9} molar concentrations. A therapeutically effective amount in vivo may range depending on the route of administration, between about 1-100 mg/kg.

In certain instances, it may be advantageous to administer the compound of the present invention in combination with at least one additional pharmaceutical (or therapeutic) agent, such as a pain killer, and combinations thereof. In particular, compositions will either be formulated together as a combination therapeutic or administered separately.

In certain instances, it may be advantageous to administer the compound of the present invention in combination with at least one additional pharmaceutical (or therapeutic) agent, such as an immunosuppressant for example corticosteroids, cyclosporine, tacrolimus, and combinations of immunosuppressants. In particular, compositions will either be formulated together as a combination therapeutic or administered separately.

The compound of the present invention may be administered either simultaneously with, or before or after, one or more other therapeutic agent. The compound of the present invention may be administered separately, by the same or different route of administration, or together in the same pharmaceutical composition as the other agents. A therapeutic agent is, for example, a chemical compound, peptide, antibody, antibody fragment or nucleic acid, which is therapeutically active or enhances the therapeutic activity when administered to a patient in combination with a compound of the invention.

In one embodiment, the invention provides a product comprising a compound of Formula A1 or subformulae thereof, and at least one other therapeutic agent as a combined preparation for simultaneous, separate or sequential use in therapy. In one embodiment, the therapy is the treatment of a disease or condition mediated by LATS1/2. Products provided as a combined preparation include a composition comprising the compound of Formula A1 and the other therapeutic agent(s) together in the same pharmaceutical composition, or the compound of Formula A1 and the other therapeutic agent(s) in separate form, *e.g.* in the form of a kit.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula A2 or subformulae thereof and another therapeutic agent(s). Optionally, the pharmaceutical composition may comprise a pharmaceutically acceptable carrier, as described above.

In one embodiment related to in vivo use, the invention provides a kit comprising two or more separate pharmaceutical compositions, at least one of which contains a compound of Formula A2 or subformulae thereof. In one embodiment, the kit comprises means for separately retaining said compositions, such as a container, divided bottle, divided tube, or divided foil packet. An example of such a kit is a foil packet, as typically used for deliver gel or ointment, and the like.

The kit of an embodiment of the invention related to in vivo use may be used for administering different dosage forms of the active agents, for example, oral and topical, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit of the invention typically comprises directions for administration.

In the combination therapies of the invention, the compound of the invention and the other therapeutic agent may be manufactured and/or formulated by the same or different manufacturers. Moreover, for embodiments of the invention related to in vivo use, the compound of the invention and the other therapeutic may be brought together into a combination therapy: (i) prior to release of the combination product to physicians (*e.g.* in the case of a kit comprising the compound of the invention and the other therapeutic agent); (ii) by the physician themselves (or under the guidance of the physician) shortly before administration; (iii) in the patient themselves, *e.g.* during sequential administration of the compound of the invention and the other therapeutic agent.

Accordingly, the invention relates to the use of a compound of Formula A1 or subformulae thereof for treating a disease or condition mediated by inhibition of LATS, wherein the medicament is prepared for administration with another therapeutic agent. The invention also relates to the use of another therapeutic agent for treating a disease or condition mediated by inhibition of LATS, wherein the medicament is administered with a compound of Formula A1 or subformulae thereof.

The invention also relates to a compound of formula A1 or subformulae thereof for use in a method of treating a disease or condition mediated by inhibition of LATS, wherein the compound of Formula A1 or subformulae thereof is prepared for administration with another therapeutic agent. The invention also relates to another therapeutic agent for use in a method of treating a disease or condition mediated inhibition of LATS, wherein the other therapeutic agent is prepared for administration with a compound of Formula A1 or subformulae thereof.

The invention also relates to a compound of Formula A1 or subformulae thereof for use in a method of treating a disease or condition mediated by inhibition of LATS, wherein the compound is administered with another therapeutic agent. The invention also provides another therapeutic agent for use in a method of treating a disease or condition mediated by inhibition of LATS, wherein the other therapeutic agent is administered with a compound of Formula A1 or subformulae thereof.

The invention also relates to the use of a compound of Formula A1 or subformulae thereof for treating a disease or condition mediated by LATS1/2, wherein the patient has previously (*e.g.* within 24 hours) been treated with another therapeutic agent. The invention also provides the use of another therapeutic agent for treating a disease or condition mediated by LATS, wherein the patient has previously (*e.g.* within 24 hours) been treated with a compound of Formula A1 or subformulae thereof.

PREPARATION OF COMPOUNDS

The compounds of the present invention can be prepared in a number of ways known to one skilled in the art of organic synthesis in view of the methods, reaction schemes and examples provided herein. The compounds of the present invention can be synthesized using the methods described below, together with synthetic methods known in the art of synthetic organic chemistry, or by variations thereon as appreciated by those skilled in the art. Preferred methods include, but are not limited to, those described below. The reactions are performed in a solvent or solvent mixture appropriate to the reagents and materials employed and suitable for the transformations being effected. It will be understood by those skilled in the art of organic synthesis that the functionality present on the molecule

should be consistent with the transformations proposed. This will sometimes require a judgment to modify the order of the synthetic steps or to select one particular process scheme over another in order to obtain a desired compound of the invention.

The starting materials are generally available from commercial sources such as Aldrich Chemicals (Milwaukee, Wis.) or are readily prepared using methods well known to those skilled in the art (e.g., prepared by methods generally described in Louis F. Fieser and Mary Fieser, Reagents for Organic Synthesis, v. 1-19, Wiley, New York (1967-1999 ed.), Larock, R.C., *Comprehensive Organic Transformations*, 2nd-ed., Wiley-VCH Weinheim, Germany (1999), or Beilsteins Handbuch der organischen Chemie, 4, Aufl. ed. Springer-Verlag, Berlin, including supplements (also available via the Beilstein online database).

For illustrative purposes, the reaction schemes depicted below provide potential routes for synthesizing the compounds of the present invention as well as key intermediates. For a more detailed description of the individual reaction steps, see the Examples section below. Those skilled in the art will appreciate that other synthetic routes may be used to synthesize the inventive compounds. Although specific starting materials and reagents are depicted in the schemes and discussed below, other starting materials and reagents can be easily substituted to provide a variety of derivatives and/or reaction conditions. In addition, many of the compounds prepared by the methods described below can be further modified in light of this disclosure using conventional chemistry well known to those skilled in the art.

In the preparation of compounds of the present invention, protection of remote functionality of intermediates may be necessary. The need for such protection will vary depending on the nature of the remote functionality and the conditions of the preparation methods. The need for such protection is readily determined by one skilled in the art. For a general description of protecting groups and their use, see Greene, T.W. et al., *Protecting Groups in Organic Synthesis*, 4th Ed., Wiley (2007). Protecting groups incorporated in making of the compounds of the present invention, such as the trityl protecting group, may be shown as one regioisomer but may also exist as a mixture of regioisomers.

Abbreviations

Abbreviations as used herein, are defined as follows: "1x" for once, "2x" for twice, "3x" for thrice, "ºC" for degrees Celsius, "aq" for aqueous, "Col" for column, "eq" for equivalent or equivalents, "g" for gram or grams, "mg" for milligram or milligrams, "nm" for nanometer or

nanometers, "L" for liter or liters, "mL" or “ml” for milliliter or milliliters, "ul", "uL", "µl", or "µL" for microliter or microliters, "nL" or “nl” for nanoliter or nanoliters, " "N" for normal, “uM” or “µM” micromolar, "nM" for nanomolar, "mol" for mole or moles, "mmol" for millimole or millimoles, "min" for minute or minutes, "h" or "hrs" for hour or hours, "RT" for room temperature, "ON" for overnight, "atm" for atmosphere, "psi" for pounds per square inch, "conc." for concentrate, "aq" for aqueous, "sat" or "sat'd" for saturated, "MW" for molecular weight, "mw" or "µwave" for microwave, "mp" for melting point, "Wt" for weight, "MS" or "Mass Spec" for mass spectrometry, "ESI" for electrospray ionization mass spectroscopy, "HR" for high resolution, "HRMS" for high resolution mass spectrometry, "LCMS" for liquid chromatography mass spectrometry, "HPLC" for high pressure liquid chromatography, "RP HPLC" for reverse phase HPLC, "TLC" or "tlc" for thin layer chromatography, "NMR" for nuclear magnetic resonance spectroscopy, "nOe" for nuclear Overhauser effect spectroscopy, "1H" for proton, "δ " for delta, "s" for singlet, "d" for doublet, "t" for triplet, "q" for quartet, "m" for multiplet, "br" for broad, "Hz" for hertz, "ee" for "enantiomeric excess" and "α", "β", "R","r", "S", "s", "E", and "Z" are stereochemical designations familiar to one skilled in the art.

The following abbreviations used herein below have the corresponding meanings:

AC	Active Control
AIBN	azobisisobutyronitrile
ATP	adenosine triphosphate
Bn	benzyl
Boc	*tert*-butoxy carbonyl
Boc_2O	di-*tert*-butyl dicarbonate
BSA	bovine serum albumin
Bu	butyl
Cs_2CO_3	cesium carbonate anhydrous
$CHCl_3$	chloroform
DAST	diethylaminosulfurtrifluoride
DBU	2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMEM	Dulbecco’s modified Eagle’s medium
DMF	dimethylformamide

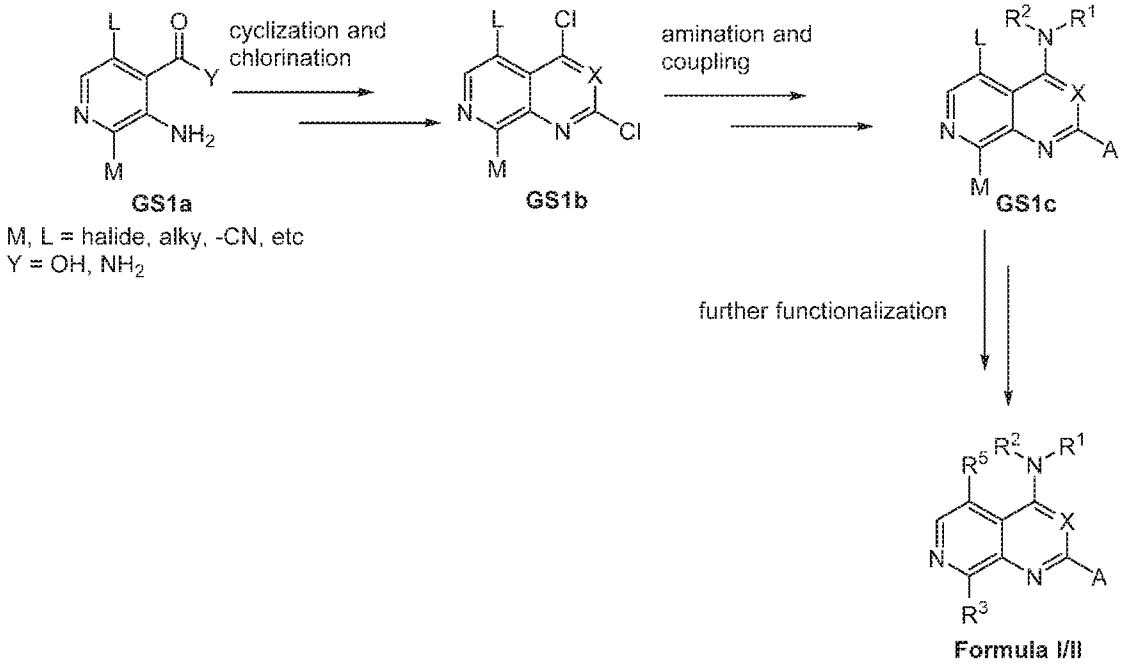
DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
DTT	dithiolthreitol
EA	ethyl acetate
EDTA	ethylenediaminetetraacetic acid
Equiv.	equivalence
Et	ethyl
Et_2O	diethyl ether
EtOH	ethanol
EtOAc	ethyl acetate
FBS	fetal bovine serum
HATU	2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCl	hydrochloric acid
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPMC	(hydroxypropyl)methyl cellulose
HTRF	homogeneous time resolved fluorescence
i-Bu	isobutyl
i-Pr	isopropyl
KOAc	potassium acetate
$LiAlH_4$	lithium aluminium hydride
Me	methyl
mCPBA	3-chloroperoxybenzoic acid
MeCN	acetonitrile
MnO_2	manganese dioxide
N_2	nitrogen
$NaBH_4$	sodium borohydride
$NaHCO_3$	sodium bicarbonate
Na_2SO_4	sodium sulfate
NBS	*N*-Bromosuccinimide
NC	Neutral Control
PBS	phosphate buffered saline
PFA	paraformaldehyde
Ph	phenyl

PPh_3	triphenylphosphine
$Ph_3P=O$	triphenylphosphine oxide
pYAP	phospho-YAP
R_f	retention factor
RT	room temperature (°C)
Ser	serine
t-Bu or Bu^t	*tert*-butyl
T3P®	Propane phosphonic acid anhydride
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
UVA	Ultraviolet A
YAP	Yes associated protein (NCBI Gene ID: 10413; official symbol: (YAP1)

I. General Synthetic Routes

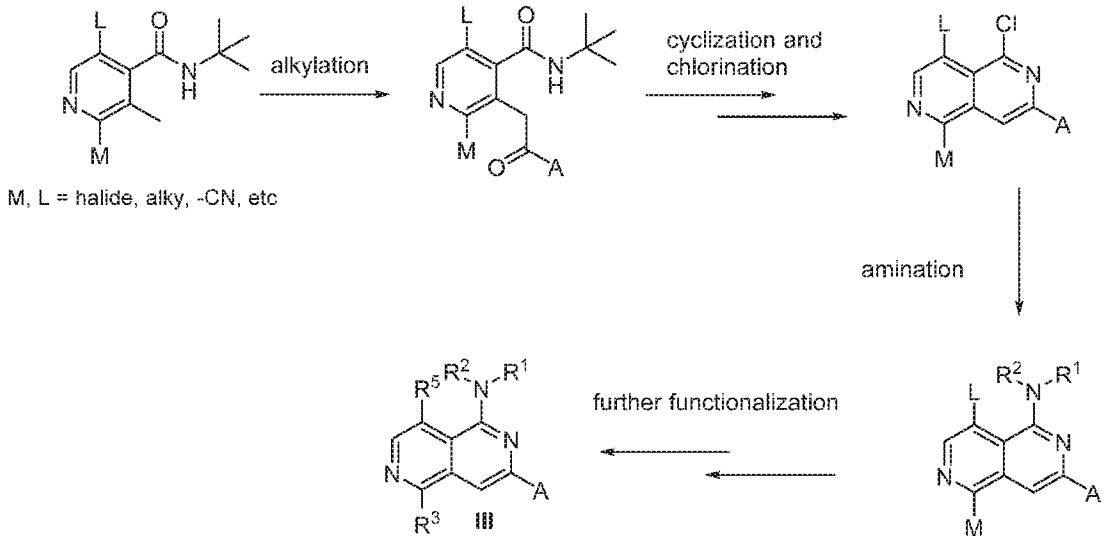
Compounds of Formulae I to VI can be prepared as illustrated in the General Schemes I to III and in greater details in Schemes 1 to 6 below. Detailed description for the synthesis of the intermediates and exemplified compounds are also disclosed below.

General Scheme I for the preparation of compounds of Formula I or II

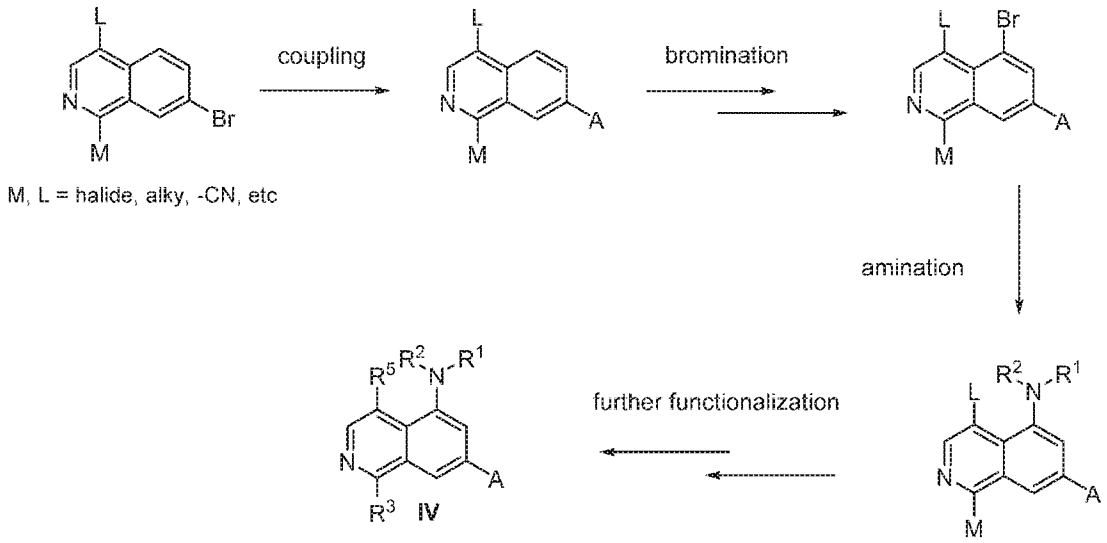


The bicyclic dichloride **GS1b** could be commercially available when X = C or could be prepared from aminoisonicotinic acid/amide **GS1a** through cyclization and chlorination. The dichloride of **GS1b** could be aminated and coupled with the appropriate agents to form **GS1c**, which further functionalized to yield Formula I or Formula II through any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, amination, coupling, etc

General Scheme II for the preparation of compounds of Formula III



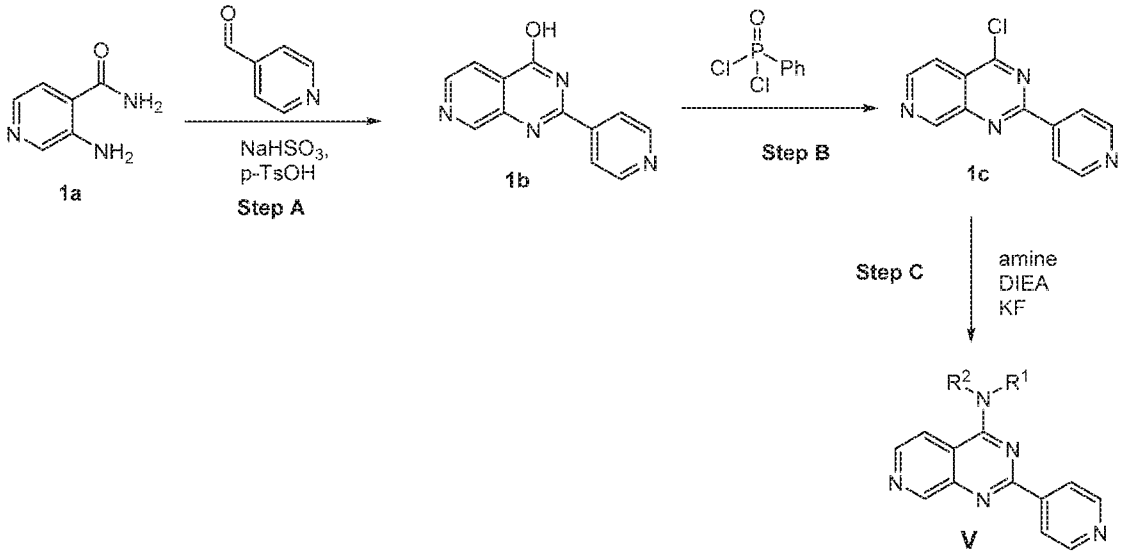
General Scheme III for the preparation of compounds of Formula IV



Scheme 1.

Compounds of Formula V can be prepared as illustrated in Scheme 1 below. Step C could include amination and any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, etc.

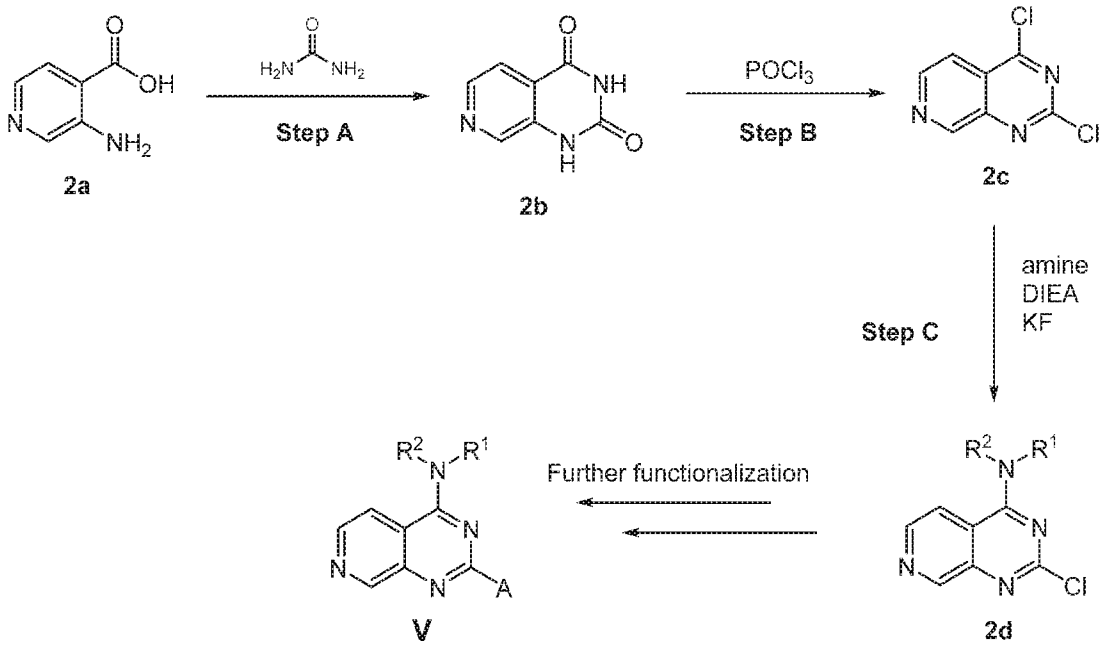
Scheme 1



Scheme 2.

Alternatively, compounds of Formula V can be prepared as illustrated in Scheme 2. Step C could include amination and any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, etc. Further functionalization of mono-chloride intermediate **2d** by but not limited to metal mediated coupling, amination, alkylation etc. and necessary protection and de-protection steps, leads to compounds of Formula V.

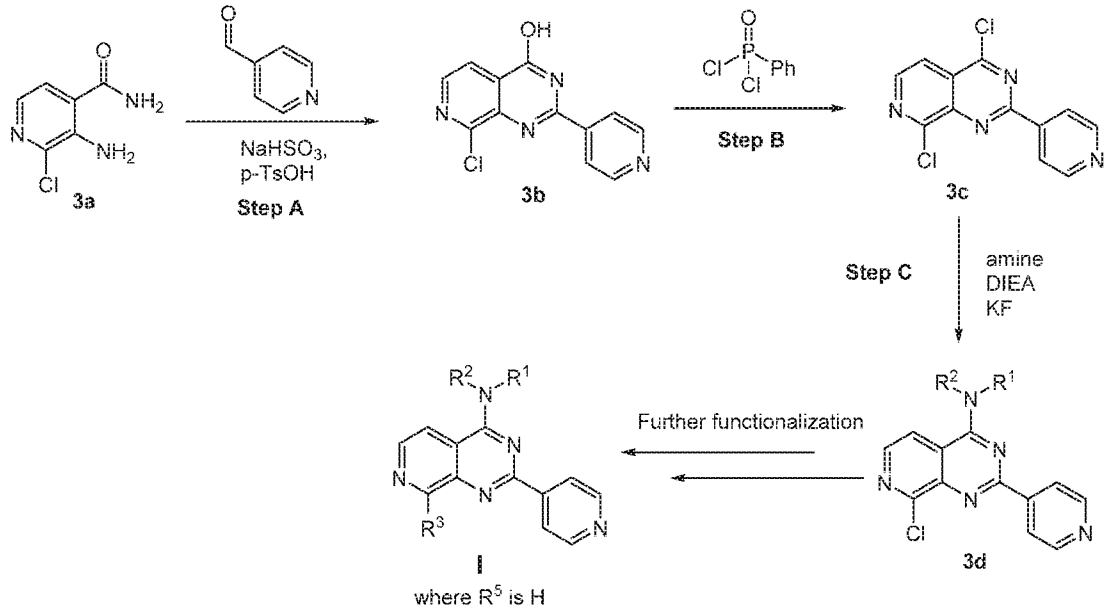
Scheme 2



Scheme 3.

Compounds of Formula I where R^5 is hydrogen can be prepared as illustrated in Scheme 3. Step C could include amination and any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, etc. Further functionalization of mono-chloride intermediate **3d** by but not limited to metal mediated coupling, amination, alkylation etc. and necessary protection and de-protection steps, leads to compounds of Formula (I) where R^5 is hydrogen.

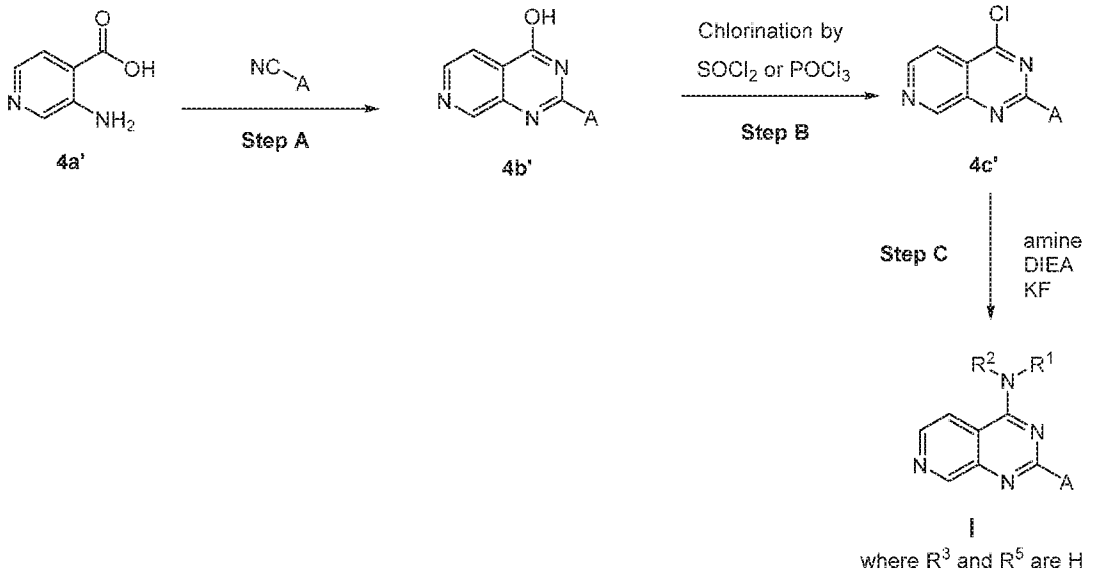
Scheme 3



Scheme 4.

Compounds of Formula I, where R^3 and R^5 are both hydrogen, can be prepared as illustrated in Scheme 4. Step C could include amination and any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, etc. leads to compounds of Formula I where R^3 and R^5 are both hydrogen.

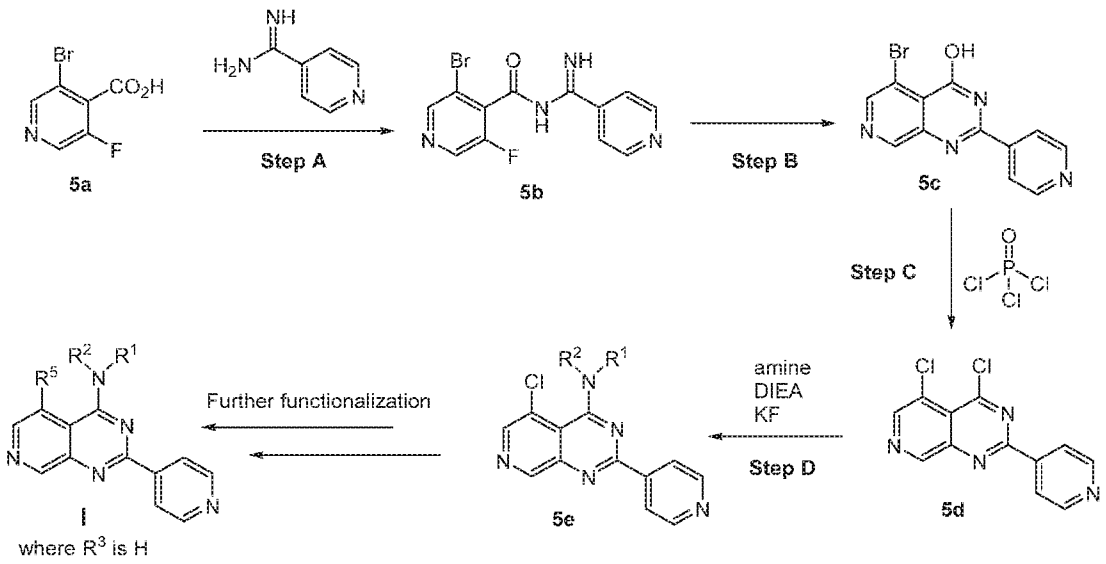
Scheme 4



Scheme 5.

Compounds of Formula I, where R^3 is hydrogen, can be prepared as illustrated in Scheme 5. Step D could include amination and any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, etc. Further functionalization of mono- chloride intermediate **5d** by, but not limited to, metal mediated coupling, amination, alkylation etc. and necessary protection and de-protection steps, leads to compounds of Formula I where R^3 is hydrogen,

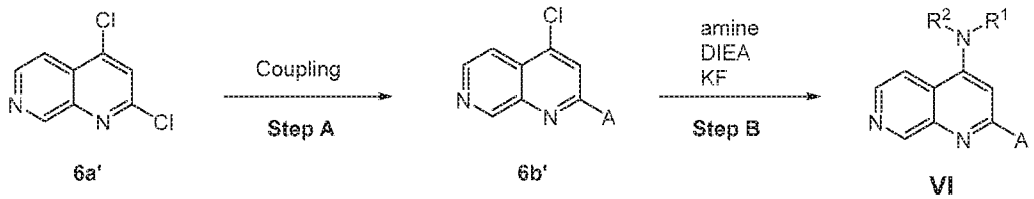
Scheme 5



Scheme 6.

Compounds of Formula VI can be prepared from commercially available dichloride **6a'** (2,4-dichloro-1,7-naphthyridine, Aquila Pharmatech) as illustrated in Scheme 6. Step A could include metal mediated coupling and any necessary functionalization, such as but not limited to protection and de-protection steps, cyclization, reduction, hydrolysis, alkylation, etc. Step B could include amination and any necessary functionalization, such as but not limited to protection and de-protection steps, reduction, hydrolysis, alkylation, etc.

Scheme 6



Preparation of Exemplified Examples

The following Examples have been prepared, isolated and characterized using the methods disclosed herein. The following examples demonstrate a partial scope of the invention and are not meant to be limiting of the scope of the invention.

Unless specified otherwise, starting materials are generally available from a non-excluding commercial sources such as TCI Fine Chemicals (Japan), Shanghai Chemhere Co., Ltd.(Shanghai, China), Aurora Fine Chemicals LLC (San Diego, CA), FCH Group (Ukraine), Aldrich Chemicals Co. (Milwaukee, Wis.), Lancaster Synthesis, Inc. (Windham, N.H.), Acros Organics (Fairlawn, N.J.), Maybridge Chemical Company, Ltd. (Cornwall, England), Tyger Scientific (Princeton, N.J.), AstraZeneca Pharmaceuticals (London, England), Chembridge Corporation (USA), Matrix Scientific (USA), Conier Chem & Pharm Co., Ltd (China), Enamine Ltd (Ukraine), Combi-Blocks, Inc. (San Diego, USA), Oakwood Products, Inc. (USA), Apollo Scientific Ltd. (UK), Allichem LLC. (USA) and Ukrorgsyntez Ltd (Latvia).

LCMS Methods Employed in Characterization of Examples 1-290

Analytical LC/MS is carried out on Agilent systems using ChemStation software. The systems consist of:

- Agilent G1312 Binary Pump
- Agilent G1367 Well Plate Autosampler

- Agilent G1316 Thermostated Column Compartment
- Agilent G1315 Diode Array Detector
- Agilent 6140/6150 Mass Spectrometer
- SOFTA Evaporative Light Scattering Detector

Typical method conditions are as follows:

- Flow Rate: 0.9mL/min
- Column: 1.8 micrometres 2.1x50mm Waters Acquity HSS T3 C18 column
- Mobile Phase A: Water+0.05% TFA
- Mobile Phase B: Acetonitrile+0.035% TFA
- Run Time: 2.25 minutes
- The system runs a gradient from 10% B to 90% B in 1.35 minutes. A 0.6 minute wash at 100% B follows the gradient. The remaining duration of the method returns the system to initial conditions.
- Typical mass spectrometer Scan range is 100 to 1000 amu.

LCMS Methods Employed in Characterization of Examples 291-335

LC-MS (method 1):

System: Waters Acquity UPLC with Waters SQ detector.
Column: Acquity HSS T3 1.8 µm 2.1x50mm.
Flow: 1.0 ml/min. Column temperature: 60 °C.
Gradient: from 5 to 98% B in 1.4 min, A = water + 0.05% formic acid + 3.75 mM ammonium acetate, B = acetonitrile + 0.04% formic acid.

LC-MS (method 2):

System: Waters Acquity H-Class UPLC with Waters SQ detector.
Column: BEH C18 1.7 µm 2.1x50mm
Flow: 3.0 ml/min. Column temperature: 30 °C.
Gradient: from 2 to 100% B in 2.7 min, A = 2 mM ammonium acetate / water + 0.1% formic acid , B = acetonitrile + 0.1% formic acid.

NMR Employed in Characterization of Examples 1-290

Proton spectra are recorded on a Bruker AVANCE II 400 MHz with 5 mm QNP Cryoprobe or a Bruker AVANCE III 500 MHz with 5 mm QNP probe unless otherwise noted. Chemical shifts are reported in ppm relative to dimethyl sulfoxide (δ 2.50), chloroform (δ 7.26), methanol (δ 3.34), or dichloromethane (δ 5.32). A small amount of the dry sample (2-5 mg) is dissolved in an appropriate deuterated solvent (1 mL).

NMR Employed in Characterization of Examples 291-335

Proton spectra are recorder on a Bruker Avance 400 NMR spectrometer (400 MHz) equipped with a cryo probe or a Bruker Avance 600 NMR spectrometer (600 MHz) equipped with a cryo probe. Chemical shifts (δ-values) are reported in ppm downfield from tetramethylsilane, spectra splitting pattern are designated as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p) multiplet, unresolved or more overlapping signals (m), broad signal (br). Solvents are given in parentheses.

Reagents and materials

Solvents and reagents were purchased from suppliers and used without any further purification. Basic ion exchange resin cartridges PoraPakTM Rxn CX 20cc (2g) were purchased from Waters. Phase separator cartridges (Isolute Phase Separator) were purchased from Biotage. Isolute absorbant (Isolute HM-N) was purchased from Biotage.

ISCO Methods Employed in Purification of Examples

ISCO flash chromatography is carried on Teledyne COMBIFLASH® system with prepacked silica RediSep® column.

Preparative HPLC Methods Employed in Purification of Examples

Preparative HPLC is carried out on Waters Autoprep systems using MassLynx and FractionLynx software. The systems consist of:

- Waters 2767 Autosampler/Fraction Collector
- Waters 2525 Binary Pump
- Waters 515 Makeup pump
- Waters 2487 Dual Wavelength UV Detector

- Waters ZQ Mass Spectrometer

Typical method conditions are as follows:

- Flow Rate: 100mL/min
- Column: 10 micrometres 19x50mm Waters Atlantis T3 C18 column
- Injection Volume: 0-1000 microlitres
- Mobile Phase A: Water+0.05% TFA
- Mobile Phase B: Acetonitrile+0.035% TFA
- Run Time: 4.25 minutes

The system runs a gradient from x% B to y% B as appropriate for the examples in 3 minutes following a 0.25 minute hold at initial conditions. A 0.5 minute wash at 100%B follows the gradient. The remaining duration of the method returns the system to initial conditions.

Fraction collection is triggered by mass detection through FractionLynx software.

Chiral Preparative HPLC Methods Employed in Purification of Examples

SFC chiral screening is carried out on a Thar Instruments Prep Investigator system coupled to a Waters ZQ mass spectrometer. The Thar Prep Investigator system consists of:

- Leap HTC PAL autosampler
- Thar Fluid Delivery Module (0 to 10 mL/min)
- Thar SFC 10 position column oven
- Waters 2996 PDA
- Jasco CD-2095 Chiral Detector
- Thar Automated Back Pressure Regulator.

All of the Thar components are part of the SuperPure Discovery Series line.

The system flows at 2mL/min (4mL/min for the WhelkO-1 column) and is kept at 30 degrees C. The system back pressure is set to 125 bar. Each sample is screened through a battery of six 3 micrometre columns:

- 3 micrometre 4.6x50 mm ChiralPak AD
- 3 micrometre 4.6x50 mm ChiralCel OD
- 3 micrometre 4.6x50 mm ChiralCel OJ

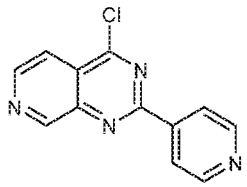
- 3 micrometre 4.6x250 mm Whelk O-1
- 3 micrometre 4.6x50 mm ChiralPak AS
- 3 micrometre 4.6x50 mm Lux-Cellulose-2

The system runs a gradient from 5% co-solvent to 50% co-solvent in 5 minutes followed by a 0.5 minute hold at 50% co-solvent, a switch back to 5% co-solvent and a 0.25 minute hold at initial conditions. In between each gradient there is a 4 minute equilibration method the flows at 5% co-solvent through the next column to be screened. The typical solvents screened are MeOH, MeOH+20mM NH_3, MeOH+0.5%DEA, IPA, and IPA+20mM NH_3.

Once separation is detected using one of the gradient methods an isocratic method will be developed and, if necessary, scaled up for purification on the Thar Prep80 system.

Synthesis of Intermediates

Intermediate 1c (Scheme 1): 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine

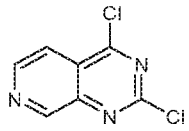


Step A: In a 20 mL microwave vial was added 3-aminoisonicotinamide (**1a**, 2 g, 14.58 mmol), isonicotinaldehyde (1.521 mL, 16.04 mmol), sodium bisulfite (1.821 g, 17.50 mmol) and 4-methylbenzenesulfonic acid hydrate (0.277 g, 1.458 mmol) in DMA (Volume: 5 mL) to give an orange suspension. The reaction was well stirred and heated in microwave at 160°C for 12 min. The reaction mixture was diluted with water and filtered. The solid was washed with water, MeOH and ether to give 2.03g off white solid as the product **1b** (59%). 1H NMR (400 MHz, DMSO-d6) δ 9.18 (d, J = 0.9 Hz, 1H), 8.88 - 8.78 (m, 2H), 8.73 (d, J = 5.2 Hz, 1H), 8.16 - 8.08 (m, 2H), 8.03 (dd, J = 5.2, 0.9 Hz, 1H).

Step B: In a 5 mL microwave reactor was 2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**1b,** 500 mg, 2.230 mmol) and phenylphosphonic dichloride (1564 microlitre, 11.15 mmol) to give a brown suspension. The reaction mixture was stirred at 170°C for 30min when LCMS indicated full conversion. The reaction mixture was quenched with ice/water and neutralized with saturated Na_2CO_3, then extracted with DCM x 3 and give the product **1c** (74%). 1H

NMR (500 MHz, DMSO-d6) δ 9.65 (d, J = 1.0 Hz, 1H), 8.96 (d, J = 5.6 Hz, 1H), 8.87 (s, 2H), 8.47 - 8.30 (m, 2H), 8.18 (dd, J = 5.6, 1.0 Hz, 1H). LCMS (m/z $[M+H]^+$): 243.1.

Intermediate 2c (Scheme 2): 2,4-dichloropyrido[3,4-d]pyrimidine

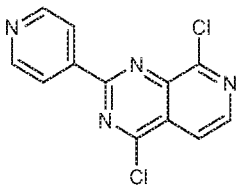


Step A: A mixture of urea (40.00 g, 666.00 mmol) and 3-aminoisonicotinic acid (**2a,** 18.40 g, 133.20 mmol) was heated at 210°C for 1 hr (NOTE: no solvent was used). NaOH (2N, 320 mL) was added, and the mixture was stirred at 90°C for 1h. The solid was collected by filtration, and washed with water. The crude product thus obtained was suspended in HOAc (400 mL), and stirred at 100°C for 1h. The mixture was cooled to RT, filtered, and the solid was washed with a large amount of water, and then dried under the vacuum to give pyrido[3,4-d]pyrimidine-2,4(1H,3H)-dione (**2b,** 17.00 g, 78% yield) without further purification. LCMS (m/z $[M+H]^+$): 164.0.

Step B: To a mixture of pyrido[3,4-d]pyrimidine-2,4(1H,3H)-dione (**2b**, 20.00 g, 122.60 mmol) and $POCl_3$ (328.03 g, 2.14 mol) in toluene (200 mL) was added DIEA (31.69 g, 245.20 mmol) dropwise and this reaction mixture stirred at 25°C overnight (18hr) to give suspension.

The solvent and $POCl_3$ was removed under vacuum, diluted with DCM (50 mL), neutralized with DIEA to pH=7 at -20°C and concentrated again, the residue was purified by column (20-50% EA/PE) to give the product (**2c**, 20.00g, 99.99 mmol, 82% yield) as a yellow solid. 1H NMR (400 MHz, CHLOROFORM-*d*) δ 9.52 (s, 1 H), 8.92 (d, *J*=5.6 Hz, 1 H), 8.04 (d, *J*=5.6 Hz, 1 H). LCMS (m/z $[M+H]^+$): 200.0.

Intermediate 3c (Scheme 3): 4,8-dichloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine

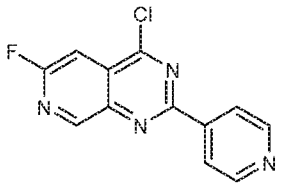


Step A: In a 20 mL microwave reactor was added 3-aminoisonicotinamide (**3a**, 650 mg, 3.79 mmol), and isonicotinaldehyde (487 mg, 4.55 mmol), sodium bisulfite (788 mg, 7.58 mmol) in DMA (Volume: 10 mL) to give a yellow suspension. The reaction mixture

was stirred under microwave at 160°C for 10min. The reaction mixture was diluted with water, filtered and washed with MeOH and ether. The solid was collected to give the product **3b** (8-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol, 300 mg, 29%). 1H NMR (400 MHz, DMSO-d6) δ 8.86 - 8.80 (m, 2H), 8.45 (d, J = 5.1 Hz, 1H), 8.18 - 8.12 (m, 2H), 8.00 (d, J = 5.1 Hz, 1H).

Step B: In a 20 mL microwave reactor was mixed (8-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**3b**, 300 mg, 1.160 mmol), and phenylphosphonic dichloride (1 mL, 7.13 mmol) to give a yellow suspension. The reaction mixture was stirred under microwave at 170°C for 60min. The reaction mixture was diluted with water and filtered. The solid was washed with water, MeOH and ether to give the title product **3c** (78%). 1H NMR (400 MHz, DMSO-d6) δ 9.05 - 8.96 (m, 2H), 8.52 (d, J = 5.1 Hz, 1H), 8.46 - 8.38 (m, 2H), 8.05 (d, J = 5.1 Hz, 1H). (NMR sample was added 1 drop of TFA, otherwise, 2 set of peaks were observed). LCMS (m/z $[M+H]^+$): 277.0.

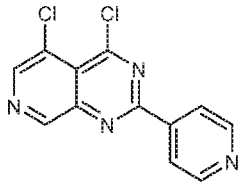
<u>Intermediate 4c (Scheme 4, intermediate 4c, wherein X is F and A is 4-pyridinyl):</u> 4-chloro-6-fluoro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine



Step A: A solution of isonicotinonitrile (800 mg, 7.69 mmol) in MeOH (30 ml) was treated with sodium methoxide (0.474 ml, 5.4M, 2.56 mmol) at r.t for 1 hr. Then 5-amino-2-fluoroisonicotinic acid (1.0 g, 6.41 mmol) was added and the resulting mixture was refluxed for 24 hours. After cooling to r.t, the solid product was collected by filteration. It was washed with EtOAc, then dried under vacuum to afford 6-fluoro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**4b**, 756 mg, 48.7%). 1H NMR (600 MHz, DMSO-d6) δ 8.89 (s, 1H), 8.81 (d, J = 5.6 Hz, 2H), 8.14 - 8.06 (m, 2H), 7.74 (d, J = 2.3 Hz, 1H). LCMS (m/z $[M+H]^+$): 243.10.

Step B: To a mixture of 6-fluoro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**4b**, 750 mg, 3.1 mmol) in DCE (40 ml) was added thionyl chloride(1.81 ml, 24.8 mmol) and DMF (0.1 ml). The mixture was then stirred for 3 hours at 85°C. The reaction mixture was concentrated under reduced pressure and dried under vacuum overnight. The crude product (950 mg) was used for next step reaction without further work up or purification. LCMS (m/z $[M+H]^+$): 261.10.

Intermediate 5d (Scheme 5): 4,5-dichloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine



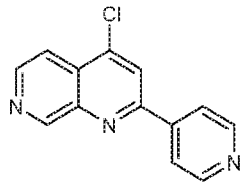
Step A: A mixture of 3-bromo-5-fluoroisonicotinic acid (**5a**, 1.87 g, 8.5 mmol) and HATU (4.85 g, 12.75 mmol) in DMF (30 ml) was added DIEA (4.5 ml), the mixture was stirred at r.t for 20 minutes, then isonicotinimidamide (1.236 g, 10.2 mmol) was added. Stirring was continued for another 15 hours at r.t. The reaction mixture was concentrated under reduced pressure. The light brown syrup crude mixture was then dissolved in DCM and purified by silica gel chromatography (eluted with 0-10% MeOH/solvent A, solvent A is mixture of 4 liter DCM and 8 ml of 7N ammonia solution in MeOH), fractions 66-80 were pooled and concentrated to afford the desired product 3-bromo-5-fluoro-N-(imino(pyridin-4-yl)methyl)isonicotinamide **5b** (566 mg, 20.6%). 1H NMR (500 MHz, DMSO-d6) δ 10.27 (s, 1H), 10.09 (s, 1H), 8.79 - 8.73 (m, 2H), 8.71 (s, 2H), 7.95 - 7.89 (m, 2H). LCMS (m/z $[M+H]^+$): 323.0.

Step B: A mixture of 3-bromo-5-fluoro-N-(imino(pyridin-4-yl)methyl)isonicotinamide (**5b**, 600 mg, 1.857 mmol), DIEA (0.33 ml, 1.857 mmol), potassium carbonate (257 mg, 1.857 mmol) and DBU (0.28 ml, 1.857 mmol) in DMA (8 ml) in a 20 ml microwave reaction vessel was heated at 150°C for 45 minutes (microwave irradiation). The reaction mixture was diluted with water (20 ml), extracted with EtOAc (3x60ml), the desired product remained in the aqueous phase. The aqueous phase was then purified by reverse phase ISCO (10-50% CH3CN/Water) to afford the desired product 5-bromo-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol **5c** (520 mg, 80% purity, 74%). LCMS (m/z $[M+H]^+$): 303.0.

Step C: 5-bromo-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**5c**, 150mg, 0.495mmol) was dissolved in anhydrous CH_3CN (2ml) and followed by addition of $POCl_3$ (759mg, 4.95mmol). The reaction mixture was then heated at 100°C for 16 hrs. LCMS showed the reaction was complete. The reaction was cooled to rt and the solvent was evaporated. The residue was diluted with ice water (40ml), and was then extracted with EtOAc (3x40ml). The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness to afford the desired product 4,5-dichloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine **5d** (86%) The product was used directly for next step without further purification. 1H NMR (400 MHz,

DMSO-d_6) δ 9.05 (s, 1H), 8.87 (s, 2H), 8.68 (s, 1H), 8.16 (d, J = 4.9 Hz, 2H). LCMS (m/z $[M+H]^+$): 277.0.

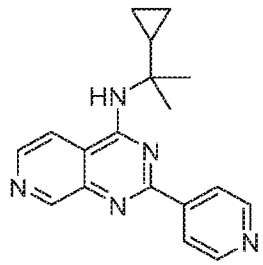
Intermediate 6b (Scheme 6, intermediate 6b', wherein A is 4-pyridinyl): 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine



Step A: In a 20 mL microwave reactor was added PalladiumTetrakis (58.1 mg, 0.050 mmol), potassium carbonate (1.256 mL, 2.51 mmol), and 2,4-dichloro-1,7-naphthyridine (**6a,** 200 mg, 1.005 mmol) and pyridin-4-ylboronic acid (130 mg, 1.055 mmol) in acetonitrile (2 mL) to give an orange suspension. The reaction mixture was stirred at 120°C for 60min under microwave. The crude mixture was diluted with DCM, H_2O, separated and extracted with DCM x3. Combined the organic layers and dried Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the product (62%). 1H NMR (400 MHz, DMSO-d6) δ 9.58 (d, J = 0.9 Hz, 1H), 8.85 - 8.78 (m, 4H), 8.32 - 8.29 (m, 2H), 8.11 (dd, J = 5.8, 0.9 Hz, 1H). LCMS (m/z $[M+H]^+$): 242.1.

Synthesis of Compounds of Formula A1

Example 1: N-(2-cyclopropylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (Compound 1)



Title compound was prepared from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (Intermediate **1c**) using **step C** as in **Scheme 1**.

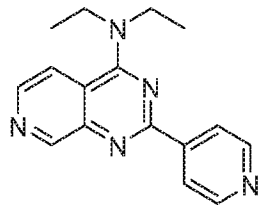
Step C: In a 20mL vial 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (30 mg, 0.12 mmol) was stirred in DMF (0.7mL) at room temperature and degassed with N_2. TEA (19uL,

0.14mmol) was added and stirred for 5 minutes then KF (7mg, 0.12mmol). This mixture was stirred at room temperature for 15 minutes then 2-cyclopropylpropan-2-amine (0.013 mL, 0.12 mmol) was added and degassed then stirred at 80°C for two hrs. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the product N-(2-cyclopropylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (50%). 1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.80(d, J = 6.1 Hz, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.40(dd, J = 5.7, 0.9 Hz, 1H), 8.29(m, 2H), 7.74(s, 1H), 1.94(m, 1H), 1.52(s, 6H), 0.49(m, 4H). LCMS (m/z $[M+H]^+$): 306.2.

Examples 2-110:

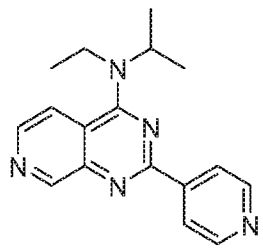
Examples 2-110 described *infra* were synthesized according to the protocol described for **Example 1** using 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (Intermediate **1c**) and various amines respectively except specially stated.

Example 2: N-(2-cyclopropylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



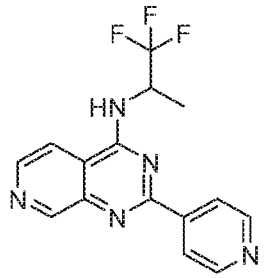
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.58(d, J = 5.9 Hz, 1H), 8.31(m, 2H), 7.89(dd, J = 5.9, 0.9 Hz, 1H), 3.90(q, J = 7.0 Hz, 4H), 1.40(t, J = 7.0 Hz, 6H). LCMS (m/z $[M+H]^+$): 280.1.

Example 3: N-ethyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



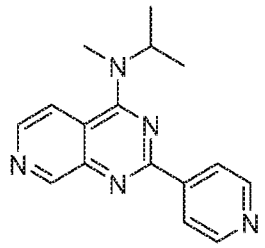
1H NMR (400 MHz, DMSO-d6) δ 9.22(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.58(d, J = 5.8 Hz, 1H), 8.30(m, 2H), 7.88(dd, J = 5.8, 0.8 Hz, 1H), 4.95-4.90(m, 1H), 3.81(q, J = 6.9 Hz, 2H), 1.40(s, 3H), 1.38(s, 3H), 1.35(m, 3H). LCMS (m/z [M+H]+): 294.2.

Example 4: 2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



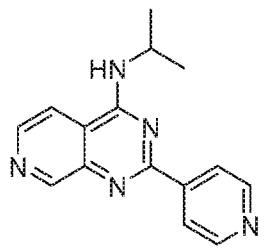
1H NMR (400MHz, CDCl3) δ 9.45(d, J = 0.9 Hz, 1H), 8.83(m, 2H), 8.78(d, J = 5.5 Hz, 1H), 8.70-8.60(m, 2H), 7.63(s, 1H), 5.98-5.92(m, 1H), 5.80-5.75(m, 1H), 1.81(d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 320.1.

Example 5: N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



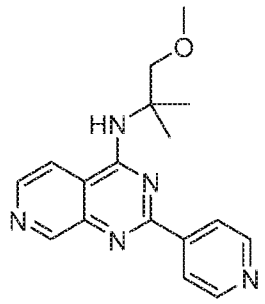
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.55(d, J = 5.8 Hz, 1H), 8.31(m, 2H), 8.03(dd, J = 5.8, 0.9 Hz, 1H), 5.15-5.10(m, 1H), 3.34(s, 3H), 1.35(d, J = 6.6 Hz, 6H). LCMS (m/z $[M+H]^+$): 280.2.

Example 6: N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



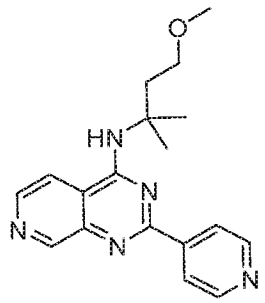
1H NMR (400 MHz, DMSO-d6) δ 9.18(d, J = 0.8 Hz, 1H), 8.76(m, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.50(d, J = 7.5 Hz, 1H), 8.32(m, 2H), 8.28(dd, J = 5.7, 0.9 Hz, 1H), 4.74-4.67(d, J = 6.7 Hz, 1H), 1.36(d, J = 6.6 Hz, 6H). LCMS (m/z $[M+H]^+$): 266.1.

Example 7: N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



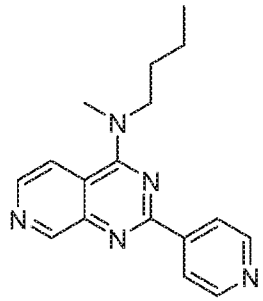
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.80(m, 2H), 8.64(d, J = 5.8 Hz, 1H), 8.40(dd, J = 5.7, 0.9 Hz, 1H), 8.29(m, 2H), 7.74(s, 1H), 3.85(s, 2H), 3.28(s, 3H), 1.60(s, 6H). LCMS (m/z $[M+H]^+$): 310.2.

Example 8: N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



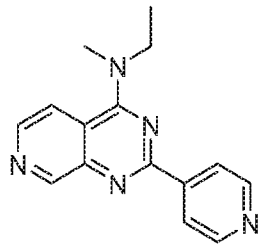
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.9 Hz, 1H), 8.80(m, 2H), 8.65(d, J = 5.6 Hz, 1H), 8.30(m, 2H), 8.28(m, 1H), 7.85(s, 1H), 3.48(t, J = 6.7 Hz, 2H), 3.20(s, 3H), 2.35(t, J = 6.8 Hz, 2H), 1.64(s, 6H). LCMS (m/z $[M+H]^+$): 324.2.

Example 9: N-butyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



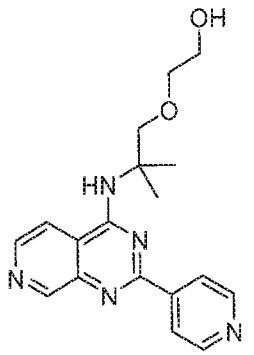
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.8 Hz, 1H), 8.77(m, 2H), 8.55(d, J = 5.9 Hz, 1H), 8.31(m, 2H), 8.08(dd, J = 5.8, 0.9 Hz, 1H), 3.92(m, 2H), 3.54(s, 3H), 1.82-1.75(m, 2H), 1.48-1.36(m, 2H), 0.98(t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 294.2.

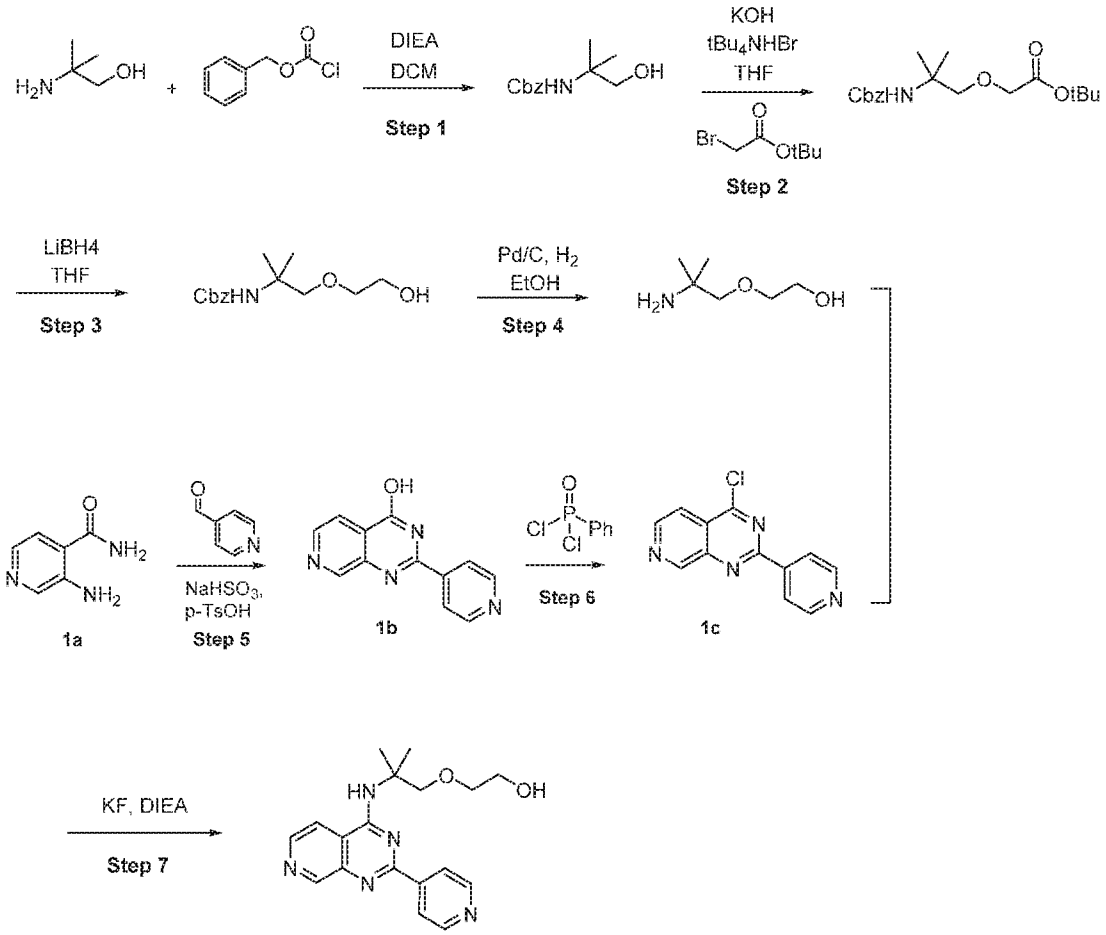
Example 10: **N-ethyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.55(d, J = 5.8 Hz, 1H), 8.31(m, 2H), 8.05(dd, J = 5.9, 0.9 Hz, 1H), 3.94(q, J = 7.1 Hz, 2H), 3.50(s, 3H), 1.39(t, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 266.1.

Example 11: **2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol**





Step 1: In a 40 mL vial at 0°C was added 2-amino-2-methylpropan-1-ol (1.5g, 16.8 mmol) in 8 mL of dry DCM. DIEA (3.2mL, 18.5mmol) was added then benzyl carbonochloridate (2.37mL, 16.8mmol) in portions. The reaction mixture was stirred for two hrs slowly warming to room temperature. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-50% EtOAc/Hexane to afford the product benzyl-(1-hydroxy-2-methylpropan-2-yl)carbamate (90%). LCMS (m/z $[M+H]^+$): 224.3.

Step 2: In a 20 mL vial was added benzyl-(1-hydroxy-2-methylpropan-2-yl)carbamate (0.63 g, 2.8 mmol) in 5mL of dry THF. Potassium hydroxide (0.16 g, 2.8 mmol) was added in 0.5 mL of H_2O, then tert-butyl 2-bromoacetate(0.62 mL, 4.2 mmol) and tetrabutylammonium bromide (90 mg, 0.28 mmol). The reaction mixture was stirred overnight at 30°C. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-40% EtOAc/Hexane to afford the product tert-butyl 2-(2-(((benzyloxy)carbonyl)amino)-2-methylpropoxy)acetate (35%). LCMS (m/z $[M+H]^+$): 338.4.

Step 3: In a 20 mL vial at 0°C was added tert-butyl 2-(2-(((benzyloxy)carbonyl)amino)-2- methylpropoxy) acetate (0.14 g, 0.17 mmol) in 1 mL of dry DMF. Lithium borohydride (0.45 mL, 0.91 mmol) was added in portions and stirred for 4 hrs at room temperature then quenched with water. DCM was used for extraction and the solvent evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-70% EtOAc/Hexane to afford the product benzyl-(1-(2-hydroxyethoxy)-2-methylpropan-2-yl)carbamate (35%). LCMS (m/z $[M+H]^+$): 268.3.

Step 4: In a 20 mL septa sealed vial was added benzyl-(1-(2-hydroxyethoxy)-2-methylpropan-2- yl)carbamate (0.03 g, 0.11 mmol) in 1.2mL of EtOH. The vial was vigorously purged with N_2 at room temperature. A small scoop of Pd/C (30%, cat. amount) was carefully added and the reaction maintained under N_2. A H_2 balloon was then used to flush the reaction vessel thoroughly and then stirred under H_2 pressure for four hrs. The H_2 was removed from the reaction then the vessel was purged with N_2. The material was then filtered through Na_2SO_4 and celite and the solvent evaporated under air flow. No further purification of the residue was necessary. 2-(2-amino-2-methylpropoxy)ethanol (95%). 1H NMR (500 MHz, Chloroform-d) δ 3.76 - 3.73 (m, 2H), 3.62 - 3.59 (m, 2H), 3.26 (s, 2H), 1.27 (s, 2H), 1.20 (d, J = 4.6 Hz, 1H), 1.11 (s, 6H).LCMS (m/z [M+H]+): 134.2.

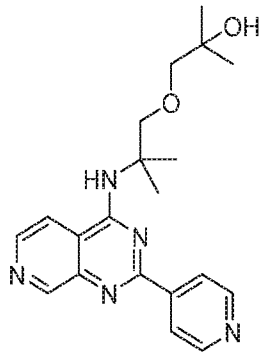
Step 5: In a 20 mL microwave vial was added 3-aminoisonicotinamide (**1a**, 2 g, 14.58 mmol), isonicotinaldehyde (1.521 mL, 16.04 mmol), sodium bisulfite (1.821 g, 17.50 mmol) and 4-methylbenzenesulfonic acid hydrate (0.277 g, 1.458 mmol) in DMA (Volume: 5 mL) to give an orange suspension. The reaction was well stirred and heated in microwave at 160 °C for 12 min. The reaction mixture was diluted with water and filtered. The solid was washed with water, MeOH and ether to give 2.03g off white solid as the product **1b** (59%). 1H NMR (400 MHz, DMSO-d6) δ 9.18 (d, J = 0.9 Hz, 1H), 8.88 - 8.78 (m, 2H), 8.73 (d, J = 5.2 Hz, 1H), 8.16 - 8.08 (m, 2H), 8.03 (dd, J = 5.2, 0.9 Hz, 1H).

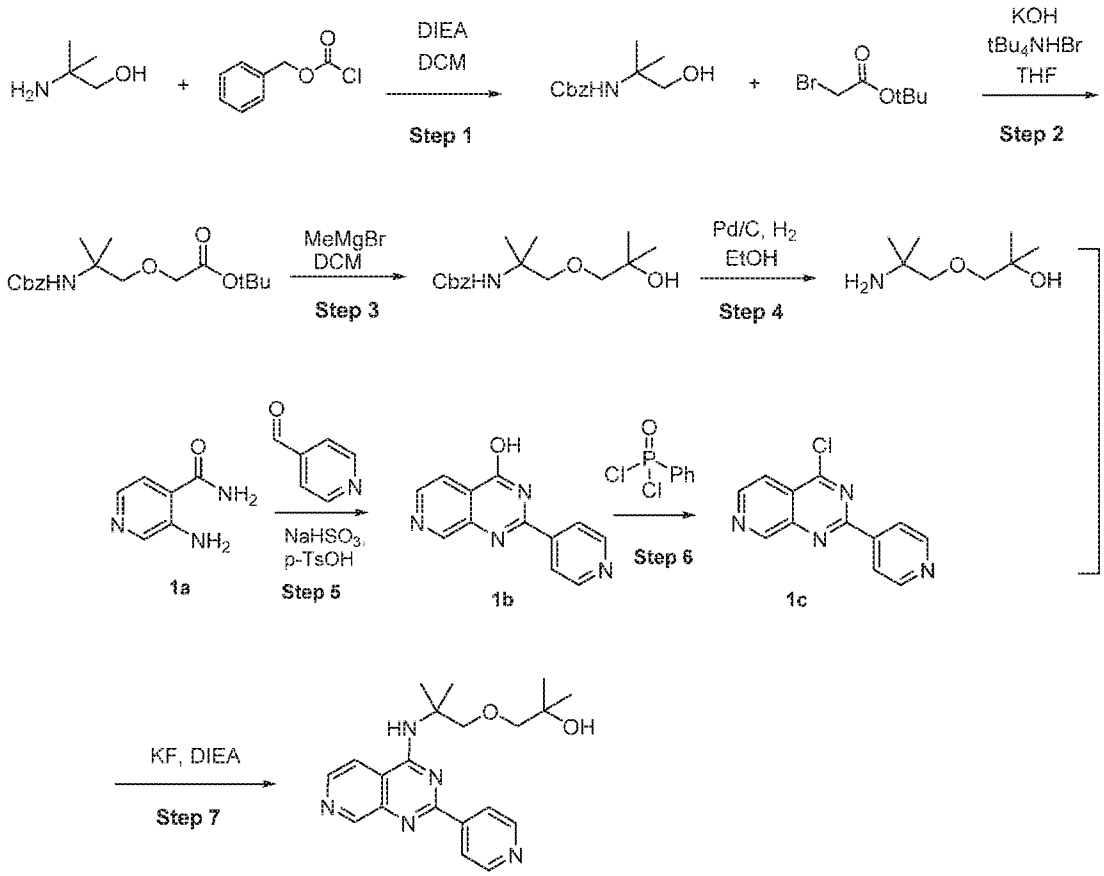
Step 6: In a 5 mL microwave reactor was 2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**1b,** 500 mg, 2.230 mmol) and phenylphosphonic dichloride (1564 microlitre, 11.15 mmol) to give a brown suspension. The reaction mixture was stirred at 170°C for 30 min when LCMS indicated full conversion. The reaction mixture was quenched with ice/water and neutralized with saturated Na_2CO_3, then extracted with DCM x 3 and give the product **1c** (74%). 1H NMR (500 MHz, DMSO-d6) δ 9.65 (d, J = 1.0 Hz, 1H), 8.96 (d, J = 5.6 Hz, 1H), 8.87 (s, 2H), 8.47 - 8.30 (m, 2H), 8.18 (dd, J = 5.6, 1.0 Hz, 1H). LCMS (m/z $[M+H]^+$): 243.1.

Step 7: In a 20 mL vial 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**intermediate 1c**) (150 mg, 0.618 mmol) was stirred in DMSO (1.5 mL) at room temperature and

degassed with N_2. DIEA (324 microlitre, 1.85 mmol) was added and stirred for 5 minutes then KF (36 mg, 0.618 mmol). This mixture was stirred at room temperature for 15 minutes then 2-(2-amino-2-methylpropoxy)ethanol (99 mg, 0.74 mmol) was added and degassed then stirred at 60°C for 30 min. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the title compound (25%). 1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.80(m, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.35(m, 1H), 8.29(m, 2H), 7.76(s, 1H), 4.64-4.59(m, 1H), 3.90(s, 2H), 3.50-3.44(m, 4H), 1.61(s, 6H). LCMS (m/z $[M+H]^+$): 340.2

Example 12: 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol





Step 1: In a 40ml vial at 0°C was added 2-amino-2-methylpropan-1-ol (1.5g, 16.8 mmol) in 8mL of dry DCM. DIEA(3.2mL, 18.5mmol) was added then benzyl carbonochloridate (2.37mL, 16.8mmol) in portions. The reaction mixture was stirred for two hrs slowly warming to room temperature. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-50% EtOAc/Hexane to afford the product benzyl-(1-hydroxy-2-methylpropan-2-yl)carbamate (90%). 1H NMR (400 MHz, Chloroform-d) δ 7.41 - 7.29 (m, 5H), 5.06 (s, 2H), 4.97 - 4.83 (m, 1H), 4.70 (s, 1H), 3.62 (s, 2H), 1.34 - 1.19 (m, 6H). LCMS (m/z $[M+H]^+$): 224.3.

Step 2: In a 20mL vial was added benzyl-(1-hydroxy-2-methylpropan-2-yl)carbamate (0.63g, 2.8 mmol) in 5mL of dry THF. Potassium hydroxide (0.16g, 2.8mmol) was added in 0.5mL of H_2O, then tert-butyl 2-bromoacetate (0.62mL, 4.2mmol) and tetrabutylammonium bromide (90mg, 0.28mmol). The reaction mixture was stirred overnight at 30°C. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-40% EtOAc/Hexane to afford the product tert-butyl 2-(2-(((benzyloxy)carbonyl)amino)-2-methylpropoxy)acetate (35%).

1H NMR (500 MHz, Chloroform-d) δ 7.39 - 7.28 (m, 5H), 5.52 (s, 1H), 5.06 (s, 2H), 3.96 (s, 2H), 3.45 (s, 2H), 1.48 (s, 9H), 1.36 (s, 6H). LCMS (m/z $[M+H]^+$): 338.4.

Step 3: tert-butyl 2-(2-(((benzyloxy)carbonyl)amino)-2-methylpropoxy) acetate (700mg, 4.15mmol) was stirred in DCM (5mL) in an ice bath for 10 minutes. Methyl magnesium bromide (30mL, 42mmol) was slowly added in portions then stirred while warming to room temp over two hrs. The reaction was then quenched with water and extracted three times with DCM. The organic layers were combined, concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-50% EtOAc/Hexane to afford the product benzyl (1-(2-hydroxy-2 methylpropoxy)-2-methylpropan-2-yl)carbamate (55%). LCMS [M+H] = 296.4.

Step 4: In a 20mL septa sealed vial was added benzyl (1-(2-hydroxy-2-methylpropoxy)-2-methylpropan-2-yl)carbamate (0.03g, 0.11mmol) in 1.2mL of EtOH. The vial was vigorously purged with N_2 at room temperature. A small scoop of Pd/C (30%, cat. amount) was carefully added and the reaction maintained under N_2. A H_2 balloon was then used to flush the reaction vessel thoroughly and then stirred under H_2 pressure for four hrs. The H_2 was removed from the reaction then the vessel was purged with N_2. The material was then filtered through Na_2SO_4 and celite and the solvent evaporated under air flow. No further purification of the residue was necessary to afford (1-(2-amino-2-methylpropoxy)-2-methylpropan-2-ol) (95%). 1H NMR (500 MHz, Chloroform-d) δ 3.32 (s, 2H), 3.26 (s, 2H), 1.22 (s, 6H), 1.12 (s, 6H). LCMS (m/z $[M+H]^+$): 162.2.

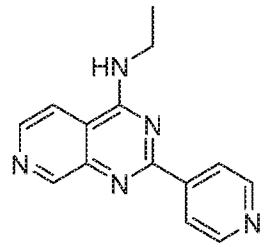
Step 5: In a 20 mL microwave vial was added 3-aminoisonicotinamide (**1a**, 2 g, 14.58 mmol), isonicotinaldehyde (1.521 mL, 16.04 mmol), sodium bisulfite (1.821 g, 17.50 mmol) and 4-methylbenzenesulfonic acid hydrate (0.277 g, 1.458 mmol) in DMA (Volume: 5mL) to give an orange suspension. The reaction was well stirred and heated in microwave at 160°C for 12 min. The reaction mixture was diluted with water and filtered. The solid was washed with water, MeOH and ether to give 2.03g off white solid as the product **1b** (59%). 1H NMR (400 MHz, DMSO-d6) δ 9.18 (d, J = 0.9 Hz, 1H), 8.88 - 8.78 (m, 2H), 8.73 (d, J = 5.2 Hz, 1H), 8.16 - 8.08 (m, 2H), 8.03 (dd, J = 5.2, 0.9 Hz, 1H).

Step 6: In a 5 mL microwave reactor was 2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-ol (**1b,** 500 mg, 2.230 mmol) and phenylphosphonic dichloride (1564 microlitre, 11.15 mmol) to give a brown suspension. The reaction mixture was stirred at 170 °C for 30min when LCMS indicated full conversion. The reaction mixture was quenched with ice/water and neutralized with saturated Na_2CO_3, then extracted with DCM x 3 and give the product **1c** (74%). 1H

NMR (500 MHz, DMSO-d6) δ 9.65 (d, J = 1.0 Hz, 1H), 8.96 (d, J = 5.6 Hz, 1H), 8.87 (s, 2H), 8.47 - 8.30 (m, 2H), 8.18 (dd, J = 5.6, 1.0 Hz, 1H). LCMS (m/z $[M+H]^+$): 243.1.

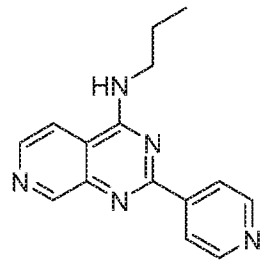
Step 7: In a 20mL vial 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (intermediate **1c**) (25 mg, 0.10 mmol) was stirred in DMSO (0.7mL) at room temperature and degassed with N_2. DIEA (43uL, 0.25 mmol) was added and stirred for 5 minutes then KF (6 mg, 0.10 mmol). This mixture was stirred at room temperature for 15 minutes then 2(1-(2-amino-2-methylpropoxy)-2-methylpropan-2-ol) (0.013 mL, 0.12 mmol) was added and degassed then stirred at 60°C for 30 min. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the title compound (50%). 1H NMR (400 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.79 (m, 2H), 8.64 (d, J = 5.6 Hz, 1H), 8.37 (dd, 5.7, 0.9 Hz, 1H), 8.30 (m, 2H), 7.80 (s, 1H), 4.40 (s, 1H), 3.90 (s, 2H), 3.17 (s, 2H), 1.61 (s, 6H), 0.99 (s, 6H). LCMS (m/z $[M+H]^+$): 368.2.

Example 13: N-ethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



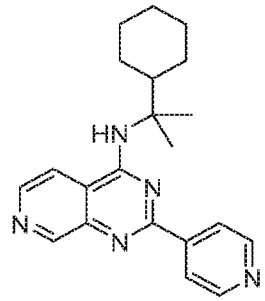
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.9 Hz, 1H), 8.84(m, 1H), 8.76(m, 2H), 8.65(d, J = 5.6 Hz, 1H), 8.34(m, 2H), 8.18(dd, J = 5.6, 0.9 Hz, 1H), 3.77-3.69(qd, J = 7.2, 5.4 Hz, 2H), 1.31(t, J = 7.2 Hz, 3H). LCMS (m/z $[M+H]^+$): 252.1.

Example 14: N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



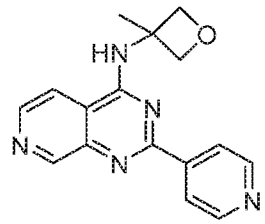
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.82(t, J = 5.5 Hz, 1H), 8.78(m, 2H), 8.64(d, J = 5.5 Hz, 1H), 8.33(m, 2H), 8.19(dd, J = 5.6, 0.9 Hz, 1H), 3.70-3.62(td, J = 7.0, 5.7 Hz, 2H), 1.80-1.70(m, 2H), 1.00(t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 266.1.

Example 15: **N-(2-cyclohexylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



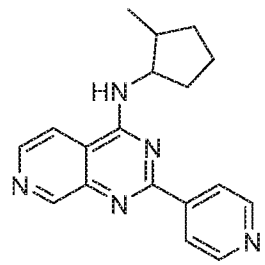
1H NMR (400 MHz, DMSO-d6) δ 9.16(d, J = 0.9 Hz, 1H), 8.80(m, 2H), 8.62(d, J = 5.6 Hz, 1H), 8.40(dd, J = 5.7, 0.9 Hz, 1H), 8.28(m, 2H), 7.62(s, 1H), 1.76-1.70(m, 4H), 1.62-1.59(m, 1H), 1.52(s, 6H), 1.18-1.04(q, J = 11.8, 10.9 Hz, 6H). LCMS (m/z $[M+H]^+$): 348.2.

Example 16: **N-(3-methyloxetan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



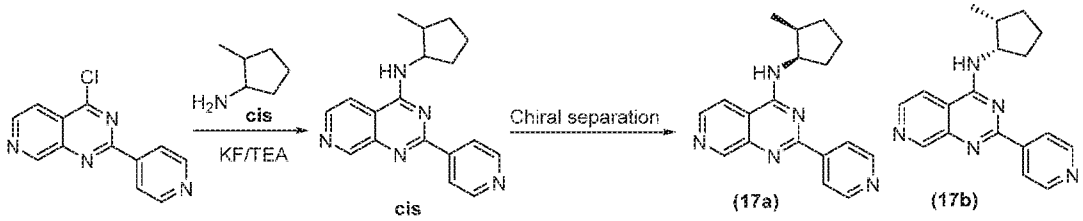
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.9 Hz, 1H), 9.14(s, 1H), 8.76(m, 2H), 8.68(d, J = 5.5 Hz, 1H), 8.25(m, 2H), 8.16(dd, J = 5.6 Hz, 1H), 4.90(d, J = 6.3 Hz, 2H), 4.64(d, J = 6.5 Hz, 2H), 1.82(s, 3H)). LCMS (m/z $[M+H]^+$): 294.1.

Example 17: **N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 1.0 Hz, 1H), 8.78(m, 2H), 8,66(m, 1H), 8.33(m, 2H), 8.32(m, 1H), 7.91(m, 1H), 4.45-4.42(m, 1H), 2.20-2.14(m, 1H), 1.98-1.82(m, 2H), 1.80-1.72(m, 2H), 1.70-1.62(m, 1H), 1.50-1.42(m, 1H), 0.91(d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 306.2.

Example 17b: N-((1R,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (17a) and N-((1S,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (17b)

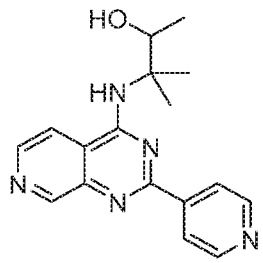


To a solution of 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (130 mg, 0.536 mmol) in DMF (10 ml) was added TEA (0.23 ml, 1.61 mmol) and KF (32.7 mg, 0.56 mmol). The mixture was stirred for 5 minute before *cis*-2-methylcyclopentanamine hydrochloride (72.7 mg, 0.536 mmol) was added. The resulting mixture was then stirred for 2 hours at 50 °C. The crude mixture was then purified by silica gel chromatography to afford 109 mg product. 100 mg of this material was subjected to chiral separation to afford two cis isomers, peak 1 (T_R = 1.46 min) isomer 35 mg, peak 2 (T_R =1.95 min) isomer 46 mg. Chiral center assignments are tentative chiral separation conditions: solvent A CO_2 (80%), solvent B MeOH+0.1% NH_4Cl (20%), flow rate 2 ml/min, column 21x250 mm AD-H, run time 6 minute stacked injections, 10 minute elution time.

Peak 1 (T_R =1.46 min) isomer: 1H NMR (500 MHz, DMSO-d6) δ 9.19 (s, 1H), 8.77 (d, J = 5.8 Hz, 2H), 8.66 (d, J = 5.6 Hz, 1H), 8.40 (dd, J = 5.7, 0.8 Hz, 1H), 8.36 - 8.29 (m, 3H), 4.86 (p, J = 7.5 Hz, 1H), 2.09 (dtd, J = 11.7, 8.1, 3.4 Hz, 1H), 1.89 (dddq, J = 29.7, 12.7, 8.4, 3.8 Hz, 3H), 1.66 - 1.53 (m, 1H), 1.51 - 1.41 (m, 1H), 0.83 (d, J = 7.1 Hz, 3H). LCMS (m/z $[M+H]^+$): 306.2.

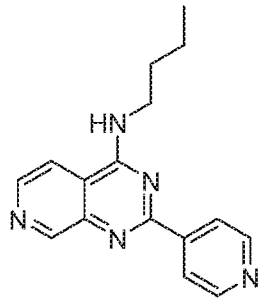
Peak 2 (T_R =1.95 min) isomer: 1H NMR (500 MHz, DMSO-d6) δ 9.19 (d, J = 0.7 Hz, 1H), 8.80 - 8.74 (m, 2H), 8.66 (d, J = 5.6 Hz, 1H), 8.40 (dd, J = 5.7, 0.8 Hz, 1H), 8.36 - 8.29 (m, 3H), 4.86 (p, J = 7.6 Hz, 1H), 2.09 (dp, J = 12.4, 4.6, 4.2 Hz, 1H), 1.98 - 1.79 (m, 3H), 1.66 - 1.54 (m, 1H), 1.50 - 1.41 (m, 1H), 0.83 (d, J = 7.1 Hz, 3H).). LCMS (m/z $[M+H]^+$): 306.2.

Example 18: 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol



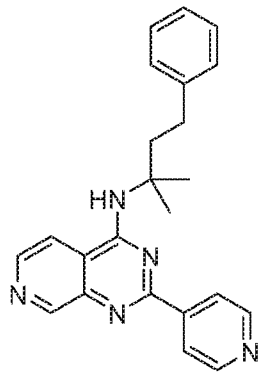
1H NMR (400 MHz, DMSO-d6) δ 9.17(d, J = 5.4 Hz, 1H), 8.79(m, 2H), 8.63(m, 1H), 8.33(m, 1H), 8.28(dd, J = 5.4, 0.8 Hz, 2H), 7.58(s, 1H), 5.00(d, J = 5.6 Hz, 1H), 4.30-4.26(m, 1H), 1.29(s, 3H), 1.27(s, 3H), 1.05(d, J = 6.7 Hz, 3H). LCMS (m/z $[M+H]^+$): 310.2.

Example 19: **N-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



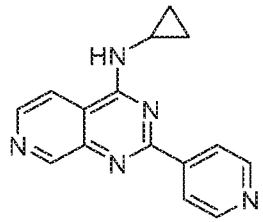
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.9 Hz, 1H), 8.82(m, 1H), 8.78(m, 2H), 8.65(d, J = 5.6 Hz, 1H), 8.34(m, 2H), 8.20(dd, J = 5.6, 0.9 Hz, 1H), 3.75-3.68(td, 7.2, 5.6 Hz, 2H), 1.78-1.69(m, 2H), 1.50-1.40(m, 2H), 0.98(t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 280.2.

Example 20: **N-(2-methyl-4-phenylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



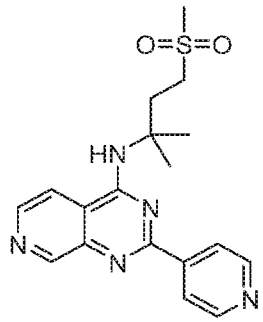
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.63(d, J = 5.6 Hz, 1H), 8.40(dd, J = 5.7, 0.9 Hz, 1H), 8.30(m, 2H), 7.79(s, 1H), 7.13-7.04(m, 5H), 2.61-2.56(dd, J = 10.7, 6.0 Hz, 2H), 2.48-2.43(m, 2H), 1.65(s, 6H). LCMS (m/z $[M+H]^+$): 370.2.

Example 21: **N-cyclopropyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



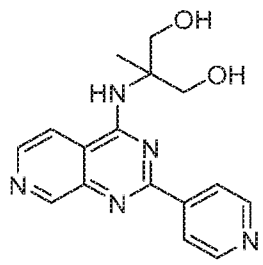
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.9 Hz, 1H), 8.83(m, 1H), 8.78(m, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.40(m, 2H), 8.18(dd, J = 5.6 1.0 Hz, 1H), 3.30-3.22(m, 1H), 0.98-0.94(m, 2H), 0.80-0.76(m, 2H). LCMS (m/z $[M+H]^+$): 264.1.

Example 22: **N-(4-methanesulfonyl-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



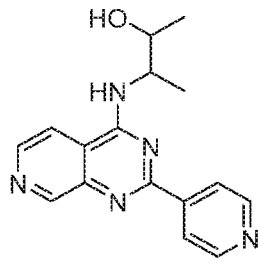
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.79(m, 2H), 8.65(d, J = 5.6 Hz, 1H), 8.39(dd, J = 5.7, 0.9 Hz, 1H), 8.30(m, 2H), 7.76(s, 1H), 3.15-3.10(m, 2H), 2.90(s, 3H), 2.65-2.60(m, 2H), 1.61(s, 6H). LCMS (m/z $[M+H]^+$): 372.1.

Example 23: **2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol**



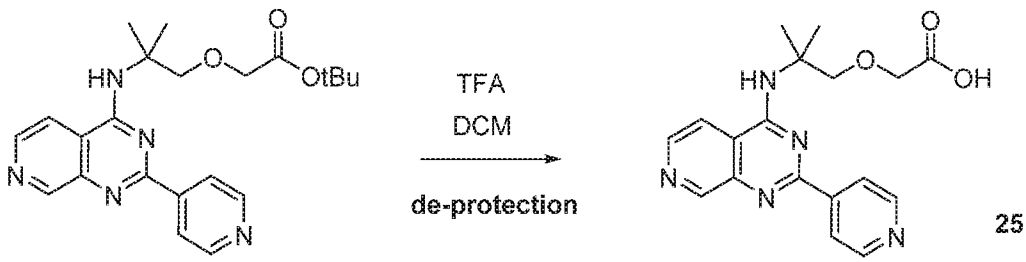
1H NMR (400 MHz, DMSO-d6) δ 9.18(d, J = 0.8 Hz, 1H), 8.79(m, 2H), 8.63(d, J = 5.6 Hz, 1H), 8.32(dd, J = 5.7, 0.9 Hz, 1H), 8.29(m, 2H), 7.38(s, 1H), 4.81(t, J = 6.0 Hz, 2H), 3.97-3.94(dd, J = 10.8, 6.0 Hz, 2H), 3.90-3.82(dd, J = 10.9, 6.2 Hz, 2H), 1.52(s, 3H). LCMS (m/z $[M+H]^+$): 312.1.

Example 24: 3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol



1H NMR (400 MHz, DMSO-d6) δ 9.18(d, J = 0.8 Hz, 1H), 8.77(m, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.38(m, 1H), 8.32(m, 2H), 8.30(s, 1H), 4.80(d, J = 5.5 Hz, 1H), 4.76-4.72(m, 1H), 3.93-3.88(m, 1H), 1.31(d, J = 6.7 Hz, 3H), 1.19(d, J = 6.3 Hz, 3H). LCMS (m/z $[M+H]^+$): 296.2.

Example 25: 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)acetic acid



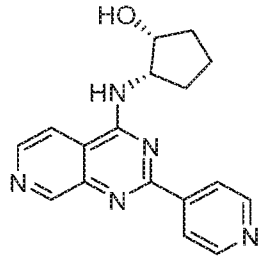
25

Title compound was prepared from tert-butyl 2-(2-amino-2-methylpropoxy)acetate and 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine as described in **Step C**, **Example 1**, followed by de-protection of the tert-butyl ester.

De-protection: tert-butyl 2-(2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino) propoxy)yl)amino)propoxy)acetate (25mg, 0.061mmol) was stirred in a 40% mixture of TFA in DCM at room temperature for two hours. No starting material was observed by TLC or LCMS. The reaction was diluted with DCM and concentrated using N_2 and mild heat then repeated three times and put on high vacuum overnight. Intermediates under these conditions were typically used without further purification. Title compounds

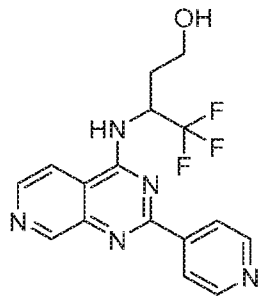
under these conditions were then also purified by flash chromatography. 1H NMR (400 MHz, DMSO-d6) δ 9.20(d, 0.8 Hz, 1H), 8.80(m, 2H), 8.63(d, J = 5.6 Hz, 1H), 8.33(ddd, J = 10.8, 5.1, 1.2 Hz, 2H), 8.30(m, 1H), 8.19(s, 1H), 4.14(s, 2H), 3.80(s, 2H), 3.16(s, 1H), 1.62(s, 6H). LCMS (m/z $[M+H]^+$): 354.2.

Example 26: (1R,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol



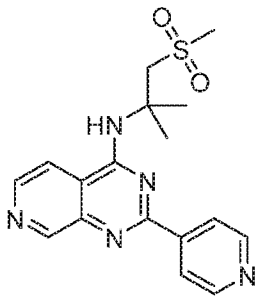
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.78(m, 1H), 8.65(m, 1H), 8.40(m, 1H), 8.37(m, 1H), 8.32(m, 2H), 4.72(d, J = 3.7 Hz, 1H), 4.60-4.52(ddd, J = 15.9, 9.2, 4.4 Hz, 1H), 4.44-4.40(m, 1H), 2.04-1.98(m, 3H), 1.90-1.82(m, 1H), 1.74-1.56(m, 2H). LCMS (m/z $[M+H]^+$): 308.1.

Example 27: 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol



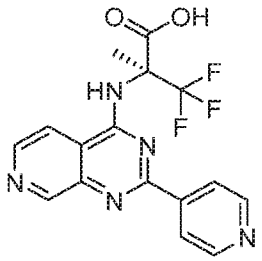
1H NMR (400 MHz, DMSO-d6) δ 9.30(d, J = 0.8 Hz, 1H), 8.85(m, 1H), 8.80(m, 2H), 8.74(m, 1H), 8.39(d, J = 4.3 Hz, 1H), 8.33(dd, J = 5.7, 0.9 Hz, 2H), 5.76-5.70(m, 1H), 4.77-4.70(m, 1H), 3.61-3.55(m, 2H), 2.11-2.08(m, 2H). LCMS (m/z $[M+H]^+$): 350.1.

Example 28: N-(1-methanesulfonyl-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



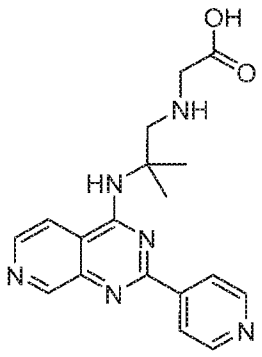
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.9 Hz, 1H), 8.79(m, 2H), 8.67(dd, J = 5.9, 4.7 Hz, 1H), 8.40(dd, J = 5.7, 0.9 Hz, 1H), 8.30(m, 2H), 8.08(s, 1H), 4.13(s, 2H), 2.90(s, 3H), 1.80(s, 6H). LCMS (m/z $[M+H]^+$): 358.1.

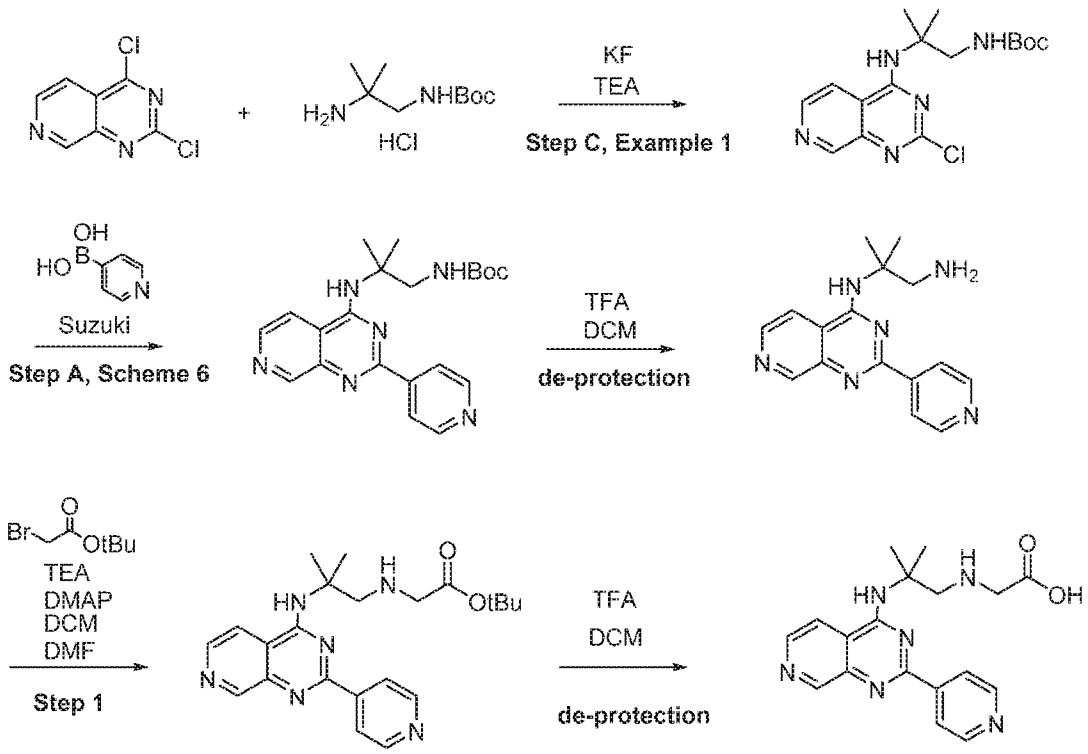
Example 29: (2S)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid



1H NMR (400 MHz, DMSO-d6) δ 9.23(d, J = 0.8 Hz, 1H), 8.79(m, 2H), 8.76(m, 1H), 8.70(m, 1H), 8.30(dd, J = 5.6, 0.8 Hz, 2H), 7.81(s, 1H), 2.02(s, 3H). LCMS (m/z $[M+H]^+$): 364.1.

Example 30: 2-[(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)amino]acetic acid

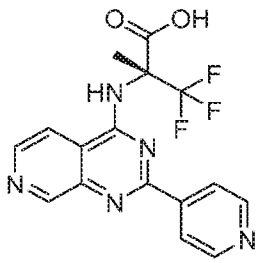




Title compound was prepared using tert-butyl 2-((2-methyl-2-((2-(pyridin-4-yl)pyrido [3,4-d]pyrimidin-4-yl)amino)propyl)amino)acetate as described in the above scheme. 1H NMR (400 MHz, DMSO-d6) δ 9.25(m, 1H), 8.82(m, 2H), 8.70(m, 1H), 8.40(m, 1H), 8.35(m, 2H), 7.84(d, J = 9.4 Hz, 1H), 3.89-3.80(d, J = 6.2 Hz, 1H), 3.60-3.56(d, J = 11.0 Hz, 4H), 1.70(s, 6H). LCMS (m/z $[M+H]^+$): 353.2.

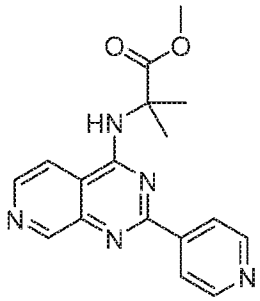
Step 1: 2-methyl-N2-(2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)propane-1,2-diamine (20mg, 0.068mmol) was stirred in DCM/DMF at room temperature. 21 microlitre of TEA (21 microlitre, 0.149mmol) was added and stirred for three minutes. tert-butyl 2-bromoacetate (11 microlitre, 0.071mmol) and a catalytic amount of DMAP was then added and stirred at room temperature for four hrs. Reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the product tert-butyl 2-((2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propyl)amino)acetate (35%). LCMS (m/z $[M+H]^+$): 409.5.

Example 31: (2R)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid



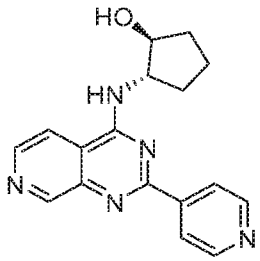
1H NMR (400 MHz, DMSO-d6) δ 9.23(d, J = 0.8 Hz, 1H), 8.79(m, 2H), 8.77(m, 1H), 8.70(m, 1H), 8.30(m, 2H), 7.83(s, 1H), 2.02(s, 3H). LCMS (m/z $[M+H]^+$): 364.1.

Example 32: Methyl 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoate



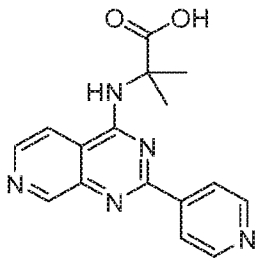
1H NMR (400 MHz, DMSO-d6) δ 9.24(d, J = 0.8 Hz, 1H), 8.85(s, 1H), 8.79(m, 2H), 8.70(d, J = 5.5 Hz, 1H), 8.39(dd, J = 5.7, 1.0 Hz, 1H), 8.25(m, 2H), 3.51(s, 3H), 1.69(s, 6H). LCMS (m/z $[M+H]^+$): 324.1.

Example 33: (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol



1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.65(d, J = 5.6 Hz, 1H), 8.51(d, J = 7.0 Hz, 1H), 8.38(m, 1H), 8.36(m, 1H), 8.31(dd, J = 5.6, 0.9, Hz, 1H), 4.92(d, J = 4.6 Hz, 1H), 4.60-4.54(d, J = 7.0 Hz, 1H), 4.26-4.20(m, 1H), 2.32-2.22(ddt, J = 13.2, 8.2, 4.3 Hz, 1H), 2.00-1.92(m, 1H), 1.85-1.72(m, 2H), 1.70-1.56(m, 2H). LCMS (m/z $[M+H]^+$): 308.1.

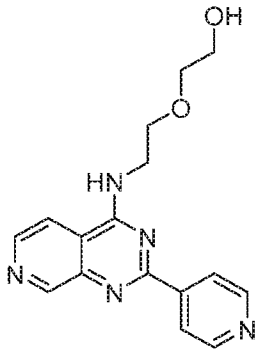
Example 34: 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid



Title compound was prepared from de-protection of methyl 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propanoate as described in **Step A, Example 25.**

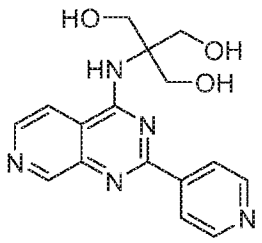
1H NMR (400 MHz, DMSO-d6) δ 12.61(s, 1H), 9.20(d, J = 0.8 Hz, 1H), 8.86-8.80(s, 1H), 8.75(m, 2H), 8.67(d, J = 5.6 Hz, 1H), 8.30(m, 2H), 8.28(m, 1H), 1.66(s, 6H). LCMS (m/z $[M+H]^+$): 310.1.

Example 35: 2-(2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethoxy)ethan-1-ol



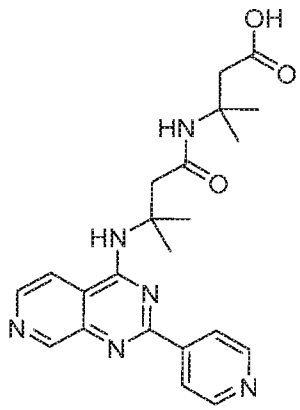
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.9 Hz, 1H), 8.89(t, J = 5.5 Hz, 1H), 8.76(m, 2H), 8.65(d, J = 5.5 Hz, 1H), 8.32(m, 2H), 8.20(dd, J = 5.7, 0.9 Hz, 1H), 4.60-4.57(m, 1H), 3.89(q, J = 5.7 Hz, 2H), 3.78(t, J = 5.8 Hz, 2H), 3.51-3.48(d, J = 2.9 Hz, 4H). LCMS (m/z $[M+H]^+$): 312.1.

Example 36: 2-(hydroxymethyl)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol



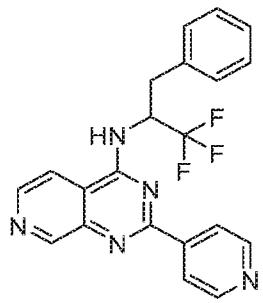
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.80(m, 2H), 8.65(d, J = 5.6 Hz, 1H), 8.30(ddd, J = 19.4, 5.1, 1.3 Hz, 1H), 8.26(m, 2H), 7.19(s, 1H), 4.72(t, J = 6.0 Hz, 3H), 4.00(d, J = 6.0 Hz, 6H). LCMS (m/z $[M+H]^+$): 328.1.

Example 37: 3-methyl-3-(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanamido) butanoic acid



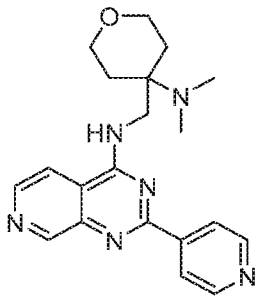
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.9 Hz, 1H), 8.80(dt, J = 4.5, 1.1 Hz, 2H), 8.64(m, 1H), 8.33(m, 2H), 8.30(m, 1H), 7.90(s, 1H), 1.72(s, 6H), 1.70(s, 2H), 1.30(s, 2H), 1.00(s, 6H). LCMS (m/z $[M+H]^+$): 423.2.

Example 38: 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-3-phenylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



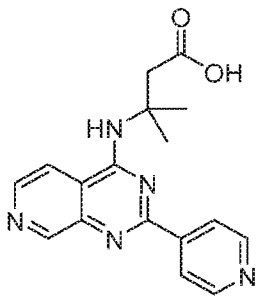
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.9 Hz, 1H), 8.99(d, J = 9.0 Hz, 1H), 8.75(m, 2H), 8.71(d, J = 5.6 Hz, 1H), 8.33(m, 1H), 8.30(m, 2H), 7.41(m, 2H), 7.11(m, 2H), 7.02(m, 1H), 5.95-5.86(t, J = 8.2 Hz, 1H), 3.38-3.34(m, 1H), 3.24-3.17(ddt, J = 13.8, 11.7, 0.7 Hz, 1H). LCMS (m/z $[M+H]^+$): 396.1.

Example 39: N-{[4-(dimethylamino)oxan-4-yl]methyl}-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



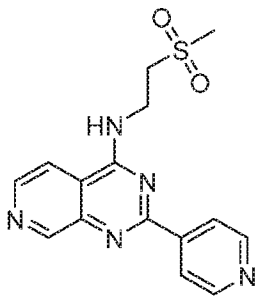
1H NMR (400 MHz, DMSO-d6) δ 9.20(s, 1H), 8.78(m, 2H), 8,67(d, J = 5.5 Hz, 1H), 8.52(s, 1H), 8.33(m, 2H), 8.28(m, 1H), 3.62(t, J = 10.6 Hz, 2H), 3.53(d, J = 10.7 Hz, 2H), 3.27(d, J = 5.3 Hz, 2H), 2.41(s, 6H), 1.80(d, J = 14.1 Hz, 2H), 1.63(t, J = 11.5 Hz, 2H). LCMS (m/z $[M+H]^+$): 365.2.

Example 40: 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid



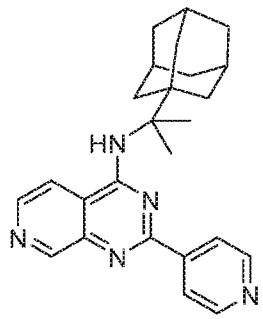
1H NMR (400 MHz, DMSO-d6) δ 12.02(s, 1H), 9.20(d, J = 0.8 Hz, 1H), 8.80(m, 2H), 8.66(m, 1H), 8.40(m, 1H), 8.30(m, 2H), 7.99(m, 1H), 3.12-3.08(q, J = 7.3 Hz, 2H), 1.70(s, 6H. LCMS (m/z $[M+H]^+$): 324.1.

Example 41: N-(2-methanesulfonylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



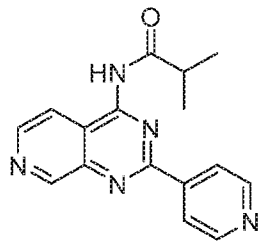
1H NMR (400 MHz, DMSO-d6) δ 9.23(s, 1H), 9.10(t, J - 5.6 Hz, 1H), 8.78(m, 2H), 8.69(d, J = 5.7 Hz, 1H), 8.39(m, 2H), 8.14(d, J = 5.7 Hz, 1H), 4.13-4.09(q, J = 6.4 Hz, 2H), 3.62(t, J = 6.7 Hz, 2H), 3.09(s, 3H). LCMS (m/z $[M+H]^+$): 330.1.

Example 42: **N-[2-(adamantan-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



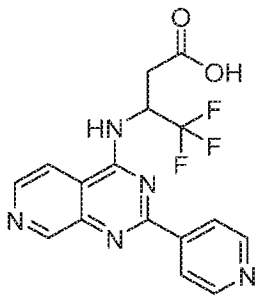
1H NMR (400 MHz, DMSO-d6) δ 9.16(d, J = 0.8 Hz, 1H), 8.78(m, 2H), 8.63(d, J = 5.6 Hz, 1H), 8.45(m, 1H), 8.25(m, 2H), 7.06(s, 1H), 1.99-1.96(d, J = 7.5 Hz, 3H), 1.80-1.76(m, 6H), 1.67-1.64(m, 6H), 1.64-1.60(m, 6H). LCMS (m/z $[M+H]^+$): 400.2.

Example 43: **2-methyl-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propanamide**



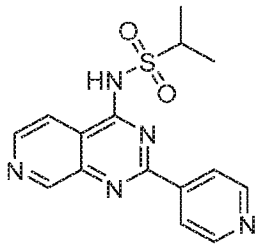
1H NMR (400 MHz, DMSO-d6) δ 11.08(s, 1H), 9.49(m, 1H), 8.84(m, 2H), 8.80(m, 1H), 8.40(m, 2H), 8.25(dd, J = 5.8, 1.0 Hz, 1H), 3.29-3.18(m, 1H), 1.26(d, J = 6.8 Hz, 6H). LCMS (m/z $[M+H]^+$): 294.1.

Example 44: **4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid**



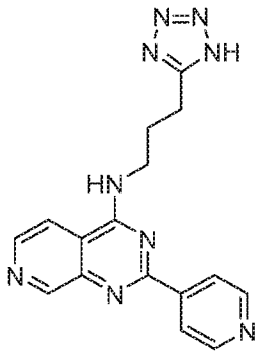
1H NMR (400 MHz, DMSO-d6) δ 9.29(d, J = 0.9 Hz, 1H), 8.80(m, 2H), 8.71(t, J = 4.9 Hz, 1H), 8.34(m, 2H), 8.23(d, J = 5.7 Hz, 1H), 5.84-5.79(m, 1H), 3.65-3.55(d, J = 4.8 Hz, 1H), 2.45-2.41(d, J = 4.7 Hz, 1H), 2.32-2.26(m, 1H). LCMS (m/z $[M+H]^+$): 364.1.

Example 45: N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propane-2-sulfonamide



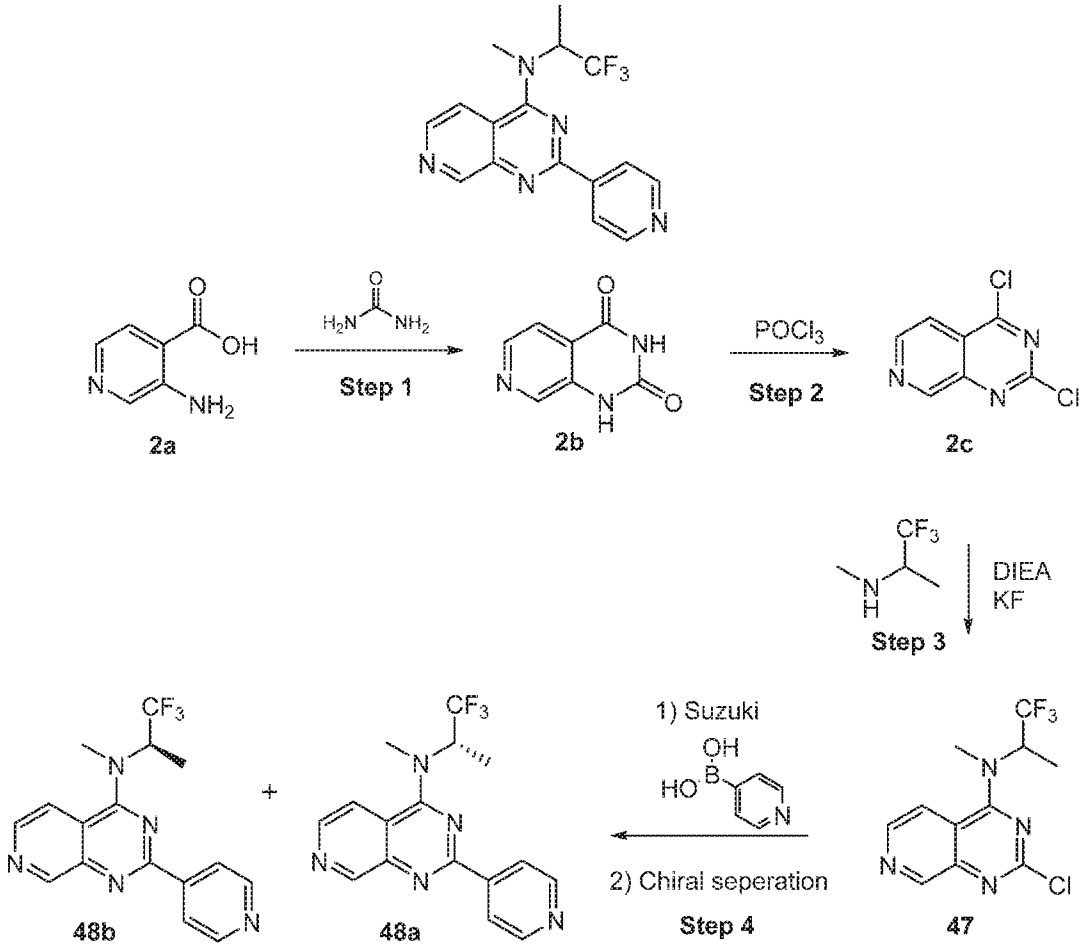
1H NMR (400 MHz, DMSO-d6) δ 9.38(s, 1H), 8.88(m, 2H), 8.76(d, J = 5.6 Hz, 1H), 8.40-8.30(m, 3H), 4.20-4.10(q, J = 7.1 Hz, 1H), 1.40(d, J = 6.9 Hz, 6H). LCMS (m/z $[M+H]^+$): 330.1.

Example 46: 2-(pyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.21(m, 1H), 9.18(s, 1H), 8.75(m, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.30(m, 2H), 8.21(dd, J = 5.6, 1.0 Hz, 1H), 3.81-3.75(td, J = 6.9, 5.3 Hz, 2H), 2.95(t, J = 7.2 Hz, 2H), 2.18-2.10(m, 2H). LCMS (m/z $[M+H]^+$): 334.2.

Example 47: **N-methyl-2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine**



Step 1: A mixture of urea (40.00 g, 666.00 mmol) and 3-aminoisonicotinic acid (**2a,** 18.40 g, 133.20 mmol) was heated at 210°C for 1 hr (NOTE: no solvent was used). NaOH (2N, 320 mL) was added, and the mixture was stirred at 90°C for 1h. The solid was collected by filtration, and washed with water. The crude product thus obtained was suspended in HOAc (400 mL), and stirred at 100°C for 1h. The mixture was cooled to RT, filtered, and the solid was washed with a large amount of water, and then dried under the vacuum to give pyrido[3,4-d]pyrimidine-2,4(1H,3H)-dione (**2b,** 17.00 g, 78% yield) without further purification. LCMS (m/z $[M+H]^+$): 164.0.

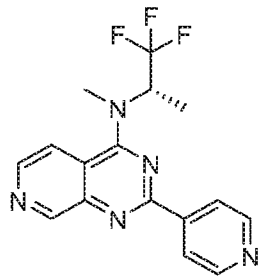
Step 2: To a mixture of pyrido[3,4-d]pyrimidine-2,4(1H,3H)-dione (**2b**, 20.00 g, 122.60 mmol) and $POCl_3$ (328.03 g, 2.14 mol) in toluene (200 mL) was added DIEA (31.69 g, 245.20 mmol) dropwise and this reaction mixture stirred at 25°C overnight (18hr) to give suspension.

The solvent and $POCl_3$ was removed under vacuum, diluted with DCM (50 mL), neutralized with DIEA to pH=7 at -20 °C and concentrated again, the residue was purified by column (20-50% EA/PE) to give 2,4-dichloropyrido[3,4-d]pyrimidine (**2c**, 20.00g, 99.99 mmol, 82% yield) as a yellow solid. 1H NMR (400 MHz, CHLOROFORM-*d*) δ 9.52 (s, 1 H), 8.92 (d, *J*=5.6 Hz, 1 H), 8.04 (d, *J*=5.6 Hz, 1 H). LCMS (m/z $[M+H]^+$): 200.0.

Step 3: In a 20mL vial 2,4-dichloropyrido[3,4-d]pyrimidine (600 mg, 3.0 mmol) was stirred in DMSO (0.7mL) at room temperature and degassed with N_2. DIEA (1 mL, 6 mmol) was added and stirred for 5 minutes then KF (174 mg, 3 mmol). This mixture was stirred at room temperature for 15 minutes then racemic 1,1,1-trifluoro-N-methylpropan-2-amine (419 mg, 3.3 mmol) was added and degassed then stirred at 60°C for 4 hours. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford 2-chloro-N-methyl-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine (680 mg, 74%). 1H NMR (500 MHz, Acetone-d6) δ 9.09 (d, J = 0.9 Hz, 1H), 8.59 (d, J = 5.9 Hz, 1H), 8.22 (dd, J = 5.9, 0.9 Hz, 1H), 5.93 (dddd, J = 15.3, 8.3, 7.0, 1.2 Hz, 1H), 3.61 (q, J = 1.0 Hz, 3H), 1.63 (d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 291.7.

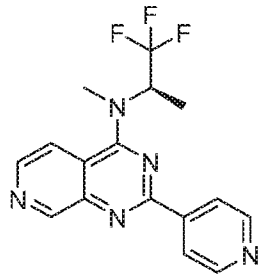
Step 4: In a 20 mL microwave reactor was added PalladiumTetrakis (99 mg, 0.086 mmol), potassium carbonate (2.15 mL, 4.3 mmol), and 2 chloro-N-methyl-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine (500 mg, 1.72 mmol) and pyridin-4-ylboronic acid (233 mg, 1.89 mmol) in acetonitrile (8 mL) to give an yellow suspension. The reaction mixture was stirred at 130 °C for 30 min under microwave. The crude mixture was diluted with DCM, H_2O, separated and extracted with DCM x3. Combined the organic layers and dried Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give **Example 47**, the racemic product, then followed by chiral HPLC (21x250mm OJ-H column with 85% CO_2 as phase A and 15% MeOH as phase B, flow rate 2mL/min, 30°C, 3.5 min elution time) to separate the enantiomers to afford **Examples 4ba and 48b**.

<u>Example 48a:</u> N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine



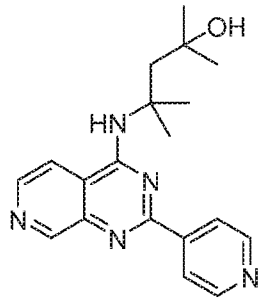
1H NMR (500 MHz, DMSO-d6) δ 9.33 (d, J = 0.8 Hz, 1H), 8.86 - 8.75 (m, 2H), 8.63 (d, J = 5.9 Hz, 1H), 8.38 - 8.30 (m, 2H), 8.20 (dd, J = 6.0, 0.9 Hz, 1H), 6.11 (qt, J = 8.5, 7.4 Hz, 1H), 3.50 (d, J = 1.1 Hz, 3H), 1.61 (d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 334.1. Chiral HPLC T_R = 1.73 min. Absolute stereochemistry was confirmed by X-ray crystal structure.

Example 48b: N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine



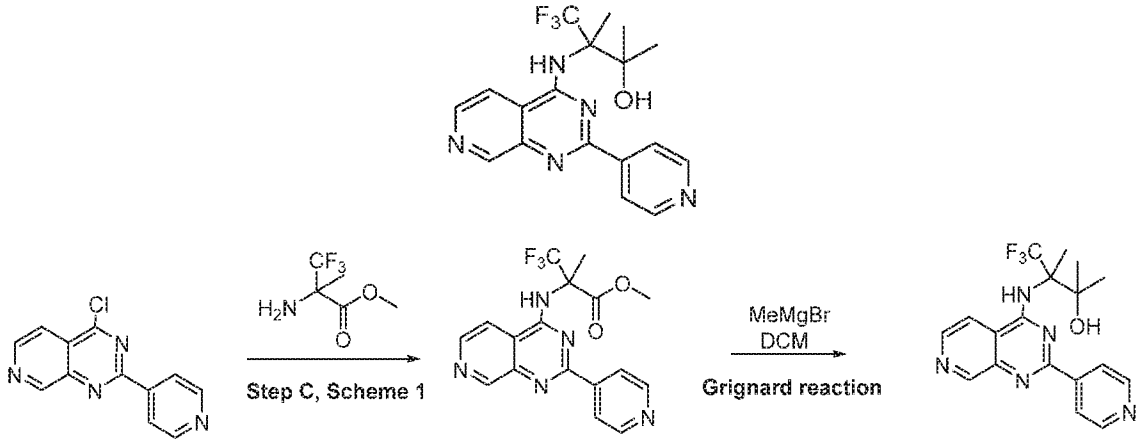
1H NMR (500 MHz, DMSO-d6) δ 9.33 (d, J = 0.8 Hz, 1H), 8.86 - 8.75 (m, 2H), 8.63 (d, J = 5.9 Hz, 1H), 8.38 - 8.30 (m, 2H), 8.20 (dd, J = 6.0, 0.9 Hz, 1H), 6.11 (qt, J = 8.5, 7.4 Hz, 1H), 3.50 (d, J = 1.1 Hz, 3H), 1.61 (d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 334.1. Chiral HPLC T_R = 1.25 min. Absolute stereochemistry was confirmed by X-ray crystal structure.

Example 49: 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol



1H NMR (400 MHz, Acetone-d6) δ 9.57 (s, 1H), 9.15 (d, J = 0.9 Hz, 1H), 8.82 - 8.72 (m, 2H), 8.56 (d, J = 5.6 Hz, 1H), 8.44 - 8.37 (m, 2H), 7.69 (dd, J = 5.6, 0.9 Hz, 1H), 2.08 (s, 2H), 1.87 (s, 6H), 1.48 (d, J = 0.8 Hz, 6H). LCMS (m/z $[M+H]^+$): 338.2.

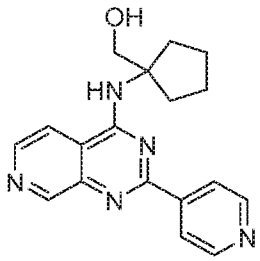
Example 50: 4,4,4-trifluoro-2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol



The title compound was prepared from methyl 2-amino-3,3,3-trifluoro-2-methylpropanoate hydrochloride and 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine as described in **Step C**, **Example 1**, followed by Grignard reaction with methylmagnesium bromide.

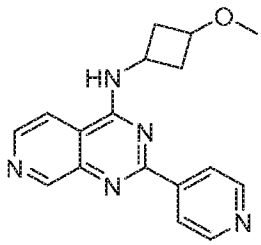
Grignard reaction: methyl 3,3,3-trifluoro-2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propanoate (7 mg, 0.019 mmol) was stirred with methylmagnesium bromide (1.4 M in hexanes, 0.133 mL) in DCM at 0°C for 1 hour. No starting material was observed by TLC or LCMS. The reaction was quenched by MeOH and concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the title compound (66%). 1H NMR (500 MHz, Methanol-d4) δ 9.28 (d, J = 0.9 Hz, 1H), 8.77 - 8.71 (m, 2H), 8.67 (d, J = 5.7 Hz, 1H), 8.44 - 8.38 (m, 2H), 7.85 (dd, J = 5.7, 0.9 Hz, 1H), 2.09 (d, J = 1.4 Hz, 3H), 1.55 (t, J = 2.2 Hz, 3H), 1.37 (s, 3H). LCMS (m/z $[M+H]^+$): 378.2.

Example 51: (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentyl)methanol



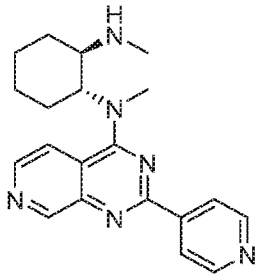
1H NMR (500 MHz, Chloroform-d) δ 9.35 (d, J = 0.9 Hz, 1H), 8.82 - 8.75 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.30 - 8.24 (m, 2H), 7.49 (dd, J = 5.8, 0.9 Hz, 1H), 3.99 (d, J = 4.0 Hz, 2H), 2.19 (td, J = 7.3, 6.7, 2.5 Hz, 4H), 1.94 - 1.78 (m, 4H). LCMS (m/z $[M+H]^+$): 322.2.

Example 52: **N-(3-methoxycyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



1H NMR (500 MHz, DMSO-d6) δ 9.19 (d, J = 0.9 Hz, 1H), 8.87 (d, J = 6.4 Hz, 1H), 8.80 - 8.72 (m, 2H), 8.66 (d, J = 5.5 Hz, 1H), 8.37 - 8.32 (m, 2H), 8.25 (dd, J = 5.6, 0.9 Hz, 1H), 4.53 - 4.42 (m, 1H), 3.84 - 3.74 (m, 1H), 3.20 (s, 3H), 2.89 - 2.79 (m, 2H), 2.11 (tdd, J = 9.0, 7.5, 2.8 Hz, 2H). LCMS (m/z $[M+H]^+$): 308.1.

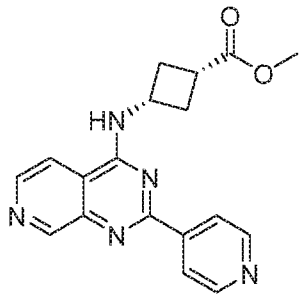
Example 53: **(1R,2R)-1-N,2-N-dimethyl-1-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]cyclohexane-1,2-diamine**



1H NMR (500 MHz, Methanol-d4) δ 9.23 (d, J = 0.8 Hz, 1H), 8.73 - 8.68 (m, 2H), 8.53 (d, J = 5.9 Hz, 1H), 8.46 - 8.41 (m, 2H), 8.22 (dd, J = 5.8, 0.9 Hz, 1H), 3.47 (s, 3H), 2.91 (dd, J = 13.0, 9.0 Hz, 1H), 2.39 (s, 3H), 2.30 (dtt, J = 12.6, 5.1, 2.5 Hz, 1H), 2.04 (dp, J = 12.4, 3.1 Hz, 1H), 1.90 (dddd, J = 23.1, 13.0, 5.6, 3.0 Hz, 2H), 1.84 - 1.74 (m, 1H), 1.58

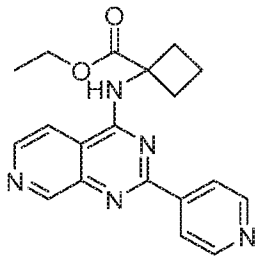
(qt, J = 12.7, 3.5 Hz, 1H), 1.42 (qt, J = 13.2, 3.3 Hz, 1H), 1.33 - 1.23 (m, 1H). LCMS (m/z $[M+H]^+$): 349.2.

Example 54: methyl (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate



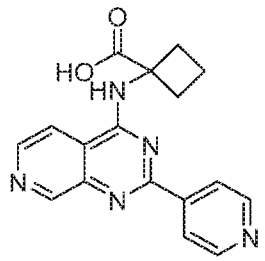
1H NMR (500 MHz, DMSO-d6) δ 9.20 (d, J = 0.9 Hz, 1H), 8.95 (d, J = 6.0 Hz, 1H), 8.82 – 8.72 (m, 2H), 8.67 (d, J = 5.5 Hz, 1H), 8.34 – 8.28 (m, 2H), 8.25 (dd, J = 5.6, 1.0 Hz, 1H), 4.95 (h, J = 7.3 Hz, 1H), 3.71 (s, 3H), 3.27 – 3.21 (m, 1H), 2.72 (dddd, J = 10.7, 8.2, 4.3, 2.5 Hz, 2H), 2.62 – 2.51 (m, 2H). LCMS (m/z $[M+H]^+$): 336.1.

Example 55: ethyl 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate



1H NMR (400 MHz, Chloroform-d) δ 9.27 (dd, J = 12.0, 4.4 Hz, 1H), 8.83 - 8.74 (m, 2H), 8.54 - 8.40 (m, 1H), 8.40 - 8.31 (m, 2H), 7.56 - 7.49 (m, 1H), 4.32 - 4.16 (m, 2H), 3.03 - 2.85 (m, 2H), 2.49 (dddd, J = 11.8, 9.4, 7.1, 2.3 Hz, 2H), 2.31 - 2.11 (m, 2H), 1.22 (dtd, J = 9.6, 7.5, 7.0, 1.8 Hz, 3H). LCMS (m/z $[M+H]^+$): 350.2.

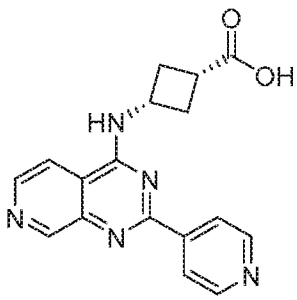
Example 56: 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid



The title compound was prepared by hydrolysis of the ethyl ester of **Example 55**.

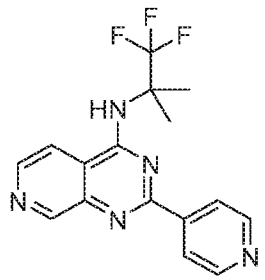
Hydrolysis: a suspension of example 55 (27 mg, 0.077 mmol) and lithium hydroxide (0.077 mL, 0.077 mmol) in MeOH (2 mL) was stirred at 90°C for 3 hr until LCMS indicated no starting material left. The reaction mixture was neutralized with 2 mL 1M HCl. The aqueous layer was back extracted with DCM x5. The combined organic layer was washed with brine, dried and concentrated. The residue was washed with DCM/ether to give the title compound (92%). 1H NMR (500 MHz, DMSO-d6) δ 12.49 (s, 1H), 9.35 (s, 1H), 9.23 (d, J = 0.9 Hz, 1H), 8.80 - 8.72 (m, 2H), 8.70 (d, J = 5.5 Hz, 1H), 8.34 (dd, J = 5.6, 1.0 Hz, 1H), 8.32 - 8.21 (m, 2H), 2.82 (ddd, J = 13.3, 8.8, 4.9 Hz, 2H), 2.49 - 2.43 (m, 2H), 2.12 - 1.96 (m, 2H). LCMS (m/z $[M+H]^+$): 322.1.

Example 57: (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid



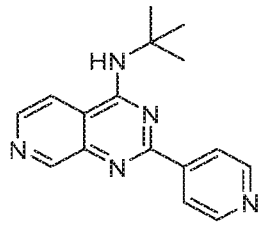
The title compound was prepared by hydrolysis of the ethyl ester of **Example 54** using the procedure as described in **Example 56.** 1H NMR (500 MHz, DMSO-d6) δ 9.26 (d, J = 0.9 Hz, 1H), 9.21 (d, J = 6.0 Hz, 1H), 9.02 - 8.93 (m, 2H), 8.73 (d, J = 5.6 Hz, 1H), 8.66 (d, J = 5.7 Hz, 2H), 8.38 (dd, J = 5.7, 0.9 Hz, 1H), 4.92 (q, J = 7.4 Hz, 1H), 3.19 - 3.11 (m, 1H), 2.76 - 2.68 (m, 2H), 2.56 (ddd, J = 12.9, 6.5, 2.7 Hz, 2H). LCMS (m/z $[M+H]^+$): 322.1.

Example 58: 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



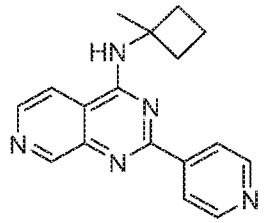
1H NMR (400 MHz, DMSO-d6) δ 9.27 (d, J = 0.8 Hz, 1H), 8.84 - 8.77 (m, 2H), 8.72 (d, J = 5.7 Hz, 1H), 8.51 (dd, J = 5.7, 0.9 Hz, 1H), 8.30 - 8.24 (m, 2H), 7.98 (s, 1H), 1.91 (s, 6H).LCMS (m/z $[M+H]^+$): 334.1.

Example 59: **N-tert-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



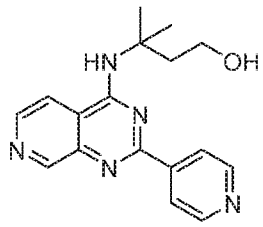
1H NMR (500 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.82 - 8.77 (m, 2H), 8.64 (d, J = 5.6 Hz, 1H), 8.39 (dd, J = 5.7, 0.9 Hz, 1H), 8.34 - 8.29 (m, 2H), 7.88 (s, 1H), 1.66 (s, 9H). LCMS (m/z $[M+H]^+$): 280.2.

Example 60: **N-(1-methylcyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



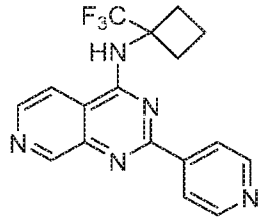
1H NMR (400 MHz, DMSO-d6) δ 9.17 (d, J = 0.8 Hz, 1H), 8.77 (dd, J = 6.3, 1.9 Hz, 2H), 8.63 (d, J = 5.6 Hz, 1H), 8.33 - 8.29 (m, 2H), 8.27 (dd, J = 5.7, 0.9 Hz, 1H), 2.70 - 2.64 (m, 1H), 2.58 - 2.53 (m, 1H), 2.35 - 2.26 (m, 2H), 2.04 - 1.81 (m, 2H), 1.71 (s, 3H). LCMS (m/z $[M+H]^+$): 292.2.

Example 61: **3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol**



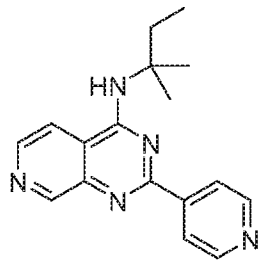
1H NMR (500 MHz, DMSO-d6) δ 9.19 (d, J = 0.8 Hz, 1H), 8.83 - 8.77 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.34 - 8.29 (m, 2H), 8.17 (s, 1H), 8.12 (dd, J = 5.7, 0.9 Hz, 1H), 4.86 (s, 1H), 3.68 - 3.61 (m, 2H), 2.19 (t, J = 6.6 Hz, 2H), 1.66 (s, 6H). LCMS (m/z $[M+H]^+$): 310.2.

Example 62: 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine



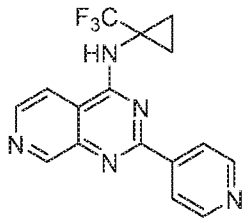
1H NMR (500 MHz, DMSO-d6) δ 9.26 (d, J = 0.9 Hz, 1H), 8.98 (s, 1H), 8.81 - 8.76 (m, 2H), 8.71 (d, J = 5.6 Hz, 1H), 8.39 (dd, J = 5.8, 0.9 Hz, 1H), 8.30 - 8.25 (m, 2H), 2.91 - 2.76 (m, 4H), 2.11 - 1.96 (m, 2H). LCMS (m/z $[M+H]^+$): 346.1.

Example 63: N-(2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



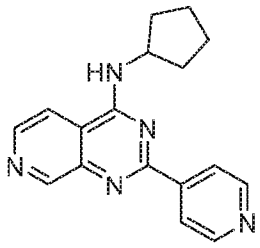
1H NMR (500 MHz, Methanol-d4) δ 9.19 - 9.12 (m, 1H), 8.72 (ddt, J = 6.3, 4.7, 1.6 Hz, 2H), 8.57 (dd, J = 5.8, 2.6 Hz, 1H), 8.43 (tt, J = 7.3, 3.4 Hz, 2H), 8.24 - 8.17 (m, 1H), 2.23 (qd, J = 6.7, 6.0, 2.6 Hz, 2H), 1.66 (s, 6H), 0.94 (td, J = 7.4, 1.6 Hz, 3H). LCMS (m/z $[M+H]^+$): 294.2.

Example 64: 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine



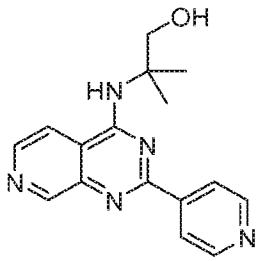
1H NMR (500 MHz, DMSO-d6) δ 9.40 (s, 1H), 9.29 (d, J = 0.9 Hz, 1H), 8.83 - 8.78 (m, 2H), 8.71 (d, J = 5.6 Hz, 1H), 8.40 - 8.35 (m, 2H), 8.27 (dd, J = 5.7, 1.0 Hz, 1H), 1.65 - 1.55 (m, 2H), 1.41 - 1.37 (m, 2H). LCMS (m/z $[M+H]^+$): 332.1.

Example 65: N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



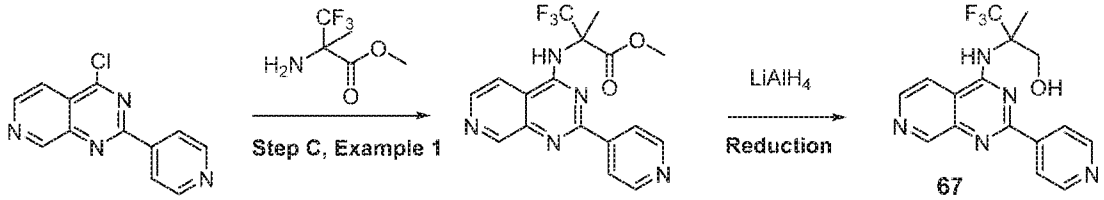
In a 20 mL vial 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**intermediate 1c**) (200 mg, 0.82 mmol) was stirred in DCM (5 mL) at room temperature and degassed with N_2. DIEA (324 microlitre, 1.85 mmol) was added and stirred for 5 minutes then KF (48 mg, 0.82 mmol). This mixture was stirred at room temperature for 15 minutes then cyclopentanamine (84 mg, 0.99 mmol) was added and degassed then stirred at 25°C for 16 hours. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the title compound (51%). 1H NMR (400 MHz, DMSO-d6) 9.20(s, 1H), 8.78(d, 2H), 8.55(d, 1H), 8.31(d, 2H), 8.03(d, 1H), 5.15-5.10(m, 1H), 3.34(s, 3H), 1.35(s, 6H). LCMS (m/z $[M+H]^+$): 292.2.

Example 66: 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol



1H NMR (500 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.82 - 8.77 (m, 2H), 8.64 (d, J = 5.6 Hz, 1H), 8.38 (dd, J = 5.7, 0.9 Hz, 1H), 8.30 (dt, J = 4.5, 1.7 Hz, 2H), 7.62 (s, 1H), 4.95 - 4.89 (m, 1H), 3.86 (d, J = 6.1 Hz, 2H), 1.57 (s, 5H). LCMS (m/z $[M+H]^+$): 296.1.

Example 67: 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol

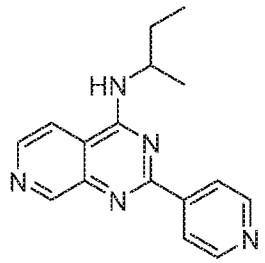


The title compound was prepared from methyl 2-amino-3,3,3-trifluoro-2-methylpropanoate hydrochloride and 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine as described in **Step C**, **Example 1**, followed by reduction of methyl ester to alcohol with lithium aluminum hydride.

Reduction: methyl 3,3,3-trifluoro-2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl)amino)propanoate (12 mg, 0.032 mmol) was stirred with lithium aluminum hydride (4.8 mg, 0.127 mmol) in THF at 0°C for 25 hours. No starting material was observed by TLC or LCMS. The reaction mixture was diluted with DCM/MeOH, washed by H_2O and brine, then concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the title compound (17%).

1H NMR (500 MHz, DMSO-d6) δ 8.37 (d, J = 1.0 Hz, 1H), 7.86 - 7.81 (m, 2H), 7.74 (d, J = 5.7 Hz, 1H), 7.54 - 7.49 (m, 2H), 7.32 (dt, J = 5.7, 1.3 Hz, 1H), 3.77 (d, J = 11.6 Hz, 1H), 3.19 - 3.12 (m, 1H), 1.02 (d, J = 1.2 Hz, 3H). LCMS (m/z $[M+H]^+$): 350.1.

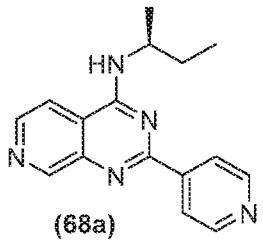
Example 68: N-(butan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine

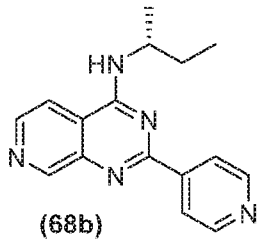


1H NMR (500 MHz, DMSO-d6) δ 9.18 (d, J = 0.9 Hz, 1H), 8.80 - 8.74 (m, 2H), 8.65 (d, J = 5.5 Hz, 1H), 8.43 (d, J = 7.8 Hz, 1H), 8.36 - 8.27 (m, 3H), 4.56 (hept, J = 6.4 Hz, 1H),

1.83 - 1.63 (m, 2H), 1.33 (d, J = 6.6 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 280.2.

Example 68a: (S)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (68a) and (R)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (68b)


(68a)

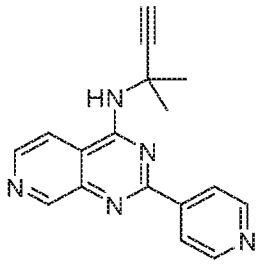

(68b)

Compound N-(butan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (26.7 mg) (Example 68) was subjected to chiral separation to get two enantiomers, peak 1 (T_R =1.44 min) isomer (11.7 mg) and peak 2 (T_R =1.94 min) isomer 11.6 mg. Chiral center assignments are tentative. Chiral separation conditions: solvent A CO_2(85%), solvent B MeOH(15%), flow rate 2 ml/min, temp 30 °C, column 21x250 mm AD-H, run time 3.5 minute stacked injections, 7 minute elution time.

Peak 1 (T_R = 1.44 min) isomer: 1H NMR (500 MHz, DMSO-d6) δ 9.18 (s, 1H), 8.77 (d, J = 5.5 Hz, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.43 (d, J = 7.8 Hz, 1H), 8.36 - 8.31 (m, 2H), 8.29 (d, J = 5.6 Hz, 1H), 4.56 (p, J = 7.2 Hz, 1H), 1.85 - 1.62 (m, 2H), 1.33 (d, J = 6.6 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 280.2.

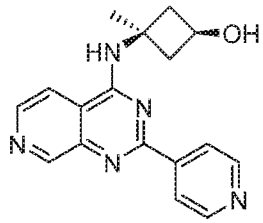
Peak 2 (T_R =1.94 min) isomer: 1H NMR (500 MHz, DMSO-d6) δ 9.18 (s, 1H), 8.80 - 8.72 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.43 (d, J = 7.8 Hz, 1H), 8.35 - 8.31 (m, 2H), 8.30 (dd, J = 5.6, 0.8 Hz, 1H), 4.57 (hept, J = 6.7 Hz, 1H), 1.82 - 1.63 (m, 2H), 1.33 (d, J = 6.6 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 280.2.

<u>Example 69:</u> N-(2-methylbut-3-yn-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



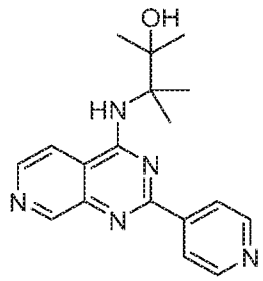
1H NMR (500 MHz, Methanol-d4) δ 9.25 (d, J = 0.9 Hz, 1H), 8.77 - 8.71 (m, 2H), 8.64 - 8.58 (m, 3H), 8.23 (dd, J = 5.7, 0.9 Hz, 1H), 2.81 (s, 1H), 1.93 (s, 6H). LCMS (m/z $[M+H]^+$): 290.1.

Example 70: (1r,3s)-3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutan-1-ol



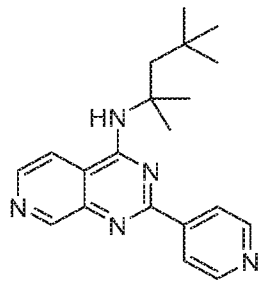
1H NMR (400 MHz, DMSO-d6) 9.21(s, 1H), 8.78(d, 2H), 8.55(d, 1H), 8.31(d, 2H), 8.05(d, 1H), 3.94(q, 2H), 3.50(s, 3H), 1.39(t, 3H). LCMS (m/z $[M+H]^+$): 308.1.

Example 71: 2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol



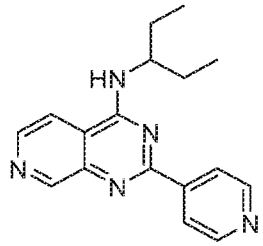
1H NMR (500 MHz, DMSO-d6) δ 9.20 (d, J = 0.8 Hz, 1H), 8.82 - 8.77 (m, 2H), 8.67 (d, J = 5.6 Hz, 1H), 8.28 - 8.23 (m, 2H), 8.07 (dd, J = 5.8, 0.9 Hz, 1H), 7.49 (s, 1H), 1.64 (s, 6H), 1.27 (s, 6H). LCMS (m/z $[M+H]^+$): 324.2.

Example 72: 2-(pyridin-4-yl)-N-(2,4,4-trimethylpentan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



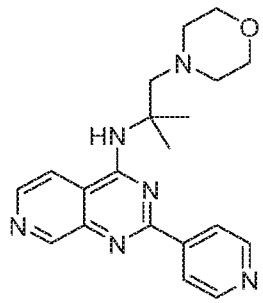
1H NMR (500 MHz, Methanol-d4) δ 9.19 (d, J = 0.9 Hz, 1H), 8.78 - 8.72 (m, 2H), 8.58 (d, J = 5.7 Hz, 1H), 8.50 - 8.45 (m, 2H), 8.22 (dd, J = 5.8, 0.9 Hz, 1H), 2.33 (d, J = 1.2 Hz, 2H), 1.77 (s, 6H), 1.01 (s, 9H). LCMS (m/z $[M+H]^+$): 336.2.

Example 73: **N-(pentan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



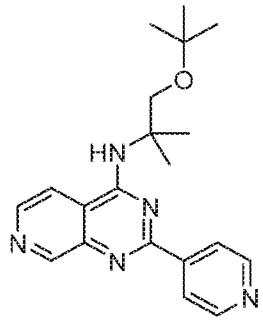
1H NMR (500 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.79 – 8.74 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.37 – 8.30 (m, 4H), 4.47 (dtd, J = 13.2, 8.2, 5.1 Hz, 1H), 1.83 – 1.63 (m, 4H), 0.95 (t, J = 7.4 Hz, 6H). LCMS (m/z $[M+H]^+$): 294.2.

Example 74: **(N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



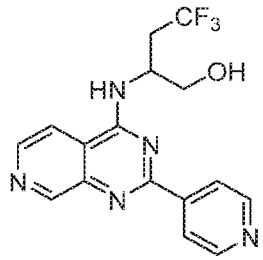
1H NMR (500 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.83 - 8.77 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.35 (dd, J = 5.8, 0.9 Hz, 1H), 8.32 - 8.28 (m, 2H), 7.79 (s, 1H), 3.55 - 3.49 (m, 4H), 2.99 (s, 2H), 2.51 - 2.45 (m, 4H), 1.62 (s, 6H). LCMS (m/z $[M+H]^+$): 365.2.

Example 75: **N-[1-(tert-butoxy)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



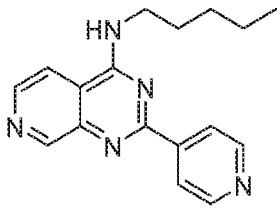
1H NMR (500 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.82 - 8.77 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.38 (dd, J = 5.7, 0.9 Hz, 1H), 8.33 - 8.28 (m, 2H), 7.66 (s, 1H), 3.84 (s, 2H), 1.60 (s, 6H), 1.05 (s, 9H). LCMS (m/z $[M+H]^+$): 352.2.

Example 76: 4,4,4-trifluoro-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol



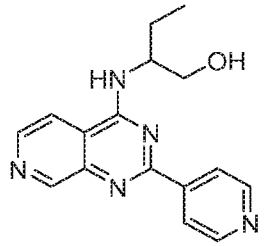
1H NMR (500 MHz, DMSO-d6) δ 9.23 (d, J = 0.8 Hz, 1H), 8.81 - 8.76 (m, 2H), 8.70 (d, J = 5.5 Hz, 1H), 8.62 (d, J = 8.2 Hz, 1H), 8.36 - 8.31 (m, 2H), 8.22 (dd, J = 5.7, 0.9 Hz, 1H), 5.19 (dd, J = 6.3, 5.4 Hz, 1H), 4.98 (tq, J = 9.6, 5.9 Hz, 1H), 3.72 (dt, J = 10.9, 5.5 Hz, 1H), 3.61 (dt, J = 11.0, 6.3 Hz, 1H), 2.87 - 2.71 (m, 2H). LCMS (m/z $[M+H]^+$): 350.1.

Example 77: N-pentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



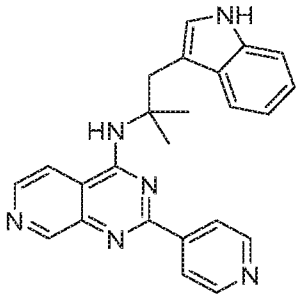
1H NMR (400 MHz, DMSO-d6) δ 9.18 (d, J = 0.8 Hz, 1H), 8.83 (t, J = 5.6 Hz, 1H), 8.79 - 8.73 (m, 2H), 8.64 (d, J = 5.5 Hz, 1H), 8.36 - 8.29 (m, 2H), 8.18 (dd, J = 5.6, 1.0 Hz, 1H), 3.70 (td, J = 7.2, 5.6 Hz, 2H), 1.81 - 1.68 (m, 2H), 1.47 - 1.30 (m, 4H), 0.93 - 0.86 (m, 3H). LCMS (m/z $[M+H]^+$): 294.2.

Example 78: **2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol**



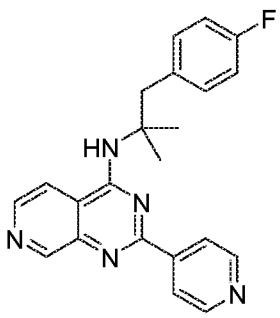
1H NMR (400 MHz, DMSO-d6) δ 9.19 (d, J = 0.8 Hz, 1H), 8.80 - 8.74 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.40 - 8.30 (m, 4H), 4.83 (t, J = 5.7 Hz, 1H), 4.54 (td, J = 8.4, 4.9 Hz, 1H), 3.72 - 3.56 (m, 2H), 1.84 (ddd, J = 14.0, 7.4, 5.1 Hz, 1H), 1.68 (ddd, J = 13.8, 8.8, 7.3 Hz, 1H), 0.96 (t, J = 7.4 Hz, 3H). LCMS (m/z $[M+H]^+$): 296.1.

Example 79: **N-[1-(1H-indol-3-yl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



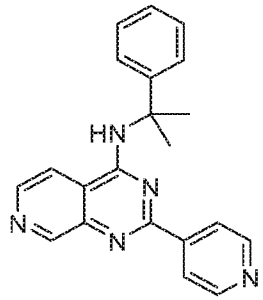
1H NMR (400 MHz, DMSO-d6) δ 10.78 (d, J = 2.6 Hz, 1H), 9.21 (d, J = 0.8 Hz, 1H), 8.86 - 8.78 (m, 2H), 8.60 (d, J = 5.6 Hz, 1H), 8.42 - 8.36 (m, 2H), 8.30 (dd, J = 5.7, 0.9 Hz, 1H), 7.68 (s, 1H), 7.47 - 7.40 (m, 1H), 7.29 (dt, J = 8.2, 0.9 Hz, 1H), 7.00 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H), 6.91 - 6.82 (m, 2H), 3.59 (s, 2H), 1.66 (s, 6H). LCMS (m/z $[M+H]^+$): 395.2.

Example 80: **N-[1-(4-fluorophenyl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



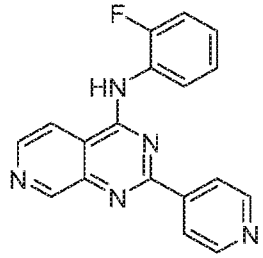
1H NMR (500 MHz, Methanol-d4) δ 9.23 (d, J = 0.9 Hz, 1H), 8.80 - 8.75 (m, 2H), 8.58 - 8.51 (m, 3H), 8.13 (dd, J = 5.7, 0.9 Hz, 1H), 7.11 - 7.04 (m, 2H), 6.95 - 6.87 (m, 2H), 3.56 (s, 2H), 1.69 (s, 6H). LCMS (m/z $[M+H]^+$): 374.2.

Example 81: **N-(2-phenylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



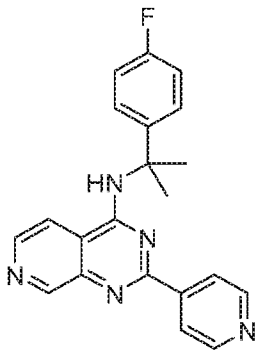
1H NMR (500 MHz, DMSO-d6) δ 9.16 (d, J = 0.9 Hz, 1H), 8.69 (d, J = 5.6 Hz, 1H), 8.66 (s, 1H), 8.61 - 8.56 (m, 2H), 8.53 (dd, J = 5.6, 0.9 Hz, 1H), 7.80 - 7.75 (m, 2H), 7.55 - 7.48 (m, 2H), 7.35 - 7.28 (m, 2H), 7.20 - 7.12 (m, 1H), 1.89 (s, 6H). LCMS (m/z $[M+H]^+$): 342.2.

Example 82: **N-(2-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



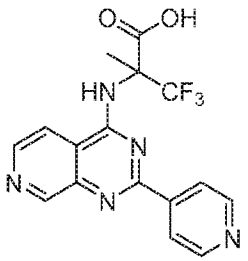
1H NMR (500 MHz, Methanol-d4) δ 9.33 (d, J = 1.0 Hz, 1H), 8.71 (d, J = 5.7 Hz, 1H), 8.68 - 8.64 (m, 2H), 8.32 (ddd, J = 9.5, 5.1, 1.3 Hz, 3H), 7.81 (td, J = 8.0, 2.0 Hz, 1H), 7.41 (d, J = 1.1 Hz, 1H), 7.38 - 7.31 (m, 2H). LCMS (m/z $[M+H]^+$): 318.1.

Example 83: **N-[2-(4-fluorophenyl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



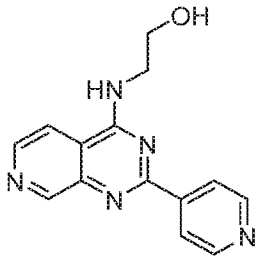
1H NMR (500 MHz, Methanol-d4) δ 9.18 (d, J = 0.9 Hz, 1H), 8.63 (d, J = 5.7 Hz, 1H), 8.59 - 8.53 (m, 2H), 8.36 (dd, J = 5.7, 0.9 Hz, 1H), 7.97 - 7.92 (m, 2H), 7.61 - 7.53 (m, 2H), 7.11 - 7.02 (m, 2H), 1.96 (s, 6H). LCMS (m/z $[M+H]^+$): 360.2.

Example 84: 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid



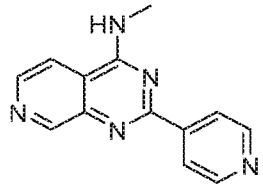
1H NMR (500 MHz, DMSO-d6) δ 9.38 (s, 1H), 9.24 (d, J = 0.8 Hz, 1H), 8.81 - 8.75 (m, 2H), 8.71 (d, J = 5.5 Hz, 1H), 8.34 - 8.29 (m, 2H), 7.84 (d, J = 5.5 Hz, 1H), 7.07 (s, 1H), 2.03 (s, 3H). LCMS (m/z $[M+H]^+$): 364.1.

Example 85: 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethan-1-ol



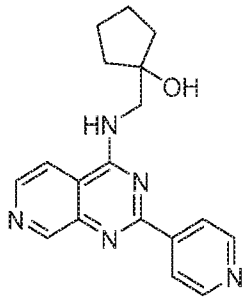
1H NMR (400 MHz, DMSO-d6) δ 9.20 (d, J = 0.8 Hz, 1H), 8.88 (t, J = 5.3 Hz, 1H), 8.82 - 8.73 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.38 - 8.31 (m, 2H), 8.21 (dd, J = 5.6, 0.9 Hz, 1H), 4.90 (t, J = 5.3 Hz, 1H), 3.76 (ttd, J = 7.9, 5.5, 2.5 Hz, 4H). LCMS (m/z $[M+H]^+$): 268.1.

Example 86: **N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



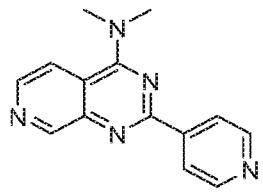
1H NMR (400 MHz, DMSO-d6) δ 9.19 (d, J = 0.9 Hz, 1H), 8.88 (q, J = 4.4 Hz, 1H), 8.80 - 8.73 (m, 2H), 8.65 (d, J = 5.5 Hz, 1H), 8.40 - 8.33 (m, 2H), 8.12 (dd, J = 5.6, 1.0 Hz, 1H), 3.18 (d, J = 4.5 Hz, 3H). LCMS (m/z $[M+H]^+$): 238.1.

Example 87: **1-({[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}methyl)cyclopentan-1-ol**



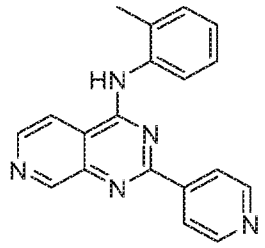
1H NMR (400 MHz, DMSO-d6) δ 9.19 (d, J = 0.8 Hz, 1H), 8.81 - 8.74 (m, 2H), 8.67 (dd, J = 11.8, 5.8 Hz, 2H), 8.37 - 8.33 (m, 2H), 8.30 (dd, J = 5.6, 0.9 Hz, 1H), 4.70 (s, 1H), 3.89 (d, J = 5.9 Hz, 2H), 1.78 - 1.50 (m, 8H). LCMS (m/z $[M+H]^+$): 322.2.

Example 88: **N,N-dimethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



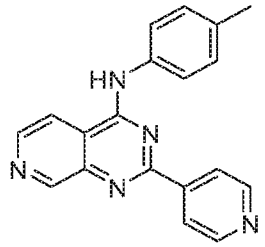
1H NMR (400 MHz, DMSO-d6) δ 9.22 (d, J = 0.8 Hz, 1H), 8.80 - 8.73 (m, 2H), 8.57 (d, J = 5.8 Hz, 1H), 8.38 - 8.31 (m, 2H), 8.15 (dd, J = 5.8, 0.8 Hz, 1H), 3.53 (s, 6H). LCMS (m/z $[M+H]^+$): 252.1.

Example 89: **N-(2-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



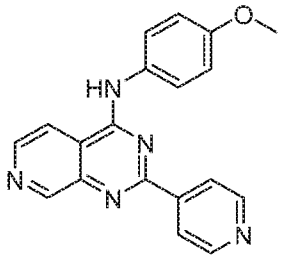
1H NMR (500 MHz, DMSO-d6) δ 10.22 (s, 1H), 9.29 (d, J = 0.9 Hz, 1H), 8.76 (d, J = 5.5 Hz, 1H), 8.72 - 8.67 (m, 2H), 8.42 (dd, J = 5.7, 1.0 Hz, 1H), 8.09 - 8.04 (m, 2H), 7.50 (dd, J = 7.7, 1.5 Hz, 1H), 7.43 (dd, J = 7.1, 1.8 Hz, 1H), 7.40 - 7.29 (m, 2H), 2.27 (s, 3H). LCMS (m/z $[M+H]^+$): 314.1..

Example 90: **N-(4-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



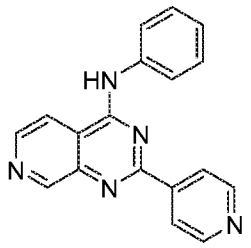
1H NMR (500 MHz, DMSO-d6) δ 10.25 (s, 1H), 9.29 (d, J = 0.8 Hz, 1H), 8.81 - 8.73 (m, 3H), 8.49 (dd, J = 5.7, 1.0 Hz, 1H), 8.30 - 8.25 (m, 2H), 7.87 - 7.80 (m, 2H), 7.36 - 7.30 (m, 2H), 2.38 (s, 3H). LCMS (m/z $[M+H]^+$): 314.1.

Example 91: **N-(4-methoxyphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



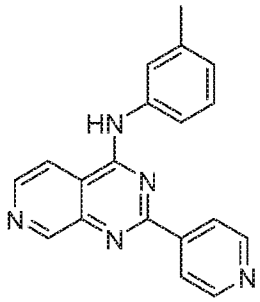
1H NMR (400 MHz, DMSO-d6) δ 10.24 (s, 1H), 9.27 (d, J = 0.8 Hz, 1H), 8.80 - 8.72 (m, 3H), 8.46 (dd, J = 5.8, 1.0 Hz, 1H), 8.29 - 8.23 (m, 2H), 7.88 - 7.79 (m, 2H), 7.14 - 7.05 (m, 2H), 3.83 (s, 3H). LCMS (m/z $[M+H]^+$): 330.1.

Example 92: **N-phenyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



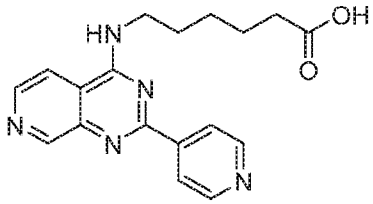
1H NMR (400 MHz, DMSO-d6) δ 10.31 (s, 1H), 9.30 (d, J = 0.8 Hz, 1H), 8.82 - 8.74 (m, 3H), 8.51 (dd, J = 5.8, 1.0 Hz, 1H), 8.31 - 8.24 (m, 2H), 8.00 - 7.92 (m, 2H), 7.58 - 7.48 (m, 2H), 7.26 (tt, J = 7.3, 1.2 Hz, 1H). LCMS (m/z $[M+H]^+$): 300.1.

Example 93: **N-(3-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



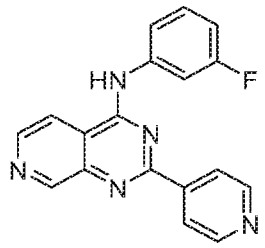
1H NMR (500 MHz, DMSO-d6) δ 10.24 (s, 1H), 9.30 (d, J = 0.8 Hz, 1H), 8.82 - 8.75 (m, 3H), 8.51 (dd, J = 5.8, 0.9 Hz, 1H), 8.31 - 8.26 (m, 2H), 7.83 - 7.76 (m, 2H), 7.41 (t, J = 7.8 Hz, 1H), 7.08 (ddt, J = 7.6, 1.7, 0.9 Hz, 1H), 2.43 (s, 3H). LCMS (m/z $[M+H]^+$): 314.1.

Example 94: **6-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}hexanoic acid**



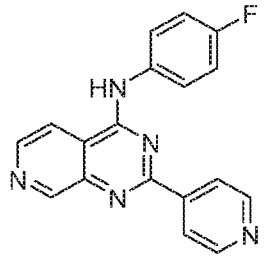
1H NMR (400 MHz, DMSO-d6) δ 12.02 (s, 1H), 9.19 (s, 1H), 8.84 (t, J = 5.6 Hz, 1H), 8.80 - 8.73 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.37 - 8.31 (m, 2H), 8.18 (dd, J = 5.6, 1.0 Hz, 1H), 3.76 - 3.66 (m, 2H), 2.23 (t, J = 7.3 Hz, 2H), 1.81 - 1.69 (m, 2H), 1.61 (p, J = 7.3 Hz, 2H), 1.43 (tt, J = 9.6, 6.1 Hz, 2H). LCMS (m/z $[M+H]^+$): 338.2.

Example 95: **N-(3-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



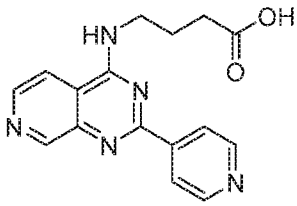
1H NMR (500 MHz, DMSO-d6) δ 10.39 (s, 1H), 9.34 (d, J = 0.8 Hz, 1H), 8.84 - 8.78 (m, 3H), 8.51 (dd, J = 5.8, 0.9 Hz, 1H), 8.31 - 8.26 (m, 2H), 7.96 (dt, J = 11.7, 2.3 Hz, 1H), 7.81 (ddd, J = 8.2, 2.0, 0.9 Hz, 1H), 7.56 (td, J = 8.2, 6.8 Hz, 1H), 7.08 (tdd, J = 8.5, 2.6, 0.9 Hz, 1H). LCMS (m/z $[M+H]^+$): 318.1.

Example 96: **N-(4-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



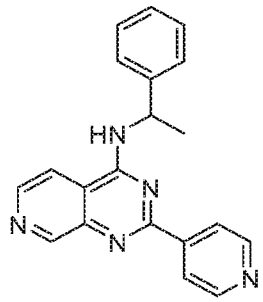
1H NMR (500 MHz, DMSO-d6) δ 10.34 (s, 1H), 9.31 (d, J = 0.9 Hz, 1H), 8.81 - 8.75 (m, 3H), 8.47 (dd, J = 5.7, 0.9 Hz, 1H), 8.31 - 8.24 (m, 2H), 7.99 - 7.92 (m, 2H), 7.41 - 7.33 (m, 2H). LCMS (m/z $[M+H]^+$): 318.1.

Example 97: **4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid**



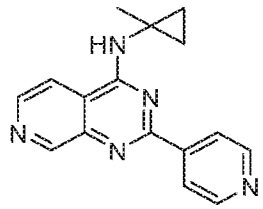
1H NMR (400 MHz, DMSO-d6) δ 10.43 (s, 1H), 9.16 (s, 1H), 8.79 - 8.72 (m, 2H), 8.62 (d, J = 5.5 Hz, 1H), 8.39 - 8.32 (m, 2H), 8.19 (dd, J = 5.6, 1.0 Hz, 1H), 3.67 (q, J = 6.0 Hz, 2H), 2.33 (t, J = 6.5 Hz, 2H), 1.94 (p, J = 6.6 Hz, 2H). LCMS (m/z $[M+H]^+$): 365.2.

Example 98: N-(1-phenylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



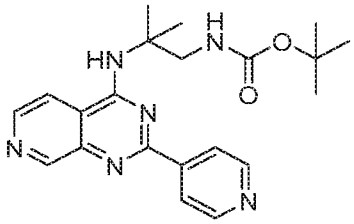
1H NMR (DMSO-d6) d 1.67 (d, J = 6.8 Hz, 3H), 5.73 (m, 1H), 7.22 (m, 1H), 7.47 (m, 2H), 7.55 (m, 2H), 8.26 (d, J = 5 Hz, 2H), 8.41 (d, J = 5.6 Hz, 1H), 8.69 (d, J = 5.6 Hz, 1H), 8.74 (d, J = 5 Hz, 1H), 9.08 (d, J = 7.2 Hz, 1H), 9.19 (s, 1H). LCMS (m/z $[M+H]^+$): 328.2.

Example 99: **N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



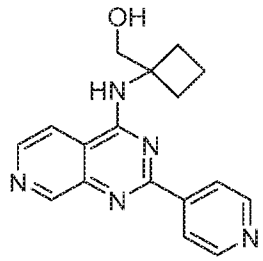
1H NMR (400 MHz, Methanol-d4) δ 9.19 (s, 1H), 8.73 (d, J = 5.7 Hz, 2H), 8.55 (m, 3H), 8.01 (dd, J = 5.7, 0.7 Hz, 1H), 1.64 (s, 3H), 0.99 (m, 2H), 0.93 (m, 2H). LCMS (m/z $[M+H]^+$): 278.1.

Example 100: **Tert-butyl N-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)carbamate**



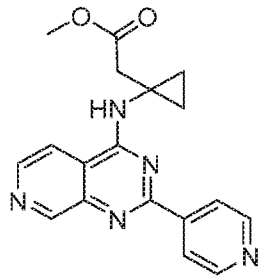
1H NMR (400 MHz, DMSO-d6) δ 9.21 (s, 1H), 8.87 (s, 2H), 8.67 (d, J = 5.6 Hz, 1H), 8.49 (d, J = 4.6 Hz, 2H), 8.24 (d, J = 5.6 Hz, 1H), 8.03 (s, 1H), 7.33 (t, J = 6.4 Hz, 1H), 3.54 (d, J = 6.4 Hz, 2H), 1.57 (s, 6H), 1.36 (s, 9H). LCMS (m/z $[M+H]^+$): 395.2.

Example 101: **(1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol**



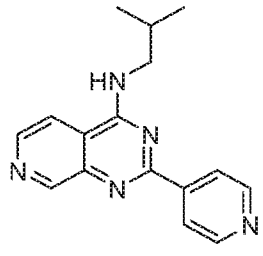
1H NMR (400 MHz, DMSO-d6) δ 9.16 (s, 1H), 8.78 – 8.72 (m, 2H), 8.68 (s, 1H), 8.62 (d, J = 5.6 Hz, 1H), 8.36 (d, J = 5.7 Hz, 1H), 8.31 – 8.20 (m, 2H), 4.94 (t, J = 6.0 Hz, 1H), 3.96 (d, J = 6.0 Hz, 2H), 2.41 (dt, J = 12.5, 7.2 Hz, 4H), 1.89 (p, J = 8.0 Hz, 2H). LCMS (m/z [M+H]+): 308.1.

Example 102: methyl 2-(1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)acetate



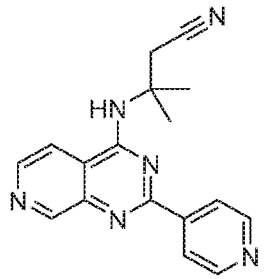
1H NMR (400 MHz, DMSO-d6) 9.25(s, 1H), 9.05(t, 1H), 8.90(d, 2H), 8.70(d, 1H), 8.60(d, 2H), 8.24(d, 1H), 3.90(m, 2H), 3.79(t, 2H), 3.59(m, 2H), 3.55-3.49(m, 4H), 3.45(m, 2H), 3.41(m, 2H), 2.40(t, 2H). LCMS (m/z [M+H]+): 336.1.

Example 103: N-(2-methylpropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



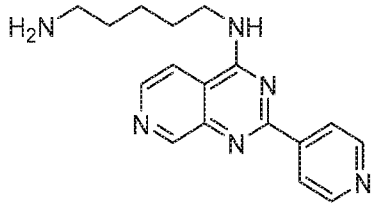
1HNMR (400 MHz, $CDCl_3$) d 9.35 (d, J = 0.8 Hz, 1H), 8.79 (d, J = 5.6 Hz, 2H), 8.64 (d, J = 5.6 Hz, 1H), 8.39 (d, J = 5.6 Hz, 2H), 7.52 (dd, J = 5.6, 0.8 Hz, 1H), 6.00 (t, J = 6.0 Hz, 1H), 3.66 (dd, J = 6.8, 6.0 Hz, 2H), 2.09 (nonet, J = 6.8 Hz, 1H), 1.12 (d, J = 6.8 Hz, 6H). LCMS (m/z [M+H]+): 280.1.

Example 104: 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanenitrile



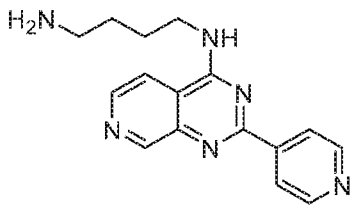
1H NMR (500 MHz, Acetone-d6) δ 9.23 (d, J = 0.9 Hz, 1H), 8.82 - 8.75 (m, 2H), 8.63 (d, J = 5.6 Hz, 1H), 8.42 - 8.33 (m, 2H), 8.17 (dd, J = 5.7, 0.9 Hz, 1H), 3.64 (s, 2H), 1.83 (s, 6H). LCMS (M/Z [M+H]+): 305.1.

Example 105: N-(6-aminohexyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



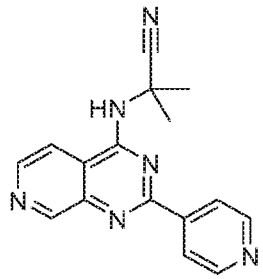
1H NMR (400 MHz, DMSO-d6) δ 9.19 (s, 1H), 8.88 - 8.80 (m, 1H), 8.82 - 8.73 (m, 2H), 8.68 - 8.61 (m, 1H), 8.37 - 8.31 (m, 2H), 8.19 (d, J = 5.6 Hz, 1H), 3.71 (q, J = 6.5 Hz, 2H), 2.53 (d, J = 1.6 Hz, 2H), 1.75 (dt, J = 14.2, 7.1 Hz, 2H), 1.47 - 1.32 (m, 6H). LCMS (M/Z [M+H]+): 323.2.

Example 106: N-(4-aminobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



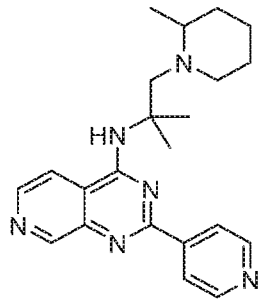
1H NMR (400 MHz, DMSO-d6) δ 9.23 (s, 1H), 8.96 (t, J = 5.7 Hz, 1H), 8.88 - 8.82 (m, 2H), 8.70 (d, J = 5.5 Hz, 1H), 8.51 - 8.44 (m, 2H), 8.20 (dd, J = 5.6, 1.0 Hz, 1H), 7.65 (s, 2H), 3.80 - 3.72 (m, 2H), 2.86 (td, J = 7.5, 5.5 Hz, 2H), 1.85 - 1.73 (m, 2H), 1.67 (ddt, J = 12.8, 9.9, 5.8 Hz, 2H). LCMS (M/Z [M+H]+): 295.2.

Example 107: 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanenitrile



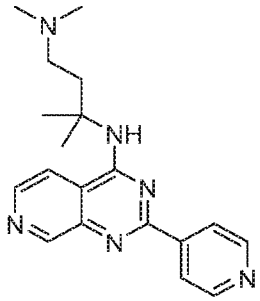
1H NMR (500 MHz, DMSO-d6) δ 9.31 (d, J = 0.8 Hz, 1H), 8.85 - 8.80 (m, 2H), 8.78 - 8.72 (m, 2H), 8.45 - 8.40 (m, 2H), 8.37 (dd, J = 5.7, 0.9 Hz, 1H), 1.93 (s, 6H). LCMS (M/Z [M+H]+): 291.1.

Example 108: N-[2-methyl-1-(2-methylpiperidin-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



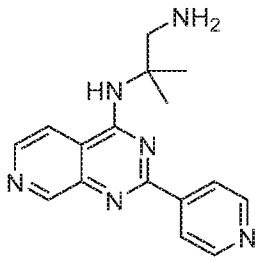
1H NMR (500 MHz, Methanol-d4) δ 9.08 (d, J = 0.9 Hz, 1H), 8.65 - 8.58 (m, 2H), 8.50 (d, J = 5.7 Hz, 1H), 8.37 - 8.29 (m, 2H), 7.94 (dd, J = 5.8, 1.0 Hz, 1H), 3.18 (d, J = 14.7 Hz, 1H), 3.00 - 2.92 (m, 2H), 2.66 (s, 1H), 2.44 (dq, J = 12.3, 5.9, 5.1 Hz, 1H), 1.86 (s, 1H), 1.69 - 1.43 (m, 10H), 1.38 - 1.25 (m, 2H), 0.97 (d, J = 6.4 Hz, 3H). LCMS (M/Z [M+H]+): 377.2.

Example 109: dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine



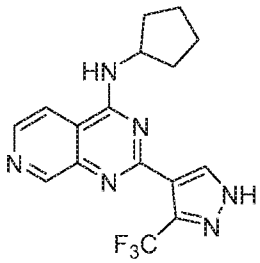
1H NMR (400 MHz, DMSO-d6) δ 9.23 (s, 1H), 8.96 (t, J = 5.7 Hz, 1H), 8.88 - 8.82 (m, 2H), 8.70 (d, J = 5.5 Hz, 1H), 8.51 - 8.44 (m, 2H), 8.20 (dd, J = 5.6, 1.0 Hz, 1H), 7.65 (s, 2H), 3.80 - 3.72 (m, 2H), 2.86 (td, J = 7.5, 5.5 Hz, 2H), 1.85 - 1.73 (m, 2H), 1.67 (ddt, J = 12.8, 9.9, 5.8 Hz, 2H). LCMS (M/Z [M+H]+): 295.2.

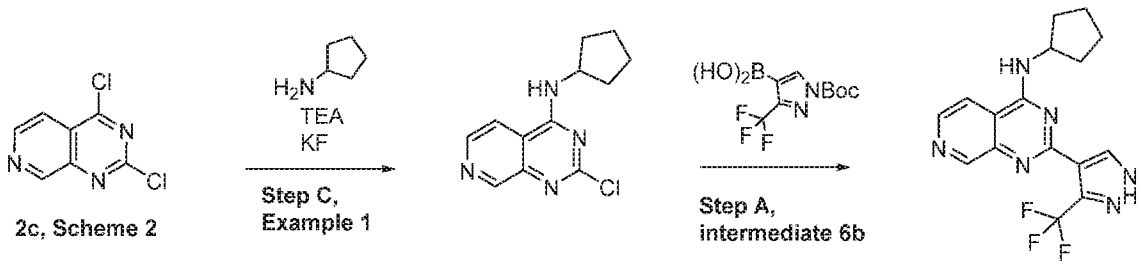
Example 110: N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.25 (s, 1H), 8.88 (s, 2H), 8.72 (d, J = 5.6 Hz, 1H), 8.45 (d, J = 5.7 Hz, 1H), 8.39 (d, J = 4.9 Hz, 2H), 7.87 (s, 1H), 7.79 (s, 2H), 3.67 (d, J = 5.9 Hz, 2H), 1.64 (s, 6H). LCMS (M/Z [M+H]+): 295.2.

Example 111: N-cyclopentyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine



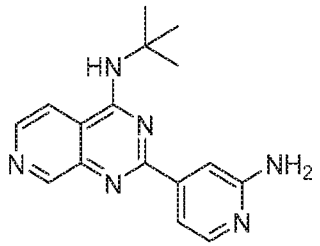


The title compound was prepared from 2,4-dichloropyrido[3,4-d]pyrimidine (intermediate 2c) as in **Scheme 2** using **Step C** for Example **1** and **Step A** for **Intermediate 6b**. 1H NMR (400 MHz, DMSO-d6) δ 9.10(d, J = 0.8 Hz, 1H), 8.60(d, J = 5.6 Hz, 1H), 8.36(dd, J = 5.8, 0.9 Hz, 1H), 8.09(dd, J = 5.3, 0.8 Hz, 1H), 7.77(s, 1H), 7.50(dd, J = 1.5, 0.8 Hz, 1H), 7.42(dd, J = 5.3, 1.5 Hz, 1H), 6.10(s, 2H), 1.60(s, 9H). LCMS (m/z [M+H]+): 349.1.

Examples 112-197:

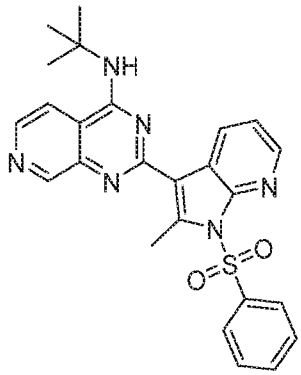
These compounds were synthesized according to the protocol described above using 2,4-dichloropyrido[3,4-d]pyrimidine (**Intermediate 2c**) and various amines and coupling partners respectively except specially stated.

Example 112: 4-[4-(tert-butylamino)pyrido[3,4-d]pyrimidin-2-yl]pyridin-2-amine



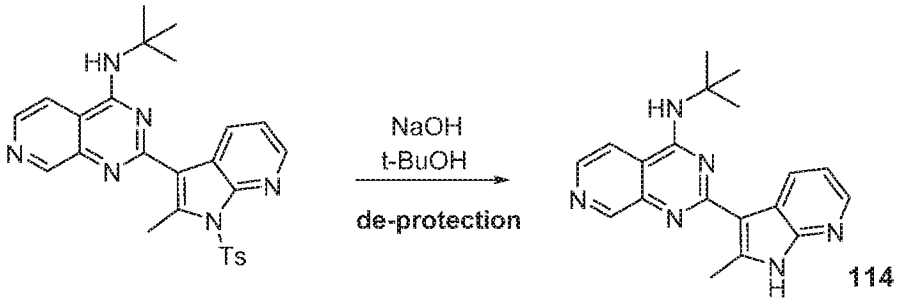
1H NMR (400 MHz, DMSO-d6) δ 9.10(d, J = 0.8 Hz, 1H), 8.60(d, J = 5.6 Hz, 1H), 8.36(dd, J = 5.8, 0.9 Hz, 1H), 8.09(dd, J = 5.3, 0.8 Hz, 1H), 7.77(s, 1H), 7.50(dd, J = 1.5, 0.8 Hz, 1H), 7.42(dd, J = 5.3, 1.5 Hz, 1H), 6.10(s, 2H), 1.60(s, 9H). LCMS (m/z [M+H]+): 295.2

Example 113: 2-[1-(benzenesulfonyl)-2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl]-N-tert-butylpyrido[3,4-d]pyrimidin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.12(d, J = 0.8 Hz, 1H), 8.70(dd, J = 7.9, 1.7 Hz, 1H), 8.58(d, J = 5.6 Hz, 1H), 8.20(m, 2H), 7.71(m, 1H), 7.66-7.59(m, 5H), 7.35(dd, J = 7.9, 4.7 Hz, 1H), 3.24(s, 3H), 1.59(s, 9H). LCMS (m/z [M+H]+): 473.2.

Example 114: N-tert-butyl-2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine

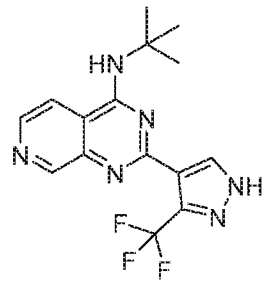


The title compound was prepared by de-protection of the tosyl group of Example **113**.

De-protection: N-(tert-butyl)-2-(2-methyl-1-(phenylsulfonyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)pyrido[3,4-d]pyrimidin-4-amine(32mg, 0.068mmol) was stirred in 1mL of t-butanol at room temperature. Sodium hydroxide (270 microlitre (5M), 1.35mmol) was then added and the reaction stirred at room temperature for two hours upon which time all starting material was consumed. The material was concentrated to an off white oil and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-20% MeOH/DCM to afford the title product N-tert-butyl-2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine (55%).

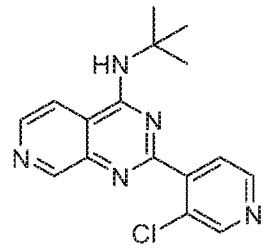
1H NMR (400 MHz, DMSO-d6) δ 12.01(s, 1H), 9.05(d, J = 0.8 Hz, 1H), 8.97(dd, J = 8.0, 1.7 Hz, 1H), 8.45(d, J = 5.5 Hz, 1H), 8.25(ddd, J = 23.7, 5.2, 1.3 Hz, 1H), 8.19(m, 1H), 7.42(s, 1H), 7.15(dd, J = 7.9, 4.7 Hz, 1H), 2.96(s, 3H), 1.63(s, 9H). LCMS (m/z [M+H]+): 333.2.

Example 115: **N-tert-butyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine**



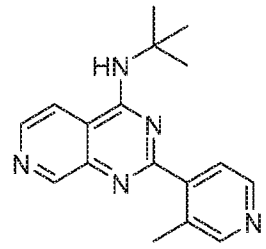
1H NMR (400 MHz, DMSO-d6) δ 8.95(d, J = 0.8 Hz, 1H), 8.54(d, J = 5.6 Hz, 1H), 8.46(s, 1H), 8.30(dd, J = 5.7, 0.9 Hz, 1H), 7.62(s, 1H), 1.58(s, 9H). LCMS (m/z [M+H]+): 337.1.

Example 116: **N-tert-butyl-2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



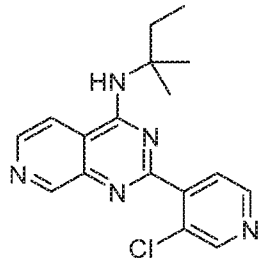
1H NMR (400 MHz, DMSO-d6) δ 9.12(d, J = 0.8 Hz, 1H), 8.79(d, J = 0.5 Hz, 1H), 8.68(m, 2H), 8.41(dd, J = 5.8, 0.9 Hz, 1H), 7.92(s, 1H), 7.77(dd, J = 4.9, 0.6 Hz, 1H), 1.55(s, 9H). LCMS (m/z [M+H]+): 314.1.

Example 117: **N-tert-butyl-2-(3-methylpyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



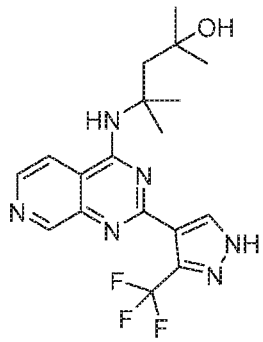
1H NMR (400 MHz, DMSO-d6) δ 9.11(d, J = 0.8 Hz, 1H), 8.63(d, J = 5.6 Hz, 1H), 8.55(m, 2H), 8.39(dd, J = 5.7, 0.9 Hz, 1H), 7.79(m, 1H), 7.76(m, 1H), 2.59(s, 3H), 1.58(s, 9H). LCMS (m/z [M+H]+): 294.2.

Example 118: 2-(3-chloropyridin-4-yl)-N-(2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



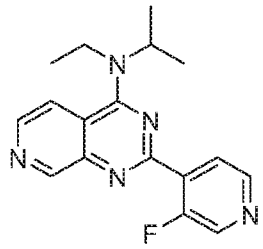
1H NMR (400 MHz, DMSO-d6) δ 9.12(d, J = 0.9 Hz, 1H), 8.78(d, J = 0.6 Hz, 1H), 8.68(dd, J = 5.3, 2.2 Hz, 1H), 8,66(m, 2H), 8.41(dd, J = 5.7, 0.9 Hz, 1H), 7.74(m, 1H), 2.00(q, J = 7.3 Hz, 2H), 1.49(s, 6H), 0.80(t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 328.1.

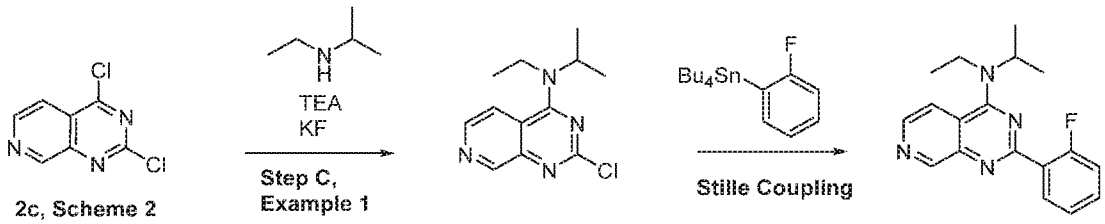
Example 119: 2,4-dimethyl-4-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)pentan-2-ol



1H NMR (400 MHz, DMSO-d6) δ 9.29(s, 1H), 8.99(d, J = 0.8 Hz, 1H), 8.55(d, J = 5.6 Hz, 1H), 8.49(s, 1H), 7.73(dd, J = 5.7, 0.9 Hz, 1H), 5.61(s, 1H), 1.97(s, 2H), 1.70(s, 6H), 1.30(s, 6H). LCMS (m/z [M+H]+): 395.2.

Example 120: N-ethyl-2-(3-fluoropyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine

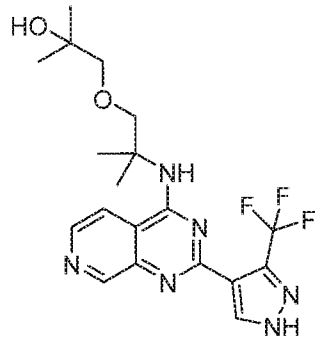




Title compound was prepared from 2,4-dichloropyrido[3,4-d]pyrimidine (intermediate **2c**) similar as described in Example **111** except using Stille coupling as the second step.

Stille coupling: 2-chloro-N-ethyl-N-isopropylpyrido[3,4-d]pyrimidin-4-amine (30 mg, 0.12mmol) was stirred in dry DMF (1 mL) at room temperature. 3-fluoro-4-(tributylstannyl)pyridine (50.8 mg, 0.13 mmol) was added then PdCl2(dppf).CH2Cl2 adduct(9.8 mg, 0.012mmol) and CuI(2.3 mg, 0.012 mmol). The reaction was stirred for 1 hour at 130°C. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the title compound. 1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.72(d, J = 2.9 Hz, 1H), 8.61(m, 1H), 8.59(m, 1H), 8.08(dd, J = 6.7, 4.9 Hz, 1H), 7.90(m, 1H), 4.92-4.88(m, 1H), 3.79(q, J = 7.0 Hz, 2H), 1.37(m, 6H), 1.32(dd, J = 23.7, 6.8 Hz, 3H). LCMS (m/z [M+H]+): 312.2.

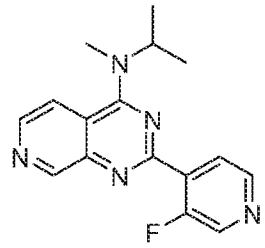
Example 121: 2-methyl-1-[2-methyl-2-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)propoxy]propan-2-ol



The title compound was prepared using procedures described in Example **111** and the amine as described in Example **12**.

1H NMR (400 MHz, DMSO-d6) δ 13.80(s, 1H), 8.99(d, J = 0.8 Hz, 1H), 8.55(d, J = 5.6 Hz, 1H), 8.44(s, 1H), 8.25(dd, J = 5.8, 0.9 Hz, 1H), 7.53(s, 1H), 4.35(s, 1H), 3.82(s, 2H), 3.17(s, 2H), 1.54(s, 6H), 1.00(s, 6H). LCMS (m/z [M+H]+): 425.2.

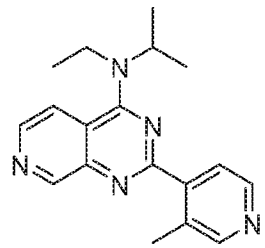
Example 122: 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



The title compound was prepared using procedures described in Example **120**.

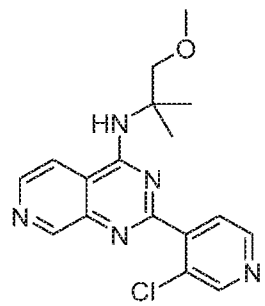
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.8 Hz, 1H), 8.74(d, J = 2.9 Hz, 1H), 8.60(m, 2H), 8.11(m, 1H), 8.09(m, 1H), 5.11-5.06(d, J = 6.6 Hz, 1H), 3.37(s, 3H), 1.34(d, J = 6.7 Hz, 6H). LCMS (m/z [M+H]+): 298.1.

Example 123: N-ethyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



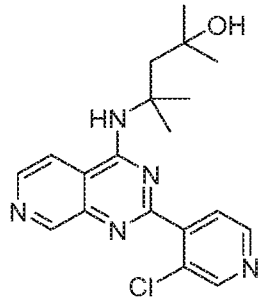
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.59(m, 1H), 8.56(m, 1H), 8.54(m, 1H), 7.89(m, 1H), 7.85(m, 1H), 4.95-4.90(d, J = 6.6 Hz, 1H), 3.79(q, J = 7.0 Hz, 2H), 2.60(s, 3H), 1.36(d, J = 6.6 Hz, 6H), 1.30(t, J = 7.0 Hz, 3H). LCMS (m/z [M+H]+): 308.2.

Example 124: 2-(3-chloropyridin-4-yl)-N-(1-methoxy-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



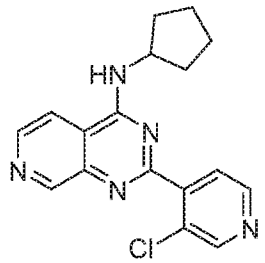
1H NMR (400 MHz, DMSO-d6) δ 9.12(s, 1H), 8.79(s, 1H), 8.69(dd, J = 5.2, 1.8 Hz, 2H), 8.40(dd, J = 5.8, 1.0 Hz, 1H), 7.78(s, 1H), 7.75(m, 1H), 3.73(s, 2H), 3.22(s, 3H), 1.50(s, 6H). LCMS (m/z [M+H]+): 344.1.

Example 125: 4-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol



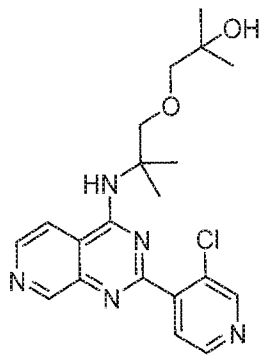
1H NMR (400 MHz, DMSO-d6) δ 9.45(s, 1H), 9.13(d, J = 0.7 Hz, 1H), 8.79(s, 1H), 8.68(dd, J = 5.3, 3.4 z, 1H), 8.66(m, 1H), 7.83(dd, J = 5.7, 0.9 Hz, 1H), 7.77(dd, J = 4.9, 0.6 Hz, 1H), 5.57(s, 1H), 1.96(s, 2H), 1.65(s, 6H), 1.29(s, 6H). LCMS (m/z [M+H]+): 372.2.

Example 126: 2-(3-chloropyridin-4-yl)-N-cyclopentylpyrido[3,4-d]pyrimidin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.14(d, J = 0.9 Hz, 1H), 8.78(d, J = 0.6 Hz, 1H), 8.69(dd, J = 9.4, 5.2 Hz, 1H), 8.67(d, J = 6.9 Hz, 1H), 8.61(d, J - 6.8 Hz, 1H), 8.32(dd, J = 5.7, 0.9 Hz, 1H), 7.81(m, 1H), 4.61-4.57(m, 1H), 2.10-2.01(m, 2H), 1.80-1.54(m, 6H). LCMS (m/z [M+H]+): 326.1.

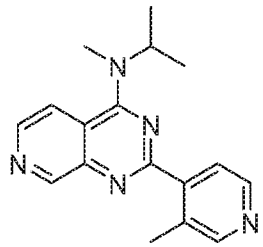
Example 127: 1-(2-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2-methylpropoxy)-2-methylpropan-2-ol



The title compound was prepared using procedures described in Example **111** and the amine as described in Example **12**.

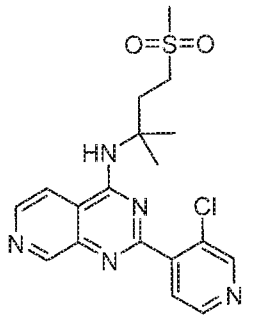
1H NMR (400 MHz, DMSO-d6) δ 9.13(m, 1H), 8.80(d, J = 0.6 Hz, 1H), 8.69(m, 2H), 8.40(dd, J = 5.8, 0.9 Hz, 1H), 7.83(dd, J = 4.9, 0.6 Hz, 1H), 7.76(dd, J = 4.9, 0.6 Hz, 1H), 4.38(s, 1H), 3.80(s, 2H), 3.16(s, 2H), 1.51(s, 6H), 1.00(s, 6H). LCMS (m/z [M+H]+): 402.2.

Example 128: N-methyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



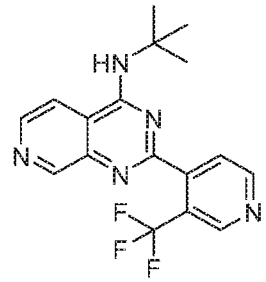
1H NMR (400 MHz, DMSO-d6) δ 9.19(d, J = 0.8 Hz, 1H), 8.58(m, 2H), 8.53(m, 1H), 8.03(dd, J = 5.8, 0.9 Hz, 1H), 7.87(d, J = 5.0 Hz, 1H), 5.09-5.00(m, 1H), 3.29(s, 3H), 2.60(s, 3H), 1.31(d, J = 6.7 Hz, 6H). LCMS (m/z [M+H]+): 294.2.

Example 129: 2-(3-chloropyridin-4-yl)-N-(4-methanesulfonyl-2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



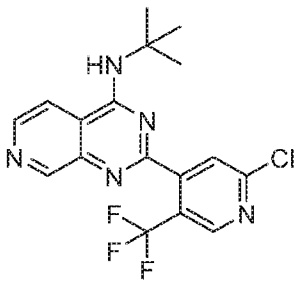
1H NMR (400 MHz, DMSO-d6) δ 9.15(d, J = 0.8 Hz, 1H), 8.79(d, J = 0.6 Hz, 1H), 8.69(dd, J = 21.3, 5.3 Hz, 1H), 8,65(m, 1H), 8.40(dd, J = 5.8, 0.9 Hz, 1H), 7.80(m, 1H), 7.78(m, 1H), 3.12-3.08(m, 2H), 2.87(s, 3H), 2.49-2.46(m, 2H), 1.53(s, 6H). LCMS (m/z [M+H]+): 406.1.

Example 130: N-tert-butyl-2-[3-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine



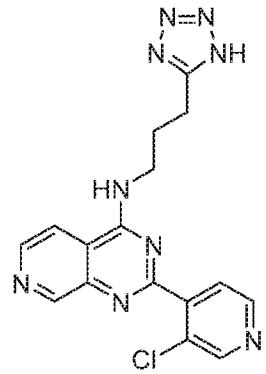
1H NMR (400 MHz, DMSO-d6) δ 8.92(m, 1H), 8.89(m, 1H), 8.08(d, J = 4.9 Hz, 1H), 7.76(d, J = 0.8 Hz, 1H), 7.70(m, 1H), 7.67(m, 1H), 7.56(dd, J = 5.0, 0.8 Hz, 1H), 1.49(s, 9H). LCMS (m/z [M+H]+): 348.1.

Example 131: N-tert-butyl-2-[2-chloro-5-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.08(d, J = 0.9 Hz, 1H), 8.87(s, 1H), 8.61(d, J = 5.8 Hz, 1H), 8.22(dd, J = 5.8, 1.0 Hz, 1H), 7.83(s, 1H), 1.20(s, 9H). LCMS (m/z [M+H]+): 382.1.

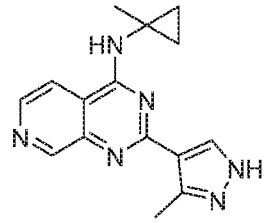
Example 132: 2-(3-chloropyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.30(s, 1H), 9.15(d, J = 0.8 Hz, 1H), 8.77(s, 1H), 8.70(dd, J = 18.4, 5.2 Hz, 1H), 8.65(m, 1H), 8.25(dd, J = 5.7, 0.9 Hz, 1H), 7.81(d, J = 4.9

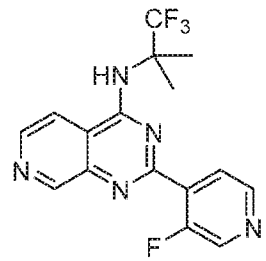
Hz, 1H), 3.70-3.65(td, J = 6.8, 5.1 Hz, 2H), 2.90(t, J = 7.4 Hz, 2H), 2.12-2.07(m, 2H). LCMS (m/z [M+H]+): 368.1.

Example 133: 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



1H NMR (500 MHz, Methanol-d4) δ 9.01 (s, 1H), 8.41 (d, J = 5.7 Hz, 1H), 8.26 (s, 1H), 7.91 (dd, J = 5.7, 0.9 Hz, 1H), 2.83 (s, 3H), 1.60 (s, 3H), 1.05 – 0.94 (m, 2H), 0.91 – 0.82 (m, 2H). LCMS (m/z [M+H]+): 281.1.

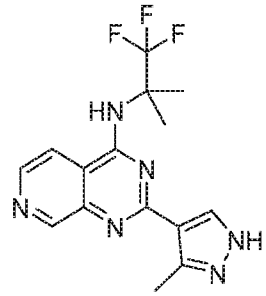
Example 134: 2-(3-fluoropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



Title compound was prepared using procedures described in Example **120**.

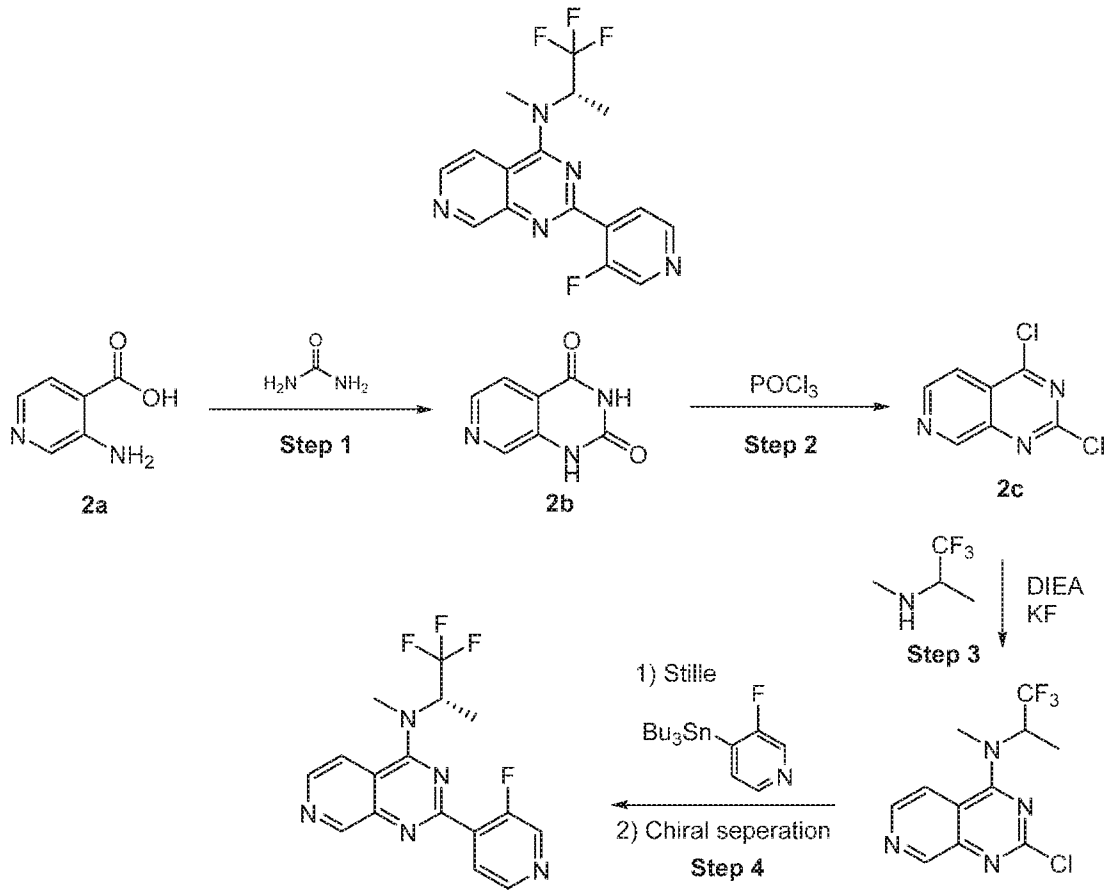
1H NMR (600 MHz, DMSO-d6) δ 9.24 (d, J = 0.8 Hz, 1H), 8.77 – 8.73 (m, 2H), 8.62 (dd, J = 4.9, 0.8 Hz, 1H), 8.52 (dd, J = 5.8, 0.9 Hz, 1H), 8.04 (dd, J = 6.7, 4.9 Hz, 1H), 7.99 (s, 1H), 1.86 (s, 6H). LCMS (m/z [M+H]+): 352.1.

Example 135: 2-(3-methyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



1H NMR (600 MHz, Methanol-d4) δ 9.06 (d, J = 0.9 Hz, 1H), 8.47 (d, J = 5.7 Hz, 1H), 8.16 (dd, J = 5.7, 0.9 Hz, 1H), 8.11 (s, 1H), 2.74 (d, J = 34.7 Hz, 3H), 1.92 (s, 6H). LCMS (m/z [M+H]+): 337.1.

Example 136: 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine



Step 1: A mixture of urea (40.00 g, 666.00 mmol) and 3-aminoisonicotinic acid (**2a,** 18.40 g, 133.20 mmol) was heated at 210°C for 1 hr (NOTE: no solvent was used). NaOH (2N, 320 mL) was added, and the mixture was stirred at 90°C for 1h. The solid was collected

by filtration, and washed with water. The crude product thus obtained was suspended in HOAc (400 mL), and stirred at 100°C for 1h. The mixture was cooled to RT, filtered, and the solid was washed with a large amount of water, and then dried under the vacuum to give pyrido[3,4-d]pyrimidine-2,4(1H,3H)-dione (**2b,** 17.00 g, 78% yield) without further purification. LCMS (m/z $[M+H]^+$): 164.0.

Step 2: To a mixture of pyrido[3,4-d]pyrimidine-2,4(1H,3H)-dione (**2b**, 20.00 g, 122.60 mmol) and $POCl_3$ (328.03 g, 2.14 mol) in toluene (200 mL) was added DIEA (31.69 g, 245.20 mmol) dropwise and this reaction mixture stirred at 25°C overnight (18hr) to give suspension.

The solvent and $POCl_3$ was removed under vacuum, diluted with DCM (50 mL), neutralized with DIEA to pH=7 at -20°C and concentrated again, the residue was purified by column (20-50% EA/PE) to give 2,4-dichloropyrido[3,4-d]pyrimidine (**2c**, 20.00g, 99.99 mmol, 82% yield) as a yellow solid. 1H NMR (400 MHz, CHLOROFORM-*d*) δ 9.52 (s, 1 H), 8.92 (d, *J*=5.6 Hz, 1 H), 8.04 (d, *J*=5.6 Hz, 1 H). LCMS (m/z $[M+H]^+$): 200.0.

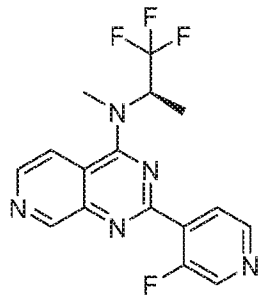
Step 3: In a 20 mL vial 2,4-dichloropyrido[3,4-d]pyrimidine (600 mg, 3.0 mmol) was stirred in DMSO (0.7 mL) at room temperature and degassed with N_2. DIEA (1 mL, 6 mmol) was added and stirred for 5 minutes then KF (174 mg, 3 mmol). This mixture was stirred at room temperature for 15 minutes then racemic 1,1,1-trifluoro-N-methylpropan-2-amine (419 mg, 3.3 mmol) was added and degassed then stirred at 60°C for 4 hours. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford 2-chloro-N-methyl-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine (680 mg, 74%). 1H NMR (500 MHz, Acetone-d6) δ 9.09 (d, J = 0.9 Hz, 1H), 8.59 (d, J = 5.9 Hz, 1H), 8.22 (dd, J = 5.9, 0.9 Hz, 1H), 5.93 (dddd, J = 15.3, 8.3, 7.0, 1.2 Hz, 1H), 3.61 (q, J = 1.0 Hz, 3H), 1.63 (d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 291.7.

Step 4: : In a 20 mL vial 2-chloro-N-methyl-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine (100 mg, 0.34 mmol) was stirred in dry DMF (1 mL) at room temperature. 3-fluoro-4-(tributylstannyl)pyridine (133 mg, 0.34 mmol) was added then $PdCl_2(dppf).CH_2Cl_2$ adduct (28.1 mg, 0.034mmol) and CuI (6.55 mg, 0.034 mmol). The reaction was stirred for 0.5 hour at 130 °C. The crude mixture was diluted with DCM, H_2O, separated and extracted with DCM x3. Combined the organic layers and dried Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the racemic product, then followed by chiral HPLC (21x250mm OJ-H column with 85% CO_2 as phase A and 15%

MeOH as phase B, flow rate 2mL/min, 30°C, 2.75 min elution time) to separate the enantiomers to afford **Examples 136** and **137**.

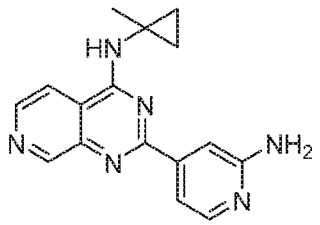
Examples 136: 1H NMR (500 MHz, DMSO-d6) δ 9.30 (d, J = 0.8 Hz, 1H), 8.75 (d, J = 3.0 Hz, 1H), 8.66 (d, J = 5.9 Hz, 1H), 8.62 (dd, J = 4.9, 0.8 Hz, 1H), 8.24 (dd, J = 5.9, 0.9 Hz, 1H), 8.15 (dd, J = 6.8, 4.9 Hz, 1H), 6.12 – 5.98 (m, 1H), 3.51 (d, J = 1.1 Hz, 3H), 1.57 (d, J = 7.0 Hz, 3H). LCMS (m/z $[M+H]^+$): 352.1. Chiral HPLC T_R = 0.88 min. .

Example 137: 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine



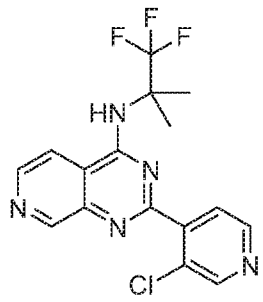
1H NMR (500 MHz, DMSO-d6) δ 9.30 (d, J = 0.8 Hz, 1H), 8.75 (d, J = 3.0 Hz, 1H), 8.66 (d, J = 5.9 Hz, 1H), 8.62 (dd, J = 4.9, 0.8 Hz, 1H), 8.24 (dd, J = 5.9, 0.9 Hz, 1H), 8.15 (dd, J = 6.8, 4.9 Hz, 1H), 6.12 – 5.98 (m, 1H), 3.51 (d, J = 1.1 Hz, 3H), 1.57 (d, J = 7.0 Hz, 3H). LCMS (m/z [M+H]+): 352.1. Chiral HPLC TR = 0.70 min.

Example 138: 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridin-2-amine



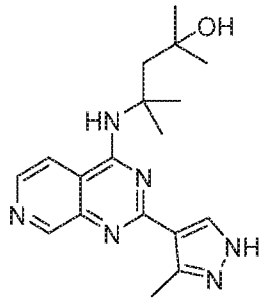
1H NMR (400 MHz, Acetone-d6) δ 9.15 (d, J = 0.9 Hz, 1H), 8.55 (d, J = 5.6 Hz, 1H), 8.17 - 8.09 (m, 2H), 7.98 (dd, J = 5.6, 1.0 Hz, 1H), 7.81 - 7.70 (m, 2H), 1.61 (s, 3H), 1.00 - 0.94 (m, 2H), 0.91 - 0.84 (m, 2H). LCMS (m/z [M+H]+): 293.1.

Example 139: 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



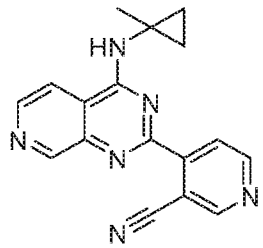
1H NMR (500 MHz, Methanol-d4) δ 9.20 (s, 1H), 8.74 (s, 1H), 8.68 (d, J = 5.8 Hz, 1H), 8.63 (d, J = 5.0 Hz, 1H), 8.33 (dd, J = 5.8, 0.9 Hz, 1H), 7.79 (d, J = 4.9 Hz, 1H), 1.88 (d, J = 1.0 Hz, 6H). LCMS (m/z [M+H]+): 368.1.

Example 140: 2,4-dimethyl-4-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol



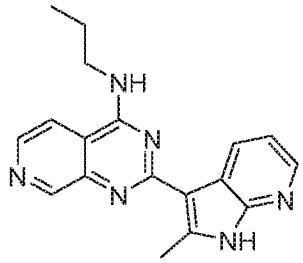
1H NMR (400 MHz, Chloroform-d) δ 9.14 (s, 1H), 8.80 (s, 1H), 8.37 (t, J = 5.8 Hz, 2H), 7.50 - 7.38 (m, 1H), 2.81 (s, 3H), 1.99 (s, 2H), 1.81 (s, 6H), 1.49 (s, 6H). LCMS (m/z [M+H]+): 341.2.

Example 141: 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile



1H NMR (400 MHz, Acetone-d6) δ 9.23 (d, J = 0.9 Hz, 1H), 9.09 (d, J = 0.8 Hz, 1H), 8.99 (d, J = 5.2 Hz, 1H), 8.65 (d, J = 5.6 Hz, 1H), 8.59 – 8.53 (m, 1H), 8.42 (s, 1H), 8.07 (dd, J = 5.7, 0.9 Hz, 1H), 1.62 (s, 3H), 1.04 – 0.97 (m, 2H), 0.90 – 0.81 (m, 2H). LCMS (m/z [M+H]+): 303.1.

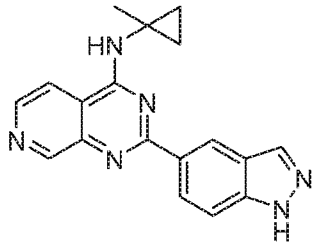
Example 142: 2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine



Title compound was prepared using procedures described in Example **114**.

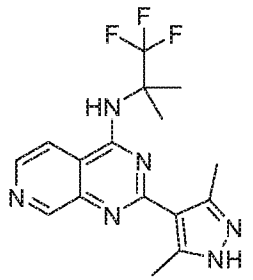
1H NMR (400 MHz, DMSO-d6) δ 12.02 (s, 1H), 9.06 (d, J = 0.8 Hz, 1H), 8.98 (dd, J = 7.9, 1.7 Hz, 1H), 8.50 (t, J = 5.5 Hz, 1H), 8.46 (d, J = 5.5 Hz, 1H), 8.19 (dd, J = 4.7, 1.7 Hz, 1H), 8.06 (dd, J = 5.6, 0.9 Hz, 1H), 7.16 (dd, J = 7.9, 4.7 Hz, 1H), 3.62 (dt, J = 8.1, 6.0 Hz, 2H), 2.96 (s, 3H), 1.90 - 1.69 (m, 2H), 1.00 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 319.2.

Example 143: 2-(1H-indazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



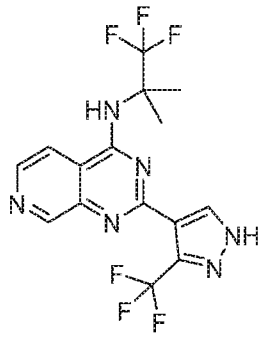
1H NMR (400 MHz, DMSO-d6) δ 9.12 (d, J = 0.9 Hz, 1H), 9.00 (dd, J = 1.5, 0.8 Hz, 1H), 8.85 (s, 1H), 8.61 (dd, J = 8.9, 1.5 Hz, 1H), 8.52 (d, J = 5.5 Hz, 1H), 8.26 (s, 1H), 8.11 (dd, J = 5.6, 0.9 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 1.62 (s, 3H), 0.97 – 0.81 (m, 4H). LCMS (m/z [M+H]+): 317.1.

Example 144: 2-(3,5-dimethyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



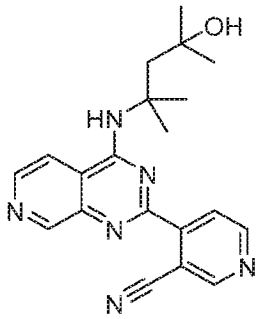
1H NMR (500 MHz, Methanol-d4) δ 9.06 (d, J = 0.9 Hz, 1H), 8.50 (d, J = 5.8 Hz, 1H), 8.17 (dd, J = 5.8, 0.9 Hz, 1H), 2.58 (s, 6H), 1.91 (d, J = 1.1 Hz, 6H). LCMS (m/z [M+H]+): 3511.

Example 145: N-(1,1,1-trifluoro-2-methylpropan-2-yl)-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine



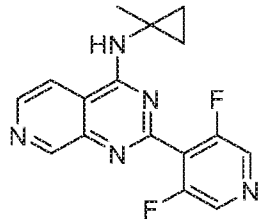
1H NMR (600 MHz, Methanol-d4) δ 9.06 (d, J = 1.0 Hz, 1H), 8.54 (d, J = 5.7 Hz, 1H), 8.38 - 8.36 (m, 1H), 8.20 (dd, J = 5.7, 0.9 Hz, 1H), 1.90 (d, J = 1.1 Hz, 6H). LCMS (m/z [M+H]+): 391.1.

Example 146: 4-{4-[(4-hydroxy-2,4-dimethylpentan-2-yl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile



1H NMR (400 MHz, Chloroform-d) δ 9.55 (s, 1H), 9.38 (s, 1H), 9.07 (d, J = 0.8 Hz, 1H), 8.95 (d, J = 5.2 Hz, 1H), 8.62 (d, J = 5.9 Hz, 1H), 8.27 (dd, J = 5.2, 0.8 Hz, 1H), 7.86 - 7.80 (m, 1H), 2.02 (s, 2H), 1.84 (s, 6H), 1.53 (s, 6H). LCMS (m/z [M+H]+): 363.2.

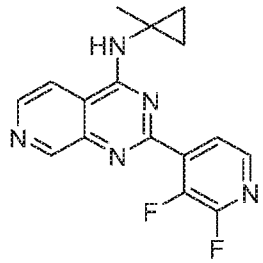
Example 147: 2-(3,5-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



Title compound was prepared using procedures described in Example **120**.

1H NMR (400 MHz, DMSO-d6) δ 9.16 (s, 1H), 9.14 (d, J = 0.8 Hz, 1H), 8.71 (s, 2H), 8.68 (d, J = 5.6 Hz, 1H), 8.19 (dd, J = 5.7, 1.0 Hz, 1H), 1.45 (s, 3H), 0.87 – 0.77 (m, 2H), 0.75 – 0.64 (m, 2H). LCMS (m/z [M+H]+): 314.1.

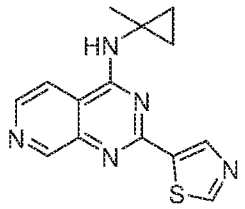
Example 148: 2-(2,3-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



Title compound was prepared using procedures described in Example **120**.

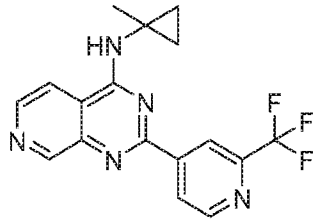
1H NMR (400 MHz, Chloroform-d) δ 9.36 (s, 1H), 8.90 (dd, J = 5.0, 0.9 Hz, 1H), 8.85 (dd, J = 1.6, 0.9 Hz, 1H), 8.69 (dd, J = 5.1, 1.6 Hz, 1H), 8.66 (d, J = 5.7 Hz, 1H), 7.64 – 7.52 (m, 1H), 1.64 (s, 3H), 1.04 – 0.94 (m, 4H). LCMS (m/z [M+H]+): 314.1.

Example 149: N-(1-methylcyclopropyl)-2-(1,3-thiazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine



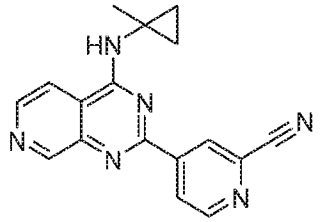
1H NMR (400 MHz, DMSO-d6) δ 9.22 (d, J = 0.8 Hz, 1H), 9.08 (d, J = 0.8 Hz, 1H), 9.01 (s, 1H), 8.69 – 8.62 (m, 1H), 8.56 (d, J = 5.5 Hz, 1H), 8.10 (dd, J = 5.7, 0.9 Hz, 1H), 1.55 (s, 3H), 0.92 – 0.86 (m, 2H), 0.86 – 0.76 (m, 2H). LCMS (m/z [M+H]+): 284.1.

Example 150: N-(1-methylcyclopropyl)-2-[2-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine



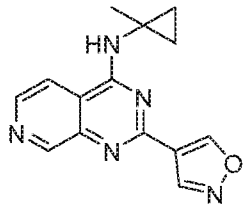
1H NMR (400 MHz, Chloroform-d) δ 9.41 (s, 1H), 8.97 – 8.89 (m, 1H), 8.88 (dd, J = 1.5, 0.8 Hz, 1H), 8.70 – 8.66 (m, 1H), 8.66 – 8.61 (m, 1H), 7.89 – 7.77 (m, 1H), 6.95 – 6.80 (m, 1H), 1.65 (s, 3H), 1.07 – 1.01 (m, 2H), 1.01 – 0.92 (m, 2H). LCMS (m/z [M+H]+): 346.1.

Example 151: 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-2-carbonitrile



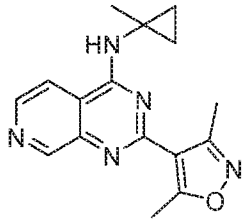
1H NMR (400 MHz, Chloroform-d) δ 9.36 (s, 1H), 8.90 (dd, J = 5.0, 0.9 Hz, 1H), 8.85 (dd, J = 1.6, 0.9 Hz, 1H), 8.69 (dd, J = 5.1, 1.6 Hz, 1H), 8.66 (d, J = 5.7 Hz, 1H), 7.64 – 7.52 (m, 1H), 1.64 (s, 3H), 1.04 – 0.94 (m, 4H). LCMS (m/z [M+H]+): 303.1.

Example 152: N-(1-methylcyclopropyl)-2-(1,2-oxazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



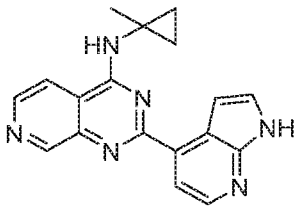
1H NMR (400 MHz, Chloroform-d) δ 9.25 (s, 1H), 8.83 (s, 1H), 8.54 (dd, J = 6.0, 0.7 Hz, 1H), 7.94 – 7.88 (br s, 1H), 7.32 – 7.27 (br s, 1H), 6.61 (dd, J = 6.0, 0.7 Hz, 1H), 0.91 (s, 3H), 0.85 – 0.73 (m, 4H). LCMS (m/z [M+H]+): 268.1.

Example 153: 2-(dimethyl-1,2-oxazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



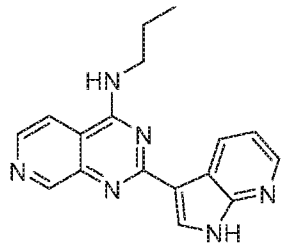
1H NMR (400 MHz, DMSO-d6) δ 9.05 (d, J = 0.8 Hz, 1H), 8.89 (s, 1H), 8.54 (d, J = 5.6 Hz, 1H), 8.10 (dd, J = 5.7, 0.9 Hz, 1H), 2.90 (s, 3H), 2.65 (s, 3H), 1.51 (s, 3H), 0.96 – 0.84 (m, 2H), 0.83 – 0.71 (m, 2H). LCMS (m/z [M+H]+): 296.1.

Example 154: N-(1-methylcyclopropyl)-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}pyrido[3,4-d]pyrimidin-4-amine



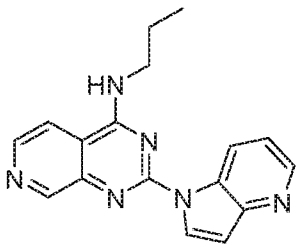
1H NMR (600 MHz, DMSO-d6) δ 11.77 (s, 1H), 9.23 (d, J = 0.8 Hz, 1H), 8.92 (s, 1H), 8.59 (d, J = 5.5 Hz, 1H), 8.38 (d, J = 5.0 Hz, 1H), 8.22 (d, J = 5.0 Hz, 1H), 8.16 (dd, J = 5.7, 0.9 Hz, 1H), 7.69 (dd, J = 3.4, 2.0 Hz, 1H), 7.61 (t, J = 2.9 Hz, 1H), 1.61 (s, 3H), 0.99 - 0.94 (m, 2H), 0.92 - 0.87 (m, 2H). LCMS (m/z [M+H]+): 317.1.

Example 155: N-propyl-2-{1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine



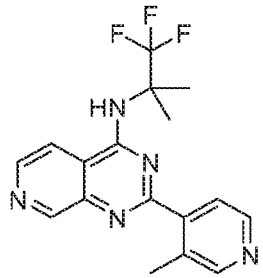
1H NMR (400 MHz, DMSO-d6) δ 12.18 (s, 1H), 9.07 (d, J = 0.8 Hz, 1H), 8.96 (dd, J = 7.9, 1.6 Hz, 1H), 8.51 (dd, J = 16.1, 5.5 Hz, 2H), 8.35 - 8.27 (m, 2H), 8.09 (dd, J = 5.7, 1.0 Hz, 1H), 7.25 (dd, J = 7.9, 4.6 Hz, 1H), 3.67 (dt, J = 7.7, 5.9 Hz, 2H), 1.78 (h, J = 7.4 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 305.1.

Example 156: N-propyl-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine



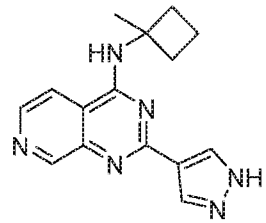
1H NMR (500 MHz, DMSO-d6) δ 9.19 (ddd, J = 8.4, 1.5, 0.8 Hz, 1H), 9.13 (d, J = 0.9 Hz, 1H), 9.02 (t, J = 5.5 Hz, 1H), 8.65 (d, J = 3.7 Hz, 1H), 8.55 (d, J = 5.5 Hz, 1H), 8.48 (dd, J = 4.6, 1.5 Hz, 1H), 8.16 (dd, J = 5.5, 0.9 Hz, 1H), 7.35 (dd, J = 8.4, 4.6 Hz, 1H), 6.88 (dd, J = 3.8, 0.8 Hz, 1H), 3.71 - 3.63 (m, 2H), 1.85 - 1.74 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 305.1.

Example 157: 2-(3-methylpyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine



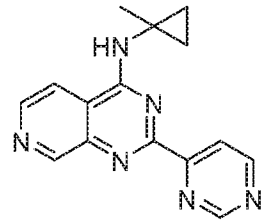
1H NMR (400 MHz, DMSO-d6) δ 9.22 (d, J = 0.8 Hz, 1H), 8.73 (d, J = 5.7 Hz, 1H), 8.58 (d, J = 5.8 Hz, 2H), 8.52 (dd, J = 5.8, 1.0 Hz, 1H), 7.91 (s, 1H), 7.75 (d, J = 5.0 Hz, 1H), 2.59 (s, 3H), 1.85 (s, 6H). LCMS (m/z [M+H]+): 348.1.

Example 158: N-(1-methylcyclobutyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



1H NMR (500 MHz, DMSO-d6) δ 13.11 (s, 1H), 8.98 (s, 1H), 8.47 (d, J = 5.5 Hz, 1H), 8.38 (s, 1H), 8.27 (s, 1H), 8.14 (d, J = 5.5 Hz, 1H), 8.07 (s, 1H), 2.48 - 2.41 (m, 2H), 2.25 (td, J = 9.0, 4.3 Hz, 2H), 1.99 - 1.81 (m, 2H), 1.67 (s, 3H). LCMS (m/z [M+H]+): 281.1.

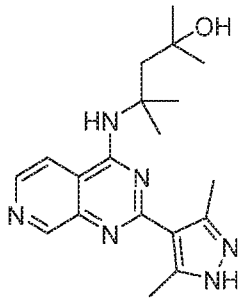
Example 159: N-(1-methylcyclopropyl)-2-(pyrimidin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



Title compound was prepared using procedures described in Example **120**

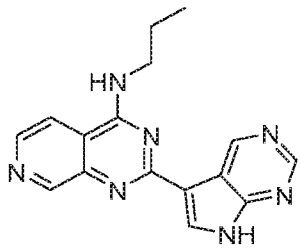
1H NMR (400 MHz, Acetone-d6) δ 9.15 (d, J = 0.9 Hz, 1H), 8.55 (d, J = 5.6 Hz, 1H), 8.17 - 8.09 (m, 2H), 7.98 (dd, J = 5.6, 1.0 Hz, 1H), 7.81 - 7.70 (m, 2H), 1.61 (s, 3H), 1.00 - 0.94 (m, 2H), 0.91 - 0.84 (m, 2H). LCMS (m/z [M+H]+): 279.1.

Example 160: 4-{[2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol



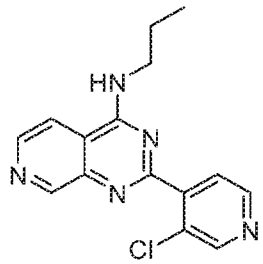
1H NMR (500 MHz, DMSO-d6) δ 12.43 (s, 1H), 9.15 (s, 1H), 8.96 (s, 1H), 8.46 (d, J = 5.5 Hz, 1H), 7.69 (dd, J = 5.7, 1.0 Hz, 1H), 5.61 (s, 1H), 2.61 (s, 3H), 2.55 (s, 3H), 1.95 (s, 2H), 1.70 (s, 6H), 1.32 (s, 6H). LCMS (m/z [M+H]+): 355.2.

Example 161: 4 N-propyl-2-{7H-pyrrolo[2,3-d]pyrimidin-5-yl}pyrido[3,4-d]pyrimidin-4-amine



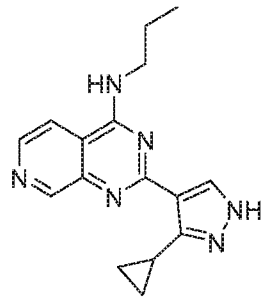
1H NMR (500 MHz, DMSO-d6) δ 12.59 (d, J = 2.5 Hz, 1H), 9.87 (s, 1H), 9.12 (d, J = 0.9 Hz, 1H), 8.86 (s, 1H), 8.60 (t, J = 5.6 Hz, 1H), 8.53 (d, J = 5.5 Hz, 1H), 8.36 (d, J = 2.6 Hz, 1H), 8.11 (dd, J = 5.6, 0.9 Hz, 1H), 3.72 - 3.64 (m, 2H), 1.85 - 1.72 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 306.1.

Example 162: 2-(3-chloropyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine



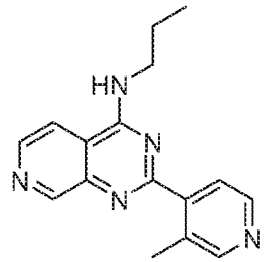
1H NMR (500 MHz, DMSO-d6) δ 9.15 (d, J = 0.8 Hz, 1H), 8.89 (t, J = 5.5 Hz, 1H), 8.78 (d, J = 0.6 Hz, 1H), 8.68 (dd, J = 12.2, 5.2 Hz, 2H), 8.22 (dd, J = 5.6, 0.9 Hz, 1H), 7.82 (dd, J = 4.9, 0.6 Hz, 1H), 3.60 - 3.52 (m, 2H), 1.76 - 1.65 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 300.1.

Example 163: 2-(3-cyclopropyl-1H-pyrazol-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine



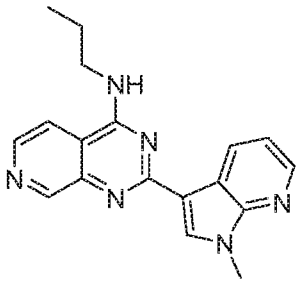
1H NMR (400 MHz, DMSO-d6) δ 12.47 (s, 1H), 8.98 (s, 1H), 8.54 (t, J = 5.4 Hz, 1H), 8.48 (d, J = 5.5 Hz, 1H), 8.06 (dd, J = 5.6, 0.9 Hz, 1H), 3.55 (dt, J = 7.8, 5.9 Hz, 2H), 3.24 (t, J = 7.1 Hz, 1H), 3.17 (d, J = 5.1 Hz, 1H), 1.77 - 1.63 (m, 2H), 0.93 (m, 7H). LCMS (m/z [M+H]+): 295.2.

Example 164: 2-(3-methylpyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine



1H NMR (500 MHz, DMSO-d6) δ 9.14 (d, J = 0.9 Hz, 1H), 8.79 (t, J = 5.6 Hz, 1H), 8.65 (d, J = 5.5 Hz, 1H), 8.58 - 8.52 (m, 2H), 8.20 (dd, J = 5.6, 1.0 Hz, 1H), 7.85 (d, J = 5.0 Hz, 1H), 3.62 - 3.54 (m, 2H), 2.60 (s, 3H), 1.77 - 1.66 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 280.2.

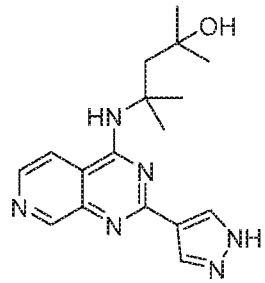
Example 165: 2-{1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine



1H NMR (500 MHz, DMSO-d6) δ 9.06 (d, J = 0.9 Hz, 1H), 8.97 (dd, J = 7.9, 1.7 Hz, 1H), 8.48 (d, J = 5.5 Hz, 2H), 8.44 (s, 1H), 8.36 (dd, J = 4.7, 1.7 Hz, 1H), 8.09 (dd, J = 5.7,

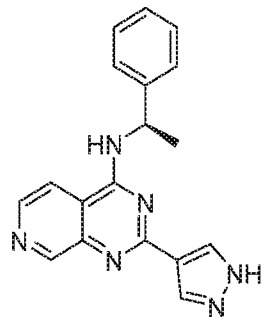
1.0 Hz, 1H), 7.29 (dd, J = 7.9, 4.6 Hz, 1H), 3.94 (s, 3H), 3.72 - 3.64 (m, 2H), 1.85 - 1.73 (m, 2H), 1.04 (t, J = 7.4 Hz, 3H). LCMS (m/z [M+H]+): 319.2.

Example 166: 2,4-dimethyl-4-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol



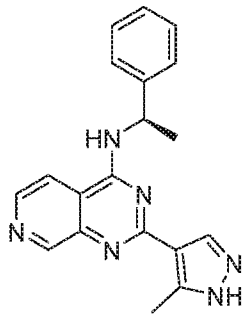
1H NMR (DMSO-d6) d 1.29 (s, 6H), 1.72 (s, 6H), 1.98 (s, 2H), 5.5 (s, 1H, NH), 7.69 (d, J = 3.6 Hz, 1H), 8.09 (s, 1H) 8.30 (s, 1H), 8.48 (d, J = 3.6 Hz, 1H), 8.99 (s, 1H), 9.13 (s, 1H). LCMS (m/z [M+H]+): 327.2.

Example 167: N-[(1R)-1-phenylethyl]-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



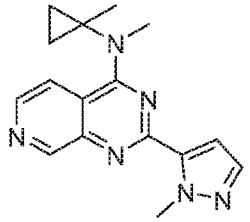
1H NMR (MeOH-d4) d 1.70 (d, J = 6.8 Hz, 3H), 5.63 (m, 1H), 7.21 (m, 1H), 7.32 (m, 2H), 7.49 (m, 2H), 8.17 (d, J = 5.6 Hz, 1H), 8.20-8.23 (2H), 8.48 (d, J = 5.6 Hz, 1H), 9.00 (s, 1H). LCMS (m/z [M+H]+): 317.2.

Example 168: 2-(5-methyl-1H-pyrazol-4-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine



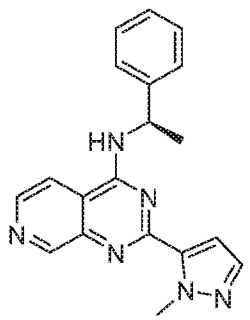
1H NMR (MeOH-d4) d1.70 (d, J = 6.8 Hz, 3H), 2.54 (s, 3H), 5.65 (m, 1H), 7.20 (m, 1H), 7.32 (m, 2H), 7.44(m, 2H), 8.14-8.18 (2H), 8.46 (s, 1H), 9.00 (s, 1H). LCMS (m/z [M+H]+): 331.2.

Example 169: N-methyl-2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



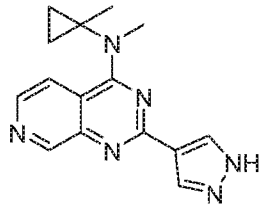
1H NMR (DMSO-d6) d 0.95 (s, 4H), 1.70 (s, 3H), 3.38 (s, 3H), 4.35 (s, 3H), 7.04 (d, J = 2.0 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 8.16 (d, J = 5.6 Hz, 1H), 8.56 (d, J = 5.6 Hz, 1H), 9.16 (s, 1H). LCMS (m/z [M+H]+): 295.2.

Example 170: 2-(1-methyl-1H-pyrazol-5-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine



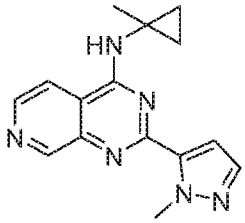
1H NMR (MeOH-d4) d 1.60 (d, J = 7.2 Hz, 3H), 4.02 (s, 3H), 5.46 (m, 1H), 6.86 (d, J = 2 Hz, 1H), 7.10 (m, 1H), 7.22 (m, 2H), 7.33 (m, 2H), 8.11 (d, J = 5.6 Hz, 1H), 8.44 (d, J = 5.6 Hz, 1H), 8.96 (s, 1H). LCMS (m/z [M+H]+): 331.2.

Example 171: **N-methyl-N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



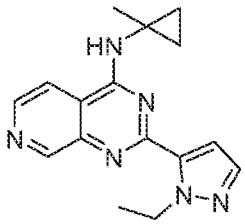
1H NMR (DMSO-d6) d 0.92 (s, 4H), 1.68 (s, 3H), 3.37(s, 3H), 8.10 (d, J = 5.6 Hz, 1H), 8.13 (s, 1H), 8.35 (s, 1H), 8.45 (m, 1H), 9.05 (s, 1H). LCMS (m/z [M+H]+): 281.1.

Example 172: **2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine**



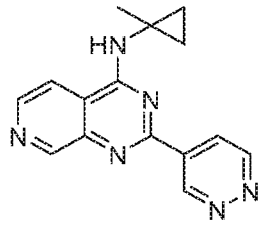
1H NMR (400 MHz, Methanol-d4) δ 8.99 (s, 1H), 8.43 (d, J = 5.7 Hz, 1H), 7.87 (dd, J = 5.7, 0.8 Hz, 1H), 7.48 (d, J = 2.0 Hz, 1H), 7.08 (d, J = 2.0 Hz, 1H), 4.42 (s, 3H), 1.55 (s, 3H), 0.98 – 0.93 (m, 2H), 0.85 – 0.80 (m, 2H). LCMS (m/z [M+H]+): 281.1.

Example 173: **2-(1-ethyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine**



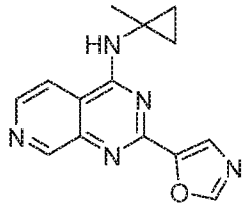
1H NMR (400 MHz, Methanol-d4) δ 9.10 (s, 1H), 8.52 (s, 1H), 7.98 (d, J = 5.5 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.15 (d, J = 2.0 Hz, 1H), 5.07 (q, J = 7.1 Hz, 2H), 1.59 (s, 3H), 1.49 (t, J = 7.1 Hz, 3H), 1.04 – 0.95 (m, 2H), 0.93 – 0.76 (m, 2H). LCMS (m/z [M+H]+): 295.2.

Example 174: **N-(1-methylcyclopropyl)-2-(pyridazin-4-yl)pyrido[3,4-d]pyrimidin-4-amine**



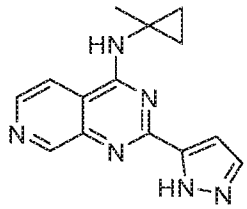
1H NMR (400 MHz, Methanol-d4) δ 10.19 (dd, J = 2.1, 1.3 Hz, 1H), 9.39 (dd, J = 5.3, 1.2 Hz, 1H), 9.20 (d, J = 0.8 Hz, 1H), 8.70 (dd, J = 5.3, 2.2 Hz, 1H), 8.57 (d, J = 5.7 Hz, 1H), 8.01 (dd, J = 5.7, 0.8 Hz, 1H), 1.64 (s, 3H), 1.04 – 0.98 (m, 2H), 0.97 – 0.91 (m, 2H). LCMS (m/z [M+H]+): 279.1.

Example 175: N-(1-methylcyclopropyl)-2-(1,3-oxazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine



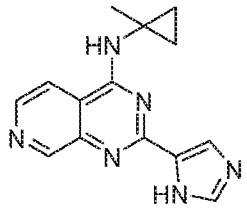
1H NMR (400 MHz, Methanol-d4) δ 9.12 (s, 1H), 8.53 (d, J = 5.7 Hz, 1H), 8.46 (s, 1H), 7.99 – 7.95 (m, 1H), 7.95 (s, 1H), 1.60 (s, 3H), 0.96 (m, 2H), 0.88 (m, 2H). LCMS (m/z [M+H]+): 268.1.

Example 176: N-(1-methylcyclopropyl)-2-(1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine



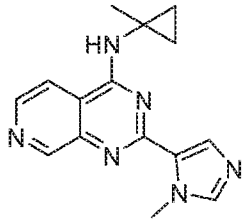
1H NMR (400 MHz, Methanol-d4) δ 9.09 (d, J = 0.8 Hz, 1H), 8.44 (s, 1H), 7.94 (dd, J = 5.7, 0.8 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.11 (d, J = 2.1 Hz, 1H), 1.60 (s, 3H), 0.94 (m, 2H), 0.90 – 0.84 (m, 2H). LCMS (m/z [M+H]+): 267.1.

Example 177: 2-(1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



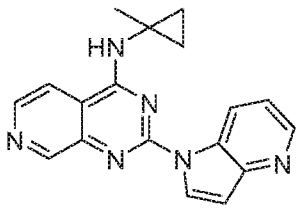
1H NMR (400 MHz, Methanol-d4) δ 9.06 (s, 1H), 8.44 (d, J = 5.7 Hz, 1H), 8.16 (s, 1H), 7.98 – 7.90 (m, 2H), 1.60 (s, 3H), 0.95 (m, 2H), 0.92 – 0.84 (m, 2H). LCMS (m/z [M+H]+): 267.1.

Example 178: 2-(1-methyl-1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



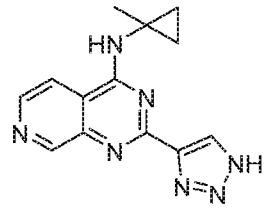
1H NMR (400 MHz, Methanol-d4) δ 9.03 (s, 1H), 8.41 (d, J = 5.7 Hz, 1H), 7.90 (dd, J = 5.7, 0.8 Hz, 1H), 7.70 (d, J = 0.8 Hz, 2H), 4.25 (s, 3H), 1.57 (s, 3H), 0.99 – 0.94 (m, 2H), 0.85 – 0.79 (m, 2H). LCMS (m/z [M+H]+): 281.1.

Example 179: N-(1-methylcyclopropyl)-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine



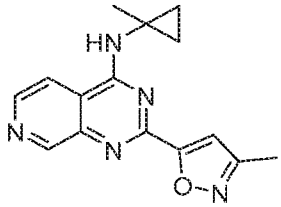
1H NMR (400 MHz, DMSO-d6) δ 9.69 (d, J = 8.3 Hz, 1H), 9.39 (s, 1H), 9.18 (s, 1H), 8.88 (d, J = 3.5 Hz, 1H), 8.72 (s, 1H), 8.58 (s, 1H), 8.18 (d, J = 5.4 Hz, 1H), 7.71 (s, 1H), 7.10 – 7.02 (m, 1H), 1.59 (s, 3H), 0.99 (m, 2H), 0.98 – 0.95 (m, 2H). LCMS (m/z [M+H]+): 317.1.

Example 180: N-(1-methylcyclopropyl)-2-(1H-1,2,3-triazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



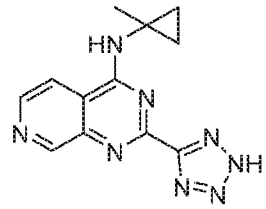
1H NMR (400 MHz, DMSO-d6) δ 9.19 (s, 1H), 8.64 (d, J = 5.0 Hz, 1H), 8.51 (s, 1H), 8.16 (d, J = 5.5 Hz, 1H), 1.55 (s, 3H), 0.90 (m, 2H), 0.87 (m, 2H). LCMS (m/z [M+H]+): 268.1.

Example 181: 2-(3-methyl-1,2-oxazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



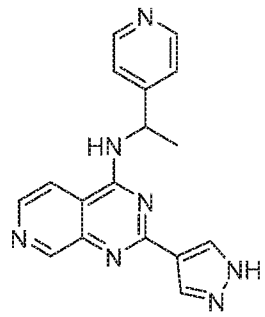
1H NMR (400 MHz, Chloroform-d) δ 9.41 (s, 1H), 8.63 (d, J = 5.6 Hz, 1H), 7.43 (d, J = 5.6 Hz, 1H), 6.98 (s, 1H), 2.44 (s, 3H), 1.60 (s, 3H), 0.99 – 0.93 (m, 2H), 0.93 – 0.85 (m, 2H). LCMS (m/z [M+H]+): 282.1.

Example 182: N-(1-methylcyclopropyl)-2-(2H-1,2,3,4-tetrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine



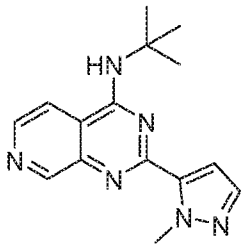
1H NMR (400 MHz, DMSO-d6) δ 9.21 (s, 2H), 8.69 (s, 1H), 8.21 (d, J = 5.2 Hz, 1H), 1.57 (s, 3H), 0.89 (m, 2H), 0.85 (m, 2H). LCMS (m/z [M+H]+): 269.1.

Example 183: 2-(1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine



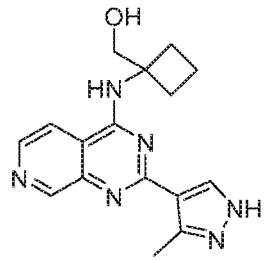
1H NMR (400 MHz, Methanol-d4) δ 9.17 (s, 1H), 8.75 (d, J = 5.6 Hz, 3H), 8.37 (d, J = 4.8 Hz, 1H), 8.32 (d, J = 1.6 Hz, 2H), 8.14 – 8.09 (m, 2H), 5.89 (q, J = 7.3 Hz, 1H), 1.84 (d, J = 7.2 Hz, 3H). LCMS (m/z [M+H]+): 318.1.

Example 184: N-tert-butyl-2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine



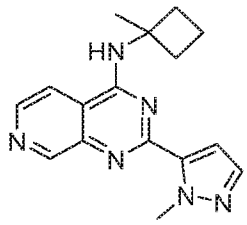
1H NMR (400 MHz, DMSO-d6) δ 9.13 (d, J = 2.5 Hz, 1H), 8.61 (d, J = 5.6 Hz, 1H), 8.38 (t, J = 5.2 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 1.9 Hz, 1H), 6.97 (d, J = 1.9 Hz, 1H), 4.37 (s, 3H), 1.59 (s, 9H). LCMS (m/z [M+H]+): 283.1.

Example 185: (1-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol



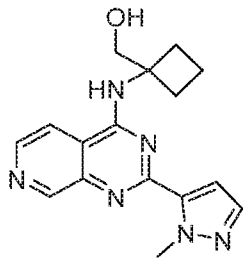
1H NMR (400 MHz, DMSO-d6) δ 9.07 (s, 1H), 8.62 (s, 1H), 8.38 (d, J = 5.6 Hz, 1H), 8.25 – 8.12 (m, 1H), 3.93 (s, 2H), 2.62 (s, 3H), 2.39 (t, J = 7.8 Hz, 4H), 1.85 (dq, J = 12.0, 8.4 Hz, 2H). LCMS (m/z [M+H]+): 311.2.

Example 186: 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclobutyl)pyrido[3,4-d]pyrimidin-4-amine



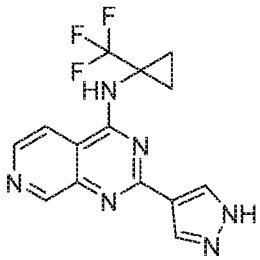
1H NMR (400 MHz, DMSO-d6) δ 9.12 (s, 1H), 8.78 (s, 1H), 8.62 (d, J = 5.6 Hz, 1H), 8.31 – 8.24 (m, 1H), 7.53 – 7.46 (m, 1H), 6.97 (d, J = 1.9 Hz, 1H), 4.38 – 4.31 (m, 3H), 2.48 – 2.41 (m, 2H), 2.22 (tt, J = 8.4, 3.2 Hz, 2H), 1.98 – 1.78 (m, 2H), 1.65 (s, 3H). LCMS (m/z [M+H]+): 295.2.

Example 187: (1-{[2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol



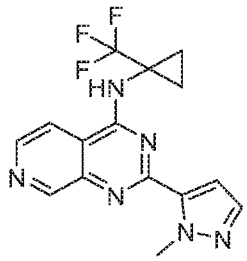
1H NMR (400 MHz, DMSO-d6) δ 9.19 – 9.11 (m, 1H), 8.76 (s, 1H), 8.63 (d, J = 5.7 Hz, 1H), 8.40 (dd, J = 5.7, 0.7 Hz, 1H), 7.54 – 7.47 (m, 1H), 6.95 (d, J = 1.9 Hz, 1H), 4.32 (s, 3H), 3.90 (s, 2H), 2.35 (t, J = 7.2 Hz, 4H), 1.92 – 1.76 (m, 2H). LCMS (m/z [M+H]+): 311.2.

Example 188: 2-(1H-pyrazol-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine



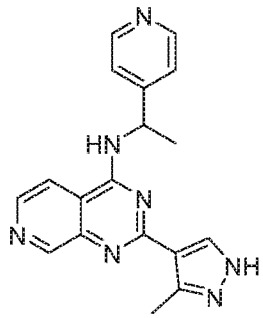
1H NMR (400 MHz, DMSO-d6) δ 9.21 (s, 1H), 9.10 (s, 1H), 8.56 (d, J = 5.6 Hz, 1H), 8.27 (s, 2H), 8.16 (d, J = 5.7 Hz, 1H), 1.61 – 1.53 (m, 2H), 1.33 (d, J = 6.0 Hz, 2H). LCMS (m/z [M+H]+): 321.1.

Example 189: 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine



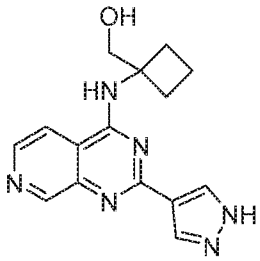
1H NMR (400 MHz, DMSO-d6) δ 9.32 (s, 1H), 9.19 (d, J = 0.7 Hz, 1H), 8.65 (d, J = 5.6 Hz, 1H), 8.23 (dd, J = 5.7, 0.8 Hz, 1H), 7.58 – 7.47 (m, 1H), 7.07 (d, J = 1.9 Hz, 1H), 4.37 (s, 3H), 1.64 – 1.49 (m, 2H), 1.37 (d, J = 5.8 Hz, 2H). LCMS (m/z [M+H]+): 335.1.

Example 190: 2-(3-methyl-1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine



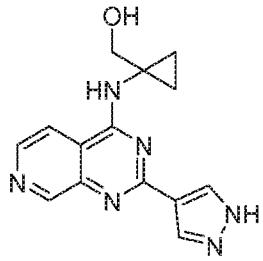
1H NMR (400 MHz, DMSO-d6) δ 9.00 (d, J = 0.7 Hz, 1H), 8.77 (s, 1H), 8.54 (d, J = 5.6 Hz, 1H), 8.51 – 8.47 (m, 2H), 8.30 (dd, J = 5.6, 0.8 Hz, 1H), 7.99 (s, 1H), 7.50 – 7.40 (m, 2H), 5.54 (d, J = 6.6 Hz, 1H), 2.48 (s, 3H), 1.63 (d, J = 7.1 Hz, 3H). LCMS (m/z [M+H]+): 332.2.

Example 191: (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol



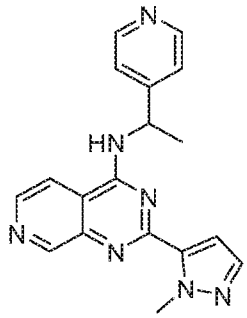
1H NMR (400 MHz, DMSO-d6) δ9.11(m, 2H), 8.69(m, 2H), 8.39 (s, 2H), 3.92 (s, 2H), 2.39 (t, J = 7.3 Hz, 4H), 1.87 (p, J = 7.7, 6.6 Hz, 2H). LCMS (m/z [M+H]+): 297.1.

Example 192: (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)methanol



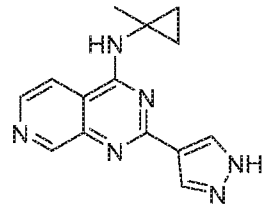
1H NMR (400 MHz, DMSO-d6) δ9.65(s, 1H), 9.12 (s, 1H), 8.65 (d, J = 5.4 Hz, 1H), 8.41 (s, 2H), 8.26 (d, J = 5.5 Hz, 1H), 3.73 (m, 2H), 1.06 – 0.96 (m, 2H), 0.94 – 0.77 (m, 2H). LCMS (m/z [M+H]+): 283.1.

Example 193: 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine



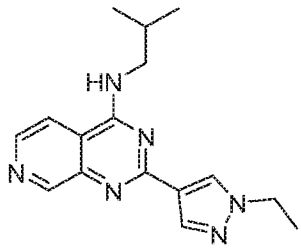
1H NMR (400 MHz, DMSO-d6) δ 8.37 (s, 1H), 7.93 (d, J = 6.4 Hz, 2H), 7.85 (d, J = 5.7 Hz, 1H), 7.51 (d, J = 5.7 Hz, 1H), 7.31 (d, J = 6.6 Hz, 2H), 6.63 (d, J = 2.0 Hz, 1H), 6.01 (d, J = 2.0 Hz, 1H), 4.89 (q, J = 7.2 Hz, 1H), 3.43 (s, 3H), 1.00 (d, J = 7.2 Hz, 3H). LCMS (m/z [M+H]+): 332.2.

Example 194: N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



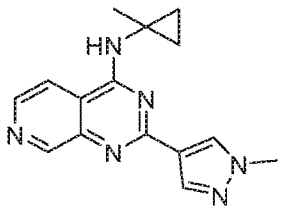
1HNMR (400 MHz, CD3OD) d 9.03 (d, J = 0.8 Hz, 1H), 8.43 (d, J = 5.6 Hz, 1H), 8.24 (d, J = 0.8 Hz, 1H), 8.20 (s, 1H), 7.93 (dd, J = 5.6, 0.8 Hz, 1H), 1.61 (s, 3H), 0.99-0.95 (m, 2H), 0.90-0.85 (m, 2H). LCMS (m/z [M+H]+): 267.1.

Example 195: 2-(1-ethyl-1H-pyrazol-4-yl)-N-(2-methylpropyl)pyrido[3,4-d]pyrimidin-4-amine



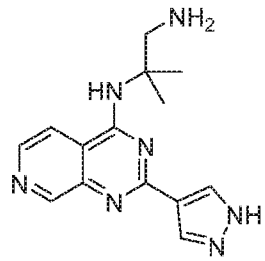
1HNMR (400 MHz, $CDCl_3$) d 9.20 (s, 1H), 8.50 (d, J = 5.6 Hz, 1H), 8.24 (s, 1H), 8.20 (s, 1H), 7.43 (d, J = 5.6 Hz, 1H), 5.87 (br s, 1H), 4.25 (q, J = 7.2 Hz, 2H), 3.58 (dd, J = 6.8, 6.0 Hz, 2H), 2.11 (nonet, J = 6.8 Hz, 1H), 1.56 (t, J = 7.2 Hz, 3H), 1.06 (d, J = 6.8 Hz, 6H). LCMS (m/z [M+H]+): 297.2.

Example 196: 2-(1-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine



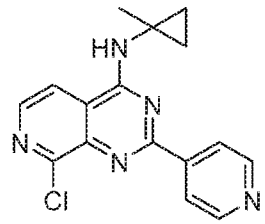
1HNMR (400 MHz, DMSO-d6) d 8.99 (s, 1H), 8.72 (s, 1H), 8.46 (d, J = 5.6 Hz, 1H), 8.33 (s, 1H), 8.05 (s, 1H), 8.03 (d, J = 5.6 Hz, 1H), 3.93 (s, 3H), 1.54 (s, 3H), 0.90-0.80 (m, 4H). LCMS (m/z [M+H]+): 281.1.

Example 197: N-(1-amino-2-methylpropan-2-yl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



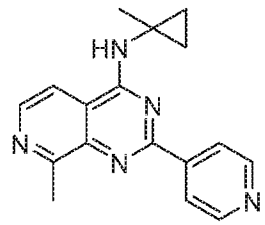
1H NMR (400 MHz, DMSO-d6) δ 9.05 (s, 1H), 8.56 (d, J = 5.4 Hz, 1H), 8.32 (d, J = 5.6 Hz, 1H), 8.28 (m, 1H), 7.76 (m, 3H),3.63 (d, J = 5.9 Hz, 2H), 1.59 (s, 6H). LCMS (M/Z [M+H]+): 284.2.

Example 198: 8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



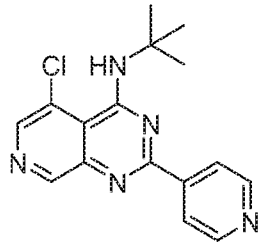
Title compound was prepared from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (intermediate **3c**) as in Scheme **3** using the procedure in Example **1** with 1-methylcyclopropanamine. 1H NMR (400 MHz, Methanol-d4) δ 8.78 - 8.66 (m, 2H), 8.61 - 8.49 (m, 2H), 8.30 (d, J = 5.6 Hz, 1H), 7.94 (d, J = 5.6 Hz, 1H), 1.63 (s, 3H), 1.04 - 0.96 (m, 2H), 0.95 - 0.86 (m, 2H). LCMS (m/z [M+H]+): 312.1.

Example 199: 8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



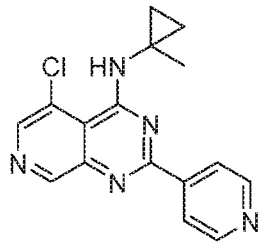
Title compound was prepared from Example **198** using the procedure for intermediate **6b** with 2,4,6-trimethyl-1,3,5,2,4,6-trioxatriborinane instead of 4-pyridine boronic acid. 1H NMR (500 MHz, DMSO-d6) δ 8.79 (d, J = 5.1 Hz, 2H), 8.44 (d, J = 5.6 Hz, 1H), 8.41 (d, J = 5.0 Hz, 2H), 7.98 (dd, J = 5.8, 0.8 Hz, 1H), 2.91 (s, 3H), 1.57 (s, 3H), 0.97 - 0.75 (m, 4H). LCMS (m/z [M+H]+): 292.2.

Example 251: N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



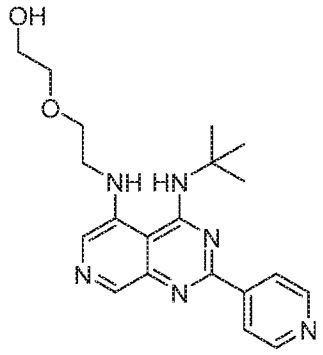
Title compound was prepared from 4,5-dichloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (intermediate 5d) as in Scheme 5 using **Step C** of Example **1** with tert-butyl amine. 1H NMR (400 MHz, Methanol-d4) δ 9.07 (s, 1H), 8.73 (s, 2H), 8.55 (s, 1H), 8.46 - 8.39 (m, 2H), 1.72 (s, 9H). LCMS (M/Z [M+H]+): 314.1.

Example 252: 5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



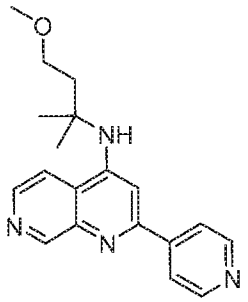
Title compound was prepared as Example **251** using 4,5-dichloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (intermediate 5d) and 1-methylcyclopropan-1-amine. 1HNMR (400 MHz, $CDCl_3$) d 9.18 (s, 1H), 8.81 (br s, 2H), 8.50 (s, 1H), 8.42 (d, J = 4.8 Hz, 2H), 8.00 (br s, 1H), 1.64 (s, 3H), 1.00-0.90 (m, 4H). LCMS (M/Z [M+H]+): 312.1.

Example 253: 5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine



N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine (Example **251**, 10 mg, 0.032 mmol) and 2-(2-aminoethoxy)ethanol (670 mg, 6.37 mmol) were dissolved in NMP (1 mL) in a 2 ml microwave reactor. The reaction was heated at 160°C for 1 hr (microwave irradiation). The reaction was cooled to rt and was diluted with water (20 ml), extracted with EtOAc (3x 20mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated. The residue was purified by mass-triggered HPLC to afford title compound (40%). 1H NMR (400 MHz, Methanol-d4) δ 8.63 (s, 1H), 8.23 (d, J = 5.7 Hz, 2H), 8.18 (d, J = 5.7 Hz, 2H), 8.08 (s, 1H), 3.4-3.8 (m, 8H), 1.72 (s, 9H). LCMS (M/Z [M+H]+): 383.2.

Example 254: N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



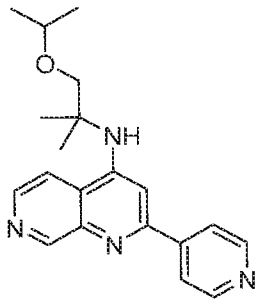
Title compound was prepared from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (intermediate **6b**) using **Step B** as in **Scheme 6**.

Step B: In a 20ml vial was added triethylamine (0.044 mL, 0.25 mmol), potassium fluoride (7.2 mg, 0.124 mmol), 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (intermediate **6b**, 30 mg, 0.124 mmol), and 4-methoxy-2-methylbutan-2-amine (16 mg, 0.137 mmol) in DMSO (1 mL) to give a yellow suspension. The reaction mixture was stirred at 130°C for 24 hrs. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the title compound (21%). 1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.7 Hz, 1H), 8.71(m, 2H), 8.48(d, J = 5.8 Hz, 1H), 8.09(ddd, J = 12.9, 5.2, 1.3 Hz, 2H), 8.05(m, 1H), 7.26(s, 1H), 6.81(s, 1H), 3.50(t, J = 6.5 Hz, 2H), 3.22(s, 3H), 2.11(t, J = 6.5 Hz, 2H), 1.52(s, 6H). LCMS (M/Z [M+H]+): 323.2.

Examples 255-268:

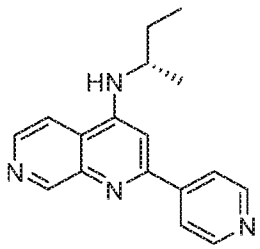
These compounds were synthesized according to the protocol described for Example **1** using 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (intermediate **6b**) and various amines respectively except specially stated.

Example 255: N-[2-methyl-1-(propan-2-yloxy)propan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



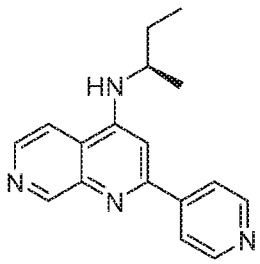
1H NMR (400 MHz, DMSO-d6) δ 9.18(dd, J = 7.4, 0.9 Hz, 1H), 8.80(m, 2H), 8.64(d, J = 5.6 Hz, 1H), 8.39(dd, J = 5.7, 0.9 Hz, 1H), 8.29(d, J = 6.1 Hz, 2H), 7.70(s, 1H), 3.90(s, 2H), 3.55-3.50(m, 1H), 1.61(s, 6H), 1.00(s, 6H). LCMS (M/Z [M+H]+): 337.2.

Example 256: N-[(2S)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



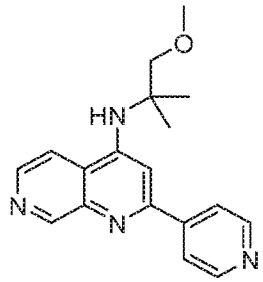
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 7.4, 0.9 Hz, 1H), 8.73(m, 2H), 8.49(m, 1H), 8.29(d, J = 6.1 Hz, 1H), 8.20(m, 2H), 7.24(s, 1H), 7.19(d, J = 0.9 Hz, 1H), 4.02-3.99(m, 1H), 1.79-1.75(m, 1H), 1.69-1.65(m, 1H), 1.29(m, 3H), 0.09(t, J = 4.9 Hz,3H). LCMS (M/Z [M+H]+): 279.1.

Example 257: N-[(2R)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



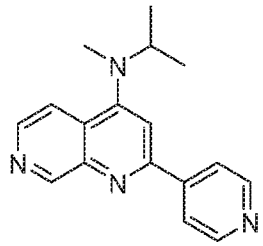
1H NMR (400 MHz, DMSO-d6) δ 9.21(d, J = 0.8 Hz, 1H), 8.73(m, 2H), 8.49(d, J = 5.8 Hz, 1H), 8.29(dd, J = 5.9, 0.9 Hz, 1H), 8.20(m, 2H), 7.24(s, 1H), 7.19(d, J = 8.3 Hz, 1H), 4.02-3.99(dt, J = 13.6, 6.5 Hz, 1H), 1.79-1.75(dq, J = 14.4, 7.2 Hz, 1H), 1.69-1.65(m, 1H), 1.29(d, J = 6.4 Hz, 3H), 0.09(t, J = 7.4 Hz, 3H). LCMS (M/Z [M+H]+): 279.3.

Example 258: **N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine**



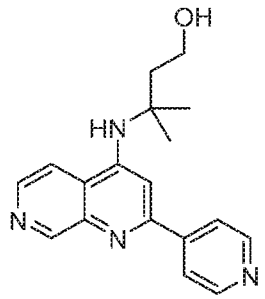
1H NMR (400 MHz, DMSO-d6) δ 9.25(dd, J = 6.6, 0.8 Hz, 1H), 8.77(m, 2H), 8.75(s, 1H), 8.24(dt, J = 4.5, 1.9 Hz, 2H), 8.21(s, 1H), 8.11(m, 1H), 7.52(s, 1H), 3.62(s, 2H), 3.34(s, 3H), 1.52(s, 6H). LCMS (M/Z [M+H]+): 309.4.

Example 259: **N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine**



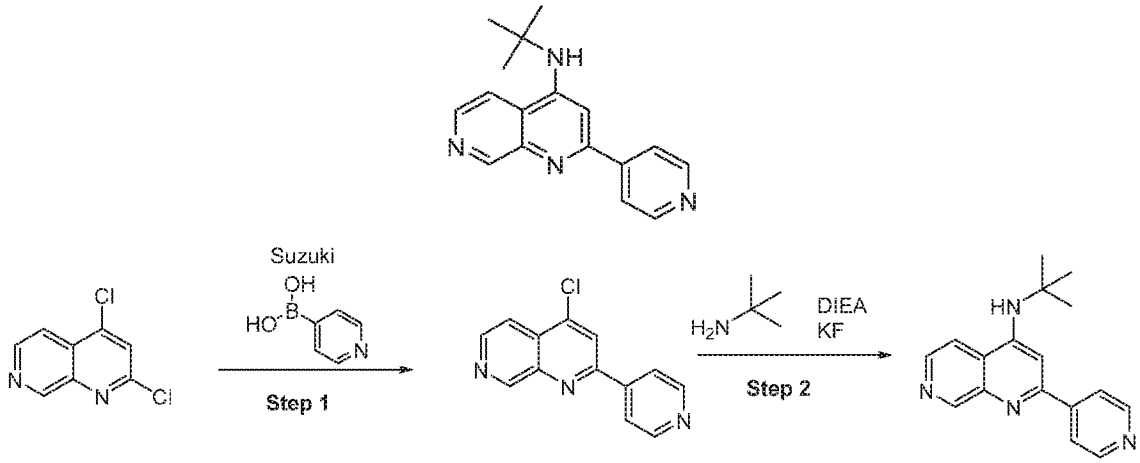
1H NMR (400 MHz, DMSO-d6) δ 9.34(dd, J = 5.2, 0.9 Hz, 1H), 8.77(ddd, J = 6.2, 4.4, 1.7 Hz, 2H), 8.50(dd, J = 6.5, 5.8 Hz, 1H), 8.20(m, 2H), 7.80(s, 1H), 7.58(s, 1H), 4.20-4.14(m, 1H), 2.97(s, 3H), 1.25(s, 6H). LCMS (M/Z [M+H]+): 279.4.

Example 260: **3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butan-1-ol**



1H NMR (400 MHz, DMSO-d6) δ 9.40(d, J = 0.9 Hz, 1H), 8.79(m, 2H), 8.60(d, J = 5.6 Hz, 1H), 8.26(m, 2H), 7.95(dd, J = 5.6, 1.0 Hz, 1H), 7.89(s, 1H), 4.55(t, J = 6.8 Hz, 2H), 4.08-4.04(q, J = 5.2 Hz, 1H), 1.95(t, J = 6.9 Hz, 2H), 1.14(s, 6H). LCMS (M/Z [M+H]+): 309.3.

Example 261: N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine

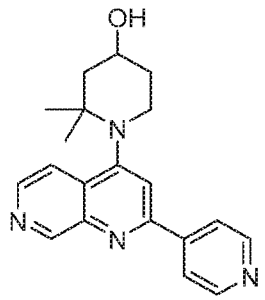


Step 1: In a 20 mL microwave reactor was added PalladiumTetrakis (58.1 mg, 0.050 mmol), potassium carbonate (1.256 mL, 2.51 mmol), and 2,4-dichloro-1,7-naphthyridine (200 mg, 1.005 mmol) and pyridin-4-ylboronic acid (130 mg, 1.055 mmol) in Acetonitrile (Volume: 2 mL) to give an orange suspension. The reaction mixture was stirred at 120°C for 60 min under microwave. The crude mixture was diluted with DCM, H_2O, separated and extracted with DCM x3. Combined the organic layers and dried Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the product (62%). 1H NMR (400 MHz, DMSO-d6) δ 9.58 (d, J = 0.9 Hz, 1H), 8.85 - 8.78 (m, 4H), 8.32 - 8.29 (m, 2H), 8.11 (dd, J = 5.8, 0.9 Hz, 1H). LCMS [M+H] = 242.

Step 2: In a 40ml vial was added potassium fluoride (11.54 mg, 0.199 mmol), 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (40 mg, 0.166 mmol), and 2-methylpropan-2-amine

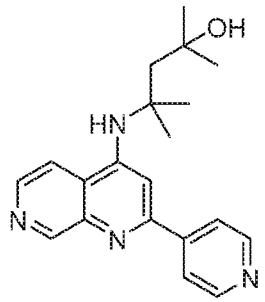
(0.035 mL, 0.331 mmol) in DMSO (Volume: 2 mL) to give a yellow suspension. The reaction mixture was stirred at 130 °C for 24 hrs. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the product (82%). 1H NMR (400 MHz, DMSO-d6) δ 9.22 (d, J = 0.7 Hz, 1H), 8.78 - 8.72 (m, 2H), 8.48 (d, J = 5.8 Hz, 1H), 8.30 (dd, J = 6.0, 0.9 Hz, 1H), 8.15 - 8.06 (m, 2H), 7.28 (s, 1H), 6.73 (s, 1H), 1.56 (s, 9H). LCMS [M+H] = 279.2.

Example 262: 2,2-dimethyl-1-[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]piperidin-4-ol



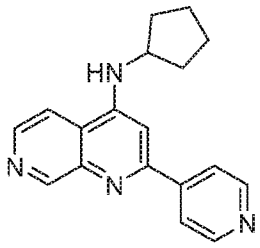
1H NMR (400 MHz, Acetone-d6) δ 9.41 (d, J = 0.9 Hz, 1H), 8.80 - 8.75 (m, 2H), 8.60 (d, J = 5.7 Hz, 1H), 8.23 - 8.20 (m, 2H), 8.18 - 8.14 (m, 1H), 8.11 (s, 1H), 4.15 - 3.99 (m, 1H), 3.52 (d, J = 16.1 Hz, 1H), 3.16 (s, 1H), 1.86 - 1.68 (m, 2H), 1.46 (d, J = 16.0 Hz, 3H), 1.16 - 1.00 (m, 3H), 0.87 (d, J = 6.8 Hz, 2H). LCMS (M/Z [M+H]+): 335.2.

Example 263: 2,4-dimethyl-4-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}pentan-2-ol



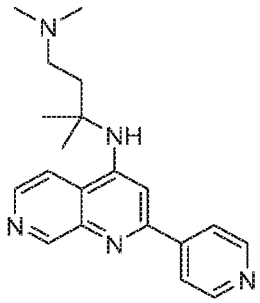
1H NMR (400 MHz, Acetone-d6) δ 9.22 (d, J = 0.8 Hz, 1H), 8.78 – 8.67 (m, 2H), 8.63 (s, 1H), 8.40 (d, J = 5.8 Hz, 1H), 8.19 – 8.09 (m, 2H), 7.81 (dd, J = 5.9, 0.9 Hz, 1H), 7.36 (s, 1H), 2.08 (s, 2H), 1.75 (s, 6H), 1.47 (d, J = 0.7 Hz, 6H). LCMS (M/Z [M+H]+): 337.2.

Example 264: N-cyclopentyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



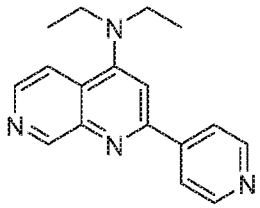
1H NMR (400 MHz, Acetone-d6) δ 9.26 (d, J = 0.9 Hz, 1H), 8.77 - 8.66 (m, 2H), 8.44 (d, J = 5.8 Hz, 1H), 8.23 - 8.15 (m, 2H), 8.06 (dd, J = 5.8, 0.9 Hz, 1H), 7.34 (s, 1H), 6.70 (d, J = 6.6 Hz, 1H), 4.40 - 4.25 (m, 1H), 2.29 - 2.19 (m, 2H), 1.86 - 1.68 (m, 6H). LCMS (M/Z [M+H]+): 279.1.

Example 265: dimethyl(3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butyl)amine



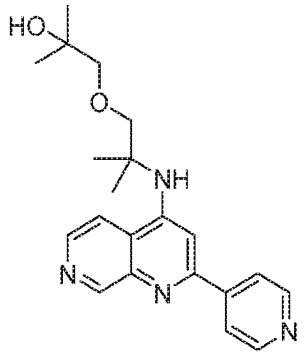
1H NMR (500 MHz, Methanol-d4) δ 9.40 (s, 1H), 8.91 (s, 2H), 8.62 (d, J = 3.5 Hz, 1H), 8.48 (s, 2H), 8.40 (d, J = 6.0 Hz, 1H), 7.46 (d, J = 2.8 Hz, 1H), 3.28 - 3.23 (m, 2H), 2.92 - 2.81 (m, 6H), 2.48 (dd, J = 8.3, 5.1 Hz, 2H), 1.71 (d, J = 2.7 Hz, 6H). LCMS (M/Z [M+H]+): 336.2.

Example 266: N,N-diethyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



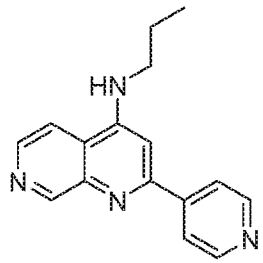
1H NMR (400 MHz, Acetone-d6) δ 9.35 (d, J = 0.9 Hz, 1H), 8.78 - 8.74 (m, 2H), 8.51 (d, J = 5.8 Hz, 1H), 8.23 - 8.19 (m, 2H), 7.90 (dd, J = 5.8, 0.9 Hz, 1H), 7.69 (s, 1H), 3.62 (q, J = 7.1 Hz, 4H), 1.29 (t, J = 7.1 Hz, 6H). LCMS (M/Z [M+H]+): 279.2.

Example 267: 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}propoxy)propan-2-ol



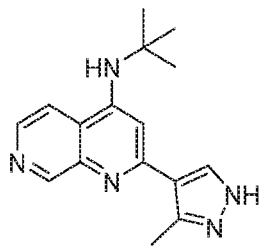
1H NMR (500 MHz, Methanol-d4) δ 9.25 (s, 1H), 8.73 (d, J = 5.2 Hz, 2H), 8.45 (d, J = 5.9 Hz, 1H), 8.14 - 8.08 (m, 3H), 7.40 (s, 1H), 3.73 (s, 2H), 3.39 (s, 2H), 1.63 (s, 6H), 1.18 (s, 6H). LCMS (M/Z [M+H]+): 367.2.

Example 268: N-propyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.22 (d, J = 0.8 Hz, 1H), 8.77 - 8.70 (m, 2H), 8.48 (d, J = 5.8 Hz, 1H), 8.22 - 8.16 (m, 3H), 7.60 (t, J = 5.5 Hz, 1H), 7.21 (s, 1H), 3.43 (ddd, J = 7.6, 6.6, 5.5 Hz, 2H), 1.79 - 1.67 (m, 2H), 1.01 (t, J = 7.4 Hz, 3H). LCMS (M/Z [M+H]+): 265.1.

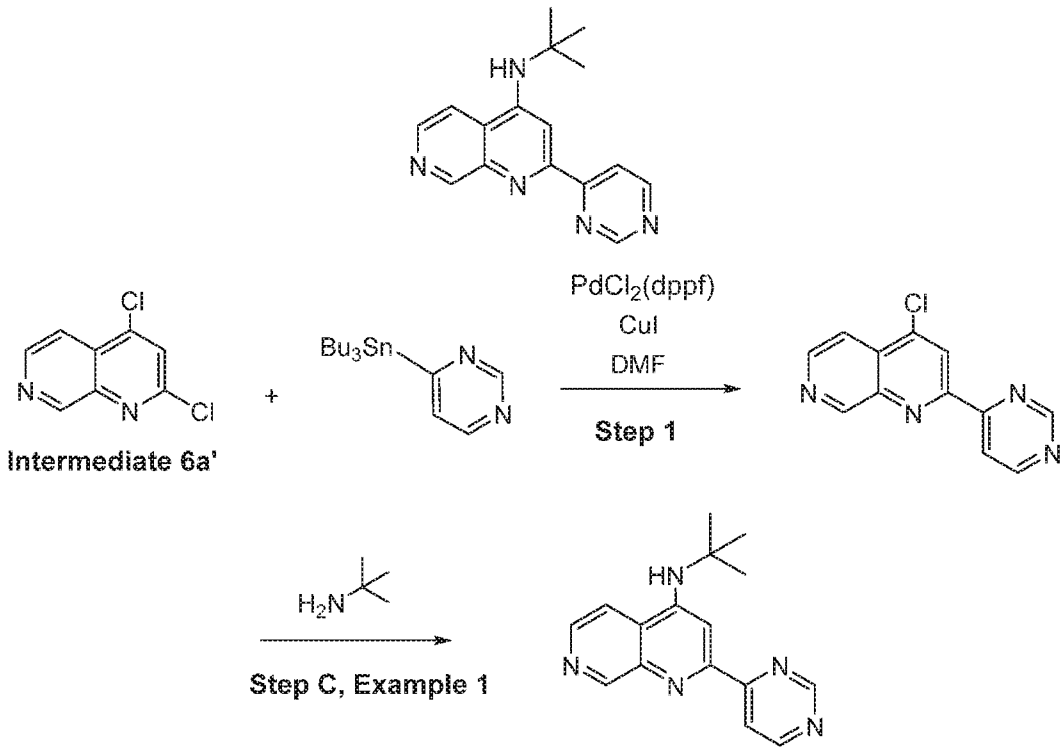
Example 269: N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine



Title compound was prepared from 2,4-dichloro-1,7-naphthyridine (intermediate **6a'**) as in **Scheme 6** using tert-butyl 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-1-carboxylate and tert-butyl amine. 1H NMR (400 MHz, DMSO-d6) 12.81(s, 1H),

9.00(d, J = 0.7 Hz, 1H), 8.31(d, J = 5.8 Hz, 1H), 8.14(dd, J = 5.9, 0.8 Hz, 1H), 8.00(s, 1H), 6.97(s, 1H), 6.36(s, 1H), 2.65(s, 3H), 1.51(s, 9H). LCMS (M/Z [M+H]+): 282.4.

Example 270: N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine

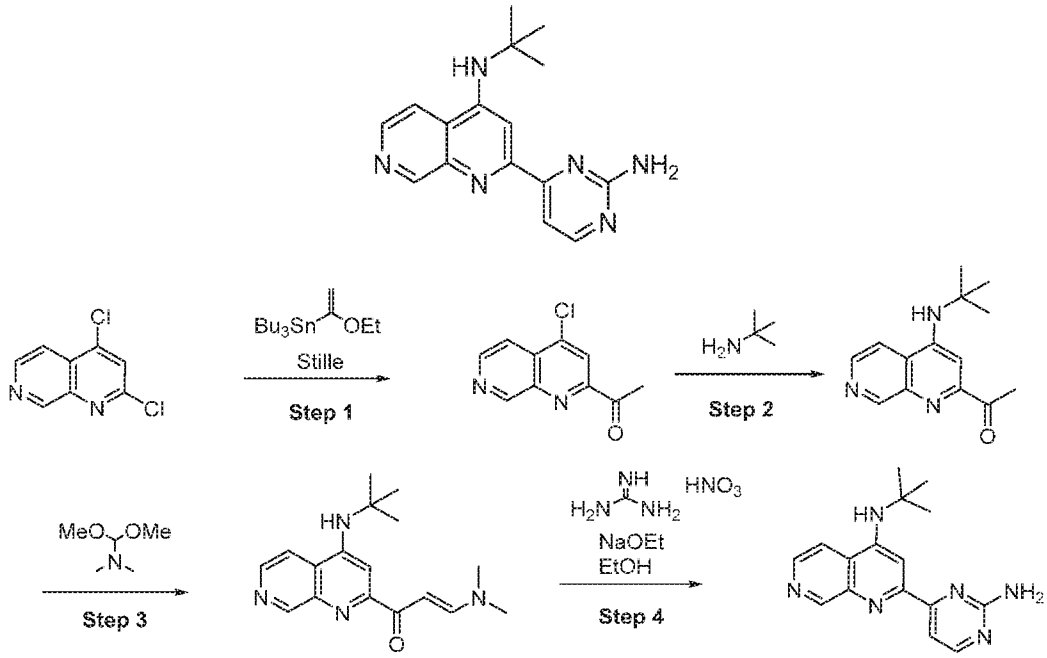


Step 1: 2,4-dichloro-1,7-naphthyridine (6a, 100mg, 0.502mmol) was stirred in dry DMF at room temperature. 4-(tributylstannyl)pyrimidine (165uL, 0.502mmol) was added then 41mg of $PdCl_2(dppf).CH_2Cl_2$ adduct (41mg, 0.05mmol, orange solid) and finally CuI (10mg, 0.05mmol, beige solid). The reaction was stirred for 1 hour at 130°C. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford the product 4-chloro-2-(pyrimidin-4-yl)-1,7-naphthyridine (30%). LCMS (m/z [M+H]+): 243.6.

Title compound: was then prepared with 4-chloro-2-(pyrimidin-4-yl)-1,7-naphthyridine using the procedure detailed in **Step C**, **Example 1**.

1H NMR (400 MHz, DMSO-d6) δ 9.48(d, J = 1.4 Hz, 1H), 9.25(d, J = 0.8 Hz, 1H), 9.00(d, J = 5.3 Hz, 1H), 8.54(m, 1H), 8.50(m, 1H), 8.31(dd, J = 6.1, 0.9 Hz, 1H), 8.01(s, 1H), 6.80(s, 1H), 1.56(s, 9H). LCMS (M/Z [M+H]+): 280.3.

Example 271: 2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine



Step 1: 2,4-dichloro-1,7-naphthyridine(**6a'**, 400mg, 2mmol), Pd2(dba)3(58mg, 0.1mmol) and PPh_3 (53 mg, 0.2 mmol) were stirred in 3mL of toluene at room temperature for 15 minutes. 680 microlitre of tributyl(1-ethoxyvinyl)stannane(680 microlitre, 2 mmol) in 1.5 mL of toluene was then added and the reaction stirred at 110°C for 1 hour. The reaction was cooled to room temperature. 4mL of 1N HCl was added and the mixture stirred overnight. The reaction was then neutralized with NaOH and extracted with ether. The crude residue was then purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-70% EtOAc/Hexane to afford the product 1-(4-chloro-1,7-naphthyridin-2-yl)ethanone (40%). LCMS (m/z [M+H]+): 207.5.

Step 2: 1-(4-chloro-1,7-naphthyridin-2-yl)ethanone (38 mg, 0.18 mmol) was stirred in DMF (2 mL) at room temperature and degassed with N_2. TEA(37 microlitre, 0.27 mmol) was added and stirred for 5 minutes then 14mg of KF(14 mg, 0.27 mmol). This mixture was stirred at room temperature for 15 minutes then 2-methylpropan-2-amine(28 microlitre, 0.27 mmol) was added and degassed then stirred at 80°C for two hrs. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-100% EtOAc/Hexane to afford the product 1-(4-(tert-butylamino)-1,7-naphthyridin-2-yl)ethanone (60%). LCMS (m/z [M+H]+): 244.3.

Step 3: 1-(4-(tert-butylamino)-1,7-naphthyridin-2-yl)ethanone(25 mg, 0.1 mmol) was stirred in 0.8mL of DMF/DMA at 110°C for 6 hrs. The reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-100%

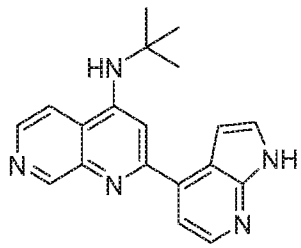
EtOAc/Hexane to afford the product (E)-1-(4-(tert-butylamino)-1,7-naphthyridin-2-yl)-3-(dimethylamino)prop-2-en-1-one (30%). LCMS (m/z [M+H]+): 299.4.

Step 4: (E)-1-(4-(tert-butylamino)-1,7-naphthyridin-2-yl)-3-(dimethylamino)prop-2-en-1-one (8 mg, 0.027 mmol) was stirred in EtOH (0.7 mL) at room temperature. Guanidine nitrate (4 mg, 0.034mmol) was added and the reaction stirred at 100°C for 20 minutes. Sodium ethoxide (in EtOH, 20 microlitre, 0.054 mmol) was then added and stirred at reflux overnight. Reaction was then concentrated and purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-20% MeOH/DCM to afford the title compound (30%). 1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.48(m, 1H), 8.41(m, 1H), 8.29(dd, J = 6.0, 0.9 Hz, 1H), 7.91(s, 1H), 7.62(d, J = 5.0 Hz, 1H), 6.74(s, 2H), 6.65(s, 1H), 1.56(s, 9H). LCMS (M/Z [M+H]+): 295.4.

Examples 272-274:

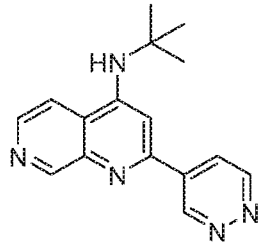
These compounds were synthesized according to the protocol used for the preparation of **Example 269** with 2,4-dichloro-1,7-naphthyridine (**6a'**) and various boronic acids or esters respectively.

Example 272: N-tert-butyl-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}-1,7-naphthyridin-4-amine



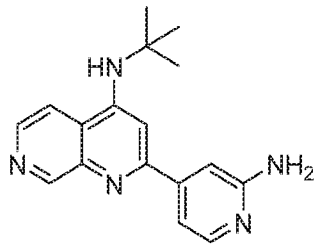
1H NMR (400 MHz, DMSO-d6) 11.86(s, 1H), 9.23(d, J = 0.8 Hz, 1H), 8.50(d, J = 5.8 Hz, 1H), 8.39(d, J = 4.9 Hz, 1H), 8.30(m, 1H), 7.63(m, 1H), 7.61(m, 1H), 7.32(s, 1H), 6.96(dd, J = 3.4, 1.9 Hz, 1H), 6.67(s, 1H), 1.56(s, 9H). LCMS (M/Z [M+H]+): 318.4.

Example 273: N-tert-butyl-2-(pyridazin-4-yl)-1,7-naphthyridin-4-amine



1H NMR (400 MHz, DMSO-d6) δ 9.97(s, 1H), 9.40(d, J = 0.8 Hz, 1H), 9.24(s, 1H), 8.50(d, J = 4.6 Hz, 1H), 8.36(d, J = 0.8 Hz, 1H), 8.31(m, 1H), 7.35(s, 1H), 6.80(s, 1H), 1.58(s, 9H). LCMS (M/Z [M+H]+): 280.3.

Example 274: 2-(2-aminopyridin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine

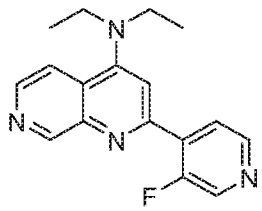


1H NMR (400 MHz, DMSO-d6) δ 9.18(d, J = 0.8 Hz, 1H), 8.45(d, J = 5.8 Hz, 1H), 8.26(m, 1H), 8.05(dd, J = 5.3 0.7 Hz, 1H), 7.20(m, 1H), 7.17(m, 1H), 7.14(m, 1H), 6.63(s, 1H), 6.09(s, 2H), 1.55(s, 9H). LCMS (M/Z [M+H]+): 294.4.

Examples 275-286:

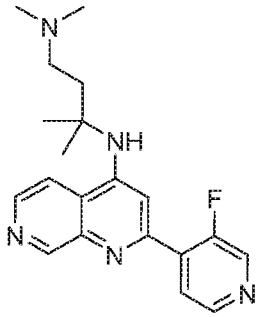
These compounds were synthesized according to the protocol used for the preparation of **Example 270** with 2,4-dichloro-1,7-naphthyridine (**Intermediate 6a'**, **Scheme 6**) and various organotin reagents and amines respectively.

Example 275: N,N-diethyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine



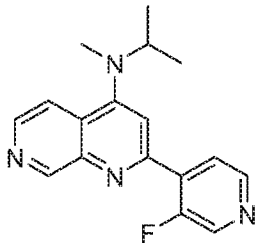
1H NMR (400 MHz, DMSO-d6) δ 9.33(d, J = 0.9 Hz, 1H), 8.78(d, J = 2.7 Hz, 1H), 8.62(dd, J = 4.9, 1.1 Hz, 1H), 8.55(d, J = 5.9 Hz, 1H), 8.02(dd, J = 6.8, 4.9 Hz, 1H), 7.88(dd, J = 5.8, 0.9 Hz, 1H), 7.42(d, J = 1.5 Hz, 1H), 3.50(q, J = 7.0 Hz, 4H), 1.21(t, J = 7.0 Hz, 6H). LCMS (M/Z [M+H]+): 297.1.

Example 276: (3-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-3-methylbutyl)dimethylamine



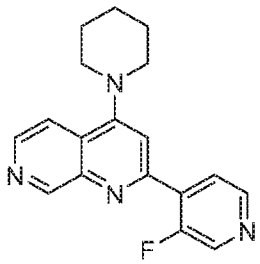
1H NMR (400 MHz, DMSO-d6) δ 9.20(d, J = 0.8 Hz, 1H), 8.74(d, J = 2.8 Hz, 1H), 8.60(dd, J = 4.9, 1.1 Hz, 1H), 8.53(d, J = 5.8 Hz, 1H), 8.04(dd, J = 6.9, 4.9 Hz, 1H), 7.91-7.83(s, 1H), 7.25(d, J = 1.4 Hz, 1H), 2.60-2.54(m, 2H), 2.33-2.24(m, 6H), 1.96-1.88(m, 2H), 1.51(s, 6H). LCMS (M/Z [M+H]+): 354.2.

Example 277: 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)-1,7-naphthyridin-4-amine



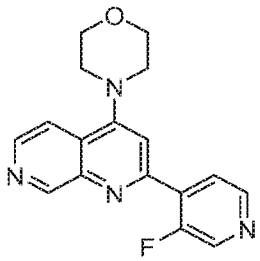
1H NMR (400 MHz, DMSO-d6) δ 9.33(m, 1H), 8.78(d, J = 2.7 Hz, 1H), 8.61(dd, J = 4.9, 1.1 Hz, 1H), 8.55(dd, J = 5.9, 2.5 Hz, 1H), 8.05-8.01(dd, J = 6.8, 4.9 Hz, 1H), 7.85(dd, J = 5.8, 0.9 Hz, 1H), 7.41(d, J = 1.4 Hz, 1H), 4.20-4.14(m, 1H), 2.93(s, 3H), 1.28(m, 6H). LCMS (M/Z [M+H]+): 297.1.

Example 278: 2-(3-fluoropyridin-4-yl)-4-(piperidin-1-yl)-1,7-naphthyridine



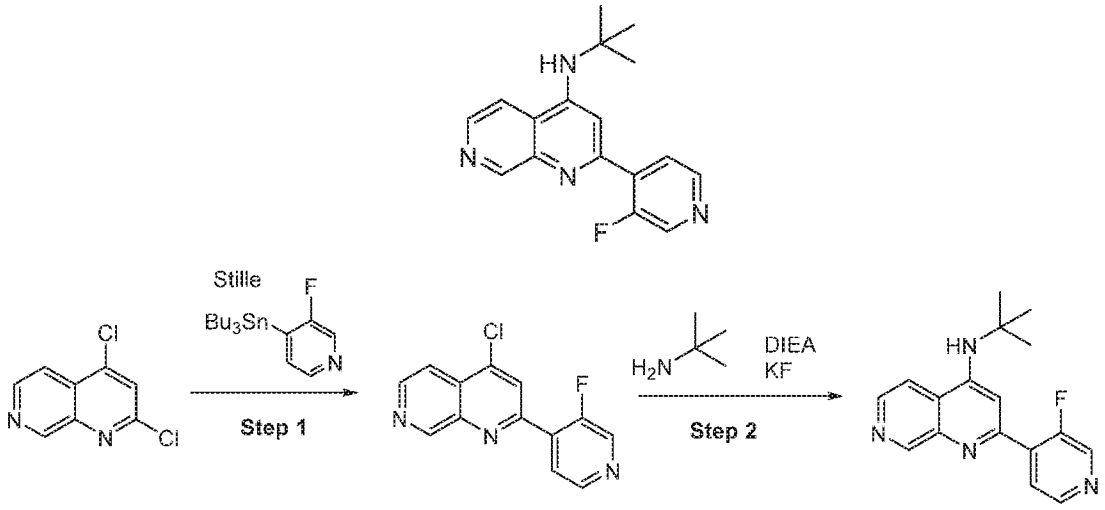
1H NMR (400 MHz, DMSO-d6) δ 9.39(d, J = 0.8 Hz, 1H), 8.78(d, J = 2.7 Hz, 1H), 8.63(m, 1H), 8.60(m, 1H), 8.03(dd, J = 6.8, 4.9 Hz, 1H), 7.85(dd, J = 5.8, 0.9 Hz, 1H), 7.50(d, J = 1.5 Hz, 1H), 3.32-3.28(m, 4H), 1.87-1.79(m, 4H), 1.72-1.65(m, 2H). LCMS (M/Z [M+H]+): 309.4.

Example 279: 2-(3-fluoropyridin-4-yl)-4-(morpholin-4-yl)-1,7-naphthyridine



1H NMR (400 MHz, DMSO-d6) δ 9.40(d, J = 0.8 Hz, 1H), 8.79(d, J = 2.6 Hz, 1H), 8.62(m, 1H), 8.60(m, 1H), 8.03(dd, J = 6.8, 4.9 Hz, 1H), 7.95(dd, J = 5.8, 0.9 Hz, 1H), 7.54(d, J = 1.4 Hz, 1H), 3.91-3.86(m, 4H), 3.36-3.33(m, 4H). LCMS (M/Z [M+H]+): 311.1.

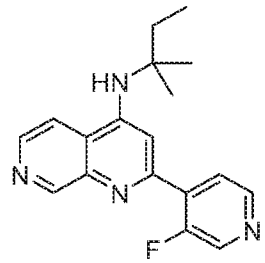
Example 280: N-tert-butyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine



Step 1: In a 20 mL microwave reactor 2,4-dichloro-1,7-naphthyridine (**6a**, 100mg, 0.502 mmol) was stirred in dry DMF (1 mL) at room temperature. 3-fluoro-4-(tributylstannyl)pyridine (194 mg, 0.502 mmol) was added then $PdCl_2(dppf).CH_2Cl_2$ adduct (41.0 mg, 0.050 mmol) and CuI (9.6 mg, 0.050 mmol). The reaction was stirred for 0.5 hour at 130°C. The crude mixture was diluted with DCM, H_2O, separated and extracted with DCM x3. Combined the organic layers and dried Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the product 4-chloro-2-(3-fluoropyridin-4-yl)-1,7-naphthyridine (71%). 1H NMR (400 MHz, Acetone-d6) δ 9.56 (d, J = 0.9 Hz, 1H), 8.83 (d, J = 5.8 Hz, 1H), 8.74 (d, J = 2.9 Hz, 1H), 8.67 (dd, J = 5.0, 1.2 Hz, 1H), 8.44 (d, J = 1.4 Hz, 1H), 8.18 (dd, J = 6.7, 4.9 Hz, 1H), 8.14 (dd, J = 5.8, 0.9 Hz, 1H). LCMS [M+H] = 260.

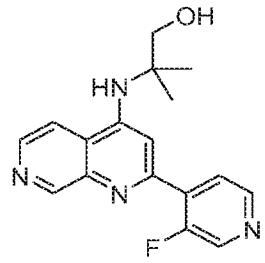
Step 2: In a 40 mL vial was added potassium fluoride (7 mg, 0.12 mmol), 4-chloro-2-(3-fluoropyridin-4-yl)-1,7-naphthyridine (26 mg, 0.10 mmol), and 2-methylpropan-2-amine (0.035 mL, 0.331 mmol) in DMSO (Volume: 1 mL) to give a yellow suspension. The reaction mixture was stirred at 130°C for 24 hrs. Solvent was evaporated under air flow. The residue was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to give the product (42%). 1H NMR (400 MHz, Acetone-d6) δ 9.24 (d, J = 0.8 Hz, 1H), 8.65 (d, J = 3.0 Hz, 1H), 8.59 (dd, J = 4.9, 1.2 Hz, 1H), 8.46 (d, J = 5.9 Hz, 1H), 8.15 (dd, J = 6.8, 4.9 Hz, 1H), 8.06 (dd, J = 5.9, 0.9 Hz, 1H), 7.48 (d, J = 1.4 Hz, 1H), 6.30 (s, 1H), 1.61 (s, 9H). LCMS (M/Z $[M+H]^+$): 297.1.

Example 281: 2-(3-fluoropyridin-4-yl)-N-(2-methylbutan-2-yl)-1,7-naphthyridin-4-amine



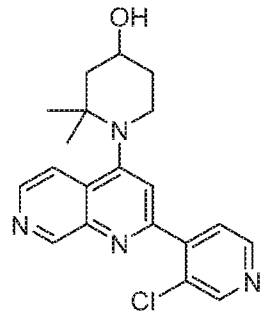
1H NMR (500 MHz, Acetone-d6) δ 9.24 (d, J = 0.9 Hz, 1H), 8.64 (d, J = 3.0 Hz, 1H), 8.58 (dd, J = 4.9, 1.2 Hz, 1H), 8.47 (d, J = 5.9 Hz, 1H), 8.15 (dd, J = 6.8, 4.9 Hz, 1H), 8.07 (dd, J = 5.9, 0.9 Hz, 1H), 7.47 (d, J = 1.4 Hz, 1H), 6.12 (s, 1H), 2.03 - 1.98 (m, 2H), 1.56 (s, 6H), 0.94 (t, J = 7.5 Hz, 3H). LCMS (M/Z [M+H]+): 311.2.

Example 282: 2-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-2-methylpropan-1-ol



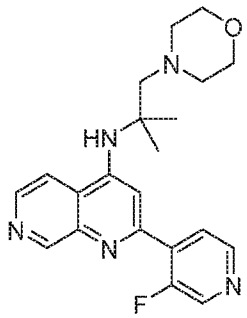
1H NMR (400 MHz, Acetone-d6) δ 9.24 (d, J = 0.8 Hz, 1H), 8.59 (dd, J = 4.9, 1.2 Hz, 1H), 8.49 (d, J = 5.9 Hz, 1H), 8.15 (dd, J = 6.8, 4.9 Hz, 1H), 7.97 (dt, J = 5.8, 1.3 Hz, 1H), 7.50 (d, J = 1.4 Hz, 1H), 6.29 (s, 1H), 3.78 (d, J = 5.6 Hz, 2H), 1.56 (s, 6H). LCMS (M/Z [M+H]+): 313.1.

Example 283: 1-[2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-yl]-2,2-dimethylpiperidin-4-ol



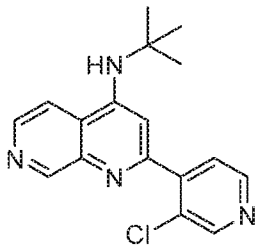
1H NMR (400 MHz, DMSO-d6) δ 9.40(s, 1H), 8.79(d, 1H), 8.62(d, 1H), 8.60(d, 1H), 8.03(dd, 1H), 7.95(d, 1H), 7.54(s, 1H), 3.91-3.86(m, 4H), 3.36-3.33(m, 4H). LCMS (M/Z [M+H]+): 311.3.

Example 284: 2-(3-fluoropyridin-4-yl)-N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-1,7-naphthyridin-4-amine



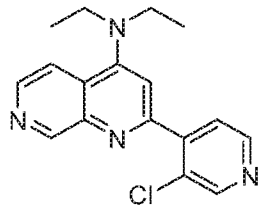
1H NMR (500 MHz, Acetone-d6) δ 9.25 (d, J = 0.9 Hz, 1H), 8.64 (d, J = 3.0 Hz, 1H), 8.59 (dd, J = 4.9, 1.2 Hz, 1H), 8.52 (d, J = 5.8 Hz, 1H), 8.15 (dd, J = 6.8, 4.9 Hz, 1H), 7.89 (dd, J = 5.9, 0.8 Hz, 1H), 7.54 (d, J = 1.4 Hz, 1H), 6.77 (s, 1H), 3.70 – 3.64 (m, 4H), 2.75 (s, 2H), 2.69 – 2.62 (m, 4H), 1.60 (s, 6H). LCMS (M/Z [M+H]+): 382.2.

Example 285: N-tert-butyl-2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-amine



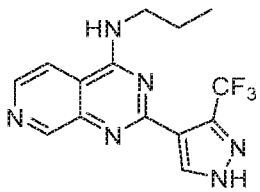
1H NMR (400 MHz, Acetone-d6) δ 9.20 (d, J = 0.9 Hz, 1H), 8.78 – 8.69 (m, 1H), 8.65 (d, J = 4.9 Hz, 1H), 8.47 (d, J = 5.9 Hz, 1H), 8.06 (dt, J = 5.9, 1.0 Hz, 1H), 7.79 – 7.70 (m, 1H), 7.25 (s, 1H), 1.60 (s, 9H). LCMS (M/Z [M+H]+): 313.1.

Example 286: 2-(3-chloropyridin-4-yl)-N,N-diethyl-1,7-naphthyridin-4-amine



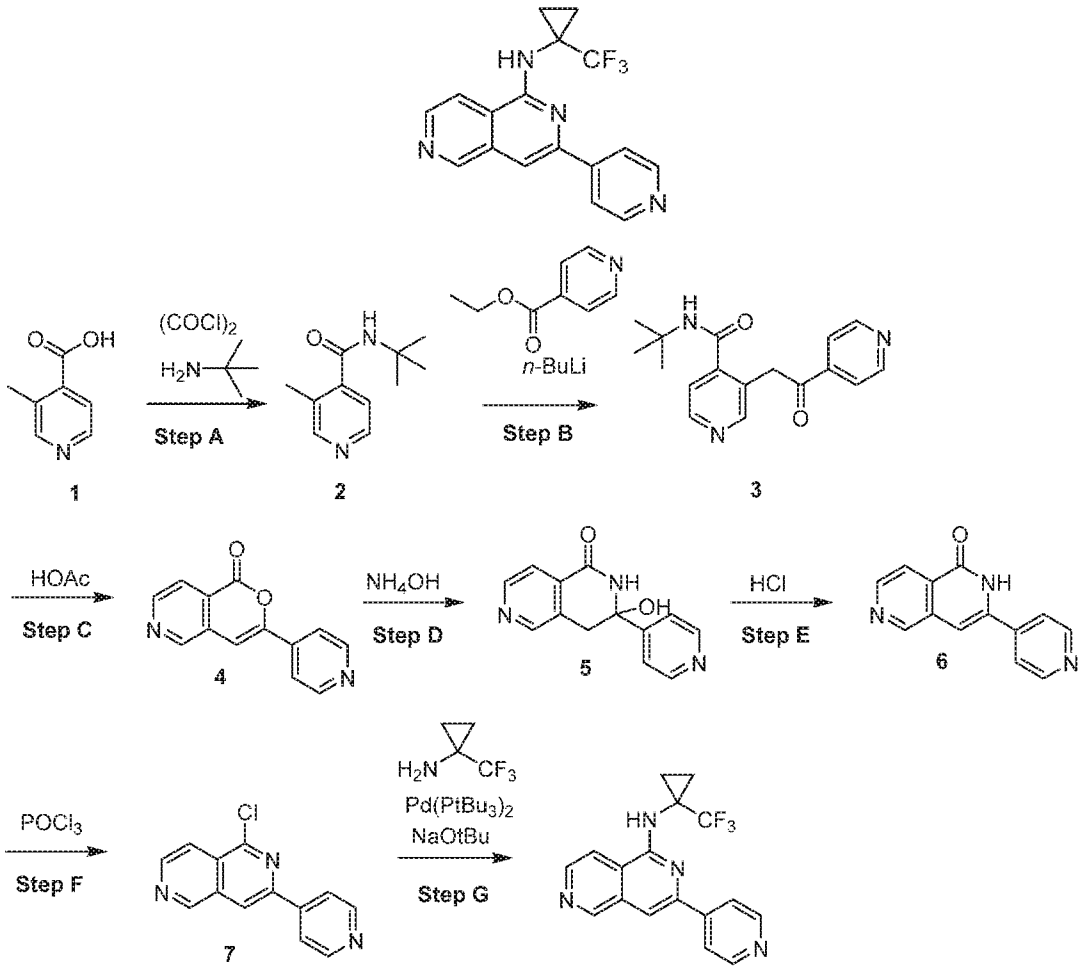
1H NMR (400 MHz, Acetone-d6) δ 9.31 (d, J = 0.9 Hz, 1H), 8.75 (d, J = 0.6 Hz, 1H), 8.67 (d, J = 4.9 Hz, 1H), 8.54 (d, J = 5.9 Hz, 1H), 7.93 (dd, J = 5.8, 0.9 Hz, 1H), 7.76 (dd, J = 4.9, 0.6 Hz, 1H), 7.39 (s, 1H), 3.58 (q, J = 7.1 Hz, 4H), 1.29 (t, J = 7.1 Hz, 6H). LCMS (M/Z [M+H]+): 313.1.

Example 287: *N*-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine



The title compound was synthesized according to the protocol described for **Example 111** using 2,4-dichloropyrido[3,4-d]pyrimidine (**intermediate 2c**) and propan-1-amine and tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)-1H-pyrazole-1-carboxylate. 1H NMR (500 MHz, DMSO-d6) δ 13.81 (s, 1H), 9.01 (d, J = 0.9 Hz, 1H), 8.64 (t, J = 5.7 Hz, 1H), 8.61 - 8.57 (m, 1H), 8.55 (d, J = 5.5 Hz, 1H), 8.11 (dd, J = 5.6, 1.0 Hz, 1H), 3.64 - 3.56 (m, 2H), 1.73 - 1.62 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). LCMS (M/Z $[M+H]^+$): 323.1.

Example 288: 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine



Step A: To a suspension of 3-methylisonicotinic acid (**1**) (10 g, 72.9 mmol) in dichloromethane (200 mL) was added DMF (200 mL) at 0°C. Ethyl chloroformate (8.6 mL, 12.5 g, 98.4 mmol) was added dropwise over 10 min at 0°C to result in a mixture which was stirred at 0°C for 15 min. *tert*-Butylamine (35.0 mL, 24.2 g, 330.0 mmol) was added dropwise over 15 min to the reaction mixture at 0°C, and the reaction mixture was concentrated under reduced pressure to result in a residue which was subjected to chromatography (hexanes:EtOAc) to give **2** (11.6 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1 H), 8.43 (d, J = 5.0 Hz, 1 H), 7.20 (d, J = 5.0 Hz, 1 H), 2.29 (s, 3H), 1.37 (s, 9H). LCMS (m/z $[M+H]^+$): 193.

Step B: To a solution of **2** (11.6 g, 60.3 mmol) in THF (440 mL) was added a solution of *n*-BuLi (58.0 mL, 145.0 mmol, 2.5 M in hexanes) dropwise over 15 min at -45°C under N_2 to result in a mixture which was stirred for 45 min at -45°C. Then a solution of ethyl isonicotinate (10.0 g, 66.3 mol) in THF (20 mL) was dropwise over 15 min at -45°C. The reaction mixture was allowed to be warmed to room temperature over 2 h, and poured

into a saturated aqueous NH_4Cl solution (1000 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (3×400 mL). The combined organic layers were washed with saturated aqueous NaCl solution (100 mL), dried over $MgSO_4$, and evaporated under reduced pressure to give crude **3**. LCMS (m/z $[M+H]^+$): 298. The crude product was used in the next step without purification.

Step C: A solution of crude **3** of the Step B in acetic acid (200 mL) was heated at 100°C for 16 h, cooled to room temperature and concentrated to ~80 mL under reduced pressure. Water (120 mL) was added, and the mixture was filtered to get a white precipitate which was washed with water (2x 50 mL), and dried under reduced pressure to afford crude **4** (8.08 g). LCMS (m/z $[M+H]^+$): 225. The crude product was used in the next step without purification.

Step D: To a suspension of crude **4** (8.08 g) in EtOH (100 mL) was added an aqueous NH_4OH solution (80 mL, 28.5%) at room temperature to result in a mixture which was stirred for 2.5 h, and evaporated to afford crude **5**. LCMS (m/z $[M+H]^+$): 242. The crude product was used in the next step without purification.

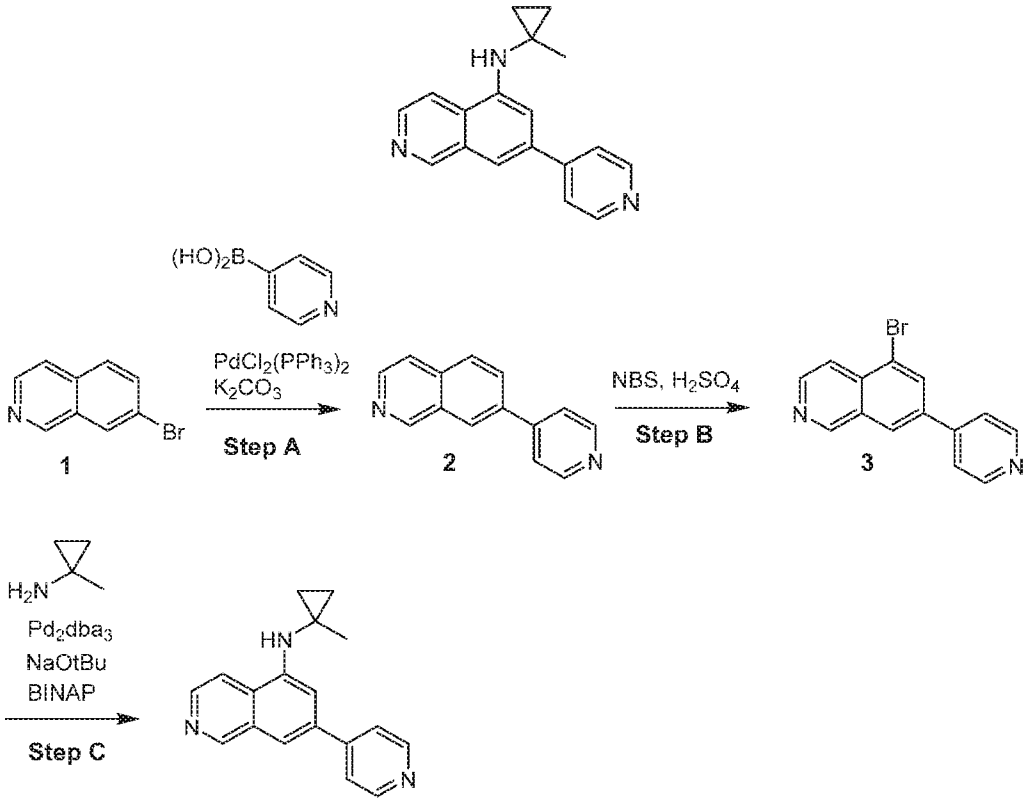
Step E: To a suspension of crude **5** of the Step D in EtOH (100 mL) was added water (20 mL) and then aqueous HCl solution (12 M, 25 mL) at 0°C. The reaction mixture was stirred for 19 h at room temperature, and filtered to get white solid which was dried under reduced pressure to afford crude **6** (10.2 g). LCMS (m/z $[M+H]^+$): 224. The crude product was used in the next step without purification.

Step F: A suspension of crude **6** (10.2 g) in $POCl_3$ (75 mL) in an open ChemGlass heavy wall round bottom pressure vessel (350 mL) was heated very slowly till the temperature reached 100°C, and stirred at 100°C for 30 min. Then the pressure vessel was sealed, and the reaction mixture was heated at 135°C for 14 h. The $POCl_3$ was removed under reduced pressure, and the residue was mixed with ice water (50 g of ice and 50 mL of water). The pH of the mixture was adjusted to ~ 7 with aqueous NaOH solution (1M), and then to ~10 with saturated aqueous Na_2CO_3 solution. Filtration of the mixture gave a while solid which was dried under reduce pressure to afford **7** (7.1 g, 49% yield for 5 steps from **2**). ^1H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H), 8.94 (s, 1H), 8.89 (d, J = 5.6 Hz, 1H), 8.78 (d, J = 4.8 Hz, 2H), 8.15 (d, J = 4.8 Hz, 2H), 8.14 (d, J = 5.6 Hz, 1H). LCMS (m/z $[M+H]^+$): 242.

Step G: To a solution of intermediate **7** (60.0 mg, 0.25 mmol) and 1-(trifluoromethyl)cyclopropan-1-amine (93.0 mg, 0.74 mmol) in dioxane (1.0 mL) was added

bis(tri-tert-butylphosphine)palladium (13.0 mg, 0.2 micromol) and sodium *tert*-butoxide (71.0 mg, 0.74 mmol). The reaction mixture was heated under N_2 at 130°C for overnight, and concentrated resulting in a residue which was subjected to chromatography (MeOH/DCM) to afford the title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.95 (d, J = 8.0 Hz, 2H), 8.75 (d, J = 4.0 Hz, 1H), 8.71 (s, 1H), 8.57 (d, J = 8.0 Hz, 2H), 8.34 (s, 1H), 8.31 d, J = 4.0 Hz, 1H), 1.57 (m, 2H), 1.31 (m, 2H). LCMS (m/z $[M+H]^+$): 331.

Example 289: **N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine**

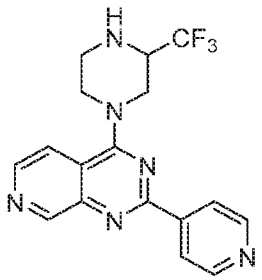


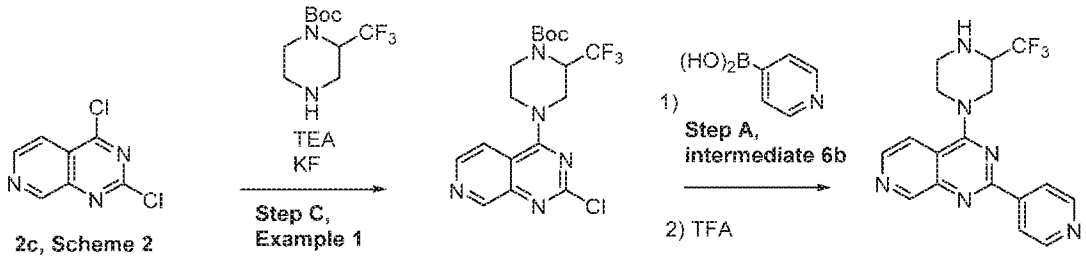
Step A: A suspension of 7-bromoisoquinoline (**1**) (300 mg, 1.44 mmol), pyridin-4-ylboronic acid (213 mg, 1.73 mmol), bis(triphenylphosphine)palladium(II) dichloride ($PdCl_2(PPh_3)_2$, 98 mg, 0.14 mmol) and potassium carbonate (2 N, 4.3 ml) in DMF (7 mL) was stirred and heated 100°C for 3 hours. The reaction mixture was filtered through celite. The solution was diluted with water and extracted with EtOA x3. The combined organic layers were washed with saturated aqueous NaCl solution, dried over $MgSO_4$, and evaporated under reduced pressure. The resulted crude was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford 7-(pyridin-4-yl)isoquinoline (**2**, 282 mg) as a pale brown oil. LCMS (m/z $[M+H]^+$): 207.

Step B: To a solution of 7-(pyridin-4-yl)isoquinoline (**2**) (282 mg, 1.37 mmol) in concentrated H_2SO_4 (3 mL) was added N-bromosuccinimide (NBS, 488 mg, 2.74 mmol) at 0°C. The reaction mixture was allowed to be warmed to room temperature over 2 h, and poured into ice-water (20 mL) and added saturated aqueous $NaHCO_3$ solution to pH ~8. The aqueous layer was extracted with EtOA x3. The combined organic layers were washed with saturated aqueous NaCl solution, dried over $MgSO_4$, and evaporated under reduced pressure. The resulted crude was purified by flash chromatography on a COMBIFLASH® system (ISCO) using 0-10% MeOH/DCM to afford 5-bromo-7-(pyridin-4-yl)isoquinoline (**3**, 110 mg). LCMS (m/z $[M+H]^+$): 285/287.

Step C: A suspension of 5-bromo-7-(pyridin-4-yl)isoquinoline (**3**) (30 mg, 0.105 mmol), 1-methylcyclopropan-1-amine hydrochloride (28.3 mg, 0.263 mmol), tris(dibenzylideneacetone)dipalladium(0) (Pd_2dba_3, 9.7 mg, 0.0105 mmol), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP, 6.5 mg, 0.0105 mmol) and sodium *tert*-butoxide (44.3 mg, 0.462) in toluene (1.5 mL) was heated under N_2 at 90°C for 16 h, cooled to room temperature and concentrated. The residue was diluted in MeOH and filtered, then purified by mass-triggered preparative reverse phase HPLC with 10-90% acetonitrile/water to afford the title compound (6 mg). 1H NMR (400 MHz, Chloroform-d) δ 9.24 (s, 1H), 8.74 (s, 2H), 8.49 (s, 1H), 7.69 – 7.60 (m, 2H), 7.59 – 7.56 (m, 1H), 7.52 – 7.46 (m, 1H), 7.39 – 7.36 (m, 1H), 1.52 (s, 3H), 0.98 – 0.92 (m, 2H), 0.88 – 0.81 (m, 2H). LCMS (m/z $[M+H]^+$): 276.

Example 290: 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine



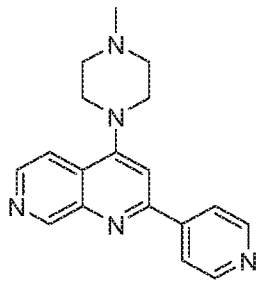


2c, Scheme 2

The title compound was synthesized according to the protocol described for example **111** using 2,4-dichloropyrido[3,4-d]pyrimidine (intermediate **2c**) with tert-butyl 2-(trifluoromethyl)piperazine-1-carboxylate and pyridin-4-ylboronic acid followed by deprotection of the N-Boc group with TFA.

1H NMR (400 MHz, Chloroform-d) δ 9.46 (d, J = 0.9 Hz, 1H), 8.83 - 8.78 (m, 2H), 8.63 (d, J = 5.8 Hz, 1H), 8.35 - 8.30 (m, 2H), 7.74 (dd, J = 5.8, 0.9 Hz, 1H), 5.80 - 5.67 (m, 1H), 4.07 (dt, J = 24.3, 12.4 Hz, 2H), 3.65 (d, J = 14.2 Hz, 1H), 3.34 (d, J = 14.6 Hz, 1H), 3.18 (d, J = 13.5 Hz, 1H), 2.89 (t, J = 11.0 Hz, 1H). LCMS (m/z $[M+H]^+$): 361.1.

Example 291: 4-(4-methylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine

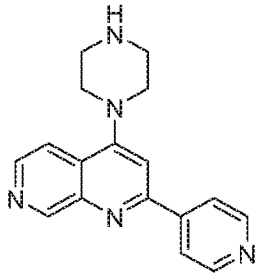


The title compound was synthesized from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and 1-methylpiperazine as follows:

A solution of 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**, 30 mg, 0.124 mmol), 1-methylpiperazine (50 mg, 0.499 mmol), potassium phosphate (132 mg, 0.621 mmol), RuPhos Pd G3 (16 mg, 0.019 mmol) and RuPhos (13 mg, 0.028 mmol) in dioxane (2 mL) was heated under argon in a microwave vial at 130°C for 10 min. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using 20-100% ethyl acetate/cyclohexane, followed by 2-30% methanol/dichloromethane to give the title compound (21%).

1H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 8.76 (d, J = 4.8 Hz, 2H), 8.55 (d, J = 5.7 Hz, 1H), 8.23 (d, J = 4.8 Hz, 2H), 7.85 (d, J = 5.7 Hz, 1H), 7.70 (s, 1H), 3.37 (s, 4H), 2.63 (s, 4H), 2.30 (s, 3H). LCMS (m/z $[M+H]^+$): 306.3, Rt_1=0.38 min.

Example 292: 4-(piperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine



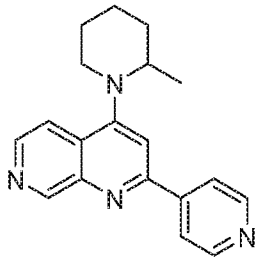
The title compound was synthesized in analogy of **Example 291** in 2 steps from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and tert-butyl piperazine-1-carboxylate (step 1) and Boc-deprotection using HCl in diethylether (step 2).

Step 2 (Boc-deprotection):

To a solution of tert-butyl 4-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)piperazine-1-carboxylate (24 mg, 0.061 mmol) in dichloromethane (5 mL) was added 6M HCl in diethyl ether (2 mL, 12 mmol). The resulting yellow suspension was stirred at r.t. for 1h. The yellow precipitate was filtered off and washed with dichloromethane. The hydrochloride salt was dissolved in MeOH and the solution was given on a PoraPak Rxn CX 20cc (2g) cartridge. The cartridge was then washed twice with 5 mL MeOH. Finally, the compound was eluted with 7N NH_3 in MeOH. The filtrate was evaporated under reduced pressure to give the title compound (76%).

1H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.76 (d, J = 4.7 Hz, 2H), 8.55 (d, J = 5.8 Hz, 1H), 8.22 (d, J = 4.8 Hz, 2H), 7.86 (d, J = 5.7 Hz, 1H), 7.67 (s, 1H), 3.28 (s, 4H), 2.99 (s, 4H). LCMS (m/z $[M+H]^+$): 292.3, Rt_1=0.38 min.

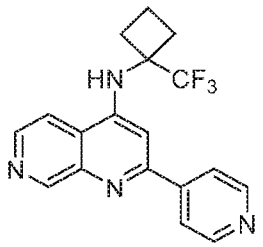
Example 293: 4-(2-methylpiperidin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine



The title compound was synthesized in analogy of **Example 291** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and 2-methylpiperidine.

1H NMR (400 MHz, DMSO-d_6) δ 9.48 - 9.32 (m, 1H), 8.86 - 8.74 (m, 2H), 8.56 (d, J = 5.7 Hz, 1H), 8.30 - 8.16 (m, 2H), 7.86 (d, J = 5.8 Hz, 1H), 7.72 (s, 1H), 4.10 (d, J = 6.3 Hz, 1H), 3.53 - 3.41 (m, 1H), 3.21 (d, J = 12.4 Hz, 1H), 2.05 (s, 1H), 1.86 - 1.74 (m, 3H), 1.62 (s, 2H), 1.06 (d, J = 6.5 Hz, 3H). LCMS (m/z $[M+H]^+$): 305.3, Rt_1=0.98 min.

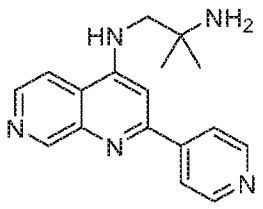
Example 294: 2-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclobutyl)-1,7-naphthyridin-4-amine



The title compound was synthesized from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and 1-(trifluoromethyl)cyclobutan-1-amine as follows:
A solution of 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (Intermediate **6b**, 100 mg, 0.414 mmol), 1-(trifluoromethyl)cyclobutan-1-amine (115 mg, 0.828 mmol), potassium tert-butylate (139 mg, 1.241 mmol), $Pd_2(dba)_3$ x $CHCl_3$ (43 mg, 0.042 mmol) and PhCPhos (36 mg, 0.085 mmol) in dioxane (5 mL) was heated under argon in a microwave vial at 100°C for 30 min. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using 20-100% ethyl acetate/cyclohexane, followed by 2-10% methanol/dichloromethane to give the title compound (9%).

1H NMR (600 MHz, DMSO-d_6) δ 9.30 (s, 1H), 8.78 - 8.74 (m, 2H), 8.57 (d, J = 5.8 Hz, 1H), 8.40 (d, J = 5.8 Hz, 1H), 8.08 - 8.04 (m, 2H), 7.89 (s, 1H), 6.94 (s, 1H), 2.85 - 2.78 (m, 2H), 2.74 (d, J = 11.6 Hz, 2H), 2.04 (dd, J = 18.6, 9.7 Hz, 2H). LCMS (m/z $[M+H]^+$): 345.3, Rt_1=0.84 min.

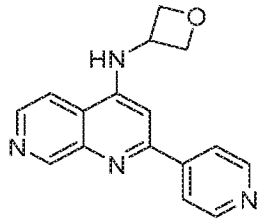
Example 295: 2-methyl-N1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,2-diamine



The title compound was synthesized in analogy of **Example 254** in 2 steps from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and tert-butyl (1-amino-2-methylpropan-2-yl)carbamate (step 1) and Boc-deprotection using HCl in diethyl ether (step 2, described in **Example 292**).

1H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.75 (d, *J*=5.14 Hz, 2H), 8.51 (d, *J*=5.75 Hz, 1H), 8.26 (d, *J*=5.75 Hz, 1H), 8.21 (d, *J*=5.26 Hz, 2H), 7.40 (s, 1H), 7.29 (br s, 1H), 3.38 (br s, 2H), 1.63-2.22 (m, 2H), 1.16 (s, 6H). LCMS (m/z $[M+H]^+$): 294.4, Rt_1=0.37 min.

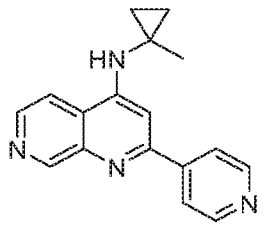
Example 296: N-(oxetan-3-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



The title compound was synthesized in analogy of **Example 254** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and oxetan-3-amine.

1H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.76 (d, *J*=5.99 Hz, 2H), 8.57 (d, *J*=5.75 Hz, 1H), 8.29 (d, *J*=5.75 Hz, 1H), 8.21 (d, *J*=5.99 Hz, 2H), 8.11 (br d, *J*=5.75 Hz, 1H), 7.03 (s, 1H), 4.99-5.17 (m, 3H), 4.73 (t, *J*=5.93 Hz, 2H). LCMS (m/z $[M+H]^+$): 279.3, Rt_1=0.49 min.

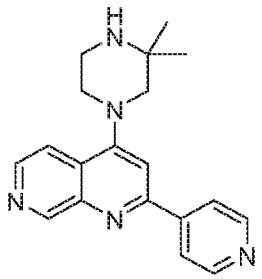
Example 297: N-(1-methylcyclopropyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



The title compound was synthesized in analogy of **Example 254** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and 1-methylcyclopropan-1-amine.

1H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.78 (d, *J*=5.75 Hz, 2H), 8.48 (d, *J*=5.87 Hz, 1H), 8.20 (d, *J*=5.87 Hz, 2H), 8.07 (d, *J*=5.87 Hz, 1H), 7.69 (s, 1H), 3.29 (s, 3H), 3.14-3.20 (m, 1H), 0.89-0.97 (m, 2H), 0.52-0.61 (m, 2H). LCMS (m/z $[M+H]^+$): 277.3, Rt_1=0.73 min.

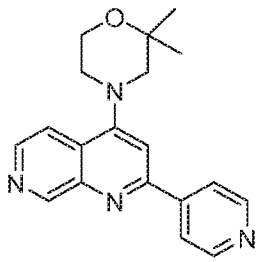
Example 298: 4-(3,3-dimethylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine



The title compound was synthesized in analogy of **Example 291** in 2 steps from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and tert-butyl 2,2-dimethylpiperazine-1-carboxylate (step 1) and Boc-deprotection using HCl in diethylether (step 2, described in **Example 292**).

1H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.79 (d, J = 6.0 Hz, 2H), 8.59 (d, J = 5.7 Hz, 1H), 8.26 (d, J = 6.0 Hz, 2H), 7.90 (d, J = 5.8 Hz, 1H), 7.70 (s, 1H), 3.31 (s, 1H), 3.25 (d, J = 4.9 Hz, 2H), 3.10 (s, 4H), 1.26 (s, 6H). LCMS (m/z $[M+H]^+$): 320.3, Rt_1=0.45 min.

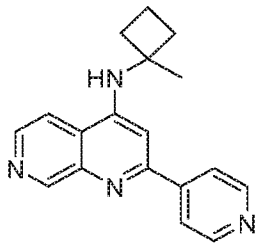
Example 299: 2,2-dimethyl-4-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)morpholine



The title compound was synthesized in analogy of **Example 291** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and 2,2-dimethylmorpholine.

1H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.80 (d, J = 5.9 Hz, 2H), 8.61 (d, J = 5.8 Hz, 1H), 8.27 (d, J = 6.0 Hz, 2H), 7.93 (d, J = 5.8 Hz, 1H), 7.75 (s, 1H), 4.01 - 3.94 (m, 2H), 3.31 (s, 2H), 3.21 (s, 2H),1.39 (s, 6H). LCMS (m/z $[M+H]^+$): 321.3, Rt_1=0.81 min.

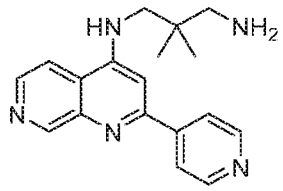
Example 300: N-(1-methylcyclobutyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



The title compound was synthesized in analogy of **Example 254** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and 1-methylcyclobutan-1-amine.

1H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.75 (d, J = 5.9 Hz, 2H), 8.49 (d, J = 5.8 Hz, 1H), 8.26 (d, J = 5.8 Hz, 1H), 8.08 (d, J = 5.9 Hz, 2H), 7.64 (s, 1H), 6.83 (s, 1H), 2.45 (t, J = 10.4 Hz, 2H), 2.32 (t, J= 9.2 Hz, 2H), 1.97 (ddd, J = 25.6, 20.0, 9.7 Hz, 2H), 1.62 (s, 3H). LCMS (m/z $[M+H]^+$): 291.3, Rt_1=0.70 min.

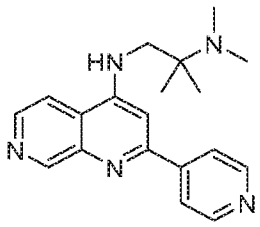
Example 301: 2,2-dimethyl-N1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,3-diamine



The title compound was synthesized in analogy of **Example 254** in 2 steps from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and tert-butyl (3-amino-2,2-dimethylpropyl)carbamate (step 1) and Boc-deprotection using HCl in diethyl ether (step 2, described in **Example 292**).

1H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.72 (d, J = 5.9 Hz, 2H), 8.47 (d, J = 5.7 Hz, 1H), 8.17 (d, J = 6.0 Hz, 2H), 8.01 (d, J = 5.8 Hz, 1H), 7.31 (s, 1H), 3.36 (s, 2H), 3.27 (s, 2H), 2.60 (s, 2H), 0.97 (s, 6H). LCMS (m/z $[M+H]^+$): 308.3, Rt_1=0.41 min.

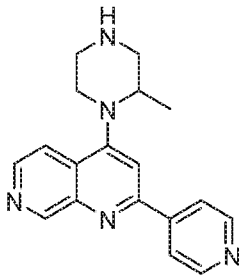
Example 302: N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,2-diamine



The title compound was synthesized in analogy of **Example 254** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and N^2,N^2,2-trimethylpropane-1,2-diamine.

1H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.76 (d, J = 5.9 Hz, 2H), 8.54 (d, J = 5.7 Hz, 1H), 8.23 (d, J = 5.7 Hz, 2H), 8.05 (s, 1H), 7.33 (s, 1H), 6.75 (s, 1H), 3.42 (s, 2H), 2.29 (d, J = 15.6 Hz, 6H), 1.16 (s, 6H). LCMS (m/z $[M+H]^+$): 322.3, Rt_1=0.39 min.

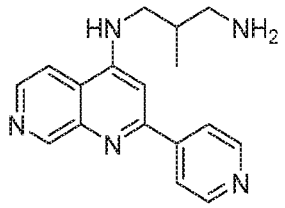
Example 303: 4-(2-methylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine



The title compound was synthesized in analogy of **Example 291** in 2 steps from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and tert-butyl 3-methylpiperazine-1-carboxylate (step 1) and Boc-deprotection using HCl in diethyl ether (step 2, described in **Example 292**).

1H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 1H), 8.80 (d, J = 5.9 Hz, 2H), 8.60 (d, J = 5.7 Hz, 1H), 8.28 (d, J = 5.9 Hz, 2H), 7.98 (d, J = 5.7 Hz, 1H), 7.84 (s, 1H), 4.08 (d, J = 5.9 Hz, 1H), 3.60 (t, J = 9.6 Hz, 1H), 3.42 (dd, J = 12.3, 2.9 Hz, 1H), 3.29 - 3.17 (m, 2H), 3.12 (t, J = 9.3 Hz, 1H), 2.98 (dd, J = 12.3, 4.1 Hz, 1H), 1.10 (d, J = 6.5 Hz, 3H). LCMS (m/z $[M+H]^+$): 306.3, Rt_1=0.43 min.

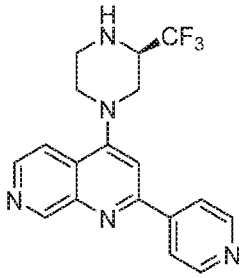
Example 304: 2-methyl-N^1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,3-diamine



The title compound was synthesized in analogy of **Example 254** in 2 steps from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and tert-butyl (3-amino-2-methylpropyl)carbamate (step 1) and Boc-deprotection using HCl in diethyl ether (step 2, described in **Example 292**).

1H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.76 (d, J = 5.9 Hz, 2H), 8.51 (d, J = 5.7 Hz, 1H), 8.24 - 8.16 (m, 3H), 7.96 (s, 1H), 7.27 (s, 1H), 5.14 (s, 2H), 3.50 (dd, J = 13.4, 6.8 Hz, 1H), 3.40 (dd, J = 13.8, 6.8 Hz, 1H), 2.80 (dd, J = 12.6, 6.0 Hz, 1H), 2.70 (dd, J = 12.5, 6.4 Hz, 1H), 2.11 (dq, J = 13.2, 6.6 Hz, 1H), 1.04 (d, J = 6.7 Hz, 3H). LCMS (m/z $[M+H]^+$): 294.3, Rt_1=0.37 min.

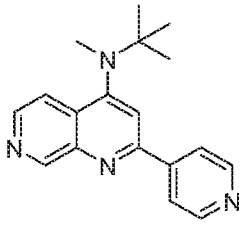
Example 305: (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)-1,7-naphthyridine



The title compound was synthesized in analogy of **Example 291** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and (*R*)-2-(trifluoromethyl)piperazine.

1H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.84 - 8.71 (m, 2H), 8.58 (d, J = 5.7 Hz, 1H), 8.35 - 8.18 (m, 2H), 7.88 (d, J = 5.8 Hz, 1H), 7.79 (s, 1H), 3.79 (s, 1H), 3.66 (d, J = 11.9 Hz, 1H), 3.53 (d, J = 10.3 Hz, 1H), 3.23 - 2.94 (m, 5H). LCMS (m/z $[M+H]^+$): 360.3, Rt_1=0.71 min.

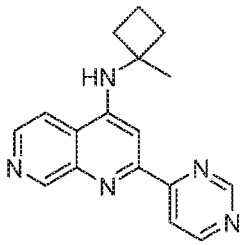
Example 306: N-(tert-butyl)-N-methyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine



The title compound was synthesized in analogy of **Example 254** from 4-chloro-2-(pyridin-4-yl)-1,7-naphthyridine (**Intermediate 6b**) and N,2-dimethylpropan-2-amine.

1H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.81 (d, J = 5.6 Hz, 2H), 8.61 (d, J = 5.7 Hz, 1H), 8.24 (d, J = 5.7 Hz, 2H), 8.13 (d, J = 6.2 Hz, 2H), 2.97 (s, 3H), 1.30 (s, 9H). LCMS (m/z $[M+H]^+$): 293.3, Rt_1=1.01 min.

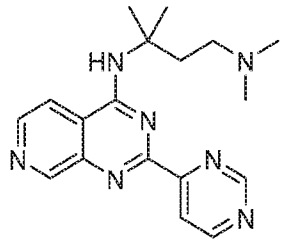
Example 307: ***N*-(1-methylcyclobutyl)-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine**



The title compound was synthesized in analogy of **Example 270** in 2 steps from 2,4-dichloro-1,7-naphthyridine (**Intermediate 6a`**) and 4-(tributylstannyl)pyrimidine (step 1) and from 4-chloro-2-(pyrimidin-4-yl)-1,7-naphthyridine and 1-methylcyclobutan-1-amine (step 2).

1H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.27 (s, 1H), 9.01 (dd, J = 5.2, 1.5 Hz, 1H), 8.59 - 8.48 (m, 2H), 8.29 (d, J = 5.9 Hz, 1H), 7.69 (s, 1H), 7.58 (d, J = 1.5 Hz, 1H), 2.46 (s, 2H),
2.27 (t, J = 9.8 Hz, 2H), 1.98 (dq, J = 31.9, 10.6, 10.2 Hz, 2H), 1.62 (s, 3H). LCMS (m/z $[M+H]^+$): 292.4, Rt_1=0.82 min.

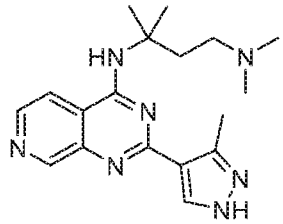
Example 308: ***N*^{1},*N*^{1},3-trimethyl-*N*^{3}-(2-(pyrimidin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine**



The title compound was synthesized in analogy of **Example 159** in 2 steps from 2,4-dichloropyrido[3,4-d]pyrimidine (**Intermediate 2c**) and N^1,N^1,3-trimethylbutane-1,3-diamine (step 1) and from N^3-(2-chloropyrido[3,4-d]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine and 4-(tributylstannyl)pyrimidine (step 2).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.29 - 9.48 (m, 2 H), 9.22 (s, 1 H), 9.05 (br d, *J*=5.01 Hz, 1 H), 8.70 (br d, *J*=5.50 Hz, 1 H), 8.38 (br d, *J*=5.01 Hz, 1 H), 7.91 (br d, *J*=4.89 Hz, 1 H), 2.21 - 2.43 (m, 8 H), 2.05 (br s, 2 H), 1.66 (s, 6 H). LCMS (m/z $[M+H]^+$): 338.3, Rt_1=0.47 min.

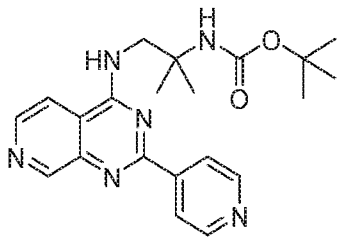
Example 309: N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine



The title compound was synthesized in analogy of **Example 111** in 2 steps from 2,4-dichloropyrido[3,4-d]pyrimidine (**Intermediate 2c**) and N^1,N^1,3-trimethylbutane-1,3-diamine (step 1) and from N^3-(2-chloropyrido[3,4-d]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine and *tert*-butyl 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-1-carboxylate (CAS no.1009071-34-4, step 2).

1H NMR (400 MHz, DMSO-d_6) δ ppm 12.57 - 12.95 (m, 1 H), 8.89 - 9.00 (m, 2 H), 8.48 (d, *J*=5.50 Hz, 1 H), 7.93 - 8.29 (m, 1 H), 7.75 (br d, *J*=5.38 Hz, 1 H), 2.55 - 2.84 (m, 5 H), 2.25 (s, 6 H), 1.99 (br s, 2 H), 1.61 (s, 6 H). LCMS (m/z $[M+H]^+$): 340.3, Rt_1=0.48 min.

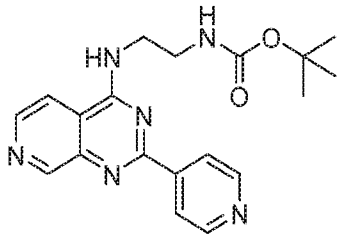
Example 310: *tert*-butyl (2-methyl-1-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propan-2-yl)carbamate



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and tert-butyl (1-amino-2-methylpropan-2-yl)carbamate.

1H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.78 (dd, J = 8.9, 4.9 Hz, 3H), 8.68 (d, J = 5.5 Hz, 1H), 8.44 - 8.34 (m, 2H), 8.22 (d, J = 5.7 Hz, 1H), 6.88 (s, 1H), 3.90 (d, J = 6.1 Hz, 2H),1.35 (s, 6H), 1.27 (d, J = 6.1 Hz, 9H). LCMS (m/z $[M+H]^+$): 395.2, Rt_1=0.97 min.

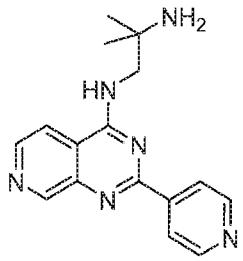
Example 311: *tert*-butyl (2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)ethyl)carbamate



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and tert-butyl (2-aminoethyl)carbamate.

1H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 8.93 - 8.81 (m, 1H), 8.79 - 8.68 (m, 2H), 8.64 (d, J = 5.5 Hz, 1H), 8.39 (d, J = 5.1 Hz, 2H), 8.13 (d, J = 5.6 Hz, 1H), 7.03 (t, J = 6.0 Hz, 1H),3.73 (q, J = 6.2 Hz, 2H), 3.40 - 3.32 (m, 2H), 1.33 (s, 9H). LCMS (m/z $[M+H]^+$): 367.2, Rt_1=0.78 min.

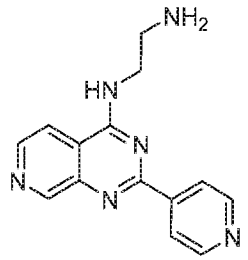
Example 312: 2-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine



The title compound was synthesized in analogy of **Example 1** in 2 steps from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and tert-butyl (1-amino-2-methylpropan-2-yl)carbamate (step 1) and Boc-deprotection using TFA (step 2).

1H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.83 - 8.74 (m, 2H), 8.65 (d, J = 5.5 Hz, 1H), 8.38 - 8.33 (m, 2H), 8.31 (d, J = 5.6 Hz, 1H), 3.70 (s, 2H), 1.13 (s, 6H). LCMS (m/z $[M+H]^+$): 295.2, Rt_1=0.41 min.

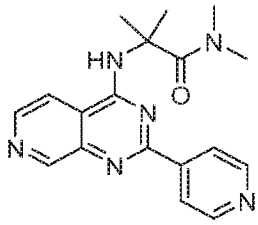
Example 313: *N*^{1}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)ethane-1,2-diamine



The title compound was synthesized in analogy of **Example 1** in 2 steps from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and tert-butyl (2-aminoethyl)carbamate (step 1) and Boc-deprotection using TFA (step 2).

1H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.81 - 8.71 (m, 2H), 8.64 (d, J = 5.5 Hz, 1H), 8.43 - 8.30 (m, 2H), 8.20 (d, J = 5.6 Hz, 1H), 3.73 (t, J = 6.4 Hz, 2H), 2.94 (t, J = 6.4 Hz, 2H). LCMS (m/z $[M+H]^+$): 267.2, Rt_1=0.38 min.

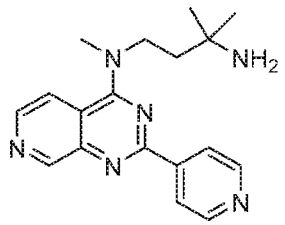
Example 314: *N,N*,2-trimethyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and 2-amino-N,N,2-trimethylpropanamide.

1H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 8.74 (dd, J = 6.9, 2.4 Hz, 3H), 8.68 (d, J = 5.6 Hz, 1H), 8.38 (d, J = 5.6 Hz, 1H), 8.36 - 8.31 (m, 2H), 2.95 - 2.69 (m, 6H), 1.6(s, 6H). LCMS (m/z $[M+H]^+$): 337.2, Rt_1=0.58 min.

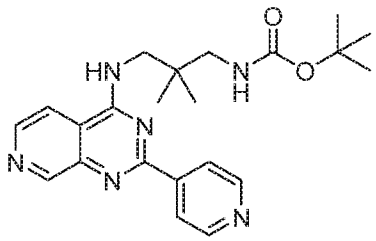
Example 315: ***N*^{1},3-dimethyl-*N*^{1}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine**



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and *N*^{1},3-dimethylbutane-1,3-diamine.

1H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.83 - 8.69 (m, 2H), 8.55 (d, J = 5.8 Hz, 1H), 8.40 - 8.29 (m, 2H), 8.12 (d, J = 5.9 Hz, 1H), 4.04 - 3.92 (m, 2H), 3.52 (s, 3H), 1.87 - 1.74 (m, 2H), 1.56 (s, 2H), 1.15 (s, 6H). LCMS (m/z $[M+H]^+$): 323.2, Rt_1=0.49 min.

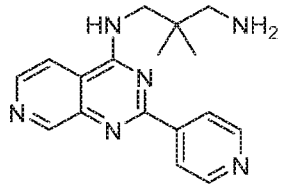
Example 316: ***tert*-butyl (2,2-dimethyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propyl)carbamate**



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and *tert*-butyl (3-amino-2,2-dimethylpropyl)carbamate.

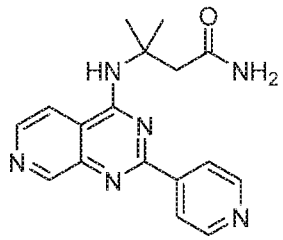
1H NMR (600 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.77 (d, J = 4.9 Hz, 2H), 8.68 (d, J = 5.4 Hz, 1H), 8.61 (t, J = 6.4 Hz, 1H), 8.43 - 8.30 (m, 2H), 8.17 (d, J = 5.5 Hz, 1H), 7.01 (t,J = 6.6 Hz, 1H), 3.62 (d, J = 6.2 Hz, 2H), 2.93 (d, J = 6.3 Hz, 2H), 1.38 (s, 9H), 0.92 (s, 6H). LCMS (m/z $[M+H]^+$): 409.3, Rt_1=1.06 min.

Example 317: 2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine



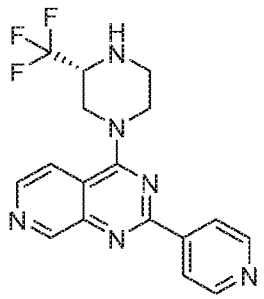
The title compound was synthesized in analogy of **Example 1** in 2 steps from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and *tert-butyl* (3-amino-2,2-dimethylpropyl)carbamate (step 1) and Boc-deprotection using TFA (step 2).
1H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.81 - 8.71 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.39 - 8.29 (m, 2H), 8.16 (d, J = 5.6 Hz, 1H), 3.67 (s, 2H), 0.95 (s, 6H). LCMS (m/z $[M+H]^+$): 309.2, Rt_1=0.43 min.

Example 318: 3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butanamide



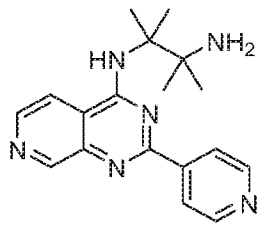
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and 3-amino-3-methylbutanamide.
1H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.94 - 8.80 (m, 2H), 8.77 - 8.60 (m, 2H), 8.48 - 8.35 (m, 2H), 8.10 (d, J = 5.6 Hz, 1H), 7.59 (s, 1H), 7.12 (s, 1H), 2.76 (s, 2H), 1.71 (s, 6H). LCMS (m/z $[M+H]^+$): 323.3, Rt_1=0.57 min.

Example 319: (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine



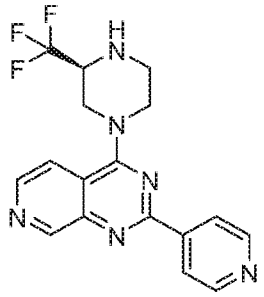
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and (*R*)-2-(trifluoromethyl)piperazine.
1H NMR (600 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.79 (d, J = 5.3 Hz, 2H), 8.63 (d, J = 5.6 Hz, 1H), 8.31 (d, J = 4.9 Hz, 2H), 7.97 (d, J = 5.7 Hz, 1H), 4.60 - 4.49 (m, 1H), 4.28 - 4.21 (m, 1H), 3.82 - 3.74 (m, 1H), 3.71 - 3.64 (m, 1H), 3.63 - 3.56 (m, 1H), 3.20 - 3.12 (m, 1H), 3.12 - 3.04 (m, 1H), 2.96 - 2.87 (m, 1H). LCMS (m/z $[M+H]^+$): 361.1, Rt_1=0.72 min.

Example 320: 2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine



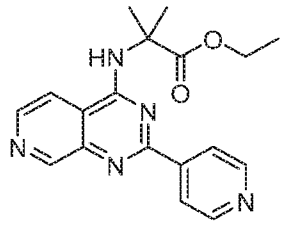
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and 2,3-dimethylbutane-2,3-diamine.
1H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.88 - 8.74 (m, 2H), 8.66 (d, J = 5.5 Hz, 1H), 8.40 - 8.19 (m, 2H), 7.74 (d, J = 5.5 Hz, 1H), 1.63 (s, 6H), 1.21 (s, 6H). LCMS (m/z $[M+H]^+$): 323.2, Rt_1=0.48 min.

Example 321: (*S*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine



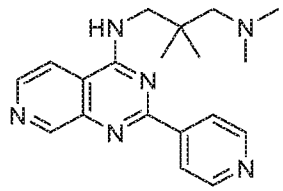
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and (*S*)-2-(trifluoromethyl)piperazine.
1H NMR (600 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.81 - 8.77 (m, 2H), 8.63 (d, J = 5.7 Hz, 1H), 8.34 - 8.29 (m, 2H), 7.97 (d, J = 5.6 Hz, 1H), 4.57 - 4.52 (m, 1H), 4.28 - 4.21 (m, 1H), 3.83 - 3.74 (m, 1H), 3.71 - 3.65 (m, 1H), 3.63 - 3.57 (m, 1H), 3.20 - 3.14 (m, 1H), 3.11 - 3.05 (m, 1H), 2.95 - 2.88 (m, 1H). LCMS (m/z $[M+H]^+$): 361.1, Rt_1=0.72 min.

Example 322: ethyl 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanoate



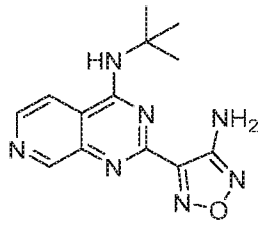
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and ethyl 2-amino-2-methylpropanoate.
1H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.83 (s, 1H), 8.80 - 8.74 (m, 2H), 8.69 (d, J = 5.6 Hz, 1H), 8.39 (d, J = 5.6 Hz, 1H), 8.28 - 8.23 (m, 2H), 3.99 (q, J = 7.1 Hz, 2H), 1.69 (s,6H), 0.91 (t, J = 7.1 Hz, 3H). LCMS (m/z $[M+H]^+$): 338.2, Rt_1=0.78 min.

Example 323: *N*^{1},*N*^{1},2,2-tetramethyl-*N*^{3}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine

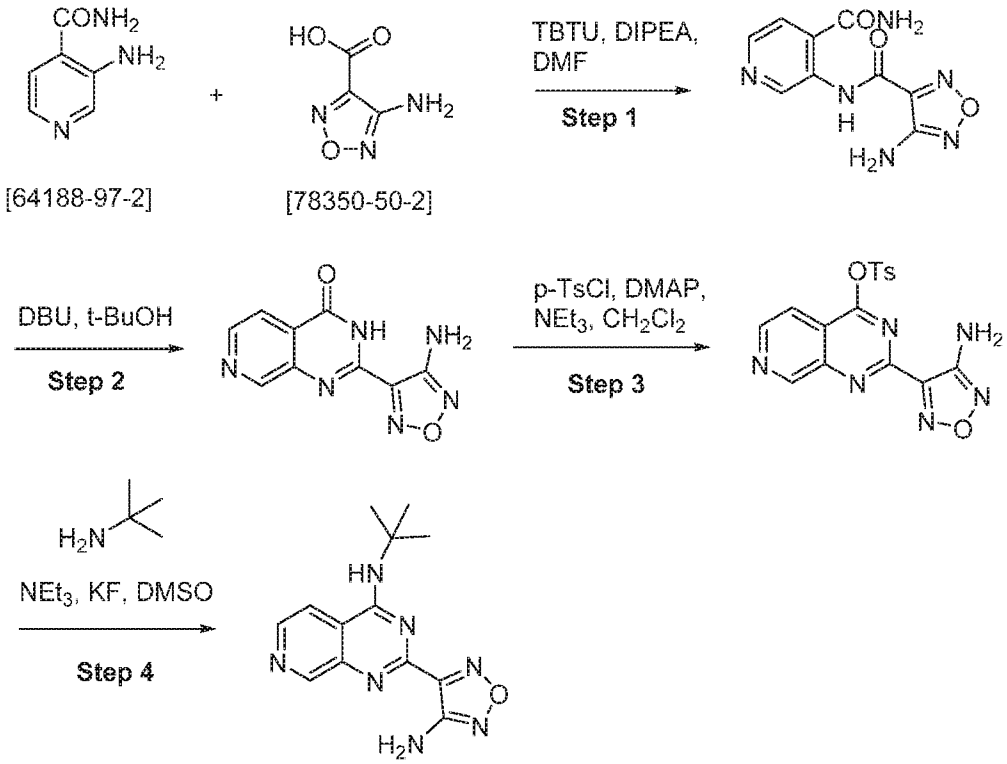


The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and *N*^{1},*N*^{1},2,2-tetramethylpropane-1,3-diamine.
1H NMR (600 MHz, DMSO-d_6) δ 9.20 - 9.17 (m, 1H), 9.04 (t, J = 5.6 Hz, 1H), 8.80 - 8.74 (m, 2H), 8.68 - 8.63 (m, 1H), 8.38 - 8.31 (m, 2H), 8.13 - 8.08 (m, 1H), 3.72 - 3.65 (m, 2H), 2.36 - 2.28 (m, 8H), 0.99 (s, 6H). LCMS (m/z $[M+H]^+$): 337.2, Rt_1=0.46 min.

Example 324: 4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine



The title compound was synthesized according to the scheme below from 3-aminoisonicotinamide and 4-amino-1,2,5-oxadiazole-3-carboxylic acid.



Step 1: 4-amino-N-(4-carbamoylpyridin-3-yl)-1,2,5-oxadiazole-3-carboxamide

In a two necked R.B. flask fitted with thermometer pocket and nitrogen inlet, TBTU (4.75 g, 14.8 mmol) and *N, N*-diisopropylethylamine (2.6 mL, 14.8 mmol) were added to a stirred solution of 3-aminoisonicotinamide (1.7 g, 12.4 mmol) and 4-amino-1,2,5-oxadiazole-3-carboxylic acid (1.92 g, 14.8 mmol) in DMF (40 mL) at 25-30 °C. Resulting reaction mixture was stirred for 72 h at 25-30 °C. Reaction mixture was poured over water (200 mL) and precipitated solid was collected by filtration. Washed with water (100 mL). Purification was done by trituration by using 20% ethyl acetate in n-Hexane to get 4-amino-N-(4-carbamoylpyridin-3-yl)-1,2,5-oxadiazole-3-carboxamide (0.84 g, 27.45%) as Off-white solid. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.60 (s, 1H, Exchangeable with D_2O), 9.70 (s, 1H), 8.64 (s, 1H, Exchangeable with D_2O), 8.52 (d, *J* = 4.4Hz, 1H), 8.81 (s, 1H, Exchangeable with

D_2O), 7.80 (d, *J* = 4.8 Hz, 1H), 6.25 (s, 2H, Exchangeable with D_2O). LCMS (m/z $[M+H]^+$): 249.1, Rt_2=1.312 min.

Step 2: 2-(4-amino-1,2,5-oxadiazol-3-yl)pyrido[3,4-d]pyrimidin-4(3H)-one

In a sealed tube, DBU (0.98 g, 6.45 mmol) was added to a stirred suspension of 4-amino-*N*-(4-carbamoylpyridin-3-yl)-1,2,5-oxadiazole-3-carboxamide (0.8 g, 3.22 mmol) in *tert*-butanol (8.0 mL). Sealed the cap and heated reaction mixture to 100 °C for 16 h. Solvent was evaporated, resulting residue was diluted with water (20 mL) and stirred for 0.5 h at 25-30 °C. Precipitated solid was collected by filtration, washed solid with water (2x10mL) and *n*-hexane (10 mL). The obtained solid were dried at a 55 ° C in rotating flask to obtain (0.421 g, 56.73 %) of 2-(4-amino-1,2,5-oxadiazol-3-yl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one as off-white solid.

^{1}H-NMR(400 MHz, DMSO-d_6): δ 13.31 (s, 1H, Exchangeable with D_2O), 9.36(s, 1H), 8.74 (d, *J* = 5.2Hz, 1H), 8.01 (d, *J* = 5.2 Hz, 1H), 6.95 (s, 2H, Exchangeable with D_2O). LCMS (m/z $[M+H]^+$): 231.2, Rt_2=1.279 min.

Step 3: 2-(4-amino-1,2,5-oxadiazol-3-yl)pyrido[3,4-d]pyrimidin-4-yl 4-methylbenzenesulfonate

In a two necked R.B. flask fitted with, thermometer pocket and nitrogen inlet, DMAP (0.015 g, 0.116 mmol) was added to a stirred solution of 2-(4-amino-1,2,5-oxadiazol-3-yl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (0.27 g, 1.168 mmol) and triethylamine (0.245 mL, 1.752 mmol) in dichloromethane (10 mL) at 0 °C. *p*-TsCl (0.245 g, 1.168 mmol) was added and stirred resulting reaction mixture for 0.5 h. Allowed reaction mixture to warm to 25-30°C by removing ice bath and stirred at 25-30°C for 1.5 h. The mixture was diluted with DCM (20 mL), washed with water (2 x 20mL) and brine (20 mL). Dried organic layers over anhydrous sodium sulphate and filtered. Filtrate was concentrated on rotavapor to get 2-(4-amino-1,2,5-oxadiazol-3-yl)pyrido[3,4-*d*]pyrimidin-4-yl 4-methylbenzenesulfonate (0.285 g, 63.21%) as crude. Crude was used for next step without purification. LCMS (m/z $[M+H]^+$): 385.1, Rt_2=1.779 min.

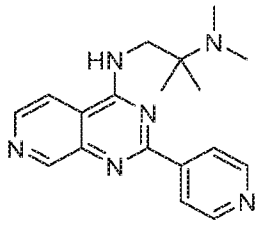
Step 4: 4-(4-(tert-butylamino)pyrido[3,4-d]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine

In a two necked R.B. flask fitted with, thermometer pocket and nitrogen inlet, *tert*-Butyl amine (0.0714 g, 0.976 mmol) was added to a stirred solution of the 2-(4-amino-1,2,5-oxadiazol-3-yl)pyrido[3,4-*d*]pyrimidin-4-yl 4-methylbenzenesulfonate (0.25 g, 0.651 mmol) in

DMSO (2.5 mL) followed by added KF (0.0566g, 0.976 mmol) and triethylamine (0.18 mL, 1.304 mmol) at 25-30°C. After being stirred at rt for 45 min reaction mixture was poured over water (25 mL). Precipitated solid was collected by filtration. Washed solid with water (25 mL) and n-hexane (10 mL). Purification was done by trituration in n-hexane: diethyl ether ; 1:1(5 mL) to get 4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine (0.135 g, 72.75%) as Off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.30 (s, 1 H), 8.67 (d, *J*=5.57 Hz, 1 H), 8.39 (d, *J*=5.58 Hz, 1 H), 8.00 (s, 1 H), 6.89 (s, 2 H), 1.60 (s, 9 H). LCMS (m/z $[M+H]^+$): 286.1, Rt_1=0.92 min.

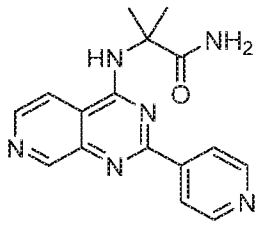
Example 325: *N*^{2},*N*^{2},2-trimethyl-*N*^{1}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and *N*^{2},*N*^{2},2-trimethylpropane-1,2-diamine.

1H NMR (400 MHz, DMSO-d6) δ 9.19 (s, 1H), 8.85 - 8.72 (m, 2H), 8.65 (d, J = 5.6 Hz, 1H), 8.36 - 8.31 (m, 2H), 8.27 (t, J = 6.4 Hz, 2H), 3.79 (d, J = 5.6 Hz, 2H), 2.27 (s, 6H), 1.09 (s, 6H). LCMS (m/z $[M+H]^+$): 323.2, Rt_1=0.44 min.

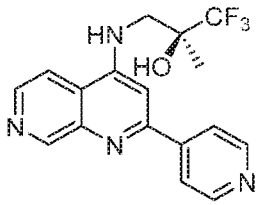
Example 326: 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide



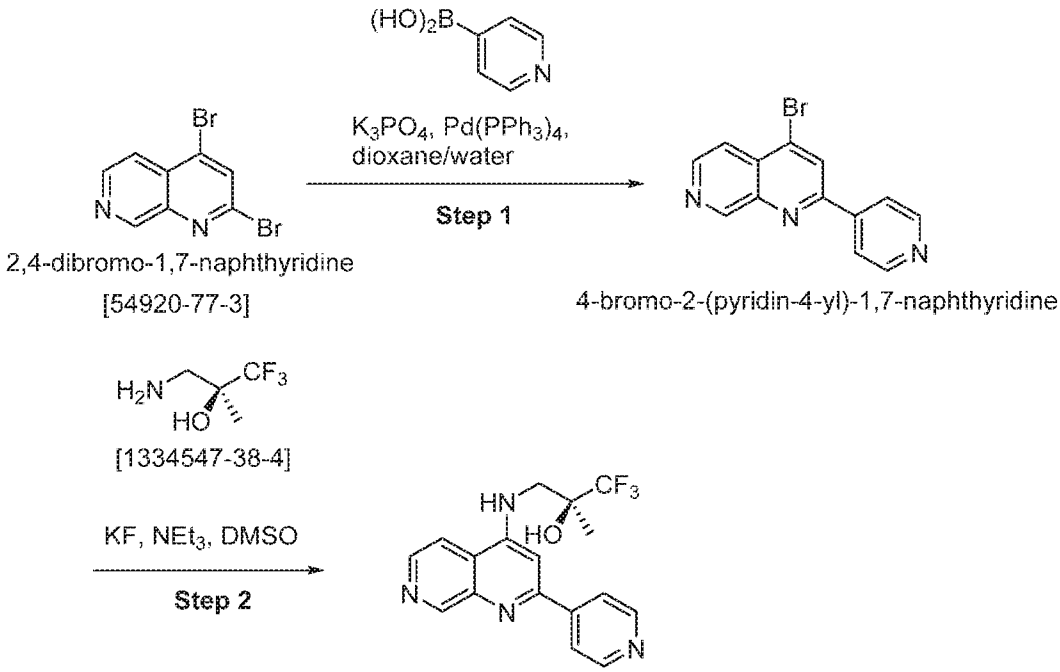
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidine (**Intermediate 1c**) and 2-amino-2-methylpropanamide.

1H NMR (400 MHz, DMSO-d6) δ 9.20 (s, 1H), 8.77 - 8.71 (m, 2H), 8.67 (d, J = 5.5 Hz, 1H), 8.49 (s, 1H), 8.39 (d, J = 5.7 Hz, 1H), 8.36 - 8.30 (m, 2H), 7.29 (s, 1H), 6.84 (s, 1H), 1.63 (s, 6H). LCMS (m/z $[M+H]^+$): 309.2, Rt_1=0.50 min.

Example 327: (*S*)-1,1,1-trifluoro-2-methyl-3-((2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)amino)propan-2-ol



The title compound was synthesized according to the scheme below from 2,4-dibromo-1,7-naphthyridine.

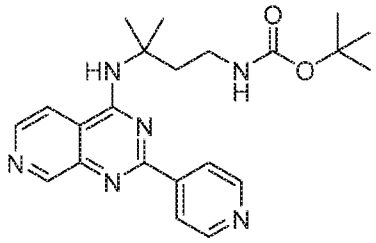


Step 1: A solution of 2,4-dibromo-1,7-naphthyridine (350 mg, 1.22 mmol), 4-pyridinylboronic acid (194 mg, 1.58 mmol), potassium phosphate (1032 mg, 4.86 mmol) and $Pd(PPh_3)_4$ (140 mg, 0.122 mmol) in dioxane (6 mL) / water (1.5 mL) was heated under argon in a microwave vial at 110°C for 60 min. Water was added at r.t. and the reaction mixture was extracted 3 times with dichloromethane. The combined organic phases were washed with brine, dried with sodium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using 0-100% ethyl acetate/cyclohexane to give 4-bromo-2-(pyridin-4-yl)-1,7-naphthyridine (44%). LCMS (m/z $[M+H]^+$): 286.0/288.0, Rt_1=0.83 min.

Step 2: The title compound was synthesized in analogy of **Example 254** from 4-bromo-2-(pyridin-4-yl)-1,7-naphthyridine and (*S*)-3-amino-1,1,1-trifluoro-2-methylpropan-2-ol [1334547-38-4].

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 9.26 (s, 1 H), 8.77 (d, *J*=5.69 Hz, 2 H), 8.53 (d, *J*=5.87 Hz, 1 H), 8.20 (d, *J*=5.69 Hz, 1 H), 8.14 - 8.18 (m, 2 H), 7.48 - 7.55 (m, 2 H), 6.34 (s, 1 H), 3.82 (dd, *J*=14.58, 6.33 Hz, 1 H), 3.69 - 3.76 (m, 1 H), 1.40 (s, 3 H). LCMS (m/z [M+H]$^+$): 349.3, Rt=0.62 min.

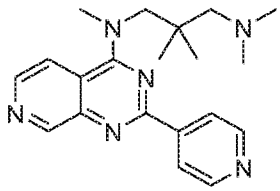
Example 328: ***tert*-butyl (3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butyl)carbamate**



The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidine (**Intermediate 1c**) and *tert*-butyl (3-amino-3-methylbutyl)carbamate.

1H NMR (400 MHz, DMSO-d6) δ 9.18 (s, 1H), 8.88 - 8.73 (m, 2H), 8.64 (d, J = 5.6 Hz, 1H), 8.37 (d, J = 5.7 Hz, 1H), 8.33 - 8.25 (m, 2H), 7.73 (s, 1H), 6.75 (s, 1H), 2.97 (t, J = 7.6 Hz, 2H), 2.31 - 2.19 (m, 2H), 1.60 (s, 6H), 1.25 (s, 9H). LCMS (m/z [M+H]$^+$): 409.2, Rt_1=0.96 min.

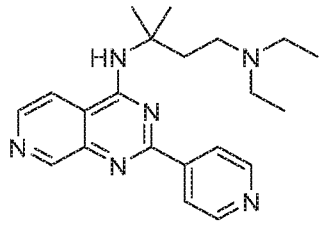
Example 329: ***N*^{1},*N*^{1},*N*^{3},2,2-pentamethyl-*N*^{3}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine**



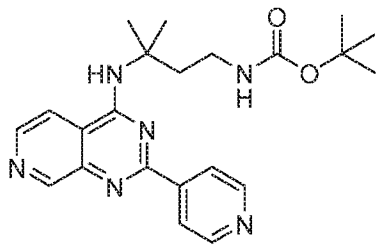
The title compound was synthesized in analogy of **Example 1** from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidine (**Intermediate 1c**) and *N*^{1},*N*^{1},*N*^{3},2,2-pentamethylpropane-1,3-diamine.

1H NMR (400 MHz, DMSO-d6) δ 9.18 (s, 1H), 8.76 (d, 2H), 8.54 (d, J = 5.9 Hz, 1H), 8.34 (d, 2H), 8.23 (d, J = 5.9 Hz, 1H), 4.11 (s, 2H), 3.66 (s, 3H), 2.30 (s, 6H), 2.23 (s, 2H), 0.96 (s, 6H). LCMS (m/z $[M+H]^+$): 351.2, Rt_1=0.50 min.

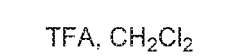
Example 330: N^1,N^1-diethyl-3-methyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine

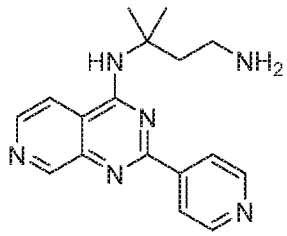


The title compound was synthesized according to the scheme below from **Example 328** (*tert*-butyl (3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butyl)carbamate).

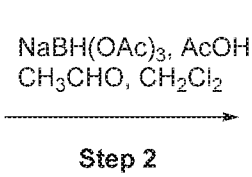


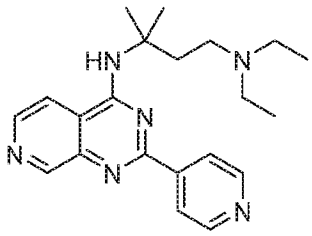
Example 328





3-methyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]-pyrimidin-4-yl)butane-1,3-diamine





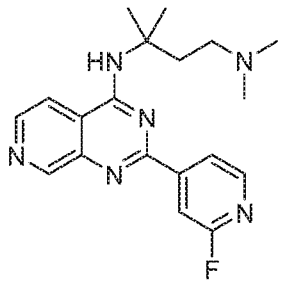
Step 1: To a solution of *tert*-butyl (3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butyl)carbamate (80 mg, 0.196 mmol) in dichloromethane (2 mL) was added TFA (0.377 mL, 4.90 mmol) and the resulting yellow solution was stirred at r.t. for 2 h. Then the

solution was evaporated to dryness. The residue was dissolved in MeOH and the solution was given on a PoraPak Rxn CX 20cc (2g) cartridge. The cartridge was then washed twice with 5 mL MeOH. Finally, the compound was eluted with 7N NH_3 in MeOH. The filtrate was evaporated under reduced pressure to give 3-methyl-*N*^{3}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine (66%). LCMS (m/z $[M+H]^+$): 309.1, Rt_1=0.53 min.

Step 2: A solution of 3-methyl-*N*^{3}-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine (40 mg, 0.130 mmol), acetic acid (0.037 mL, 0.649 mmol) and acetaldehyde (57 mg, 1.297 mmol) in dichloromethane (5 mL) was stirred at r.t. for 1h. Then $NaBH(OAc)_3$ (137 mg, 0.649 mmol) was added and the mixture was stirred for further 1 h at r.t. Saturated $NaHCO_3$-solution was added and the aqueous phase was extracted 3 times with dichloromethane (3 x 20 mL). The combined organic phases were washed with brine, dried with sodium sulfate and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography using a gradient of 12-100% ethyl acetate/cyclohexane followed by a gradient of 2-30% methanol (+10% 7N NH_3 in methanol)/dichloromethane to give a yellow oil. The oil was taken up in dichloromethane (2 mL) and 4N HCL in dioxane (0.2 mL) was added. The precipitate was filtered off and washed with pentane to give the title compound as HCL salt (17%).

1H NMR (400 MHz, Methanol-d4) δ 9.52 (s, 1H), 9.22 - 9.02 (m, 4H), 8.82 (d, J = 6.2 Hz, 1H), 8.74 (dd, J = 6.2, 0.9 Hz, 1H), 3.27 (dd, J = 8.4, 4.7 Hz, 2H), 3.21 (q, J = 7.3 Hz, 4H), 2.84 -2.70 (m, 2H), 1.79 (s, 6H), 1.23 (t, J = 7.3 Hz, 6H). LCMS (m/z $[M+H]^+$): 365.2, Rt_1=0.59 min.

Example 331: *N*^{3}-(2-(2-fluoropyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-*N*^{1},*N*^{1},3-trimethylbutane-1,3-diamine

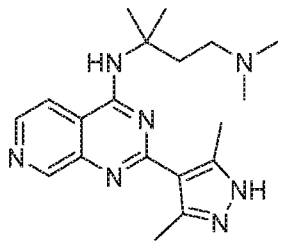


The title compound was synthesized in analogy of **Example 111** in 2 steps from 2,4-dichloropyrido[3,4-*d*]pyrimidine (**Intermediate 2c**) and *N*^{1},*N*^{1},3-trimethylbutane-1,3-diamine

(step 1) and from N^3-(2-chloropyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine and (2-fluoropyridin-4-yl)boronic acid (CAS no. 401815-98-3, step 2).

1H NMR (400 MHz, DMSO-d6) δ 9.53 (s, 1H), 9.18 (s, 1H), 8.69 (d, J = 5.5 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.26 (dd, J = 4.7, 2.0 Hz, 1H), 7.92 (s, 1H), 7.85 (d, J = 5.6 Hz, 1H), 2.26 (s, 6H), 2.00 (t, J = 6.4 Hz, 2H), 1.66 (s, 6H), remark: one CH2 signal is overlapping with DMSO signal. LCMS (m/z $[M+H]^+$): 355.2, Rt_1=0.67 min.

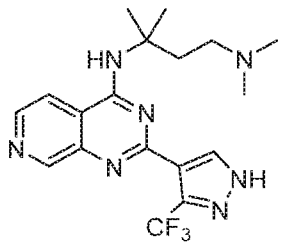
Example 332: N^3-(2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine



The title compound was synthesized in analogy of **Example 111** in 2 steps from 2,4-dichloropyrido[3,4-*d*]pyrimidine (**Intermediate 2c**) and N^1,N^1,3-trimethylbutane-1,3-diamine (step 1) and from N^3-(2-chloropyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine and *tert*-butyl 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole-1-carboxylate (CAS no. 1073354-70-7, step 2).

1H NMR (400 MHz, DMSO-d6) δ 12.41 (s, 1H), 9.11 (s, 1H), 8.95 (s, 1H), 8.49 (d, J = 5.5 Hz, 1H), 7.68 (d, J = 5.6 Hz, 1H), 2.56 (s, 6H), 2.27 (s, 6H), 1.92 (t, J = 6.1 Hz, 2H), 1.61 (s, 6H). remark: one CH2 signal is overlapping with DMSO signal. LCMS (m/z $[M+H]^+$): 354.2, Rt_1=0.50 min.

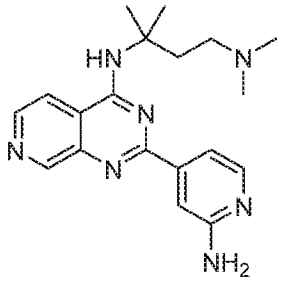
Example 333: N^1,N^1,3-trimethyl-N^3-(2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine



The title compound was synthesized in analogy of **Example 111** in 2 steps from 2,4-dichloropyrido[3,4-*d*]pyrimidine (**Intermediate 2c**) and N^1,N^1,3-trimethylbutane-1,3-diamine (step 1) and from N^3-(2-chloropyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)-1H-pyrazole (CAS no. 1218790-40-9, step 2).

1H NMR (400 MHz, DMSO-d6) δ 13.80 (s, 1H), 9.25 (s, 1H), 8.96 (s, 1H), 8.57 (d, J = 5.5 Hz, 1H), 8.46 (s, 1H), 7.75 (d, J = 5.6 Hz, 1H), 2.25 (s, 6H), 1.93 (t, J = 6.3 Hz, 2H), 1.60 (s, 6H). remark: one CH2 signal is overlapping with DMSO signal. LCMS (m/z $[M+H]^+$): 394.3, Rt_1=0.62 min.

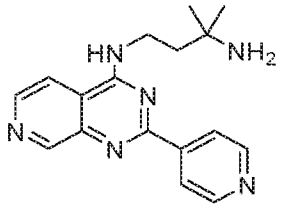
Example 334: N^3-(2-(2-aminopyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine



The title compound was synthesized in analogy of **Example 111** in 3 steps from 2,4-dichloropyrido[3,4-*d*]pyrimidine (**Intermediate 2c**) and N^1,N^1,3-trimethylbutane-1,3-diamine (step 1), from N^3-(2-chloropyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine and *tert*-butyl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)carbamate CAS no. 1095708-32-9 (step 2) and Boc-deprotection using TFA (step 3).

1H NMR (400 MHz, DMSO-d6) δ 9.30 (s, 1H), 9.11 (s, 1H), 8.62 (d, J = 5.6 Hz, 1H), 8.07 (d, J = 5.3 Hz, 1H), 7.82 (d, J = 5.6 Hz, 1H), 7.49 (s, 1H), 7.43 (dd, J = 5.3, 1.4 Hz, 1H), 6.11 (s, 2H), 2.47 (d, J = 8.1 Hz, 2H), 2.24 (s, 6H), 1.99 (t, J = 6.4 Hz, 2H), 1.66 (s, 6H). LCMS (m/z $[M+H]^+$): 352.2, Rt_1=0.48 min.

Example 335: 3-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine



The title compound was synthesized in analogy of **Example 1** in 2 steps from 4-chloro-2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidine (**Intermediate 1c**) and *tert*-butyl (4-amino-2-methylbutan-2-yl)carbamate (step 1) and Boc-deprotection using TFA (step 2).
1H NMR (400 MHz, DMSO-d6) δ 9.18 (s, 1H), 8.80 - 8.71 (m, 2H), 8.63 (d, J = 5.5 Hz, 1H), 8.37 - 8.31 (m, 2H), 8.07 (d, J = 5.5 Hz, 1H), 3.83 - 3.73 (m, 2H), 1.80 - 1.68 (m, 2H), 1.15 (s, 6H), 3 exchangable protons. LCMS (m/z $[M+H]^+$): 309.2, Rt_1=0.45 min.

Ex-vivo cell population expansion and use in therapy

Starting material to prepare an expanded population of cells:

Autologous method

The seeding population of cells for use in the method of cell population expansion to obtain an expanded population of cells may be obtained from a recipient himself/herself. In patients where tissue, organ, or cell deficiency is partial, for example healthy cells are present, the seeding population of cells may be obtained from non-affected tissue or organ or cell source. For example, in the case of unilateral ocular cell deficiency, the seeding population may be obtained from a biopsy on the non-affected eye. It may also be obtained from healthy tissue remaining in an organ that is partially damaged.

Allogenic method

In a preferred embodiment, the seeding population of cells for use in the method of cell population expansion to obtain an expanded population of cells may be obtained from cells originally derived from donor tissue (e.g. human, rabbit, monkey etc, preferably human). For example a source of human tissue is from cadaveric donors or tissues from living donors, including living relatives.

From autologous or allogenic tissue derived as described above under autologous and allogenic methods which has been removed from the body, the cells may, for example, be

extracted and prepared as follows: The desired area may be dissected, for example, using scalpels and the cells then dissociated (e.g. using collagenase, dispase, trypsin, accutase or TripLE; for example 1 mg/ml collagenase at 37^{0}C), until cell detachment becomes apparent by microscopic observation (e.g. using a Zeiss Axiovert inverted microscope) from 45 minutes to 3 hours.

For use in the cell population expansion method according to the invention the isolated cells are then added to medium, for example by pipetting, as described below in the section "Cell population expansion".

In a preferred embodiment according to the invention, an assessment of the quality of cellular material harvested from the donor is performed. For example, approximately 24 hours after harvesting the cells and beginning culturing in medium (growth or cell proliferation medium, as described below), a visual assessment under brightfield microscope to look for floating cells present (as an indicator of dead cells) may be performed. Ideally this assessment is to show that there is approximately less than 10% as floating cells for the material to be suitable for use to generate an expanded population of cells according to the invention.

The number of cells suitable for use in the method of cell population expansion according to the invention is not limited, but as an example for illustrative purposes, the seeding cell population suitable for use in the method of cell population expansion according to the invention may comprise approximately 1000 cells.

If it is desired to measure the cell numbers in the seeding cell population, this may be done for example by manual or automated cell counting using a light microscope, immunohistochemistry or FACS according to standard protocols well known in the art.

Ex-vivo ocular cell population expansion and use in therapy

Described below in more detail is a description of the methodology relating to expansion of ocular cell populations (preparation of starting material, followed by cell population expansion phase, storage of cells) as applied to ocular cells with the specific examples of limbal stem cells and corneal endothelial cells.

Starting material to prepare an expanded population of limbal stem cells: Corneal epithelial and limbal cells

Autologous method

The seeding population of cells for use in the method of cell population expansion to obtain an expanded population of limbal stem cells may be obtained from the recipient himself/herself. In patients where limbal stem cell deficiency is partial, the seeding population of cells may be obtained from non-affected parts of the limbus. For example, in the case of unilateral limbal stem cell deficiency, the seeding population may be obtained from a biopsy on the non-affected eye. It may also be obtained from healthy tissue remaining in a limbus that is partially damaged.

Allogenic method

In a preferred embodiment, the seeding population of cells for use in the method of cell population expansion to obtain an expanded population of limbal stem cells may be obtained from cells originally derived from donor mammalian corneal tissue (e.g. human, rabbit, monkey etc, preferably human).

For example a source of human corneal tissue is from cadaveric donors (for example sourced through eye banks) or tissues from living donors, including living relatives. A range of donor limbal tissue is suitable for use according to the invention. In a preferred embodiment limbal tissue is obtained from living relatives or donors with a compatible HLA profile.

The tissue that is used to obtain the LSCs may, for example, be a ring of limbal tissue of approximately 4mm in width and 1 mm in height.

From the corneal tissue as described above under autologous and allogenic methods which has been removed from the body, the LSCs may, for example, be extracted and prepared as follows: The limbal epithelial area may be dissected, for example, using scalpels and the cells then dissociated (e.g. using collagenase, dispase, trypsin, accutase or TripLE; for example 1 mg/ml collagenase at 37^{0}C), until cell detachment becomes apparent by

microscopic observation (e.g. using a Zeiss Axiovert inverted microscope) from 45 minutes to 3 hours.

For use in the cell population expansion method according to the invention the isolated cells are then added to medium, for example by pipetting, as described below in the section "Cell population expansion".

In a preferred embodiment according to the invention, an assessment of the quality of cellular material harvested from the donor cornea is performed. For example, approximately 24 hours after harvesting the cells and beginning culturing in medium (growth or cell proliferation medium, as described below), a visual assessment under brightfield microscope to look for floating cells present (as an indicator of dead cells) may be performed. Ideally this assessment is to show that there is approximately less than 10% as floating cells for the material to be suitable for use to generate an expanded population of cells according to the invention.

The number of cells suitable for use in the method of cell population expansion according to the invention is not limited, but as an example for illustrative purposes, the seeding cell population suitable for use in the method of cell population expansion according to the invention may comprise approximately 1 000 limbal stem cells.

If it is desired to measure the cell numbers in the seeding cell population, this may be done for example by manual or automated cell counting using a light microscope, immunohistochemistry or FACS according to standard protocols well known in the art.

Starting material to prepare an expanded population of corneal endothelial cells

The seeding population of corneal endothelial cells (CECs) for use in the method of cell population expansion may be obtained from cells originally derived from mammalian corneal tissue (e.g. human, rabbit, monkey etc, preferably human). For example, a source of human corneal tissue is from cadaveric human donors (which may be sourced through eye banks).

The age of the donors can range, for example, from infancy to 70 years of age. Preferably also suitable donors are those who have no history of corneal disease or trauma. In one embodiment according to the invention, preferred donor corneas are those where the corneal endothelial cell count is above 2 000 cells/mm^2 (area). In a more preferred embodiment according to the invention the corneal endothelial cell count is 2 000 to 3 500 cells/mm^2 (area). This is measured for example by examining the cornea of the donor material under a direct light microscope or a specular microscope as per standard Eye Bank techniques known in the art for evaluation of donor tissue before transplantation to patients (see Tran et al (2016) Comparison of Endothelial Cell Measurements by Two Eye Bank Specular Micorscopes; International Journal of Eye Banking; vol 4., no 2; 1-8, which is herein incorporated by reference).

The surface of cornea that is used to obtain the CECs is not limited, but may, for example, be an area of approx. 8-10mm in diameter.

The CECs may, for example, be extracted and prepared as follows from the donor corneal tissue: The corneal endothelial cell layer and Descemet's membrane (DM) are scored, for example with a surgical-grade reverse Sinsky endothelial stripper. The DM-endothelial cell layer is peeled off the corneal stroma and cells are dissociated from the DM, for example using 1 mg/ml collagenase at 37^0C until cell detachment becomes apparent by microscopic observation (e.g. using a Zeiss Axiovert inverted microscope) (from 45 minutes to 3 hours). As the DM only carries corneal endothelial cells in the cornea, the cell population isolated in this manner is a population of CECs, which is suitable for use as a seeding population of cells according to the invention.

For use in the method of cell population expansion according to the invention the isolated corneal endothelial cells may be added to medium as described below in the section "Cell population expansion".

In a preferred embodiment according to the invention, an assessment of the quality of cellular material harvested from the donor cornea is performed. For example, approximately 24 hours after harvesting the cells and beginning culturing in medium (growth or cell proliferation medium, as described below), a visual assessment under brightfield microscope to look for floating cells present (as an indicator of dead cells) may be performed. Ideally this

assessment is to show that there is approximately less than 10% as floating cells for the material to be suitable for use to generate an expanded population of cells according to the invention.

The starting number of cells suitable for use in the method of cell population expansion according to the invention is not limited, but as an example for illustrative purposes, the seeding population of corneal endothelial cells suitable for use in the method of cell population expansion according to the invention may be 100 000 to 275 000 cells.

If it is desired to measure the cell numbers in the seeding cell population, this may be done for example by taking an aliquot and performing immunocytochemistry (e.g. to count nuclei stained with Sytox Orange) or by live cell imaging under brightfield microscope to count the number of cells.

The Sytox Orange assay may be performed according to standard protocols known in the art. In brief, after cells have attached to the cell culture dish (typically 24h after cell plating), the cells are fixed in paraformaldehyde. The cells are then permeabilized (e.g. using a solution of 0.3% Triton X-100) and they are then labeled in a solution of Sytox Orange (e.g. using 0.5 micromolar of Sytox Orange in PBS). The number of nuclei stained with Sytox Orange per surface area are then counted under a Zeiss epifluorescence microscope.

Cell population expansion

In one embodiment of the invention, a population of cells comprising cells from a patient or a donor, can be grown in medium in a culture container known in the art, such as plates, multi-well plates, and cell culture flasks. For example, a culture dish may be used which is non-coated or coated with collagen, synthemax, gelatin or fibronectin. A preferred example of a suitable culture container is a non-coated plate. Standard culturing containers and equipment such as bioreactors known in the art for industrial use may also be used.

The medium used may be a growth medium or a cell proliferation medium. In general, a growth medium is a culture medium supporting the growth and maintenance of a population of cells. Those of skill in art can readily determine an appropriate growth medium for a particular type of cell population. Suitable growth mediums are known in the art for stem cell

culture or epithelial cell culture are for example: DMEM (Dulbecco's Modified Eagle's Medium) supplemented with FBS (Fetal Bovine Serum) (Invitrogen), human endothelial SF (serum free) medium (Invitrogen) supplemented with human serum, X-VIVO15 medium (Lonza), or DMEM/F12 (Thermo Fischer Scientific) (optionally supplemented with calcium chloride). These may be additionally supplemented with growth factors (e.g. bFGF), and/or antibiotics such as penicillin and streptomycin.

Alternatively, isolated cells may be added first to a cell proliferation medium according to the invention. The cell proliferation medium as defined herein comprises a growth medium and a LATS inhibitor according to the invention.

In certain embodiments, a cell proliferation medium of the invention comprises a growth medium and a LATS inhibitor according to the invention. The LATS inhibitor is preferably selected from the group comprising compounds according to Formula A1 or subformulae thereof and as further described under the section "LATS inhibitors".

In a preferred embodiment the LATS inhibitors according to Formula A1 or subformulae thereof are added at a concentration of about 0.5 to 100 micromolar, preferably about 0.5 to 25 micromolar, more preferably about 1 to 20 micromolar. In a specific embodiment the LATS inhibitors according to Formula A1 or subformulae thereof are added at a concentration of about 3 to 10 micromolar.

In one embodiment, the stock solution of the compound according to Formula A1 or subformulae thereof may be prepared by dissolving the compound powder to a stock concentration of 10mM in DMSO.

In one aspect of the invention the LATS inhibitor according to the invention inhibits LATS1 and/or LATS2 activity in the cell population. In a preferred embodiment the LATS inhibitor inhibits LATS1 and LATS2.

The cells may go through a round or rounds of addition of fresh growth medium and/or cell proliferation medium. The cells do not need to be passaged in order for fresh medium to be added, but passaging cells is also a way to add fresh medium.

A series of mediums may be also used, in various combinations of orders: for example a cell proliferation medium, followed by addition of a growth medium (which is not supplemented with LATS inhibitors according to the invention, and may be different to the growth medium used as the base for the cell proliferation medium).

The cell population expansion phase according to the invention occurs during the period the cells are exposed to the cell proliferation medium.

Standard temperature conditions known in the art for culturing cells may be used, for example preferably about 30°C to 40°C. Particularly preferably cell growth, as well as the cell population expansion phase is carried out at about 37°C. A conventional cell incubator with 5-10% CO_2 levels may be used. Preferably the cells are exposed to 5% CO_2.

The cells may be passaged during the culturing in the growth or cell proliferation medium as necessary. Cells may be passaged when they are sub-confluent or confluent. Preferably the cells are passaged when they reach approximately 90%-100% confluency, although lower percentage confluency levels may also be performed. The passaging of cells is done according to standard protocols known in the art. For example, in brief cells are passaged by treating cultures with Accutase (e.g. for 10 minutes), rinsing the cell suspension by centrifugation and plating cells in fresh growth medium or cell proliferation medium as desired. Cell splitting ratios range, for example, from 1:2 to 1:5.

For the cell population expansion phase of the method of cell population expansion according to the invention, the expansion of the seeding cell population in the cell proliferation medium may be performed until the required amount of cellular material is obtained.

The cells may be exposed to the cell proliferation medium for a range of time periods in order to expand the cell population.

In a preferred embodiment the seeding cell population is exposed to the LATS inhibitors according to the invention (such as those compounds according to Formula A1 or subformulae thereof) directly after cell isolation from the patient or donor tissue and maintained for the entire time that cell proliferation is required, for example 12 to 16 days.

In one embodiment according to the invention, a gene editing technique may optionally be performed to genetically modify cells and/or to express a biotherapeutic compound. For example, the cells may be modified to reduce or eliminate the expression and/or function of an immune response mediating gene, which may otherwise contribute to immune rejection when the cell population is delivered to the patient. The application of gene editing techniques in the method of cell population expansion according to the invention is optional, and the administration to the patient of topical immunosuppressants and/or anti-inflammatory agents (as described further under the section Immunosuppressant and Anti-inflammatory agent) may instead be used if desired to mitigate issues with immunorejection of the transplanted material in the patient.

According to one aspect of the invention, genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response. In a preferred embodiment, genetically modifying comprises introducing into an isolated stem cell or stem cell population a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. In a specific embodiment, said gene editing system is selected from the group consisting of CRISPR (CRISPR: clustered regularly interspaced short palindromic repeats, also known as CRISPR/Cas systems), ZFN (Zinc-finger nucleases), TALEN (transcription activator-like effector based nucleases), engineered meganucleases (e.g. ARCUS nucleases, such as the ARC nuclease), AAV vector (adeno-associated virus) and lentiviral vectors-based genome editing technologies.

A gene editing technique, if it is to be used, may be performed at different points, such as for example (1) on tissue, before cell isolation or (2) at the time of cell isolation or (3) during the cell population expansion phase in vitro (when the cells are exposed to a LATS inhibitor according to the invention in vitro) or (4) in vitro at the end of the cell population expansion phase (after the cells are exposed to a LATS inhibitor according to the invention in vitro). In a specific embodiment, CRISPR is used after two weeks of in vitro expansion of the cell population in the presence of the LATS inhibitor according to the invention.

The gene editing techniques suitable for use in the method of cell population expansion are further described under the section “reduction of immunorejection”.

In the method of cell population expansion according to the invention the LATS inhibitors, which are preferably compounds, produce greater than 2 fold expansion of the seeded population of cells.

In one aspect of the method of cell population expansion according to the invention the compounds according to Formula A1 or subformulae thereof produce greater than 30 fold expansion of the seeded population of isolated cells (i.e., cells obtained from a patient or a donor). In a specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce 100 fold to 2200 fold expansion of the seeded population of isolated cells. In a more specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce 600 fold to 2200 fold expansion of the seeded population of isolated cells. The fold expansion factor achieved by the method of cell population expansion according to the invention may be achieved in one or more passages of the cells. In another aspect of the invention the fold expansion factor achieved by the method of cell population expansion according to the invention may be achieved after exposure to the compound according to Formula A1 or subformulae thereof for about 12 to 16 days, preferably about 14 days.

If it is desired to measure the cell number or expansion of the cell population, this may be done for example by taking an aliquot and performing immunocytochemistry (e.g. to count nuclei stained with Sytox Orange) or by live cell imaging under brightfield microscope to count the number of cells or by performing real-time quantitative live-cell analysis of cell confluence at various time points during the cell population expansion phase of the method according to the invention.

The Sytox Orange assay may be performed according to standard protocols known in the art. In brief, after cells have attached to the cell culture dish (typically 24h after cell plating), the cells are fixed in paraformaldehyde. The cells are then permeabilized (e.g. using a solution of 0.3% Triton X-100) and they are then labeled in a solution of Sytox Orange (e.g. using 0.5 micromolar of Sytox Orange in PBS). The number of nuclei stained with Sytox Orange per surface area are then counted under a Zeiss epifluorescence microscope. The cell population expanded by the method of cell population expansion according to the invention may be added to a solution and then stored, for example in a preservation or

cryopreservation solution (such as those described below), or added directly to a composition suitable for delivery to a patient. The preservation, cryopreservation solution or composition suitable for ocular delivery may optionally comprise a LATS inhibitor according to the invention.

In a more preferred embodiment according to the invention, the cell population preparation which is delivered to a patient comprises very low to negligible levels of a LATS inhibitor compound. Thus in a specific embodiment, the method of cell population expansion according to the invention comprises the further step of rinsing to substantially remove the compound according to the invention (such as the compound according to Formula A1 or subformulae thereof). This may involve rinsing the cells after the cell population expansion phase according to the invention. To rinse the cells, the cells are detached from the culture dish (e.g. by treating with Accutase), the detached cells are then centrifuged, and a cell suspension is made in PBS or growth medium according to the invention. This step may be performed multiple times, e.g. one to ten times, to rinse out the cells. Finally the cells may be resuspended in a preservation solution, cryopreservation solution, a composition suitable for ocular delivery, growth medium or combinations thereof as desired.

The expanded population of cells prepared by the method of cell population expansion and rinsed of cell proliferation medium comprising a LATS inhibitor according the invention may be transferred to a composition suitable for delivery to a patient, such as for example a localising agent. Optionally the cell population is stored for a period before addition to a localising agent suitable for delivery to a patient. In a preferred embodiment, the expanded cell population may first be added to a solution suitable for preservation or cryopreservation, which preferably does not comprise a LATS inhibitor, and the cell population stored (optionally with freezing) before addition to a localising agent suitable for delivery to a patient, which also preferably does not comprise a LATS inhibitor.

Typical solutions suitable for cryopreservation, glycerol, dimethyl sulfoxide, propylene glycol or acetamide may be used in the cryopreservation solution of the present invention. The cryopreserved preparation of cells is typically kept at -20°C or -80°C.

Cell population expansion : To prepare an expanded population of limbal stem cells

In one embodiment of the invention, a population of cells comprising corneal epithelial and limbal cells, including limbal stem cells, for example obtained as described in the section "Starting material to prepare an expanded population of limbal stem cells: Corneal epithelial and limbal cells", can be grown in medium in a culture container known in the art, such as plates, multi-well plates, and cell culture flasks. For example, a culture dish may be used which is non-coated or coated with collagen, synthemax, gelatin or fibronectin. A preferred example of a suitable culture container is a non-coated plate. Standard culturing containers and equipment such as bioreactors known in the art for industrial use may also be used.

The medium used may be a growth medium or a cell proliferation medium. A growth medium is defined herein as a culture medium supporting the growth and maintenance of a population of cells. Suitable growth mediums are known in the art for stem cell culture or epithelial cell culture are for example: DMEM (Dulbecco's Modified Eagle's Medium) supplemented with FBS (Fetal Bovine Serum) (Invitrogen), human endothelial SF (serum free) medium (Invitrogen) supplemented with human serum, X-VIVO15 medium (Lonza), or DMEM/F12 (Thermo Fischer Scientific) (optionally supplemented with calcium chloride). These may be additionally supplemented with growth factors (e.g. bFGF), and/or antibiotics such as penicillin and streptomycin. A preferred growth medium according to the invention is X-VIVO15 medium (which is not additionally supplemented with growth factors).

Alternatively, the isolated cells may be added first to a cell proliferation medium according to the invention. The cell proliferation medium as defined herein comprises a growth medium and a LATS inhibitor according to the invention. In the cell proliferation medium according to the invention the growth medium component is selected from the group consisting of DMEM (Dulbecco's Modified Eagle's Medium) supplemented with FBS (Fetal Bovine Serum) (Invitrogen), human endothelial SF (serum free) medium (Invitrogen) supplemented with human serum, X-VIVO15 medium (Lonza or DMEM/F12 (Thermo Fischer Scientific) (optionally supplemented with calcium chloride). These may be additionally supplemented with growth factors (e.g. bFGF), and/or antibiotics such as penicillin and streptomycin.

A preferred cell proliferation medium according to the invention is X-VIVO15 medium (Lonza) with a LATS inhibitor according to the invention. This cell proliferation medium has the advantage that it does not need additional growth factors or feeder cells to facilitate the proliferation of the LSCs. X-VIVO medium comprises inter alia pharmaceutical grade human

albumin, recombinant human insulin, and pasteurized human transferrin. Optionally antibiotics may be added to X-VIVO15 medium. In a preferred embodiment, X-VIVO15 medium is used without the addition of antibiotics.

The cell proliferation medium comprises a growth medium and a LATS inhibitor according to the invention. The LATS inhibitor is preferably selected from the group comprising compounds according to Formula A1 or subformulae thereof and as further described under the section “LATS inhibitors”.

In a preferred embodiment the LATS inhibitors according to Formula A1 or subformulae thereof are added at a concentration of about 0.5 to 100 micromolar, preferably about 0.5 to 25 micromolar, more preferably about 1 to 20 micromolar. In a specific embodiment the LATS inhibitors according to Formula A1 or subformulae thereof are added at a concentration of about 3 to 10 micromolar.

In one embodiment, the stock solution of the compound according to Formula A1 or subformulae thereof may be prepared by dissolving the compound powder to a stock concentration of 10mM in DMSO.

In one aspect of the invention the LATS inhibitor according to the invention inhibits LATS1 and/or LATS2 activity in the limbal cells. In a preferred embodiment the LATS inhibitor inhibits LATS1 and LATS2.

The cells may go through a round or rounds of addition of fresh growth medium and/or cell proliferation medium. The cells do not need to be passaged in order for fresh medium to be added, but passaging cells is also a way to add fresh medium.

A series of mediums may be also used, in various combinations of orders: for example a cell proliferation medium, followed by addition of a growth medium (which is not supplemented with LATS inhibitors according to the invention, and may be different to the growth medium used as the base for the cell proliferation medium).

The cell population expansion phase according to the invention occurs during the period the cells are exposed to the cell proliferation medium.

Standard temperature conditions known in the art for culturing cells may be used, for example preferably about 30°C to 40°C. Particularly preferably cell growth, as well as the cell population expansion phase is carried out at about 37°C. A conventional cell incubator with 5-10% CO_2 levels may be used. Preferably the cells are exposed to 5% CO_2.

The cells may be passaged during the culturing in the growth or cell proliferation medium as necessary. Cells may be passaged when they are sub-confluent or confluent. Preferably the cells are passaged when they reach approximately 90%-100% confluency, although lower percentage confluency levels may also be performed. The passaging of cells is done according to standard protocols known in the art. For example, in brief cells are passaged by treating cultures with Accutase (e.g. for 10 minutes), rinsing the cell suspension by centrifugation and plating cells in fresh growth medium or cell proliferation medium as desired. Cell splitting ratios range, for example, from 1:2 to 1:5.

For the cell population expansion phase of the method of cell population expansion according to the invention, the expansion of the seeding cell population in the cell proliferation medium may be performed until the required amount of cellular material is obtained.

The cells may be exposed to the cell proliferation medium for a range of time periods in order to expand the cell population. For example this may include the entire time that the LSCs are kept in culture, or for the first week after LSC isolation or for 24 hours after dissection of the limbus from the cornea.

In a preferred embodiment the seeding cell population is exposed to the LATS inhibitors according to the invention (such as those compounds according to Formula A1 or subformulae thereof) directly after cell isolation from the cornea and maintained for the entire time that LSC proliferation is required, for example 12 to 16 days.

In one embodiment according to the invention, a gene editing technique may optionally be performed to genetically modify cells, to reduce or eliminate the expression and/or function of an immune response mediating gene which may otherwise contribute to immune rejection when the cell population is delivered to the patient. The application of gene editing techniques in the method of cell population expansion according to the invention is optional,

and the administration to the patient of topical immunosuppressants and/or anti-inflammatory agents (as described further under the section Immunosuppressant and Anti-inflammatory agent) may instead be used if desired to mitigate issues with immunorejection of the transplanted material in the patient.

According to one aspect of the invention, genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response. In a preferred embodiment, genetically modifying comprises introducing into a limbal stem cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. In a specific embodiment, said gene editing system is selected from the group consisting of CRISPR (CRISPR: clustered regularly interspaced short palindromic repeats, also known as CRISPR/Cas systems), ZFN (Zinc-finger nucleases), TALEN (transcription activator-like effector based nucleases), engineered meganucleases (e.g. ARCUS nucleases, such as the ARC nuclease), AAV vector (adeno-associated virus) gene editing (e.g., AAV vector driven homologous recombination) and lentiviral vectors-based genome editing technologies. AAV vector driven gene delivery, such as driven by homologous recombination, can be achieved by an AAV selected from the group consisting of: AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or a derivative thereof.

A gene editing technique, if it is to be used, may be performed at different points, such as for example (1) on limbal epithelial tissue, before LSC isolation or (2) at the time of cell isolation or (3) during the cell population expansion phase in vitro (when the cells are exposed to a LATS inhibitor according to the invention in vitro) or (4) in vitro at the end of the cell population expansion phase (after the cells are exposed to a LATS inhibitor according to the invention in vitro). In a specific embodiment CRISPR is used after two weeks of in vitro expansion of the cell population in the presence of the LATS inhibitor according to the invention.

The gene editing techniques suitable for use in the method of cell population expansion are further described under the section “reduction of immunorejection”.

In the method of cell population expansion according to the invention the LATS inhibitors, which are preferably compounds, produce greater than 2 fold expansion of the seeded population of cells.

In one aspect of the method of cell population expansion according to the invention the compounds according to Formula A1 or subformulae thereof produce greater than 30 fold expansion of the seeded population of limbal cells. In a specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce 100 fold to 2200 fold expansion of the seeded population of limbal cells. In a more specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce 600 fold to 2200 fold expansion of the seeded population of limbal cells. The fold expansion factor achieved by the method of cell population expansion according to the invention may be achieved in one or more passages of the cells. In another aspect of the invention the fold expansion factor achieved by the method of cell population expansion according to the invention may be achieved after exposure to the compound according to Formula A1 or subformulae thereof for about 12 to 16 days, preferably about 14 days.

In one aspect of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce a cell population with more than 6% of p63alpha positive cells compared to the total amount of cells. In a specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce a cell population with more than 20% of p63alpha positive cells compared to the total amount of cells. In another specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce a cell population with more than 70% of p63alpha positive cells compared to the total amount of cells. In yet another specific embodiment of the method of cell population expansion according to the invention the LATS inhibitors according to Formula A1 or subformulae thereof produce a cell population with more than 95% of p63alpha positive cells compared to the total amount of cells. The increase in the percentage of p63alpha positive cells achieved by the method of cell population expansion according to the invention may be achieved in one or more passages of the cells. In another aspect of the invention the

increase in the percentage of p63alpha positive cells achieved by the method of cell population expansion according to the invention may be achieved after exposure to the compound according to Formula A1 or subformulae thereof for about 12 to 16 days, preferably about 14 days.

If it is desired to measure the cell number or expansion of the cell population, this may be done for example by taking an aliquot and performing immunocytochemistry (e.g. to count nuclei stained with Sytox Orange) or by live cell imaging under brightfield microscope to count the number of cells or by performing real-time quantitative live-cell analysis of cell confluence at various time points during the cell population expansion phase of the method according to the invention.

The Sytox Orange assay may be performed according to standard protocols known in the art. In brief, after cells have attached to the cell culture dish (typically 24h after cell plating), the cells are fixed in paraformaldehyde. The cells are then permeabilized (e.g. using a solution of 0.3% Triton X-100) and they are then labeled in a solution of Sytox Orange (e.g. using 0.5 micromolar of Sytox Orange in PBS). The number of nuclei stained with Sytox Orange per surface area are then counted under a Zeiss epifluorescence microscope.

In one aspect according to the invention the LSC population obtainable or obtained by the method of cell population expansion according to the invention preferably shows at least one of the following characteristics. More preferably, it shows two or more, more preferably all, of the following characteristics.

(1) The cell preparation is positive for p63alpha cells. The expression of p63alpha may be estimated by standard techniques known in the art, such as for example immunohistochemistry and quantitative RT-PCR.

(2) The cell preparation comprises more than 6% p63alpha positive cells. Preferably the cell preparation comprises more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% p63alpha positive cells. In a preferred embodiment the cell preparation comprises more than 95% p63alpha positive cells. The percentage of p63alpha cells may be measured by immunohistochemistry or FACS.

(3) The cells express one or more of ABCB5, ABCG2, and C/EBPδ. The expression of ABCB5, ABCG2, and C/EBPδ may be estimated by standard techniques known in the art, such as for example immunohistochemistry and quantitative RT-PCR.

(4) The cells can differentiate into corneal epithelium cells as observed by keratin-12 expression. These characteristics can be observed by immunohistochemistry or FACS.

The cell population expanded by the method of cell population expansion according to the invention may be added to a solution and then stored, for example in a preservation or cryopreservation solution (such as those described below), or added directly to a composition suitable for ocular delivery. The preservation, cryopreservation solution or composition suitable for ocular delivery may optionally comprise a LATS inhibitor according to the invention.

In a more preferred embodiment according to the invention, the cell population preparation which is delivered to the eye comprises very low to neglible levels of a LATS inhibitor compound. Thus in a specific embodiment, the method of cell population expansion according to the invention comprises the further step of rinsing to substantially remove the compound according to the invention (such as the compound according to Formula A1 or subformulae thereof). This may involve rinsing the cells after the cell population expansion phase according to the invention. To rinse the cells, the cells are detached from the culture dish (e.g. by treating with Accutase), the detached cells are then centrifuged, and a cell suspension is made in PBS or growth medium according to the invention. This step may be performed multiple times, e.g. one to ten times, to rinse out the cells. Finally the cells may be resuspended in a preservation solution, cryopreservation solution, a composition suitable for ocular delivery, growth medium or combinations thereof as desired.

The expanded population of cells prepared by the method of cell population expansion and rinsed of cell proliferation medium comprising a LATS inhibitor according the invention may be transferred to a composition suitable for ocular delivery, such as for example a localising agent. Optionally the cell population is stored for a period before addition to a localising agent suitable for ocular delivery. In a preferred embodiment, the expanded cell population may first be added to a solution suitable for preservation or cryopreservation, which preferably does not comprise a LATS inhibitor, and the cell population stored (optionally with

freezing) before addition to a localising agent suitable for ocular delivery, which also preferably does not comprise a LATS inhibitor.

Typical solutions suitable for preservation of LSCs are Optisol or PBS, preferably Optisol. Optisol is a corneal storage medium comprising chondroitin sulfate and dextran to enhance corneal dehydration during storage (see for example Kaufman et al., (1991) Optisol corneal storage medium; Arch Ophthalmol Jun; 109(6): 864-8). For cryopreservation, glycerol, dimethyl sulfoxide, propylene glycol or acetamide may be used in the cryopreservation solution of the present invention. The cryopreserved preparation of cells is typically kept at -20°C or -80°C.

In one aspect the invention relates to a preserved or cryopreserved preparation of limbal stem cells obtainable by the method of cell population expansion according to the invention. In an alternative aspect the invention relates to a fresh cell preparation where limbal stem cells obtainable by the method of cell population expansion according to the invention are in suspension in PBS and/or growth medium or combined with a localising agent. The fresh cell preparation is typically kept at about 15 to 37°C. Standard cell cultures containers known in the art may be used to store the cells, such as a vial or a flask.

In a preferred embodiment according to the invention, before use in the eye, a cryopreserved preparation of cells is thawed (for example by incubating at a temperature of about 37°C in an incubator or waterbath). Preferably 10 volumes of PBS or growth medium may be added to rinse off the cells from the cryopreservant solution. Cells may then be rinsed by centrifugation, and a cell suspension may be made in PBS and/or growth medium, before combination with a localising agent for ocular delivery, which also preferably does not comprise a LATS inhibitor.

In one aspect of the invention the expanded population of cells prepared by the method of cell population expansion, are prepared as a suspension (for example in PBS and/or growth medium, such as for example X-VIVO medium) and combined with a localising agent suitable for ocular delivery, (such as a biomatrix like GelMA). In a specific embodiment of the method of treatment according to the invention, this combination of cells, PBS and/or growth medium, and biomatrix is delivered to the eye via a carrier (such as a contact lens).

In yet another specific embodiment this combination of cells, PBS and/or growth medium, and biomatrix comprises at most only trace levels of a LATS inhibitor.

The term "trace levels" as used herein means less than 5% w/v (e.g., no more than 5% w/v, 4% w/v, 3% w/v, 2% w/v, or 1% w/v), and preferably less than 0.01% w/v (e.g., no more than 0.01% w/v, 0.009% w/v, 0.008% w/v, 0.007% w/v, 0.006% w/v, 0.005% w/v, 0.004% w/v, 0.003% w/v, 0.002% w/v, or 0.001% w/v), which can be measured, for example using high-resolution chromatography as described in the Examples herein. In certain embodiments, trace levels of a LATS inhibitor compound of the invention are the levels of residual compounds present after one or more wash steps, which collectively are below the cellular potency of such compounds, and accordingly they do not induce biological effect in vivo. Accordingly, residual levels of compounds are below the amount expected to have a biological effect on cell population expansion in cell culture or in a subject (e.g., after transplantation of an expanded cell population to the subject). Trace levels can be measured, for example, as the wash-off efficiency, which can be calculated as follows: Wash-off efficiency = 100 - (average concentration in post-wash pellet x pellet volume x molecule weight) / (compound concentration x culture media volume x molecule weight). As used herein, “rinsing to substantially remove” a LATS inhibitor compound of the invention from cells refers to steps for establishing trace levels of the LATS inhibitor compound.

Alternatively, the cells may be cultured and the cell population proliferation phase may occur in cell proliferation medium on a localising agent suitable for cell delivery to the ocular surface (for example fibrin, collagen).

Cell population expansion: To prepare an expanded population of corneal endothelial cells

In a preferred embodiment of the invention, corneal endothelial cells, for example isolated and obtainable as described in the section “Starting material to prepare an expanded population of corneal endothelial cells”, can be grown in medium in a culture container known in the art, such as plates, multi-well plates, and cell culture flasks. For example, a culture dish may be used which is non-coated or coated with collagen, synthemax, gelatin or fibronectin. A preferred example of a suitable culture container is a non-coated plate.

Standard culturing containers and equipment such as bioreactors known in the art for industrial use may also be used.

The medium used may be a growth medium or a cell proliferation medium. A growth medium is defined herein as a culture medium supporting the growth and maintenance of a population of cells. Suitable growth mediums are known in the art for corneal endothelial cell culture are for example: DMEM (Dulbecco's Modified Eagle's Medium) supplemented with FBS (Fetal Bovine Serum) (Invitrogen), human endothelial SF (serum free) medium (Invitrogen) supplemented with human serum, X-VIVO15 medium (Lonza) or mesenchymal stem cell-conditioned medium. These may be additionally supplemented with growth factors (e.g. bFGF), and/or antibiotics such as penicillin and streptomycin. A preferred growth medium according to the invention is X-VIVO15 medium (which is not additionally supplemented with growth factors).

Alternatively, the isolated cells may be added first to a cell proliferation medium according to the invention. The cell proliferation medium as defined herein comprises a growth medium and a LATS inhibitor according to the invention. In the cell proliferation medium according to the invention the growth medium component is selected from the group consisting of DMEM (Dulbecco's Modified Eagle's Medium) supplemented with FBS (Fetal Bovine Serum) (Invitrogen), human endothelial SF (serum free) medium (Invitrogen) supplemented with human serum, X-VIVO15 medium (Lonza) or mesenchymal stem cell-conditioned medium. These may be additionally supplemented with growth factors (e.g. bFGF), and/or antibiotics such as penicillin and streptomycin.

A preferred cell proliferation medium according to the invention is X-VIVO15 medium (Lonza) with a LATS inhibitor according to the invention. This cell proliferation medium has the advantage that it does not need additional growth factors or feeder cells to facilitate the proliferation of the CECs. X-VIVO medium comprises *inter alia* pharmaceutical grade human albumin, recombinant human insulin, and pasteurised human transferrin. Optionally antibiotics may be added to X-VIVO15 medium. In a preferred embodiment, X-VIVO15 medium is used without the addition of antibiotics.

The cell proliferation medium comprises a growth medium and a LATS inhibitor according to the invention. The LATS inhibitor is preferably selected from the group comprising

compounds according to Formula A1 or subformulae thereof and as further described under the section “LATS Inhibitors”.

In a preferred embodiment the LATS inhibitors according to Formula A1 or subformulae thereof are added at a concentration of about 0.5 to 100 micromolar, preferably about 0.5 to 25 micromolar, more preferably about 1 to 20 micromolar. In a specific embodiment the LATS inhibitors according to Formula A1 or subformulae thereof are added at a concentration of about 3 to 10 micromolar.

In one embodiment, the stock solution of the compound according to Formula A1 or subformulae thereof may be prepared by dissolving the compound powder to a stock concentration of 10mM in DMSO.

In one aspect of the invention the LATS inhibitor according to the invention inhibits LATS1 and/or LATS2 activity in the corneal endothelial cells. In a preferred embodiment the LATS inhibitor inhibits LATS1 and LATS2.

The cells may go through a round or rounds of addition of fresh growth medium and/or cell proliferation medium. The cells do not need to be passaged in order for fresh medium to be added, but passaging cells is also a way to add fresh medium.

A series of mediums may be also used, in various combinations of orders: for example a cell proliferation medium, followed by addition of a growth medium (which is not supplemented with LATS inhibitors according to the invention, and may be different to the growth medium used as the base for the cell proliferation medium).

The cell population expansion phase according to the invention occurs during the period the cells are exposed to the cell proliferation medium.

Standard temperature conditions known in the art for culturing cells may be used, for example preferably about 30°C to 40°C. Particularly preferably cell growth, as well as the cell population expansion phase is carried out at about 37°C. A conventional cell incubator with 5-10% CO_2 levels may be used. Preferably the cells are exposed to 5% CO_2.

The cells may be passaged during the culturing in the growth or cell proliferation medium as necessary. Cells may be passaged when they are sub-confluent or confluent. Preferably the cells are passaged when they reach approximately 90%-100% confluency, although lower percentage confluency levels may also be performed. The passaging of cells is done according to standard protocols known in the art. For example, in brief the cells are detached from the culture container, for example using collagenase. The cells are then centrifuged and rinsed in PBS or the cell growth medium according to the invention and plated in fresh growth or cell proliferation medium as desired at a dilution of, for example, 1:2 to 1:4.

For the cell population expansion phase of the method of cell population expansion according to the invention, the expansion of the seeding cell population in the cell proliferation medium may be performed until the required amount of cellular material is obtained.

The cells may be exposed to the cell proliferation medium for a range of time periods in order to expand the cell population. For example, this may include the entire time that the CECs are kept in culture, or only for the first one to two weeks after CEC isolation or only for 24 hours after dissection of the cornea.

In a preferred embodiment, the corneal endothelial cells are exposed to the LATS inhibitors according to the invention (such as those compounds according to Formula A1 or subformulae thereof) directly after cell isolation from the cornea, and maintained for the entire time that CEC proliferation is required, for example one to two weeks.

In a more preferred embodiment of the invention, after the cell population expansion phase in vitro (i.e. after the cells are exposed to a LATS inhibitor according to the invention for a period of time to expand the population of cells), the method of cell population expansion according to the invention comprises a further step wherein the cells may be grown for a period of time (e.g. two weeks) in growth medium without supplementation of a LATS inhibitor, to enable a mature corneal endothelium to form. A mature corneal endothelium is defined herein as a monolayer of CECs with hexagonal morphology, ZO-1-positive tight junctions and expression of Na/K ATPase. In a preferred embodiment the cells are not passaged while the mature corneal endothelium is formed.

In one embodiment according to the invention, a gene editing technique may optionally be performed to genetically modify cells, to reduce or eliminate the expression and/or function of an immune response mediating gene which may otherwise contribute to immune rejection when the cell population is delivered to the patient. The application of gene editing techniques in the method of cell population expansion according to the invention is optional, and the administration to the patient of topical immunosuppressants and/or anti-inflammatory agents (as described further under the section Immunosuppressant and Anti-inflammatory agent) may instead be used if desired to mitigate issues with immunorejection of the transplanted material in the patient.

According to one aspect of the invention, for the scenario that a gene editing technique is used, genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response. In a preferred embodiment, genetically modifying comprises introducing into a corneal endothelial cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response. In a specific embodiment, said gene editing system is selected from the group consisting of CRISPR (CRISPR: clustered regularly interspaced short palindromic repeats, also known as CRISPR/Cas systems), ZFN (Zinc-finger nucleases), TALEN (transcription activator-like effector based nucleases), engineered meganucleases (e.g. ARCUS nucleases, such as the ARC nuclease), AAV vector (adeno-associated virus) gene editing (e.g. AAV vector driven homologous recombination) and lentiviral vectors-based genome editing technologies. AAV vector driven gene delivery, such as driven by homologous recombination, can be achieved by an AAV selected from the group consisting of: AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or a derivative thereof.

A gene editing technique, if it is to be used, may be performed at different points, such as for example (1) on corneal tissue, before CEC isolation or (2) at the time of cell isolation or (3) during the cell population expansion phase in vitro (when the cells are exposed to a LATS inhibitor according to the invention in vitro) or (4) in vitro at the end of the cell population expansion phase (after the cells are exposed to a LATS inhibitor according to the invention in vitro).

The gene editing techniques suitable for use in the method of cell population expansion are further described under the section “reduction of immunorejection”.

In the method of cell population expansion according to the invention the LATS inhibitors, which are preferably compounds, produce greater than 2 fold expansion of the seeded population of cells.

In one aspect of the method of cell population expansion according to the invention the compounds according to Formula A1 or subformulae thereof produce greater than 10 fold expansion of the seeded population of corneal endothelial cells. In a specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce 15 fold to 600 fold expansion of the seeded population of corneal endothelial cells. In a more specific embodiment of the method of cell population expansion according to the invention, the LATS inhibitors according to Formula A1 or subformulae thereof produce 20 fold to 550 fold expansion of the seeded population of corneal endothelial cells. The fold expansion factor achieved by the method of cell population expansion according to the invention may be achieved in one or more passages of the cells. In another aspect of the invention the fold expansion factor achieved by the method of cell population expansion according to the invention may be achieved after exposure to the compound according to Formula A1 or subformulae thereof for one to two weeks, preferably after about 10 days.

If it is desired to measure the cell number or expansion of the cell population, this may be done for example by taking an aliquot and performing immunocytochemistry (e.g. to count nuclei stained with Sytox Orange) or by live cell imaging under brightfield microscope to count the number of cells or by performing real-time quantitative live-cell analysis of cell confluence at various time points during the cell population expansion phase of the method according to the invention.

In one aspect according to the invention the CEC population obtainable or obtained by the method of cell population expansion according to the invention preferably shows at least one of the following characteristics. More preferably, it shows two or more, particularly preferably all, of the following characteristics.

(1) The cells express Na/K ATPase. The expression of Na/K ATPase may be estimated by standard techniques known in the art, such as for example immunohistochemistry, quantitative RT-PCR or by FACS analysis.

(2) The cells express one or more of Collagen 8a2, AQP1 (aquaporin 1) and SLC4A11 (Solute Carrier Family 4 Member 11). Preferably the relative expression levels are higher than cells which do not typically express collagen 8a2, AQP1 and SLC4A11, such as, for example, in dermal fibroblasts. The expression of Collagen 8a2, AQP1 or SLC4A11 may be estimated by standard techniques known in the art, such as for example immunohistochemistry, quantitative RT-PCR or by FACS analysis.

(3) The cells do not express (or at most express relatively low levels of) RPE65 (a marker of retinal pigmented epithelium) and/or CD31 (a marker of vascular endothelium). The relative expression levels are similar to cells which do not typically express RPE65, CD31, such as in dermal fibroblasts. The expression of RPE65 and CD31 may be estimated by standard techniques known in the art, such as for example quantitative RT-PCR, immunohistochemistry or FACS analysis.

(4) The cells express relatively low levels of CD73. The relative expression levels are lower than cells which have undergone endothelial to mesenchymal transition. The expression of CD73 may be estimated by standard techniques known in the art, such as for example FACS analysis or immunohistochemistry.

In another aspect according to the invention, when in a layer, for example when cultured on a plate, the CEC population obtainable by the method of cell population expansion according to the invention preferably shows at least one of the following characteristics. More preferably, it shows two or more, particularly preferably all, of the following characteristics:

(1) The cells are able to form a single layer structure. This is one of the characteristics of the corneal endothelial cell layer in the body. This may be observed by nuclear staining (e.g. with nuclear dye such as Sytox, Hoechst) followed by examination by microscopy.

(2) The cells are able to form tight junctions. This may be checked by a standard technique known in the art, immunofluorescence staining of tight-junction marker Zonula Occludens-1 (ZO-1).

(3) The cells are able to be regularly arranged in the cell layer. This may be checked by a standard technique known in the art, immunofluorescence staining of tight-junction marker

Zonula Occludens-1 (ZO-1). In the healthy corneal endothelial cell layer in the body, the cells constituting the layer are regularly arrayed, due to which corneal endothelial cells are considered to maintain normal function and high transparency and the cornea is considered to appropriately exhibit water control function.

The cell population expanded by the method of cell population expansion according to the invention may be added to a solution and then stored, for example in a preservation or cryopreservation solution (such as those described below), or added directly to a composition suitable for ocular delivery. The preservation, cryopreservation solution or composition suitable for ocular delivery may optionally comprise a LATS inhibitor according to the invention.

In a more preferred embodiment according to the invention, the cell population preparation which is delivered to the eye comprises very low to negligible levels of a LATS inhibitor compound. Thus in a specific embodiment, the method of cell population expansion according to the invention comprises the further step of rinsing to substantially remove the compound according to the invention (such as the compound according to Formula A1 or subformulae thereof). This may involve rinsing the cells after the cell population expansion phase according to the invention (directly after the cell population expansion phase and/or after the cells have been cultured to form a mature corneal endothelium in growth medium which has not been supplemented by a LATS inhibitor). To rinse the cells, the cells are centrifuged, and a cell suspension is made in PBS or growth medium according to the invention. This step may be performed multiple times, e.g. one to ten times, to rinse out the cells. Finally the cells may be resuspended in a preservation solution, cryopreservation solution, a composition suitable for ocular delivery, growth medium or combinations thereof as desired.

The expanded population of cells prepared by the method of cell population expansion and rinsed of cell proliferation medium comprising a LATS inhibitor according the invention may be transferred to a composition suitable for ocular delivery, such as for example a localising agent. Optionally the cell population is stored for a period before addition to a localising agent suitable for ocular delivery. In a preferred embodiment, the expanded cell population may first be added to a solution suitable for preservation or cryopreservation, which preferably does not comprise a LATS inhibitor, and the cell population stored (optionally with

freezing) before addition to a localising agent suitable for ocular delivery, which also preferably does not comprise a LATS inhibitor.

Typical solutions for suitable for preservation of CECs are Optisol or PBS, preferably Optisol. Optisol is a corneal storage medium comprising chondroitin sulfate and dextran to enhance corneal dehydration during storage (see for example Kaufman et al., (1991) Optisol corneal storage medium; Arch Ophthalmol Jun; 109(6): 864-8). For cryopreservation, glycerol, dimethyl sulfoxide, propylene glycol or acetamide may be used in the cryopreservation solution of the present invention. The cryopreserved preparation of cells is typically kept at -20°C or -80°C.

In one aspect the invention relates to a preserved or cryopreserved preparation of corneal endothelial cells obtainable by the method of cell population expansion according to the invention. In an alternative aspect the invention relates to a fresh cell preparation where corneal endothelial cells obtainable by the method of cell population expansion according to the invention are in suspension in PBS and/or growth medium or combined with a localising agent. The fresh cell preparation is typically kept at about 37°C. Standard cell cultures containers known in the art may be used to store the cells, such as a vial or a flask.

In a preferred embodiment according to the invention, before use in the eye, a cryopreserved preparation of cells is thawed (for example by incubating at a temperature of about 37°C in an incubator or waterbath). Preferably 10 volumes of PBS or growth medium may be added to rinse off the cells from the cryopreservant solution. Cells may then be rinsed by centrifugation, and a cell suspension may be made in PBS and/or growth medium, before combination with a localising agent for ocular delivery, which also preferably does not comprise a LATS inhibitor.

In one aspect of the invention the expanded population of cells prepared by the method of cell population expansion, (preferably also including the step of growth in medium without supplementation with LATS inhibitor to form a mature corneal endothelium), are prepared as a suspension (for example in PBS and/or growth medium, such as for example X-VIVO medium) and combined with a localising agent suitable for ocular delivery, (such as a biomatrix like GelMA). In a specific embodiment of the method of treatment according to the invention, this combination of cells, PBS and/or growth medium, and biomatrix is delivered

as a suspension to the eye. In yet another specific embodiment this combination of cells, PBS and/or growth medium, and biomatrix comprises at most only trace levels of a LATS inhibitor.

Alternatively, the cells may be cultured and the cell population proliferation phase may occur in cell proliferation medium on a localising agent suitable for cell delivery to the ocular surface.

In an embodiment of the invention the cell population expanded according to the invention may be isolated as a contiguous cell sheet for delivery to the cornea, using methods known in the art (for examples, see Kim et al, *JSM Biotechnol. Bioeng.*, 2016, p.1047). Cell sheets may be mechanically supported on a material or materials for delivery to the cornea.

Reduction of Immunorejection

Upon transplantation, allogeneic limbal stem cells are at risk of rejection by the recipient's immune system. Immunosuppression regimens can be used to reduce the risk of immunorejection of transplanted cells, such as LSCs.

Suitable systemic immunosuppressant agents used in recipients of allogeneic LSCs include tacrolimus, mycophenolate mofetil, prednisone and prophylactic valganciclovir and trimethoprim/sulfamethoxazole. (See: Holland EJ, Mogilishetty G, Skeens HM, Hair DB, Neff KD, Biber JM, Chan CC (2012) Systemic immunosuppression in ocular surface stem cell transplantation: results of a 10-year experience. Cornea. 2012 Jun;31(6):655-61).

As the methods of cell population expansion according the present invention provide high expansion capabilities of a population of cells, optionally gene-editing technologies may be used to remove drivers of immunorejection or add genes that reduce the recipient's immune response.

In one aspect of the invention gene editing is carried out on a cell population "ex vivo". In another aspect of the invention gene-editing technologies may optionally be used to reduce or eliminate the expression of a gene associated with facilitating a host versus graft immune response. In a preferred embodiment the gene is selected from the group consisting of: B2M, HLA-A, HLA-B and HLA-C. In a specific embodiment the gene is B2M. B2M is beta 2 microglobulin and is a component of the class I major histocompatibility complex (MHC). It

has the HUGO Gene Nomenclature Committee (HGNC) identifier 914. HLA-A is major histocompatibility complex, class I, A (HGNC ID 4931). HLA-B is major histocompatibility complex, class I, B (HGNC ID 4932). HLA-C is major histocompatibility complex, class I, C (HGNC ID 4933).

Several gene editing methods may be used, including, but not limited to methods selected from the group consisting of CRISPR (CRISPR: clustered regularly interspaced short palindromic repeats, also known as CRISPR/Cas systems), ZFN (Zinc-finger nucleases), TALEN (transcription activator-like effector based nucleases), engineered meganucleases (e.g. ARCUS nucleases, such as the ARC nuclease), AAV vector (adeno-associated virus) gene delivery (e.g., AAV vector driven homologous recombination) and lentiviral vectors-based genome editing technologies. AAV vector driven gene delivery, such as driven by homologous recombination, can be achieved by an AAV selected from the group consisting of: AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or a derivative thereof.
In one aspect of the invention gene editing is carried out on a cell population “ex vivo”.

Gene Editing System

As used herein, the term “gene editing system” refers to a system comprising one or more DNA-binding domains or components and one or more DNA-modifying domains or components, or isolated nucleic acids, e.g., one or more vectors, encoding said DNA-binding and DNA-modifying domains or components. Gene editing systems are used for modifying the nucleic acid of a target gene and/or for modulating the expression of a target gene. In known gene editing systems, for example, the one or more DNA-binding domains or components are associated with the one or more DNA-modifying domains or components, such that the one or more DNA-binding domains target the one or more DNA-modifying domains or components to a specific nucleic acid site. As described herein, gene editing can be carried out ex vivo.

AAV Gene Editing Systems

The use of vectors derived from AAVs to transfer genes in vitro and in vivo is well known in the art (see US Pat. No. 9,707,304, for example). The use of viral vectors for editing genes in a cell has been described, for example, in Chen et al., 2016, Molecular Therapy, 24:447-457; Gornalusse et al., Nature Biotechnology, 2017, 35(8):765-772; and WO2017087961.

An AAV vector can be made using methods known in the art. Such methods are described, for example, in Flotte T R. Adeno-associated virus-based gene therapy for inherited disorders. Pediatr Res. 2005 December; 58(6):1143-7; Goncalves M A. Adeno-associated virus: from defective virus to effective vector, Virol J. 2005 May 6; 2:43; Surace E M, Auricchio A. Adeno-associated viral vectors for retinal gene transfer. Prog Retin Eye Res. 2003 November; 22(6):705-19; Mandel R J, Manfredsson F P, Foust K D, Rising A, Reimsnider S, Nash K, Burger C. Recombinant adeno-associated viral vectors as therapeutic agents to treat neurological disorders. Mol Ther. 2006 March; 13(3):463-83.

CRISPR Gene Editing Systems

"CRISPR" as used herein refers to a set of clustered regularly interspaced short palindromic repeats, or a system comprising such a set of repeats. "Cas," as used herein, refers to a CRISPR-associated protein. The diverse CRISPR-Cas systems can be divided into two classes according to the configuration of their effector modules: class 1 CRISPR systems utilize several Cas proteins and the crRNA to form an effector complex, whereas class 2 CRISPR systems employ a large single-component Cas protein in conjunction with crRNAs to mediate interference. One example of class 2 CRISPR-Cas system employs Cpf1 (CRISPR from Prevotella and Francisella 1). See, e.g., Zetsche et al., Cell 163:759-771 (2015), the content of which is herein incorporated by reference in its entirety. The term "Cpf1" as used herein includes all orthologs, and variants that can be used in a CRISPR system.

Naturally-occurring CRISPR systems are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. Grissa *et al.* (2007) *BMC Bioinformatics* 8: 172. This system is a type of prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Barrangou *et al.* (2007) *Science* 315: 1709-1712; Marragini *et al.* (2008) *Science* 322: 1843-1845.

The CRISPR system has been modified for use in gene editing (silencing, enhancing or changing specific genes) in eukaryotes such as mice, primates and humans. Wiedenheft *et al.* (2012) *Nature* 482: 331-8. This is accomplished by, for example, introducing into the eukaryotic cell one or more vectors encoding a specifically engineered guide RNA (gRNA) (e.g., a gRNA comprising sequence complementary to sequence of a eukaryotic genome) and one or more appropriate RNA-guided nucleases, e.g., Cas proteins. The RNA guided

nuclease forms a complex with the gRNA, which is then directed to the target DNA site by hybridization of the gRNA's sequence to complementary sequence of a eukaryotic genome, where the RNA-guided nuclease then induces a double or single-strand break in the DNA. Insertion or deletion of nucleotides at or near the strand break creates the modified genome.

As these naturally occur in many different types of bacteria, the exact arrangements of the CRISPR and structure, function and number of Cas genes and their product differ somewhat from species to species. Haft *et al.* (2005) *PLoS Comput. Biol.* 1: e60; Kunin *et al.* (2007) *Genome Biol.* 8: R61; Mojica *et al.* (2005) *J. Mol. Evol.* 60: 174-182; Bolotin *et al.* (2005) *Microbiol.* 151: 2551-2561; Pourcel et al. (2005) *Microbiol.* 151: 653-663; and Stern *et al.* (2010) *Trends. Genet.* 28: 335-340. For example, the Cse (Cas subtype, E. coli) proteins (e.g., CasA) form a functional complex, Cascade, that processes CRISPR RNA transcripts into spacer-repeat units that Cascade retains. Brouns et al. (2008) *Science* 321: 960-964. In other prokaryotes, Cas6 processes the CRISPR transcript. The CRISPR-based phage inactivation in E. coli requires Cascade and Cas3, but not Cas1 or Cas2. The Cmr (Cas RAMP module) proteins in Pyrococcus furiosus and other prokaryotes form a functional complex with small CRISPR RNAs that recognizes and cleaves complementary target RNAs.

A simpler CRISPR system relies on the protein Cas9, which is a nuclease with two active cutting sites, one for each strand of the double helix. Combining Cas9 and modified CRISPR locus RNA can be used in a system for gene editing. Pennisi (2013) *Science* 341: 833-836.

In some embodiments, the RNA-guided nuclease is a Cas molecule, e.g., a Cas9 molecule. The "Cas9 molecule," can interact with a gRNA molecule (e.g., sequence of a domain of a tracr, also known as tracrRNA or trans activating CRISPR RNA) and, in concert with the gRNA molecule, localize (e.g., target or home) to a site which comprises a target sequence and PAM (protospacer adjacent motif) sequence.

According to the present invention, Cas9 molecules of, derived from, or based on the Cas9 proteins of a variety of species can be used in the methods and compositions described herein. For example, Cas9 molecules of, derived from, or based on, e.g., S. pyogenes, S. thermophilus, Staphylococcus aureus and/or Neisseria meningitidis Cas9 molecules, can be used in the systems, methods and compositions described herein. Additional Cas9 species include: Acidovorax avenae, Actinobacillus pleuropneumoniae, Actinobacillus succinogenes, Actinobacillus suis, Actinomyces sp., cycliphilus denitrificans, Aminomonas paucivorans,

Bacillus cereus, Bacillus smithii, Bacillus thuringiensis, Bacteroides sp., Blastopirellula marina, Bradyrhiz' obium sp., Brevibacillus latemsporus, Campylobacter coli, Campylobacter jejuni, Campylobacter lad, Candidatus Puniceispirillum, Clostridiu cellulolyticum, Clostridium perfringens, Corynebacterium accolens, Corynebacterium diphtheria, Corynebacterium matruchotii, Dinoroseobacter sliibae, Eubacterium dolichum, gamma proteobacterium, Gluconacetobacler diazotrophicus, Haemophilus parainfluenzae, Haemophilus sputorum, Helicobacter canadensis, Helicobacter cinaedi, Helicobacter mustelae, Ilyobacler polytropus, Kingella kingae, Lactobacillus crispatus, Listeria ivanovii, Listeria monocytogenes, Listeriaceae bacterium, Methylocystis sp., Methylosinus trichosporium, Mobiluncus mulieris, Neisseria bacilliformis, Neisseria cinerea, Neisseria flavescens, Neisseria lactamica. Neisseria sp., Neisseria wadsworthii, Nitrosomonas sp., Parvibaculum lavamentivorans, Pasteurella multocida, Phascolarctobacterium succinatutens, Ralstonia syzygii, Rhodopseudomonas palustris, Rhodovulum sp., Simonsiella muelleri, Sphingomonas sp., Sporolactobacillus vineae, Staphylococcus lugdunensis, Streptococcus sp., Subdoligranulum sp., Tislrella mobilis, Treponema sp., or Verminephrobacter eiseniae.

In some embodiments, the ability of an active Cas9 molecule to interact with and cleave a target nucleic acid is PAM sequence dependent. A PAM (protospacer adjacent motif) sequence is a sequence in the target nucleic acid. It is typically short, for example 2 to 7 base pairs long. In an embodiment, cleavage of the target nucleic acid occurs upstream from the PAM sequence. Active Cas9 molecules from different bacterial species can recognize different sequence motifs (e.g., PAM sequences). In an embodiment, an active Cas9 molecule of S. pyogenes recognizes the sequence motif NGG and directs cleavage of a target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from that sequence. See, e.g., Mali el al, SCIENCE 2013; 339(6121): 823- 826. In an embodiment, an active Cas9 molecule of S. thermophilus recognizes the sequence motif NGGNG (SEQ ID NO: 4) and NNAG AAW (SEQ ID NO: 5) (W = A or T and N is any nucleobase) and directs cleavage of a core target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from these sequences. See, e.g., Horvath et al., SCIENCE 2010; 327(5962): 167- 170, and Deveau et al, J BACTERIOL 2008; 190(4): 1390- 1400. In an embodiment, an active Cas9 molecule of S. mutans recognizes the sequence motif NGG or NAAR (R - A or G) and directs cleavage of a core target nucleic acid sequence 1 to 10, e.g., 3 to 5 base pairs, upstream from this sequence. See, e.g., Deveau et al., J BACTERIOL 2008; 190(4): 1390-1400.

In an embodiment, an active Cas9 molecule of S. aureus recognizes the sequence motif NNGRR (SEQ ID NO: 6) (R = A or G) and directs cleavage of a target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from that sequence. See, e.g., Ran F. et al., NATURE, vol. 520, 2015, pp. 186-191. In an embodiment, an active Cas9 molecule of N. meningitidis recognizes the sequence motif NNNNGATT (SEQ ID NO: 7) and directs cleavage of a target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from that sequence. See, e.g., Hou et al., PNAS EARLY EDITION 2013, 1 -6. The ability of a Cas9 molecule to recognize a PAM sequence can be determined, e.g., using a transformation assay described in Jinek et al , SCIENCE 2012, 337:816.

Exemplary naturally occurring Cas9 molecules are described in Chylinski et al , RNA Biology 2013; 10:5, 727-737. Such Cas9 molecules include Cas9 molecules of a cluster 1 bacterial family, cluster 2 bacterial family, cluster 3 bacterial family, cluster 4 bacterial family, cluster 5 bacterial family, cluster 6 bacterial family, a cluster 7 bacterial family, a cluster 8 bacterial family, a cluster 9 bacterial family, a cluster 10 bacterial family, a cluster 11 bacterial family, a cluster 12 bacterial family, a cluster 13 bacterial family, a cluster 14 bacterial family, a cluster 15 bacterial family, a cluster 16 bacterial family, a cluster 17 bacterial family, a cluster 18 bacterial family, a cluster 19 bacterial family, a cluster 20 bacterial family, a cluster 21 bacterial family, a cluster 22 bacterial family, a cluster 23 bacterial family, a cluster 24 bacterial family, a cluster 25 bacterial family, a cluster 26 bacterial family, a cluster 27 bacterial family, a cluster 28 bacterial family, a cluster 29 bacterial family, a cluster 30 bacterial family, a cluster 31 bacterial family, a cluster 32 bacterial family, a cluster 33 bacterial family, a cluster 34 bacterial family, a cluster 35 bacterial family, a cluster 36 bacterial family, a cluster 37 bacterial family, a cluster 38 bacterial family, a cluster 39 bacterial family, a cluster 40 bacterial family, a cluster 41 bacterial family, a cluster 42 bacterial family, a cluster 43 bacterial family, a cluster 44 bacterial family, a cluster 45 bacterial family, a cluster 46 bacterial family, a cluster 47 bacterial family, a cluster 48 bacterial family,. a cluster 49 bacterial family, a cluster 50 bacterial family, a cluster 51 bacterial family, a cluster 52 bacterial family, a cluster 53 bacterial family, a cluster 54 bacterial family, a cluster 55 bacterial family, a cluster 56 bacterial family, a cluster 57 bacterial family, a cluster 58 bacterial family, a cluster 59 bacterial family, a cluster 60 bacterial family, a cluster 61 bacterial family, a cluster 62 bacterial family, a cluster 63 bacterial family, a cluster 64 bacterial family, a cluster 65 bacterial family, a cluster 66 bacterial family, a cluster 67 bacterial family, a cluster 68 bacterial family, a cluster 69 bacterial family, a cluster 70 bacterial family, a cluster 71 bacterial family, a

cluster 72 bacterial family, a cluster 73 bacterial family, a cluster 74 bacterial family, a cluster 75 bacterial family, a cluster 76 bacterial family, a cluster 77 bacterial family, or a cluster 78 bacterial family.

Exemplary naturally occurring Cas9 molecules include a Cas9 molecule of a cluster 1 bacterial family. Examples include a Cas9 molecule of: S. pyogenes (e.g., strain SF370, MGAS 10270, MGAS 10750, MGAS2096, MGAS315, MGAS5005, MGAS6180, MGAS9429, NZ131 and SSI- 1), S. thermophilus (e.g., strain LMD-9), S. pseudoporcinus (e.g., strain SPIN 20026), S. mutans (e.g., strain UA 159, NN2025), S. macacae (e.g., strain NCTC1 1558), S. gallolylicus (e.g., strain UCN34, ATCC BAA-2069), S. equines (e.g., strain ATCC 9812, MGCS 124), S. dysdalactiae (e.g., strain GGS 124), S. bovis (e.g., strain ATCC 700338), S. cmginosus (e.g.; strain F021 1), S. agalactia* (e.g., strain NEM316, A909), Listeria monocytogenes (e.g., strain F6854), Listeria innocua (L. innocua, e.g., strain Clip l1262), EtUerococcus italicus (e.g., strain DSM 15952), or Enterococcus faecium (e.g., strain 1,23 ,408). Additional exemplary Cas9 molecules are a Cas9 molecule of Neisseria meningitidis (Hou et al. PNAS Early Edition 2013, 1-6) and a S. aureus Cas9 molecule.

In an embodiment, a Cas9 molecule, e.g., an active Cas9 molecule comprises an amino acid sequence: having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology with; differs at no more than 1%, 2%, 5%, 10%, 15%, 20%, 30%, or 40% of the amino acid residues when compared with; differs by at least 1, 2, 5, 10 or 20 amino acids but by no more than 100, 80, 70, 60, 50, 40 or 30 amino acids from; or is identical to; any Cas9 molecule sequence described herein or a naturally occurring Cas9 molecule sequence, e.g., a Cas9 molecule from a species listed herein or described in Chylinski et al. , RNA Biology 2013, 10:5, 121-T,1 Hou et al. PNAS Early Edition 2013, 1-6.

In an embodiment, a Cas9 molecule comprises an amino acid sequence having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology with; differs at no more than 1%, 2%, 5%, 10%, 15%, 20%, 30%, or 40% of the amino acid residues when compared with; differs by at least 1, 2, 5, 10 or 20 amino acids but by no more than 100, 80, 70, 60, 50, 40 or 30 amino acids from; or is identical to; *S. pyogenes* Cas9 (UniProt Q99ZW2). In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant, such as a variant described in Slaymaker et al., *Science Express*, available online December 1, 2015 at Science DOI: 10.1126/science.aad5227; Kleinstiver et al., *Nature*, 529, 2016, pp. 490-495, available online January 6, 2016 at doi:10.1038/nature16526; or US2016/0102324, the contents of which are incorporated herein in their entirety.

In some embodiments, the Cas9 molecule, e.g., a Cas9 of *S. pyogenes*, may additionally comprise one or more amino acid sequences that confer additional activity. In some aspects, the Cas9 molecule may comprise one or more nuclear localization sequences (NLSs), such as at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. Typically, an NLS consists of one or more short sequences of positively charged lysines or arginines exposed on the protein surface, but other types of NLS are known. Non-limiting examples of NLSs include an NLS sequence comprising or derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV (SEQ ID NO: 8). Other suitable NLS sequences are known in the art (e.g., Sorokin, Biochemistry (Moscow) (2007) 72:13, 1439-1457; Lange J Biol Chem. (2007) 282:8, 5101-5). In any of the aforementioned embodiments, the Cas9 molecule may additionally (or alternatively) comprise a tag, e.g., a His tag, e.g., a His(6) tag (SEQ ID NO: 25) or His(8) tag (SEQ ID NO: 26), e.g., at the N terminus or the C terminus.

Thus, engineered CRISPR gene editing systems, e.g., for gene editing in eukaryotic cells, typically involve (1) a guide RNA molecule (gRNA) comprising a targeting domain (which is capable of hybridizing to the genomic DNA target sequence), and a sequence which is capable of binding to a Cas, e.g., Cas9 enzyme, and (2) a Cas, e.g., Cas9, protein. The sequence which is capable of binding to a Cas protein may comprise a domain referred to as a tracr domain or tracrRNA. The targeting domain and the sequence which is capable of binding to a Cas, e.g., Cas9 enzyme, may be disposed on the same (sometimes referred to as a single gRNA, chimeric gRNA or sgRNA) or different molecules (sometimes referred to as a dual gRNA or dgRNA). If disposed on different molecules, each includes a hybridization domain which allows the molecules to associate, e.g., through hybridization.

gRNA molecule formats are known in the art. An exemplary gRNA molecule, e.g., dgRNA molecule, as disclosed herein comprises, e.g., consists of, a first nucleic acid having the sequence:

5'nnnnnnnnnnnnnnnnnnnnGUUUUAGAGCUAUGCUGUUUUG 3' (SEQ ID NO: 9),
where the "n"'s refer to the residues of the targeting domain, e.g., as described herein, and may consist of 15-25 nucleotides, e.g., consists of 20 nucleotides;
and a second nucleic acid sequence having the exemplary sequence:
5'AACUUACCAAGGAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGC 3', optionally with 1, 2, 3, 4, 5, 6, or 7 (e.g., 4 or 7, e.g., 7) additional U nucleotides at the 3' end (SEQ ID NO: 10).

The second nucleic acid molecule may alternatively consist of a fragment of the sequence above, wherein such fragment is capable of hybridizing to the first nucleic acid. An example of such second nucleic acid molecule is:

5'AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC CGAGUCGGUGC 3', optionally with 1, 2, 3, 4, 5, 6, or 7 (e.g., 4 or 7, e.g., 7) additional U nucleotides at the 3' end (SEQ ID NO:11).

Another exemplary gRNA molecule, e.g., a sgRNA molecule, as disclosed herein comprises, e.g., consists of a first nucleic acid having the sequence:

5'nnnnnnnnnnnnnnnnnnnGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCC GUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC 3'(SEQ ID NO:12), where the "n"'s refer to the residues of the targeting domain, e.g., as described herein, and may consist of 15-25 nucleotides, e.g., consist of 20 nucleotides, optionally with 1, 2, 3, 4, 5, 6, or 7 (e.g., 4 or 7, e.g., 4) additional U nucleotides at the 3' end.

Additional components and/or elements of CRISPR gene editing systems known in the art, e.g., are described in U.S. Publication No.2014/0068797, WO2015/048577, and Cong (2013) Science 339: 819-823, the contents of which are hereby incorporated by reference in their entirety. Such systems can be generated which inhibit a target gene, by, for example, engineering a CRISPR gene editing system to include a gRNA molecule comprising a targeting domain that hybridizes to a sequence of the target gene. In embodiments, the gRNA comprises a targeting domain which is fully complementarity to 15-25 nucleotides, e.g., 20 nucleotides, of a target gene. In embodiments, the 15-25 nucleotides, e.g., 20 nucleotides, of the target gene, are disposed immediately 5' to a protospacer adjacent motif (PAM) sequence recognized by the RNA-guided nuclease, e.g., Cas protein, of the CRISPR gene editing system (e.g., where the system comprises a *S. pyogenes* Cas9 protein, the PAM sequence comprises NGG, where N can be any of A, T, G or C).

In some embodiments, the gRNA molecule and RNA-guided nuclease, e.g., Cas protein, of the CRISPR gene editing system can be complexed to form a RNP (ribonucleoprotein) complex. Such RNP complexes may be used in the methods described herein. In other embodiments, nucleic acid encoding one or more components of the CRISPR gene editing system may be used in the methods described herein.

In some embodiments, foreign DNA can be introduced into the cell along with the CRISPR gene editing system, e.g., DNA encoding a desired transgene, with or without a promoter active in the target cell type. Depending on the sequences of the foreign DNA and target

sequence of the genome, this process can be used to integrate the foreign DNA into the genome, at or near the site targeted by the CRISPR gene editing system. For example, 3' and 5' sequences flanking the transgene may be included in the foreign DNA which are homologous to the gene sequence 3' and 5' (respectively) of the site in the genome cut by the gene editing system. Such foreign DNA molecule can be referred to "template DNA."

In an embodiment, the CRISPR gene editing system of the present invention comprises Cas9, e.g., S. pyogenes Cas9, and a gRNA comprising a targeting domain which hybridizes to a sequence of a gene of interest. In an embodiment, the gRNA and Cas9 are complexed to form a RNP(ribonucleoprotein). In an embodiment, the CRISPR gene editing system comprises nucleic acid encoding a gRNA and nucleic acid encoding a Cas protein, e.g., Cas9, e.g., S. pyogenes Cas9. In an embodiment, the CRISPR gene editing system comprises a gRNA and nucleic acid encoding a Cas protein, e.g., Cas9, e.g., S. pyogenes Cas9.

In some embodiments, inducible control over Cas9, sgRNA expression can be utilized to optimize efficiency while reducing the frequency of off-target effects thereby increasing safety. Examples include, but are not limited to, transcriptional and post-transcriptional switches listed as follows; doxycycline inducible transcription Loew et al. (2010) BMC Biotechnol. 10:81, Shield1 inducible protein stabilization Banaszynski et al. (2016) Cell 126: 995-1004, Tamoxifen induced protein activation Davis et al. (2015) Nat. Chem. Biol. 11: 316-318, Rapamycin or optogenetic induced activation or dimerization of split Cas9 Zetsche (2015) Nature Biotechnol. 33(2): 139-142, Nihongaki et al. (2015) Nature Biotechnol. 33(7): 755-760, Polstein and Gersbach (2015) Nat. Chem. Biol. 11: 198-200, and SMASh tag drug inducible degradation Chung et al. (2015) Nat. Chem. Biol. 11: 713-720.

In general, the CRISPR-Cas or CRISPR system refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or "RNA(s)" as that term is herein used (e.g., RNA(s) to guide Cas9, e.g. CRISPR RNA and transactivating (tracr) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the

formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). In the context of formation of a CRISPR complex, "target sequence" refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments it may be preferred in a CRISPR complex that the tracr sequence has one or more hairpins and is 30 or more nucleotides in length, 40 or more nucleotides in length, or 50 or more nucleotides in length; the guide sequence is between 10 to 30 nucleotides in length, the CRISPR/Cas enzyme is a Type II Cas9 enzyme. In embodiments of the invention the terms guide sequence and guide RNA are used interchangeably. In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith- Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies); ELAND (Illumina, San Diego, CA), and SOAP. In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 , 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. Preferably the guide sequence is 10 - 30 nucleotides long. The ability of a guide sequence to direct sequence- specific binding of a CRISPR complex to a target sequence may be assessed by any suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by

providing the target sequence, components of a CRISPR complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art.A guide sequence may be selected to target any target sequence. In some embodiments, the target sequence is a sequence within a genome of a cell. Exemplary target sequences include those that are unique in the target genome. For example, for the S. pyogenes Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MM M MMMNNNNNNNNNNNNXGG (SEQ ID NO: 13), where NNN NNN NN XGG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. A unique target sequence in a genome may include an S. pyogenes Cas9 target site of the form MMM MMMMMNNNNNNNNNNNXGG (SEQ ID NO: 14), where N N N N XGG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. For the S. thermophilus CRISPRI Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMMNN N N NN XXAGAAW (SEQ ID NO: 15), where NNN NN N XXAGAAW (SEQ ID NO: 29) (N is A, G, T, or C; X can be anything; and W is A or T) has a single occurrence in the genome. A unique target sequence in a genome may include an S. thermophilus CRISPRI Cas9 target site of the form MMMMMM MN N NNN NNXXAGAAW (SEQ ID NO: 16), where NNNNNNNNNNNXXAGAAW (SEQ ID NO: 30) (N is A, G, T, or C; X can be anything; and W is A or T) has a single occurrence in the genome. For the S. pyogenes Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMMNNNN NNNNNNXGGXG (SEQ ID NO: 17), where NNNNNNNNNNNNXGGXG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. A unique target sequence in a genome may include an S. pyogenes Cas9 target site of the form MMMMMMMMMNNNNNNNNNNNXGGXG (SEQ ID NO: 31) where NNNNNNNNNNNXGGXG (SEQ ID NO: 18), (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. In each of these sequences, N is any nucleobase and "M " may be A, G, T, or C, and need not be considered in identifying a sequence as unique. In some embodiments, a guide sequence is selected to reduce the degree secondary structure within the guide sequence. In some embodiments, about or less than about 75%, 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 1%, or fewer of the nucleotides of the guide sequence participate in self-complementary base pairing when optimally folded. Optimal folding may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An

example of one such algorithm is mFold, as described by Zuker and Stiegler (Nucleic Acids Res. 9 (1981), 133-148). Another example folding algorithm is the online webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see e.g. A.R. Gruber et al., 2008, Cell 106(1): 23-24; and PA Carr and GM Church, 2009, Nature Biotechnology 27(12): 1 151-62).

Methods for Designing gRNA Molecules

Methods for selecting, designing, and validating targeting domains for use in the gRNAs described herein are provided. Exemplary targeting domains for incorporation into gRNAs are also provided herein.

Methods for selection and validation of target sequences as well as off-target analyses have been described (see, e.g., Mali 2013 ; Hsu 2013; Fu 2014; Heigwer 2014; Bae 2014; and Xiao 2014). For example, target sequences can be chosen by identifying the PAM sequence for a Cas9 molecule (for example, relevant PAM e.g., NGG PAM for S. pyogenes, NNNNGATT (SEQ ID NO: 19), or NNNNGCTT PAM (SEQ ID NO: 20), for N. meningitides, and NNGRRT (SEQ ID NO: 21), or NNGRRV PAM (SEQ ID NO: 22), for S. aureus), and identifying the adjacent sequence as the target sequence for a CRISPR system using that Cas9 molecule. A software tool can be used to further refine the choice of potential targeting domains corresponding to a user's target sequence, e.g., to minimize total off-target activity across the genome. Candidate targeting domains and gRNAs comprising those targeting domains can be functionally evaluated by using methods known in the art and/or as set forth herein.

As a non-limiting example, targeting domains for use in gRNAs for use with S. pyogenes, N. meningiitidis and S. aureus Cas9s are identified using a DNA sequence searching algorithm. 17-mer, 18-mer, 19-mer, 20-mer, 21-mer, 22-mer, 23-mer, and/or 24-mer targeting domains are designed for each Cas9. With respect to S. pyogenes Cas9, preferably, the targeting domain is a 20-mer. gRNA design is carried out using a custom gRNA design software based on the public tool cas-offinder (Bae 2014). This software scores guides after calculating their genome-wide off-target propensity.

Functional Analysis of Candidate Molecules

Candidate Cas9 molecules, candidate gRNA molecules, candidate Cas9 molecule/gRNA molecule complexes, can be evaluated by art-known methods or as described herein. For example, exemplary methods for evaluating the endonuclease activity of Cas9 molecule have been described previously (Jinek 2012). Each technique described herein may be used alone or in combination with one or more techniques to evaluate the candidate molecule. The techniques disclosed herein may be used for a variety of methods including, without limitation, methods of determining the stability of a Cas9 molecule/gRNA molecule complex, methods of determining a condition that promotes a stable Cas9 molecule/gRNA molecule complex, methods of screening for a stable Cas9 molecule/gRNA molecule complex, methods of identifying an optimal gRNA to form a stable Cas9 molecule/gRNA molecule complex, and methods of selecting a Cas9/gRNA complex for administration to a subject.

Binding and Cleavage Assay: Testing the endonuclease activity of Cas9 molecule The ability of a Cas9 molecule/gRNA molecule complex to bind to and cleave a target nucleic acid can be evaluated in a plasmid cleavage assay. In this assay, synthetic or in vitro-transcribed gRNA molecule is pre-annealed prior to the reaction by heating to 95°C and slowly cooling down to room temperature. Native or restriction digest-linearized plasmid DNA (300 ng (~8 nM)) is incubated for 60 min at 37°C with purified Cas9 protein molecule (50-500 nM) and gRNA (50-500 nM, 1: 1) in a Cas9 plasmid cleavage buffer (20 mM HEPES pH 7.5, 150 mM KC1, 0.5 mM DTT, 0.1 mM EDTA) with or without 10 mM MgCl2. The reactions are stopped with 5X DNA loading buffer (30% glycerol, 1.2% SDS, 250 mM EDTA), resolved by a 0.8 or 1% agarose gel electrophoresis and visualized by ethidium bromide staining. The resulting cleavage products indicate whether the Cas9 molecule cleaves both DNA strands, or only one of the two strands. For example, linear DNA products indicate the cleavage of both DNA strands. Nicked open circular products indicate that only one of the two strands is cleaved.

Alternatively, the ability of a Cas9 molecule/gRNA molecule complex to bind to and cleave a target nucleic acid can be evaluated in an oligonucleotide DNA cleavage assay. In this assay, DNA oligonucleotides (10 pmol) are radiolabeled by incubating with 5 units T4 polynucleotide kinase and -3-6 pmol (-20-40 mCi) [γ-32P]-ATP in IX T4 polynucleotide kinase reaction buffer at 37°C for 30 min, in a 50 microlitre reaction. After heat inactivation (65°C for 20 min), reactions are purified through a column to remove unincorporated label. Duplex localising agents (100 nM) are generated by annealing labeled oligonucleotides with

equimolar amounts of unlabeled complementary oligonucleotide at 95°C for 3 min, followed by slow cooling to room temperature. For cleavage assays, gRNA molecules are annealed by heating to 95°C for 30 s, followed by slow cooling to room temperature. Cas9 (500 nM final concentration) is pre-incubated with the annealed gRNA molecules (500 nM) in cleavage assay buffer (20 mM HEPES pH 7.5, 100 mM KCl, 5 mM MgC12, 1 mM DTT, 5% glycerol) in a total volume of 9 microlitre. Reactions are initiated by the addition of 1 microlitre target DNA (10 nM) and incubated for 1 h at 37°C. Reactions are quenched by the addition of 20 microlitre of loading dye (5 mM EDTA, 0.025% SDS, 5% glycerol in formamide) and heated to 95°C for 5 min. Cleavage products are resolved on 12% denaturing polyacrylamide gels containing 7 M urea and visualized by phosphorimaging. The resulting cleavage products indicate that whether the complementary strand, the non-complementary strand, or both, are cleaved.

One or both of these assays can be used to evaluate the suitability of a candidate gRNA molecule or candidate Cas9 molecule.

Indel Detection and Identification. Targeted genome modifications can also be detected by either Sanger or deep sequencing. For the former, genomic DNA from the modified region can be amplified with either primers flanking the target sequence of the gRNA. Amplicons can be subcloned into a plasmid such as pUC19 for transformation, and individual colonies should be sequenced to reveal the clonal genotype.

Alternatively, deep sequencing is suitable for sampling a large number of samples or target sites. NGS primers are designed for shorter amplicons, typically in the 100–200-bp size range. For the detection of indels, it is important to design primers situated at least 50 bp from the Cas9 target site to allow for the detection of longer indels. Amplicons may be assessed using commercially-available instruments, for example, the Illumina system. Detailed descriptions of NGS optimization and troubleshooting can be found in the Illumina user manual.

TALEN gene editing systems

TALENs are produced artificially by fusing a TAL effector DNA binding domain to a DNA cleavage domain. Transcription activator-like effects (TALEs) can be engineered to bind any desired DNA sequence, e.g., a target gene. By combining an engineered TALE with a

DNA cleavage domain, a restriction enzyme can be produced which is specific to any desired DNA sequence. These can then be introduced into a cell, wherein they can be used for genome editing. Boch (2011) *Nature Biotech*. 29: 135-6; and Boch et al. (2009) *Science* 326: 1509-12; Moscou et al. (2009) *Science* 326: 3501.

TALEs are proteins secreted by Xanthomonas bacteria. The DNA binding domain contains a repeated, highly conserved 33-34 amino acid sequence, with the exception of the 12th and 13th amino acids. These two positions are highly variable, showing a strong correlation with specific nucleotide recognition. They can thus be engineered to bind to a desired DNA sequence.

To produce a TALEN, a TALE protein is fused to a nuclease (N), which is, for example, a wild-type or mutated FokI endonuclease. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. Cermak et al. (2011) *Nucl. Acids Res.* 39: e82; Miller et al. (2011) *Nature Biotech*. 29: 143-8; Hockemeyer et al. (2011) *Nature Biotech*. 29: 731-734; Wood et al. (2011) *Science* 333: 307; Doyon et al. (2010) *Nature Methods* 8: 74-79; Szczepek et al. (2007) *Nature Biotech.* 25: 786-793; and Guo et al. (2010) *J. Mol. Biol.* 200: 96.

The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011) *Nature Biotech.* 29: 143-8.

A TALEN (or pair of TALENs) can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, foreign DNA can be introduced into the cell along with the TALEN, e.g., DNA encoding a transgene, and depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to integrate the transgene at or near the site targeted by the TALEN. TALENs specific to a target gene can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) *Nature Biotech.* 29: 149-53; Geibler et al. (2011) *PLoS ONE* 6: e19509; US 8,420,782 ; US 8,470,973, the contents of which are hereby incorporated by reference in their entirety.

Zinc finger nuclease (ZFN) gene editing system

"ZFN" or "Zinc Finger Nuclease" refer to a zinc finger nuclease, an artificial nuclease which can be used to modify, e.g., delete one or more nucleic acids of, a desired nucleic acid sequence.

Like a TALEN, a ZFN comprises a FokI nuclease domain (or derivative thereof) fused to a DNA-binding domain. In the case of a ZFN, the DNA-binding domain comprises one or more zinc fingers. Carroll et al. (2011) *Genetics Society of America* 188: 773-782; and Kim et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 1156-1160.

A zinc finger is a small protein structural motif stabilized by one or more zinc ions. A zinc finger can comprise, for example, Cys2His2, and can recognize an approximately 3-bp sequence. Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15 or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells.

Like a TALEN, a ZFN must dimerize to cleave DNA. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. Bitinaite et al. (1998) *Proc. Natl. Acad. Sci. USA* 95: 10570-5.

Also like a TALEN, a ZFN can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression and amount of the target gene in a cell. ZFNs can also be used with homologous recombination to mutate the target gene or locus, or to introduce nucleic acid encoding a desired transgene at a site at or near the targeted sequence.

ZFNs specific to sequences in a target gene can be constructed using any method known in the art. See, e.g., Provasi (2011) Nature Med. 18: 807-815; Torikai (2013) Blood 122: 1341-1349; Cathomen et al. (2008) Mol. Ther. 16: 1200-7; and Guo et al. (2010) J. Mol. Biol. 400: 96; U.S. Patent Publication 2011/0158957; and U.S. Patent Publication 2012/0060230, the contents of which are hereby incorporated by reference in their entirety. In embodiments, The ZFN gene editing system may also comprise nucleic acid encoding one or more components of the ZFN gene editing system.

Meganuclease Gene Editing System

"Meganuclease" refers to a meganuclease, an artificial nuclease which can be used to edit a target gene.

Meganucleases are derived from a group of nucleases which recognize 15-40 base-pair cleavage sites. Meganucleases are grouped into families based on their structural motifs which affect nuclease activity and/or DNA recognition. Members of the LAGLIDADG (SEQ ID NO: 23) family are characterized by having either one or two copies of the conserved LAGLIDADG motif (SEQ ID NO: 23) (see Chevalier et al. (2001), Nucleic Acids Res. 29(18): 3757-3774). The LAGLIDADG (SEQ ID NO: 23) meganucleases with a single copy of the LAGLIDADGmotif (SEQ ID NO: 23) form homodimers, whereas members with two copies of the LAGLIDADG motif (SEQ ID NO: 23) are found as monomers. The GIY-YIG family members have a GIY-YIG module, which is 70-100 residues long and includes four or five conserved sequence motifs with four invariant residues, two of which are required for activity (see Van Roey et al. (2002), Nature Struct. Biol. 9: 806-811). The His-Cys box meganucleases are characterized by a highly conserved series of histidines and cysteines over a region encompassing several hundred amino acid residues (see Chevalier et al. (2001), Nucleic Acids Res. 29(18): 3757-3774). The NHN family, the members are defined by motifs containing two pairs of conserved histidines surrounded by asparagine residues (see Chevalier et al. (2001), Nucleic Acids Res. 29(18): 3757-3774).

Strategies for engineering a meganuclease with altered DNA-binding specificity, e.g., to bind to a predetermined nucleic acid sequence are known in the art. E.g., Chevalier et al. (2002), Mol. Cell., 10:895-905; Epinat et al. (2003) Nucleic Acids Res 31: 2952-62; Silva et al. (2006) J Mol Biol 361: 744-54; Seligman et al. (2002) Nucleic Acids Res 30: 3870-9; Sussman et al. (2004) J Mol Biol 342: 31-41; Rosen et al. (2006) Nucleic Acids Res; Doyon et al. (2006) J. Am Chem Soc 128: 2477-84; Chen et al. (2009) Protein Eng Des Sel 22: 249-56; Arnould S (2006) J Mol Biol. 355: 443-58; Smith (2006) Nucleic Acids Res. 363(2): 283-94.

A meganuclease can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, e.g., via non-homologous end joining, leading to a decrease in the expression of a target gene in a cell. Alternatively, foreign DNA can be introduced into the cell along with the Meganuclease; depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to modify a target gene,

e.g., correct a defect in the target gene, thus causing expression of a repaired target gene, or e.g., introduce such a defect into a wt gene, thus decreasing expression of a target gene, e.g., as described in Silva et al. (2011) Current Gene Therapy 11:11-27.

Ocular administration of the expanded cell population

In one aspect of the invention the expanded cell population obtainable by the methods according to the invention as described above is delivered to the eye. The delivery is performed under aseptic conditions.

In one embodiment relating to use for limbal stem cell therapy after a 360° limbal peritomy the fibrovascular corneal pannus may be carefully removed from the surface.

In one aspect of the invention, the cell population is combined with a localising agent suitable for ocular delivery (as described further below) and delivered to the eye. In a preferred embodiment the cells and localising agent suitable for ocular delivery are combined and administered to the eye via a carrier such as for example a therapeutic contact lens or amniotic membrane. In an alternative embodiment the cells and localising agent suitable for use in the eye, such as a light curable biomatrix, like GelMA, are delivered to the eye via bioprinting.

In one embodiment, the invention provides a method of transplanting a population of cells comprising corneal endothelial cells onto the cornea of a subject, the method comprising expanding a population of cells comprising corneal endothelial cells by culturing said population with cell proliferation medium comprising a LATS inhibitor according to the invention, rinsing the expanded population of cells to substantially remove the LATS inhibitor, and administering said cells onto the cornea of said subject. Preferably said cells are combined with a biomatrix prior to said administration. In a specific embodiment said cells are combined with a biomatrix which is GelMA prior to said administration. In a more specific embodiment said corneal endothelial cells are combined with a biomatrix which is bioprinted onto the ocular surface. Particularly preferably said corneal endothelial cells are combined with a biomatrix which is GelMA and bioprinted onto the ocular surface by polymerising the GelMA by a light triggered reaction.

In another embodiment, the invention provides a method of transplanting a population of cells to the eye of a subject, comprising combining the cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject or applying the mixture onto the surface of the eye of the subject, and bioprinting the cells in or on the eye by guiding and fixing the cells, such as on the cornea, using a light source, such as an Ultraviolet A or white light source. In certain embodiments, the light source produces light of a wavelength that is at least 350 nm. In certain embodiments, the light source produces light in the 350 nm to 420 nm range. For example, an LED light source can be used to produce a light having a wavelength of 365 nm or 405 nm, or any other wavelength above 350 nm, or a mercury lamp with a bandpass filter can be used to produce a light having a wavelength of 365 nm. In another embodiment, the light source produces visible, white light having a wavelength, for example, in the 400 nm to 700 nm range. In certain embodiments, the cells are ocular cells, such as corneal cells (e.g., corneal endothelial cells), lens cells, trabecular mesh cells, or cells found in the anterior chamber. In a particular embodiment, the cells are corneal endothelial cells. Certain embodiments of such method include:

Embodiment x1. A method of transplanting a population of isolated cells to the eye of a subject, comprising combining the cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject, (e.g., into the anterior chamber) and bioprinting the cells in the eye by guiding and fixing the cells in the eye using a light source.

Embodiment x2. The method of Embodiment x1, wherein the isolated cells are combined with a biomatrix which is GelMA and bioprinted onto the cornea by polymerising the GelMA by a light triggered reaction.

Embodiment x3. The method of Embodiment x1 or Embodiment x2, wherein the light source produces a light having a wavelength in the 350 nm to 700 nm range.

Embodiment x4. The method of any one of Embodiments x1 to x3, wherein the wavelength is 350 nm to 420 nm.

Embodiment x5. The method of any one of Embodiments x1 to x4, wherein the wavelength is 365 nm.

Embodiment x6. The method of any one of Embodiments x1 to x5, wherein the isolated cells are corneal endothelial cells.

Embodiment x7. A method of transplanting a population of isolated cells to the eye of a subject, comprising combining the cells with a biomatrix to form a cell/biomatrix mixture, applying the mixture onto the eye of the subject, and bioprinting the cells on the eye by guiding and fixing the cells on the eye using a light source.

Embodiment x8. The method of Embodiment x7, wherein the isolated cells are combined with a biomatrix which is GelMA and bioprinted onto the ocular surface by polymerising the GelMA by a light triggered reaction.

Embodiment x9. The method of Embodiment x7 or Embodiment x8, wherein the light source produces a light having a wavelength in the 350 nm to 700 nm range.

Embodiment x10. The method of any one of Embodiments x7 to x9, wherein the wavelength is 350 nm to 420 nm.

Embodiment x11. The method of any one of Embodiments x7 to x10, wherein the wavelength is 365 nm.

Embodiment x12. The method of any one of Embodiments x7 to x11, wherein the isolated cells are limbal stem cells.

In an alternative embodiment the expanded cell population obtainable by the methods according to the invention as described above may be delivered directly via a therapeutic contact lens to the eye, without use of a localising agent suitable for ocular delivery (such as GelMA).

Localising agent suitable for ocular delivery

In an embodiment of the invention the cell preparation may be delivered to the eye via a localising agent suitable for ocular use. The cells may be embedded within the localising agent or adhered to the surface of the localising agent, or both.

The type of localising agent is not limited as long as it is able to carry LSCs or CECs and is suitable for use in the eye. In a preferred embodiment, the localising agent is degradable and biocompatible. Where CECs are delivered, preferably the localising agent can facilitate CEC attachment to the cornea after surgical delivery to the surface of the eye.

In a preferred embodiment the cells are only combined with the localising agent after cell population expansion. In a particularly preferred embodiment the expanded cell population is combined with the localising agent suitable for ocular delivery after rinsing the cell population to substantially remove the presence of the LATs inhibitor according to the invention. In one embodiment, the LSCs or CECs and localising agent are combined and stored in a form suitable for ocular use. In another embodiment, the LSCs or CECs and localising agent are stored separately and combined immediately prior to ocular use.

The localising agent is preferably selected from the list consisting of fibrin, collagen, gelatin, cellulose, amniotic membrane, fibrin glue, polyethylene (glycol) diacrylate (PEGDA), GelMA, (which is methacrylamide modified gelatin, and is also known as gelatin methacrylate), localising agents comprising a polymer, cross-linked polymer, or hydrogel comprising one or more of hyaluronic acid, polyethylene glycol, polypropylene glycol, polyethylene oxide, polypropylene oxide, poloxamer, polyvinyl alcohol, polyacrylic acid, polymethacrylic acid, polyvinyl pyrrolidone, poly(lactide-co-glycolide), alginate, gelatin, collagen, fibrinogen, cellulose, methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropylmethylcellulose, hydroxypropyl-guar, gellan gum, guar gum, xanthan gum and carboxymethylcellulose, as well as derivatives thereof, co-polymers thereof, and combinations thereof.

In a more preferred embodiment the localising agent is selected from the list consisting of fibrin, collagen, gelatin, amniotic membrane, fibrin glue, polyethylene (glycol) diacrylate (PEGDA), GelMA, localising agents comprising a polymer, cross-linked polymer, or hydrogel

comprising one or more of hyaluronic acid, polyethylene glycol, polypropylene glycol, polyethylene oxide, polypropylene oxide, poloxamer, polyacrylic acid, poly(lactide-co-glycolide), alginate, gelatin, collagen, fibrinogen, hydroxypropylmethylcellulose and hydroxypropyl-guar, as well as derivatives thereof, co-polymers thereof, and combinations thereof.

In a preferred embodiment the expanded cell population according to the invention may be delivered to a recipient via a localising agent which is a biomatrix. In a more preferred embodiment the localising agent is a light curable, degradable biomatrix. Preferably this is able to be injected into the eye. A specific example of a biomatrix is GelMA, which is methacrylamide modified gelatin, and is also known as gelatin methacrylate.

GelMA may be prepared according to standard protocols known in the art (Van Den Bulcke et al., *Biomacromolecules*, 2000, p.31-38; Yue et al., *Biomaterials*, 2015, p.254-271). For example, gelatin from porcine skin (gel strength 300 g Bloom, Type A) is dissolved in PBS without calcium and magnesium (Dulbeccos PBS), and methacrylic anhydride may be added with strong agitation into the gelatin solution to reach the desired concentration (e.g. 8% (vol/vol). The mixture may be stirred before and after adding further DPBS. The diluted mixture may be purified via dialysis against Milli-Q water using dialysis tubing to remove methacrylic acid. The purified samples may optionally be lyophilized and the solid stored at -80°C, -20°C, or 4°C until further use.
A GelMA stock solution is prepared by dissolving lyophilized GelMA in a formulation suitable for ocular use comprising pharmaceutically acceptable excipients. To prepare a GelMA stock solution, lyophilized GelMA may be dissolved in DPBS. After the GelMA is fully dissolved, a photoinitiator (for example such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate) may be introduced into the GelMA solution. To adjust the pH to neutral, NaOH may be added to the solution before filtering using 0.22 micrometre sterile membranes. The final filtrate may be separated into aliquots and stored at 4°C until further use.

In one aspect according to the invention, the cells are encapsulated within the biomatrix using a photoinitiator to polymerise the biomatrix, which is preferably GelMa. Suitable photoinitiator agents are Irgacure 2959, lithium phenyl-2,4,6-trimethylbenzoylphosphinate, sodium phenyl-2,4,6-trimethylbenzoylphosphinate, lithium bis(2,4,6-

trimethylbenzoyl)phosphinate, sodium bis(2,4,6-trimethylbenzoyl)phosphinate, Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, eosin Y, riboflavin phosphate, camphorquinone, Quantacure BPQ, Irgacure 819, Irgacure 1850, and Darocure 1173. In a preferred embodiment, the photoiniator is lithium phenyl-2,4,6-trimethylbenzoylphosphinate, sodium phenyl-2,4,6-trimethylbenzoylphosphinate, riboflavin phosphate. In another embodiment the photoinitiator is lithium phenyl-2,4,6-trimethylbenzoylphosphinate.

Prior to polymerization, the light curable biomatrix is combined with a suitable photoinitiator in a formulation suitable for ocular use comprising pharmaceutically acceptable excipients in suitable containers known in the art such as vials. The photoinitiator may be combined with the biomatrix prior to mixing with cells; alternatively the photoinitiator may be combined with the biomatrix after mixing with cells; alternatively the photoiniator may be added to the cells first, then combined with the biomatrix. The concentration of biomatrix and photoinitiator is dependent on the specific biomatrix and specific photoinitiator used, but is chosen to provide polymerization within a convenient light exposure duration, typically less than about 5 minutes; preferably less than about 2 minutes; more preferably less than about one minute. In one embodiment the photoinitiator is lithium phenyl-2,4,6-trimethylbenzoylphosphinate and its concentration in the formulation for cell delivery to the eye is about 0.01% w/v to about 0.15% w/v. In another aspect the lithium phenyl-2,4,6-trimethylbenzoylphosphinate concentration in the formulation for cell delivery to the eye is about 0.05% w/v or about 0.075% w/v. LAP may be synthesized using published procedure (Biomaterials 2009, 30, 6702-6707) and is also available from TCI (Prod. # L0290) and Biobots (BioKey).

The cells may be added to the GelMA in suitable containers known in the art such as vials or tubes. The cells may for example be added by pipetting into the GelMA and mixing by gentle pipetting up and down. In one embodiment the GelMA concentration in the composition suitable for ocular delivery is about 10 to about 200 mg/mL, or about 25 to about 150 mg/mL, or about 25 to about 75 mg/mL. In a preferred embodiment the GelMA concentration in the composition suitable for ocular delivery is about 25 mg/mL, about 50 mg/mL or about 75 mg/mL.

To polymerise the light curable biomatrix, the biomatrix, photoinitiator, and cells are exposed to a light source for a preferred duration, as described above. The wavelength of light used for polymerization will depend on the photochemical properties of the specific photoinitiator

used. For example, photoinitation of polymerization for Irgacure 2959 will occur with light of wavelength between 300-370nm; photoinitation of polymerization for lithium phenyl-2,4,6-trimethylbenzoylphosphinate will occur with light of wavelength between 300-420nm; photoinitation of polymerization for riboflavin-5'-phosphate will occur with light of wavelength between 300-500nm. The light source used may emit a range of wavelengths, like that achieved with incandescent lamps, gas discharge lamps, or metal vapor lamps; alternatively, the light source used may emit a narrow range of wavelengths, like that achieved with optical filters or with an light emitting diode (LED). Preferably, the light source used does not emit light with wavelength less than 315nm to avoid the damaging effects of UV irradiation on cells. In one embodiment, the light source is a white light source with a spectral range of 415-700nm. In another embodiment the light source is a LED light source with spectral range of about 365±5nm, about 375±5nm, about 385±5nm, about 395±5nm, about 405±5nm, about 415±5nm, about 425±5nm, about 435±5nm, about 445±5nm, about 455±5nm, or about 465±5nm. The intensity of light is chosen to minimize phototoxicity and provide polymerization within a convenient light exposure duration, typically less than about 5 minutes; preferably less than about 2 minutes; more preferably less than about one minute. One indication of polymerization is an increase in solution viscosity. Another indication of polymerization is the onset of gelation.

The polymerization of the biomatrix may occur on the ocular surface via bioprinting techniques, or alternatively on a carrier that is then transplanted to the ocular surface. Optionally the polymerization of the biomatrix may occur on the cornea surface in the anterior chamber, or alternatively on a carrier that is then transplanted to the cornea surface in the anterior chamber.

Carrier

The cells and localising agent suitable for ocular delivery are preferably delivered via a carrier such as a contact lens or amniotic membrane.

Contact lenses suitable for use according to the invention are preferably those which conform to the patient's corneal curvature and are able to be well tolerated by the patient in clinical practice for continuous use as bandage contact lenses for several days.

Examples of suitable types of contact lens according to the invention are consistent with what has been extensively validated in clinical use for long-term bandage contact lens use with Boston keratoprosthesis type 1 (which can be also used in patients with limbal stem cell deficiency) and described in : Thomas, Merina M.D.; Shorter, Ellen O.D.; Joslin, Charlotte E. O.D., Ph.D.; McMahon, Timothy J. O.D.; Cortina, M. Soledad M.D.Contact Lens Use in Patients With Boston Keratoprosthesis Type 1: Fitting, Management, and Complications. Eye Contact Lens. 2015 Nov;41(6):334-40.

A contact lens can be of any appropriate material known in the art or later developed, and can be a soft lens, a hard lens, or a hybrid lens, preferably a soft lens, more preferably a conventional hydrogel contact lens or a silicone hydrogel (SiHy) contact lens.

A “conventional hydrogel contact lens” refers to a contact lens comprising a hydrogel bulk (core) material which is a water-insoluble, crosslinked polymeric material, is theoretically free of silicone, and can contain at least 10% by weight of water within its polymer matrix when fully hydrated. A conventional hydrogel contact lens typically is obtained by copolymerization of a conventional hydrogel lens formulation (i.e., polymerizable composition) comprising silicone-free, hydrophilic polymerizable components known to a person skilled in the art.

Examples of conventional hydrogel lens formulation for making commercial hydrogel contact lenses include, without limitation, alfafilcon A, acofilcon A, deltafilcon A, etafilcon A, focofilcon A, helfilcon A, helfilcon B, hilafilcon B, hioxifilcon A, hioxifilcon B, hioxifilcon D, methafilcon A, methafilcon B, nelfilcon A, nesofilcon A, ocufilcon A, ocufilcon B, ocufilcon C, ocufilcon D, omafilcon A, phemfilcon A, polymacon, samfilcon A, telfilcon A, tetrafilcon A, and vifilcon A.

A “SiHy contact lens” refers to a contact lens comprising a silicone hydrogel bulk (core) material which is a water-insoluble, crosslinked polymeric material containing silicone and can contains at least 10% by weight of water within its polymer matrix when fully hydrated. A silicone hydrogel contact lens typically is obtained by copolymerization of a silicone hydrogel lens formulation comprising at least silicone-containing polymerizable component and hydrophilic polymerizable components known to a person skilled in the art.

Examples of SiHy lens formulation for making commercial SiHy contact lenses include, without limitation, asmofilcon A, balafilcon A, comfilcon A, delefilcon A, efrofilcon A, enfilcon A, fanfilcon A, galyfilcon A, lotrafilcon A, lotrafilcon B, narafilcon A, narafilcon B, senofilcon A, senofilcon B, senofilcon C, smafilcon A, somofilcon A, and stenfilcon A.

In a preferred embodiment the carrier is a contact lens selected from the group consisting of Balafilcon A, Lotrafilcon A, Lotrafilcon B, Senofilcon A and methafilcon A.

In a particularly preferred embodiment the carrier is a contact lens, which is Lotrafilcon B.

The carrier may be held in place on the ocular surface using fibrin glue or sutures to prevent eye movements from dislodging the construct.

The carrier combined with biomatrix and cells may be left on the eye for a range of times in order to deliver the cells, for example a few days to one week, preferably one week.

Other delivery methods:

In an alternative embodiment the LSCs may be delivered as a cell suspension to the ocular surface (without a localising agent such as a biomatrix and with/or without a carrier such as a contact lens). Compounds and excipients known in the art to improve tissue adhesion such as mucoadhesive agents, viscosity enhancers, or reverse thermal gelators may be included in the formulation.

Bioprinting step

The population of ocular cells, e.g. corneal endothelial cells, obtainable according to the method of cell population expansion according to the invention may be grafted to the eye of a subject, e.g., to the cornea of a subject.

The cell population according to the invention may be delivered via a localising agent suitable for ocular use which is a light curable, degradable biomatrix such as GelMA. The following methods describe procedures for controlling the delivery to the inner wall of the cornea.

Method 1. Bubble depression method (shown in Figure 22)

The dysfunctional endothelial cells may first be detached from the inner wall of the cornea by peeling/scraping or in a controlled manner using photodisruption with a femtosecond

laser. A small bolus of the cell-laden biomatrix is then injected near the interior surface of the cornea. This may be done manually using a standard syringe or custom applicator. It can also be controlled through a surgical system (e.g. constellation) or syringe pumps. A gas bubble is then injected beneath the bolus. The gas bubble squeezes the bolus against the posterior cornea, creating a thin coating. The entire gel is then cured using a using a UV or near UV light source, or any other spectral band needed to cure the biomatrix. Alternatively, the dysfunctional tissue may be left, and the biomatrix cured over top of it. The light source can be focused into different sizes using other optical focusing methods to control the curing area. The remaining uncured area can be flushed out using irrigating/aspirating canula.

Method 2. Subtractive method using femtosecond laser (shown in Figure 23)
The dysfunctional endothelial cells may first be detached from the inner wall of the cornea by peeling/scraping or in a controlled manner using photodisruption with a femtosecond laser. Alternatively, they may be left in place. The cell-laden biomatrix is then injected onto the interior surface of the cornea covering the void where tissue was removed or over the dysfunctional tissue. This may be done manually using a standard syringe or custom applicator. It can also be controlled through a surgical system (e.g. constellation) or syringe pumps. The biomatrix is then cured using a using a UV or near UV light source, or any other spectral band needed to cure the biomatrix. The femtosecond laser is then used to detach excess material, controlling the thickness and area to a desired distribution. The excess material is then removed with forceps through a corneal incision.

Method 3. Stain mask and absorption based thickness control (shown in Figure 24)
A biocompatible stain (Trypan Blue, Brilliant Blue, etc.) is firstly used to dye the inner surface of the cornea. The dysfunctional endothelial cells are then detached from the inner wall of the cornea by peeling/scraping. The cell-laden biomatrix containing the biocompatible stain is then injected onto the interior surface of the cornea covering the void where tissue was removed. The biomatrix is then cured using a using a UV or near UV light source, or any other spectral band needed to cure the biomatrix. The stain in the corneal tissue increases the light absorption acting as a mask to control the area of the cured biomatrix. Similarly, the stain in the biomatrix increases the absorption of light thereby controlling the depth/thickness of the cured material. Uncured gel material is then flushed from the anterior chamber using an irrigating/aspirating cannula.

Method 4. Dry anterior chamber application (shown in Figure 25)

The dysfunctional endothelial cells may first be detached from the inner wall of the cornea by peeling/scraping or in a controlled manner using photodisruption with a femtosecond laser. Alternatively it may be left in place. The anterior chamber of the anterior segment is then drained of aqueous and replaced with gas (e.g. air). The cell-laden biomatrix is then applied to interior surface of the cornea in small controlled droplets (allowing surface tension to disperse the drops), or painted using a brush or soft tip cannula. Hyaluronic acid may be applied to the biomatrix to alter its viscous properties and enable better control over dispensing/application. The entire biomatrix is then cured using a using a UV or near UV light source, or any other spectral band needed to cure the biomatrix. Finally, the anterior chamber is then filled again with balanced salt solution.

Method 5. Naturally Buoyant Formulation

The dysfunctional endothelial cells may first be detached from the inner wall of the cornea by peeling/scraping or in a controlled manner using photodisruption with a femtosecond laser. A small bolus of the cell-laden biomatrix is then injected near the interior surface of the cornea. The biomatrix is formulated to be naturally buoyant relative to aqueous humor or aerated to achieve the same effect. This causes the biomatrix to naturally rise to posterior cornea, creating a thin coating. The entire biomatrix is then cured using a using a UV or near UV light source, or any other spectral band needed to cure the biomatrix. Alternatively, the dysfunctional tissue may be left, and the biomatrix cured over top of it. The UV light source can be focused into different sizes using optical focusing methods to control the curing area. The remaining uncured area can be flushed out using aspiration canula.

<u>Other delivery methods</u>

In an alternative embodiment an expanded cell population, such as CECs as described herein, may be delivered as a cell suspension (without a localising agent such as a light curable, degradable biomatrix) and left to attach by gravity by having the patient look down for 3 hours. Compounds and excipients known in the art to improve tissue adhesion such as adhesive agents, viscosity enhancers, or reverse thermal gelators may be included in the formulation.

In yet another alternative embodiment an expanded cell population, such as CECs as described herein can also be delivered by using magnetic beads. A suspension of

CECs/beads in a medium suitable for ocular delivery is prepared and this is then injected into the eye. Cell attachment is promoted by a magnet applied to the eye. (Magnetic field-guided cell delivery with nanoparticle-loaded human corneal endothelial cells. Moysidis SN, Alvarez-Delfin K, Peschansky VJ, Salero E, Weisman AD, Bartakova A, Raffa GA, Merkhofer RM Jr, Kador KE, Kunzevitzky NJ, Goldberg JL.Nanomedicine. 2015 Apr;11(3):499-509. doi: 10.1016/j.nano.2014.12.002.)

Other methods of use of the LATS inhibitors according to the invention

In one aspect according to the invention it is possible to directly add the LATS inhibitors according to the invention directly to the surface of the eye to achieve cell population expansion. For example this can be done if the cell loss was not total and there is a remaining seeding population of cells to be expanded. For example if there are remaining limbal stem cells in the eye of the patient or if there are remaining corneal endothelial cells in the cornea of the patient as the case may be. For example, this can be achieved by preparing the compounds as disclosed herein in a formulation suitable for ocular use comprising pharmaceutically acceptable excipients and applying by eye drops to the eye. Alternatively, compounds as disclosed herein in a formulation suitable for ocular use comprising pharmaceutically acceptable excipients can be applied intraocularly, for example, intracamerally.

Pharmaceutically acceptable excipients enhance or stabilize the composition, or facilitate preparation of the composition. Pharmaceutically acceptable excipients include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible.

The LATS inhibitor according to the invention may be combined with ophthalmologically acceptable preservatives, co-solvents, surfactants, viscosity enhancers, penetration enhancers, buffers, sodium chloride, or water to form an aqueous ophthalmic suspension or solution. Topical ophthalmic products may be packaged, for example, in multidose form. Preservatives may thus be required to prevent microbial contamination during use. Suitable preservatives include: chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% w/v.

In an embodiment of the invention the LATS inhibitors according to the invention could be used to preserve cells, e.g. corneal epithelial and limbal cells until transplantation. After postmortem dissection by eye-bank specialists, corneas are preserved in media such as Optisol or PBS until transplantation. In a specific embodiment of the invention a LATS inhibitor may be added to a solution generally used for corneal transplantation, such as Optisol or PBS. Preferably the concentration of the LATS inhibitor in the preservation solution is 0.5 to 100 micromolar, more preferably 0.5 to 25 micromolar, particularly preferably 1 to 20 micromolar. In a specific embodiment the LATS inhibitors according to Formula I are added at a concentration of about 3 to10 micromolar.

Immunosuppressant, anti-inflammatory and antibiotic agents

In addition to, or as an alternative to, the use of a gene editing technique performed in vitro on a cell population, as described for limbal stem cells or corneal endotheal cells, to mitigate the risk of immune rejection of the transplanted cells to the patient, the cell population according to the invention may be administered simultaneously or sequentially with an immunosuppressant and/or anti-inflammatory agents or agents. Standard agents for immunosuppression or anti-inflammatory agents or agents may be used including, but not limited to, dexamethasone or cyclosporine.

Antibiotics may also be administered to the patient in conjunction with an expanded cell population (e.g. the limbal stem cells or corneal endotheal cells as described herein), such as the antibiotics cefazolin and tobramycin.

When the cell population of the present invention is administered together with another agent or agents, the cell population and the agent(s) can be administered sequentially in any order or simultaneously. In some embodiments, an expanded cell population according to the invention is administered in conjunction with surgical treatments. In other embodiments, the limbal stem cell population according to the invention is administered in conjunction with surgical treatments. In other embodiments, the corneal endothelial cell population according to the invention is administered in conjunction with surgical treatments.

Therapeutic uses

The ocular cell population according to the invention may be used in a method of treatment or prophylaxis of an ocular disease or disorder comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population comprising ocular cells.

The limbal stem cell population according to the invention may be used in a method of treatment or prophylaxis of an ocular disease or disorder comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population comprising limbal stem cells. Preferably the ocular disease or disorder is associated with limbal stem cell deficiency.

Limbal stem cell deficiency may arise as a result of several diverse conditions including but not limited to:

- direct stem cell damage from chemical or thermal burns or radiation injury;
- congenital conditions such as aniridia, sclerocornea, multiple endocrine neoplasia;
- autoimmune disorders such as Stevens Johnson syndrome or ocular cicatricial pemphigoid or collagen vascular diseases;
- chronic non-auto-immune inflammatory disorders such as contact lens use, dry eye disease, rosacea, staph marginal, keratitis (bacterial, fungal & viral), pterygia or neoplasm;
- iatrogenic, such as after multiple eye surgeries, excision of pterygia or neoplasm, cryotherapy;
- as a result of medication toxicity such as preservatives (thimerosal, benzalkonium), topical anesthetics, pilocarpine, beta blockers, mitomycin, 5-fluorouracil, silver nitrate, and oral medications causing Stevens Johnson syndrome.

(See: Dry Eye: a practical guide to ocular surface disorders and stem cell surgery. SLACK 2006 - Rzany B, Mockenhaupt M, Baur S et al. J. Clin. Epidemiol. 49, 769–773 (1996)).

The most commonly encountered causes of limbal stem cell deficiency in clinical practice are chemical burns, aniridia, Stevens Johnson Syndrome and contact lens use.

More preferably the ocular disease or disorder is limbal stem cell deficiency which arises due an injury or disease or disorder selected from the group consisting of chemical burns, thermal burns, radiation injury, aniridia, sclerocornea, multiple endocrine neoplasia, Stevens Johnson syndrome, ocular cicatricial pemphigoid, collagen vascular diseases, chronic non-

auto-immune inflammatory disorders arising from contact lens use, dry eye disease, rosacea, staph marginal, keratitis (including bacterial, fungal & viral keratitis), pterygia or neoplasm, limbal stem cell deficiency arising after multiple eye surgeries or excision of pterygia or neoplasm or cryotherapy; and limbal stem cell deficiency arising as a result of medication toxicity from a medication selected from the group consisting of preservatives (thimerosal, benzalkonium), topical anaesthetics, pilocarpine, beta blockers, mitomycin, 5-fluorouracil, silver nitrate, and oral medications causing Stevens Johnson syndrome.

In a specific embodiment, the present invention provides a method of treating limbal stem cell deficiency by administering to a subject in need thereof an effective amount of a limbal stem cell population obtainable by the method of cell population expansion according to the invention.

In a more specific embodiment, the present invention provides a method of treating limbal stem cell deficiency which arises due an injury or disorder selected from the group consisting of chemical burns, thermal burns, radiation injury, aniridia, sclerocornea, multiple endocrine neoplasia, Stevens Johnson syndrome, ocular cicatricial pemphigoid, collagen vascular diseases, chronic non-auto-immune inflammatory disorders arising from contact lens use, dry eye disease, rosacea, staph marginal, keratitis (including bacterial, fungal & viral keratitis), pterygia or neoplasm, limbal stem cell deficiency arising after multiple eye surgeries, or excision of pterygia or neoplasm or cryotherapy; and limbal stem cell deficiency arising as a result of medication toxicity from a medication selected from the group consisting of preservatives (thimerosal, benzalkonium), topical anesthetics, pilocarpine, beta blockers, mitomycin, 5-fluorouracil, silver nitrate, and oral medications causing Stevens Johnson syndrome by administering to a subject in need thereof a therapeutically effective amount of a limbal stem cell population obtainable by the method of cell population expansion according to the invention.

In yet a more specific embodiment, the present invention provides a method of treating limbal stem cell deficiency which arises due an injury or disease or disorder selected from the group consisting of chemical burns, aniridia, Stevens Johnson Syndrome and contact lens use by administering to a subject in need thereof a thereapeutically effective amount of a limbal stem cell population obtainable by the method of cell population expansion according to the invention.

When an adult is a recipient (transplant recipient), preferably greater than 1 000 p63alpha expressing cells may be administered to a patient in the methods of treamtent according to the invention. In a preferred embodiment, 1 000 to 100 000 p63alpha expressing cells may be administered to a patient in the methods of treatment according to the invention.

The corneal endothelial cell population according to the invention may be used in a method of treatment or prophylaxis of an ocular disease or disorder comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population comprising corneal endothelial cells. Preferably the ocular disease or disorder is associated with decreased corneal endothelial cell density. In a preferred embodiment the ocular disease or disorder is corneal endothelial dysfunction.

More preferably the ocular disease or disorder is corneal endothelial dysfunction which is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy), corneal transplant failure, posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, X-linked endothelial corneal dystrophy, aniridia, and corneal endothelitis. In a specific embodiment the ocular disease or disorder is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy) and corneal transplant failure.

In a specific embodiment, the present invention provides a method of treating corneal endothelial dysfunction by administering to a subject in need thereof an effective amount of a corneal endothelial cell population obtainable by the method of cell population expansion according to the invention.

In a more specific embodiment, the present invention provides a method of treating corneal endothelial dysfunction which is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy), corneal transplant failure, posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, X-linked endothelial corneal dystrophy, aniridia, and corneal endothelitis by administering to a subject in need thereof an

effective amount of a corneal endothelial cell population obtainable by the method of cell population expansion according to the invention.

In yet a more specific embodiment, the present invention provides a method of treating corneal endothelial dysfunction selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy) and corneal transplant failure by administering to a subject in need thereof an effective amount of a corneal endothelial cell population obtainable by the method of cell population expansion according to the invention.

When an adult is a recipient (transplant recipient), the corneal endothelial cell layer for use in the method of treatment according to the invention preferably has a final cell density in the eye of about at least 500 cells/mm^2 (area), preferably 1 000 to 3 500 cells/mm^2 (area), more preferably 2 000 to about 4 000 cells/mm^2 (area).

EXAMPLE SECTIONS A-C

The following examples are provided to further illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

Unless defined otherwise, the technical and scientific terms used herein have the same meaning as that usually understood by a specialist familiar with the field to which the disclosure belongs.

Example A1: LATS1 biochemical assay: Homogenous Time Resolved Fluorescence (HTRF) assay (compound examples 1-290)

The LATS1 biochemical HTRF assay was performed using the HTRF KinEASE-STK S1 kit (CisBio, catalogue number 62ST1PEC) according to manufacturer's instructions. Human LATS1 kinase domain protein was purchased from Carnabio (catalogue number 01-123), which harbors the catalytic domain of amino acids 589-1130, and was co-purified with human His-tagged MOBKL1A (NP_775-739). Compounds were added by ECHO liquid handler (Labcyte) into 384-well plates. Then 5 microlitre of the following solution was added into the wells (50 mM HEPES, 0.01% BSA, 100 nM Orthaovanadate, 1 mM MgCl2, 1 mM DTT, 0.6ng/microlitre LATS1 enzyme (Carnabio, 01-123), 2 micromolar STK1 localising aid

(CisBio)), followed with 5 microlitre of 2mM ATP in the kinase buffer (50 mM HEPES, 0.01% BSA, 100 nM Orthaovanadate, 1 mM MgCl2, 1 mM DTT) and incubated for 30 minutes at room temperature. 10 microlitre of detection mix (50 mM HEPES, 0.01% BSA, 100 nM Orthaovanadate, 1 mM MgCl2, 1 mM DTT, STK Antibody-Cryptate (CisBio), Streptavidin-XL665 (CisBio), 500 micromolar potassium fluoride, 50nM EDTA) was added to each well and incubated for 1 hour at room temperature. Plates were read on Pherastar (BMG Labtech) for HTRF (665nm/620nm). The IC_{50} is measured when the effect of the compound reduces the HTRF signal by 50%. LATS1 IC_{50} values for the exemplified compounds are shown in Table 1A.

Example A2: LATS1 biochemical Caliper Assay

Human LATS1 kinase domain protein was purchased from Carnabio (catalogue number 01-123), which harbors the catalytic domain of amino acids 589-1130, and was co-purified with human His-tagged MOBKL1A (NP_775-739). 5 microlitre of enzyme buffer, 100 nL of compounds and 5 microlitre of localising aid buffer were added into 384-well plates. The final assay reaction mix contains 100 mM HEPES, pH7.5, 0.1% BSA, 0.01% Triton X-100, 1 mM DTT, 10 mM MgCl2, 10 micromolar Sodium Orthovanadate, 10 micromolar Beta-Glycerophosphate, 400 micromolar ATP, 1% DMSO, 1.1 nM LATS1 enzyme (Carnabio, 01-123), 1 micromolar localising aid of FAM-KKLRRTLSVA-COOH (SEQ ID NO: 24) (NanoSyn). The plates were incubated at 25°C for 3 hours. 40 microlitre of stop buffer with 25 mM EDTA (NanoSyn) was added to each well to terminate the reaction. Localising aids and products were separated electrophoretically using the microfluidic-based Caliper Labchip 3000 Drug Discovery System (Caliper Life Sciences). Plates were read using blue laser excitation and green fluorescence detection and quantified by fluorescence intensity. The IC_{50} is measured when the effect of the compound reduces the product fluorescence signal by 50%. According to this assay the LATS1 IC_{50} values in micromolar for the following compounds are :

Ex. 49: 0.012, Ex. 14: 0.034, Ex. 65: 0.022, Ex, 139: 0.028, Ex. 133: 0.004.

Example A3: LATS2 biochemical Caliper Assay (compound Examples 1-290)

Human LATS2 kinase domain protein was purchased from Carnabio (catalogue number 01-124), which harbors the catalytic domain of amino acids 553-1088, and was co-purified with human His-tagged MOBKL1A (NP_775-739). 5 microlitre of enzyme buffer, 100 nL of compounds and 5 microlitre of localising aid buffer were added into 384-well plates. The

final assay reaction mix contains 100 mM HEPES, pH7.5, 0.1% BSA, 0.01% Triton X-100, 1 mM DTT, 10 mM MgCl2, 10 micromolar Sodium Orthovanadate, 10 micromolar Beta-Glycerophosphate, 400 micromolar ATP, 1% DMSO, 1.1 nM LATS2 enzyme (Carnabio, 01-124), 1 micromolar localising aid of FAM-KKLRRTLSVA-COOH (SEQ ID NO: 24) (NanoSyn). The plates were incubated at 25°C for 3 hours. 40 microlitre of stop buffer with 25 mM EDTA (NanoSyn) was added to each well to terminate the reaction. Localising aids and products were separated electrophoretically using the microfluidic-based Caliper Labchip 3000 Drug Discovery System (Caliper Life Sciences). Plates were read using blue laser excitation and green fluorescence detection and quantified by fluorescence intensity. The IC_{50} is measured when the effect of the compound reduces the product fluorescence signal by 50%. LATS2 IC_{50} values for the exemplified compounds 1-290 are shown in Table 1A.

Table 1A: Inhibitory Activity against LATS1 and LATS2

Example No.	LATS1 IC_{50} (μM)	LATS2 IC_{50} (μM)		Example No.	LATS1 IC_{50} (μM)	LATS2 IC_{50} (μM)
1	0.001	n.d.		117	0.004	n.d.
2	0.001	n.d.		118	0.005	n.d.
3	0.001	n.d.		119	0.009	n.d.
4	0.001	0.001		120	0.01	n.d.
5	0.002	n.d.		121	0.014	n.d.
6	0.001	0.008		122	0.015	n.d.
7	0.002	n.d.		123	0.018	n.d.
8	0.003	n.d.		124	0.022	n.d.
9	0.003	n.d.		125	0.023	n.d.
10	0.003	n.d.		126	0.053	n.d.
11	0.003	0.012		127	0.055	n.d.
12	0.004	0.015		128	0.077	n.d.
13	0.004	n.d.		129	0.187	n.d.
14	0.002	0.05		130	1.153	n.d.
15	0.004	n.d.		131	>0.833	n.d.
16	0.005	n.d.		132	>2.5	n.d.
17	0.005	n.d.		133	0.002	0.004

17a	0.003	n.d.		134	0.001	n.d.
17b	0.0009	n.d.		135	0.002	n.d.
17c	0.003	n.d.		136	0.002	n.d.
17d	0.001	n.d.		137	0.022	n.d.
18	0.006	n.d.		138	0.002	n.d.
19	0.006	n.d.		139	0.003	0.02
20	0.006	n.d.		140	0.004	n.d.
21	0.007	n.d.		141	0.004	n.d.
22	0.007	n.d.		142	0.006	n.d.
23	0.01	n.d.		143	0.007	n.d.
24	0.017	n.d.		144	0.01	n.d.
25	0.03	n.d.		145	0.012	n.d.
26	0.031	n.d.		146	0.033	n.d.
27	0.035	n.d.		147	0.069	n.d.
28	0.036	n.d.		148	0.168	n.d.
29	0.036	n.d.		149	0.171	n.d.
30	0.039	n.d.		150	4.27	n.d.
31	0.041	n.d.		151	>10	n.d.
32	0.047	n.d.		152	>2.5	n.d.
33	0.071	n.d.		153	>5	n.d.
34	0.095	n.d.		154	0.0	n.d.
35	0.118	n.d.		155	0.001	n.d.
36	0.199	n.d.		156	0.006	n.d.
37	0.233	n.d.		157	0.006	n.d.
38	0.24	n.d.		158	0.008	n.d.
39	0.244	n.d.		159	0.016	n.d.
40	0.328	n.d.		160	0.024	n.d.
41	0.708	n.d.		161	0.025	n.d.
42	0.711	n.d.		162	0.049	n.d.
43	>1.97	n.d.		163	0.051	n.d.
44	>2.5	n.d.		164	0.159	n.d.
45	>2.5	n.d.		165	>2.5	n.d.
46	>2.5	n.d.		166	0.014	n.d.

47	0.002	0.006		167	0.143	n.d.
48a	0.002	0.002		168	0.028	n.d.
48b	0.006	0.018		169	0.098	n.d.
49	0.001	0.021		170	1.28	n.d.
50	0.005	n.d.		171	0.054	n.d.
51	0.006	n.d.		172	0.017	n.d.
52	0.012	n.d.		173	0.061	n.d.
53	0.04	n.d.		174	0.197	n.d.
54	0.078	n.d.		175	0.324	n.d.
55	0.46	n.d.		176	>2.5	n.d.
56	1.16	n.d.		177	>2.5	n.d.
57	1.44	n.d.		178	>2.5	n.d.
58	0.001	0.17		179	0.002	n.d.
59	0.001	0.004		180	0.322	n.d.
60	0.001	n.d.		181	9.0	n.d.
61	0.001	n.d.		182	>10	n.d.
62	0.001	0.002		183	0.797	n.d.
63	0.001	n.d.		184	0.004	n.d.
64	0.001	n.d.		185	0.009	n.d.
65	0.002	0.03		186	0.009	0.05
66	0.002	0.011		187	0.016	n.d.
67	0.002	n.d.		188	0.017	n.d.
68	0.002	n.d.		189	0.083	n.d.
68a	0.001	n.d.		190	0.099	n.d.
68b	0.002	n.d.		191	0.189	n.d.
69	0.003	n.d.		192	0.201	n.d.
70	0.003	n.d.		193	>10	n.d.
71	0.004	n.d.		194	0.005	n.d.
72	0.004	n.d.		195	>10	n.d.
73	0.006	n.d.		196	>2.5	n.d.
74	0.006	n.d.		197	0.023	n.d.
75	0.008	n.d.		198	0.001	n.d.
76	0.009	n.d.		199	0.001	n.d.

77	0.009	n.d.		251	0.004	n.d.
78	0.011	n.d.		252	0.002	n.d.
79	0.011	n.d.		253	0.085	n.d.
80	0.011	n.d.		254	0.005	n.d.
81	0.011	n.d.		255	0.008	n.d.
82	0.013	n.d.		256	0.008	n.d.
83	0.017	n.d.		257	0.022	n.d.
84	0.021	n.d.		258	0.023	n.d.
85	0.025	n.d.		259	0.061	n.d.
86	0.026	n.d.		260	0.459	n.d.
87	0.03	n.d.		261	0.001	0.004
88	0.037	n.d.		262	0.003	n.d.
89	0.044	n.d.		263	0.007	n.d.
90	0.126	n.d.		264	0.007	n.d.
91	0.148	n.d.		265	0.008	n.d.
92	0.304	n.d.		266	0.009	n.d.
93	0.809	n.d.		267	0.013	n.d.
94	1.15	n.d.		268	0.018	n.d.
95	>1.44	n.d.		269	0.001	n.d.
96	>1.70	n.d.		270	0.002	n.d.
97	>2.5	n.d.		271	0.002	n.d.
98	1.35	n.d.		272	0.002	n.d.
99	0.001	n.d.		273	0.463	n.d.
100	0.004	n.d.		274	0.013	n.d.
101	0.005	0.03		275	0.011	n.d.
102	0.126	n.d.		276	0.017	n.d.
103	0.002	n.d.		277	0.065	n.d.
104	0.005	n.d.		278	0.09	n.d.
105	0.001	n.d.		279	>2.5	n.d.
106	0.003	n.d.		280	0.003	n.d.
107	0.008	n.d.		281	0.004	n.d.
108	0.027	n.d.		282	0.01	n.d.
109	0.001	n.d.		283	0.02	n.d.

110	0.002	n.d.		284	0.03	n.d.
111	0.001	n.d.		285	0.19	n.d.
112	0.002	n.d.		286	0.56	n.d.
113	0.235	n.d.		287	0.002	0.015
114	0.002	n.d.		288	0.004	0.034
115	0.002	n.d.		289	0.004	n.d.
116	0.003	n.d.		290	0.004	0.008

n.d. means not determined

Example A4: LATS1 biochemical Caliper Assay (compound Examples 291-335)

The LATS1 biochemical Caliper assay was performed as following.

Human LATS1 kinase domain protein was purchased from Carnabio (catalogue number 01-123; lot 15CBS-0098D). Human LATS1, catalytic domain [589-1130(end) amino acids of accession number NP_004681.1] was co-expressed as N-terminal GST-fusion protein (90 kDa) with human His-tagged MOBKL1A [1-216(end) amino acids of accession number NP_775739.1] using baculovirus expression system. GST-LATS1 was purified by using glutathione sepharose chromatography. The substrate (Fluo-SGKtide; Peptide for LATS1; lot BS-41067) has the following sequence: 5-Fluo-Nva-KKRNRRLSVA-amide (SEQ ID NO: 27) x TFA and was purchased from Biosyntan.

The reaction is performed in reaction buffer containing 50mM Hepes pH 7,5; 0.02% Tween20; 0.02% BSA; 1mM DTT; 10uM Na_3VO_4 and 10mM beta-Glycerolphosphat and fresh added 1mM $MgCl_2$ and qsp H_2O.

The substrate solution (2 x conc.) in Reaction Buffer contains 300 µM ATP and 4 µM Fluo-SGKtide.

The kinase solution (2 x conc.) in Reaction Buffer contains 20nM LATS1 kinase.

4.5 µL of 2x conc. Kinase solution, 50 nL of 1,8mM compounds and 4.5 µL of substrate solution were added into 384-well plates black small volume from Greiner and incubated at 32°C for 1 hour. 15 µL of stop buffer containing 100mM Hepes pH 7,5; 5% DMSO; 0.1% Coating reagent; 10mM EDTA and 0.02% Brij35 and qsp H_2O to each well to terminate the reaction.

Substrates and products were electrophoretically separated using the microfluidic-based

Caliper EZ Reader System (Caliper Life Sciences) using a 12 sipper chip (cat 760404). The separation takes place in Coating Buffer (idem Stop buffer) and containing 0.1% Coating reagent CR3 and 0.5% coating reagent CR8 (Perkin Elmer).

Plates were read using a LED with an excitation at 488 nm and a detection at 520 nm to quantify the fluorescence intensity. The IC_{50} is measured when the effect of the compound reduces the product fluorescence signal by 50%. LATS1 IC_{50} values for the exemplified compounds 291-335 are shown in Table1B.

Table 1B. Inhibitory Activity against LATS1

Example No.	LATS1 IC_{50} (μM)
291	0.4570
292	0.0433
293	0.0160
294	0.0045
295	0.0100
296	0.2089
297	0.0075
298	0.0590
299	0.0971
300	0.0012
301	0.0052
302	0.0595
303	0.0102
304	0.0096
305	0.6629
306	0.0012
307	0.0019
308	0.0687
309	0.0008
310	0.1639
311	1.0336
312	0.0024

313	0.0038
314	0.4667
315	0.0050
316	0.0425
317	0.0008
318	0.0018
319	0.0581
320	0.0007
321	0.0038
322	0.0292
323	0.0045
324	0.0104
325	0.0037
326	0.0572
327	0.1251
328	0.0189
329	0.0030
330	0.0031
331	0.0105
332	0.0076
333	0.0023
334	0.0061
335	n.t.

n.t.: not tested

Example A5: Mouse hepatic progenitor cell proliferation assay

The proliferation assay consists in measuring the compound induced three-dimensional growth of hepatic progenitor cells (HPC) into small organoids by means of high content imaging methods. The progenitor cells were isolated from C57Bl/6 wild type mice and expanded as described (Lu, W. Y. *et al.,* Nat. Cell Biol. 17, 971–983 (2015)). Cells are stocked in liquid nitrogen, thawed on need and tested using the following protocol:

HPC are harvested from their collagen I coated culture vessel (Corning; cat. Number 354487), counted and assessed for viability using a CEDEX cell counter (Roche). After centrifugation, 12 million cells are re-suspended in 2 ml of William's E Medium (Life Technologies; cat. Number 22551089) supplemented with 10% Fetal Bovine Serum (Amimed; cat. Number 2-01F10-I); 1% Penicillin/Streptomycin (Sigma; cat. Number 15140-122); 17.6 mM of NaHCO3 (Sigma; cat. Number S8761); 20 mM HEPES (Gibco; cat. Number H3375); 10 mM Nicotinamide (Sigma; cat. Number N0636-100); 14 mM glucose (Sigma; cat. Number G7021); 1mM Sodium Pyruvate (Sigma; cat. Number TMS-005); Insulin Transferrin Selenium (ITS) diluted 100 times from stock solution (Sigma; cat. Number I3148); 100nM Dexamethasone (Sigma; cat. Number D4902); 0.2 mM ascorbic acid (Sigma; cat. Number A7506-100G); 10 ng/ml recombinant human IL-6 (Preprotech; cat. Number 200-06); 10 ng/ml murine HGF (Preprotech; cat. Number 315-23; Lot. 0711S527); 10 ng/ml murine EGF (Preprotech; cat. Number 315-09; Lot. 0217179-1). Six milliliters of Matrigel (Corning; cat. Number 354277; Lot: 5187006) are added to the cell/media solution, thus the final cell concentration is 1.5 million HPC per milliliter, in 25% culture medium and 75% Matrigel. Five microliter (µl) of the cell/Matrigel suspension are transferred in each well of the clear bottom 96 well assay plate (Corning; cat. Number 356649) using the Star dispenser (Hamilton). After 20 minutes incubation at 37°C and 5%CO_2 to allow for Matrigel polymerization, 45 µl of cell culture media are dispensed on top.

In the compound source plate, 300 µl of cell culture media are dispensed on 1.2 µl of compounds dissolved in 90% DMSO. After proper mixing, 50 µl of the compound solution is transferred to the assay plate using a CyBi Well (CyBio). Each compound is tested in 8-point concentration curves having a dilution factor of 3.16 between each concentration. The maximum compound concentration tested is 20 µM and the lowest is 9nM. The assay plates are incubated for 4 days at 37°C and 5%CO_2.

Following the incubation, the organoids are fixed with phosphate buffer saline (PBS) (Gibco; cat. Number 10010-015) containing 4% Paraformaldehyde (Electron Microscopy Sciences; cat. Number 15714-S) and Hoechst 33342 (Life Technologies; cat. Number H3570) diluted 1/5000 of the stock solution to stain the nuclei. The plates are incubated 90 minutes at room temperature and washed once with 300 µl PBS containing 1% Penicillin/Streptomycin.

Plates are imaged with the CV7000 imager (Yokogawa) at a 10 fold magnification using the bright field lamp (lamp power: 10%; exposure time: 20ms) and the 405 nanometer laser

(laser power: 100%; exposure time 200ms). Four different images per well were acquired and analyzed with the Yokogawa image analysis software (YAS). The output feature used to determine organoid size is the mean number of nuclei per organoid, averaged on the well. The mean number of nuclei per organoid (x) is normalized to the neutral control treatment (DMSO), using the following calculation: [xn = +100 (x - NC) / NC]. The neutral control-normalized data are used for automated curve fitting to derive curve parameters per compound, including EC50 values.

The mouse hepatic progenitor cell proliferation assay may easily be used to test whether a compound is effective at inducing hepatic progenitor cell proliferation. Certain compounds of the invention were tested in the mouse hepatic progenitor cell proliferation assay and found to have EC_{50} values of less than 20 μM, e.g. Examples 48a and 58 were found to have EC_{50} values of 0.26 and 0.84 μM respectively. Certain other compounds of the invention were tested in the mouse hepatic progenitor cell proliferation assay and found to have EC_{50} values of greater than 20 μM, e.g. Example 303.

Example A6 : pYAP HTRF Assay (HaCaT cells)

The pYAP HTRF assay was performed using the Phospho-YAP (SER127) 10000 test kit (CisBio, catalogue number 64YAPPEH) according to manufacturer's instructions. Briefly, HaCaT cells were suspended at $1.1x10^6$ cells/mL in DMEM (no phenol red) +10% FBS with penicillin-streptomycin-glutamine. 5 μL of cells were dispensed into 1536 well white solid bottom, tissue culture treated plates (5500 cells/well), and incubated for 48 hours at 37°C. 50 nL test compound was transferred into the assay plates containing HaCaT cells using Pintool (GNF) and incubated for 2 hours. Lysis buffer (CisBio) was added to the assay wells and incubated for 5 minutes at room temperature, followed by the addition of 2 μL detection antibodies (1:40 dilution of each pYAP d2 ab and pYAP cryptate antibody stocks). The plates were incubated overnight (20 hrs) at room temperature covered with metal lids. Plates were read on Pherastar (BMG Labtech) for HTRF (665 nm/620 nm). The IC_{50} is measured when the effect of the compound reduces the HTRF signal by 50%. Results are shown in Table 1C.

Example A7: YAP Translocation Assay (HaCaT cells)

HaCaT cells were suspended at $0.4x10^6$ cells/ml in DMEM +10% FBS with penicillin-streptomycin-glutamine. 50 μl of cells were dispensed into 384-well clear bottom assay plates (20,000 cells/well), and incubated overnight at 37°C. 100 nL test compound was

transferred into the assay wells containing HaCaT cells using ECHO (Labcyte). After 24 hours incubation at 37°C, 7 µL of 32% PFA was dispensed into each well and incubated for 45 minutes at room temperature. Plates were washed 3 times with PBS, leaving 20 µL of PBS per well, then treated with 0.1% Triton and 1.5% BSA in PBS for 45 minutes at room temperature. Plates were washed for 5 times with PBS, leaving 20 µL of PBS per well. 20 µL of 1:1000 primary YAP ab (Santa Cruz Biotechnology, catalogue number 63.7) and 1:1500 Draq5 in PBS with 1.5% BSA was added to each well and incubated for 4hrs at room temperature. After 3 times PBS wash, leaving 20 µL of PBS per well, each well was incubated with 20 µL of 1:2000 AlexaFluor 488_A21202 secondary antibody (Molecular Probes) for 4hrs at room temperature. Plates were further washed 3 time with PBS, sealed and imaged on the high-content Opera imaging system (PerkinElmer). Results are shown in Table 1C.

Table 1C. Inhibitor of phosphorylation of YAP and YAP Nuclear Translocation

Example No.	HaCaT pYAP IC_{50} (µM)	HaCaT nuclear translocation EC_{50} (µM)		Example No.	HaCaT pYAP IC_{50} (µM)	HaCaT nuclear translocation EC_{50} (µM)
1	0.019	0.326		117	2.31	4.47
2	0.067	0.584		118	2.05	5.01
3	0.853	0.641		119	n.d.	5.18
4	1.15	2.53		120	2.14	2.93
5	2.19	2.48		121	n.d.	8.17
6	1.24	1.70		122	7.4	5.8
7	0.165	0.733		123	9.45	9.2
8	0.198	1.20		124	8.01	12.0
9	2.10	2.42		125	n.d.	8
10	2.96	3.55		126	n.d.	>20
11	0.646	1.43		127	n.d.	>20
12	1.15	1.75		128	n.d.	>20
13	13.45	8.51		129	n.d.	>20

14	7.31	4.25		130	n.d.	>20
15	0.66	3.48		131	n.d.	>20
16	9.79	15.8		132	n.d.	>20
17	0.606	2.63		133	0.414	1.67
17a	4.95	4.1		134	0.539	1.19
17b	0.765	1.22		135	0.488	0.85
17c	4.28	4.68		136	1.14	2.96
17d	0.402	1.72		137	35.5	>17.8
18	1.52	3.46		138	3.37	9.07
19	24.8	17.6		139	0.38	2.66
20	1.77	5.18		140	1.24	2.26
21	19.2	8.81		141	13.56	>10
22	11.4	13.0		142	>100	>28.02
23	9.58	11.8		143	>10	>20
24	34.7	33.1		144	0.606	4.44
25	n.d.	>20		145	>100	9.31
26	54.6	>100		146	n.d.	>20
27	n.d.	>14.4		147	>10	>20
28	n.d.	>20		148	n.d.	>20
29	n.d.	>20		149	>10	>20
30	n.d.	>20		150	n.d.	n.d.
31	n.d.	>20		151	n.d.	n.d.
32	>10	>20		152	n.d.	>20
33	>20	>20		153	>10	>20
34	n.d.	>20		154	2.53	1.06
35	n.d.	>20		155	1.58	1.05
36	n.d.	>20		156	>100	90.1
37	n.d.	>6.67		157	4.05	3.78
38	>10	>20		158	11.2	6.29
39	n.d.	>11.6		159	>10	>20
40	>10	>20		160	6.8	>20
41	>10	>20		161	>100	>20
42	n.d.	11.6		162	n.d.	>20

43	n.d.	>20		163	n.d.	>20
44	>10	>20		164	n.d.	>20
45	n.d.	>20		165	n.d.	>20
46	n.d.	>20		166	n.d.	8.45
47	0.55	1.31		167	n.d.	>20
48a	0.37	0.80		168	26.5	11.6
48b	7.25	7.22		169	n.d.	n.d.
49	0.607	1.17		170	>10	>20
50	17.0	3.81		171	n.d.	17.2
51	57.8	4.39		172	>10	>20
52	11.3	>20		173	n.d.	>20
53	n.d.	>20		174	>10	>20
54	n.d.	>20		175	>10	>20
55	>10	>20		176	n.d.	>20
56	>10	>20		177	n.d.	>20
57	n.d.	>20		178	n.d.	>20
58	0.185	0.302		179	>10	>20
59	0.019	0.341		180	>10	>20
60	n.d.	0.44		181	n.d.	>10
61	0.141	0.517		182	n.d.	>20
62	0.040	0.538		183	n.d.	>20
63	0.131	0.338		184	2.51	2.81
64	0.063	0.479		185	n.d.	11.5
65	0.895	1.99		186	n.d.	>20
66	0.534	1.01		187	>100	>20
67	n.d.	0.685		188	>100	12.0
68	1.23	3.39		189	n.d.	>10
68a	1.43	1.68		190	n.d.	>20
68b	4.27	2.92		191	n.d.	n.d.
69	4.72	11.5		192	>10	>20
70	0.422	1.68		193	n.d.	n.d.
71	0.026	1.57		194	11.3	4.24
72	1.17	3.65		195	n.d.	n.d.

73	9.07	8.26		196	>10	>20
74	3.4	12.7		197	n.d.	n.d.
75	3.8	6.91		198	2.18	3.23
76	6.42	9.41		199	n.d.	9.1
77	>100	13.4		251	1.96	10.87
78	12.1	14.3		252	6.98	>10
79	0.765	3.87		253	16.67	>20
80	21.6	12.9		254	2.91	4.63
81	42.8	17.0		255	3.18	5.39
82	>100	>20		256	11.07	15.6
83	42.1	28.2		257	n.d.	>20
84	65.2	>20		258	n.d.	9.92
85	31.0	9.81		259	n.d.	17.31
86	56.0	>20		260	n.d.	>20
87	51.6	>20		261	0.202	1.22
88	69.8	29.3		262	n.d.	0.311
89	>100	>100		263	3.22	5.99
90	n.d.	>20		264	14.8	11.2
91	>10	>20		265	22.3	>20
92	n.d.	>20		266	n.d.	5.35
93	n.d.	>20		267	7.19	9.75
94	n.d.	>20		268	n.d.	>20
95	>10	>20		269	0.294	0.438
96	n.d.	>20		270	0.423	0.873
97	n.d.	>20		271	0.187	1.07
98	n.d.	n.d.		272	3.51	5.97
99	0.113	0.606		273	>100	>20
100	11.6	4.83		274	n.d.	17.3
101	5.4	>20		275	n.d.	15.1
102	n.d.	n.d.		276	n.d.	>20
103	2.64	2.27		277	n.d.	>20
104	2.6	5.19		278	n.d.	>20
105	19.9	>20		279	n.d.	>20

106	14.2	>20		280	0.476	2.73
107	31.2	20.0		281	0.656	2.89
108	11.8	>19.3		282	8.27	11.32
109	0.977	4.71		283	8.25	14.19
110	2.97	15.29		284	46.46	>41.19
111	0.33	2.70		285	n.d.	>20
112	1.21	3.52		286	n.d.	>20
113	n.d.	>20		287	2.99	3.88
114	0.565	1.33		288	18.3	7.42
115	1.05	1.66		289	34.1	6.50
116	0.782	2.18		290	n.d.	1.39

n.d. means not determined

Example A8: Target Identification

siRNA against MST1/2 or LATS1/2 were transfected into human HaCaT cells. Forty-eight hours later, cells were treated with 1 μM of Okadaic acid for two hours to enhance pYAP signals. Cell lysates were subjected to western blot analysis for pYAP (Ser127) described below. The results of the experiment are reported in Figures 33A to 33C.

Western Blot Analysis

Cell lysates (25 μg per lane) were mixed with XT sample buffer and reducing agent (Bio-Rad), separated by 4-12% gradient SDS Criterion precast gel (Bio-Rad), and transferred to a nitrocellulose membrane (Bio-Rad). Proteins were detected with primary antibodies and horseradish peroxidase-conjugated secondary antibodies by using an enhanced chemiluminescence kit (ThermoFisher). Primary antibodies used were anti-pYAP antibody (Cell Signaling, catalogue number 13008S) and anti ACTIN antibody (Abcam, catalogue number ab8227)

Example A9: *In vivo* Pharmacodynamics (PD)

8-10 week old male C57BL6 mice (Envigo) were used for the in vivo PD study. A sterile surgical 6 mm punch biopsy tool was used to make two full-thickness excisional wounds on the dorsum of the anesthetized mouse. LATS inhibitors were formulated in 49.5% propylene glycol / 0.5% Tween80 / 49% PBS / 1% HPMC, and administered topically at a dosing

volume of 3 µL. The wound area was covered with an adhesive dressing (Tegaderm). The animals were then wrapped with self-adhesive bandage. After two daily doses, the skin samples around the wound edge (2 mm ring-shaped samples) were collected using scissors 7 hour after the last dose.

The harvested samples were subjected to RNA extraction using the RNeasy kit (Qiagen), according to the manufacturer's instruction. Two-Step TaqMan RT-PCR analysis was performed on a PTC-200 peltier thermal cycler (MJ Research) and an ABI PRISM 7900HT Sequence Detection system (Applied Biosystems). cDNA was synthesized using a High-Capacity cDNA Archive kit (Applied Biosystems) according to the manufacturer's instructions. TaqMan analyses were performed using TaqMan Universal Master mix (Applied Biosystems) and *Cyr61* and *Gapdh* probes (Applied Biosystems) according to the manufacturer's instructions. mRNA expression levels for the target genes were normalized to *Gapdh* mRNA levels and data analyzed using SDS 2.0 software (Applied Biosystems) to calculate relative RNA quantities.

All animal studies were conducted at the Genomics Institute of the Novartis Research Foundation (GNF). The experimental protocols were in compliance with animal welfare regulations and approved by the Institutional Animal Care and Use Committee at GNF.

The resulted relative *Cyr61*/*Gapdh* expression levels against test compound concentrations were plotted as a bar graph in Figure 34.

Example A10: *In vivo* histology and Ki67 staining

7 week old male C57BL6 mice were used for the in vivo histology study. LATS inhibitors were formulated in 70% PG / 30% EtOH, and administered topically at a dosing volume of 25 µL (250 µg per dosge) to the shaved dorsal surface. After 3 days of twice a day dosing, the skin samples were collected, subjected to immuno-histology staining for Ki67 (ThermoFisher Scientific, Catalogue number RM-9106). The sections were observed visually, and Ki67 postive cells were counted by an in-house imaging algorithm. Representative micrographs of Ki67 staining were shown in Figure 35A. The abundance of

Ki67 positive cells in untreated and treated mouse skin cell were plotted as a scatter plot in Figure 35B.

Example A11: pYAP HTRF Assay (JHH-5 cells)

The pYAP HTRF assay is performed using compound-treated JHH-5 cells (Fujise et al., Hepatogastroenterology. 1990 Oct;37(5):457-60) that are analyzed with the Phospho-YAP (Ser127) 50000 test kit (CisBio catalog number 64YAPPEI) according to manufacturer's instructions. Briefly, JHH-5 cells are suspended at 0.48x10^6 cells/ml in William's E medium without phenol-red (Thermo Fisher Scientific Gibco catalog number A1217601) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Thermo Fisher Scientific Gibco catalog number 16140071) and penicillin-streptomycin at 100 U/ml each (Thermo Fisher Scientific Gibco catalog number 15140122). Cells are dispensed into 384well clear bottom culture plates (50ul/well, at 24000 cells per well, Greiner catalog number 781091), and incubated for 24 hours in a cell culture incubator at 37°C. Test compounds are transferred into culture plates containing JHH-5 cells using an Echo550 (Labcyte) acoustic dispenser (50nl/well), followed by incubation for 2 hours at 37°C. Lysis buffer (part of CisBio kit catalog number 64YAPPEI) is added to the assay plates as a four-fold concentrate (corresponding to 16ul/well) and incubated for 30 minutes at room temperature with agitation. Lysate is transferred from culture plates into white low-volume 384well assay plates (12ul/well, Greiner catalog number 784075), followed by the addition of 3ul/well of detection antibodies (part of CisBio kit catalog number 64YAPPEI: 1:40 dilution of each pYAP d2 antibody and pYAP cryptate antibody stocks). The plates are sealed and incubated overnight (18 hours) at room temperature protected from light. Plates are read on an EnVision plate reader (Perkin Elmer) at emission wavelengths of 665nm and 620nm. The HTRF signal ratio x per well is calculated as [x=Signal(665nm)/Signal(620nm)x10000] as specified in the manufacturer's protocol. The signal ratio x per well is normalized to active control and neutral control treatment (DMSO), scoring median NC as 0% and median AC as -100% to calculate normalized signal ratio xn per well as [xn = ±100 (x - NC) / (AC - NC)]. The control-normalized data is used for automated curve fitting to derive curve parameters per compound, including IC_{50} values. pYAP IC_{50} values in JHH-5 cells for the exemplified compounds are shown in Table 1D.

Table 1D. Inhibition of phosphorylation of YAP in JHH5 cells

Example No.	JHH-5 pYAP IC_{50} (μM)	Example No.	JHH-5 pYAP IC_{50} (μM)
1		**150**	n.d.
2	> 10	**151**	n.d.
3	1.0	**152**	n.d.
4	> 10	**153**	n.d.
5	2.3	**154**	n.d.
6	2.6	**155**	2.6
7	1.6	**156**	n.d.
8	1.9	**157**	9.2
9	n.d.	**158**	n.d.
10	8.7	**159**	n.d.
11	n.d.	**160**	n.d.
12	2.9	**161**	n.d.
13	n.d.	**162**	n.d.
14	> 10	**163**	n.d.
15	4.1	**164**	n.d.
16	n.d.	**165**	n.d.
17	n.d.	**166**	n.d.
18	n.d.	**167**	n.d.
19	n.d.	**168**	n.d.
20	n.d.	**169**	n.d.
21	n.d.	**170**	n.d.
22	n.d.	**171**	n.d.
23	n.d.	**172**	n.d.
24	n.d.	**173**	n.d.
25	n.d.	**174**	n.d.
26	n.d.	**175**	n.d.
27	n.d.	**176**	n.d.
28	n.d.	**177**	n.d.
29	n.d.	**178**	n.d.
30	> 10	**179**	n.d.
31	n.d.	**180**	n.d.

32	n.d.	**181**	n.d.
33	n.d.	**182**	n.d.
34	n.d.	**183**	n.d.
35	n.d.	**184**	6.5
36	n.d.	**185**	n.d.
37	n.d.	**186**	n.d.
38	n.d.	**187**	n.d.
39	n.d.	**188**	n.d.
40	> 10	**189**	n.d.
41	n.d.	**190**	n.d.
42	n.d.	**191**	n.d.
43	n.d.	**192**	n.d.
44	n.d.	**193**	n.d.
45	n.d.	**194**	n.d.
46	n.d.	**195**	n.d.
47	n.d.	**196**	n.d.
48a	0.6	**197**	n.d.
48b	n.d.	**198**	1.3
49	1.6	**199**	3.2
50	> 10	**251**	1.5
51	n.d.	**252**	n.d.
52	n.d.	**253**	n.d.
53	n.d.	**254**	1.3
54	n.d.	**255**	7.1
55	n.d.	**256**	> 10
56	n.d.	**257**	> 10
57	n.d.	**258**	> 10
58	0.6	**259**	n.d.
59	1.1	**260**	n.d.
60	n.d.	**261**	0.6
61	1.4	**262**	n.d.
62	0.6	**263**	> 10
63	n.d.	**264**	> 10

64	2.1	**265**	> 10
65	5.8	**266**	n.d.
66	6.2	**267**	5.9
67	n.d.	**268**	> 10
68	3.5	**269**	0.8
69	n.d.	**270**	0.8
70	4.0	**271**	0.8
71	> 10	**272**	8.1
72	4.6	**273**	> 10
73	n.d.	**274**	> 10
74	n.d.	**275**	> 10
75	n.d.	**276**	> 10
76	n.d.	**277**	n.d.
77	n.d.	**278**	n.d.
78	n.d.	**279**	n.d.
79	2.1	**280**	3.8
80	n.d.	**281**	5.9
81	n.d.	**282**	n.d.
82	n.d.	**283**	> 10
83	n.d.	**284**	n.d.
84	> 10	**285**	n.d.
85	> 10	**286**	n.d.
86	n.d.	**287**	9.0
87	n.d.	**288**	n.d.
88	n.d.	**289**	n.d.
89	n.d.	**290**	3.0
90	n.d.	**291**	n.d.
91	n.d.	**292**	n.d.
92	n.d.	**293**	n.d.
93	n.d.	**294**	1.1
94	n.d.	**295**	> 10
95	n.d.	**296**	n.d.
96	n.d.	**297**	> 10

97	n.d.	**298**	n.d.
98	n.d.	**299**	n.d.
99	5.7	**300**	0.9
100	n.d.	**301**	> 10
101	3.3	**302**	n.d.
102	n.d.	**303**	> 10
103	n.d.	**304**	> 10
104	n.d.	**305**	n.d.
105	n.d.	**306**	1.0
106	n.d.	**307**	1.3
107	n.d.	**308**	> 10
108	n.d.	**309**	2.0
109	1.5	**310**	n.d.
110	1.5	**311**	n.d.
111	2.4	**312**	7.7
112	3.2	**313**	7.8
113	n.d.	**314**	n.d.
114	n.d.	**315**	5.7
115	3.4	**316**	n.d.
116	2.2	**317**	3.2
117	> 10	**318**	3.1
118	4.0	**319**	> 10
119	n.d.	**320**	1.0
120	n.d.	**321**	n.d.
121	n.d.	**322**	n.d.
122	8.0	**323**	n.d.
123	n.d.	**324**	n.d.
124	n.d.	**325**	n.d.
125	n.d.	**326**	n.d.
126	n.d.	**327**	n.d.
127	n.d.	**328**	n.d.
128	n.d.	**329**	n.d.
129	n.d.	**330**	n.d.

130	n.d.	**331**	n.d.
131	n.d.	**332**	n.d.
132	n.d.	**333**	n.d.
133	1.7	**334**	n.d.
134	1.0	**335**	n.d.
135	n.d.		
136	1.3		
137	n.d.		
138	n.d.		
139	1.7		
140	2.2		
141	n.d.		
142	n.d.		
143	n.d.		
144	n.d.		
145	n.d.		
146	n.d.		
147	n.d.		
148	n.d.		
149	n.d.		

n.d. means not determined

Example A12: YAP nuclear translocation assay (JHH-5 cells)

The YAP nuclear translocation assay is performed using compound-treated JHH-5 cells (Fujise et al., Hepatogastroenterology. 1990 Oct;37(5):457-60) that are analyzed by high-content imaging following an anti-YAP1 antibody stain. Briefly, JHH-5 cells are suspended at 0.48x10^6 cells/ml in William's E medium without phenol-red (Thermo Fisher Scientific Gibco catalog number A1217601) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Thermo Fisher Scientific Gibco catalog number 16140071) and penicillin-streptomycin at 100 U/ml each (Thermo Fisher Scientific Gibco catalog number 15140122). Cells are dispensed into 384well clear bottom culture plates (50ul/well, at 24000 cells per well, Greiner catalog number 781091), and incubated for 24 hours in a cell culture incubator at 37°C. Test compounds are transferred into culture plates containing JHH-5 cells using an

Echo550 (Labcyte) acoustic dispenser (50nl/well). Following an incubation of 4 hours at 37°C, 12.5ul of 20% PFA stock solution (Electron Microscopy Sciences catalog number 15713S) is dispensed into each well for a final concentration of 4% (v/v) and incubated for 20 minutes at room temperature. Plates are washed with TBS (prepared from ten-fold concentrate, Sigma Aldrich catalog number T5912) and then permeabilized with 0.1% (v/v) Triton X-100 solution (Sigma Aldrich catalog number 93443) in DPBS for 15 minutes at room temperature. Plates are again washed with TBS, followed by addition of anti-YAP1 antibody (Novus Biologicals catalog number NB110-58358) to each well at a dilution of 1:500 in 3% (w/v) BSA in DPBS (prepared from 35% stock solution, Sigma Aldrich catalog number A7979) and incubation overnight at 4°C. After removal of primary antibody solution and washing with TBS, each well is incubated for 2 hours at room temperature with a 1:1000 dilution of goat anti-rabbit secondary antibody conjugated to Alexa Fluor 647 (Thermo Fisher Scientific catalog number A-21244) in 3% (w/v) BSA in DPBS (prepared from 35% stock solution, Sigma Aldrich catalog number A7979) supplemented with Hoechst 33342 solution (Thermo Fisher Scientific catalog number 3570) at a 1:10000 dilution. Plates are again washed with TBS and then sealed and imaged on the IN Cell Analyzer High-Content Analysis System (GE Healthcare Life Sciences). By comparison of Hoechst nuclear stain to total intracellular YAP Alexa Flour 647 stain using the CellProfiler image analysis software (Carpenter et al., Genome Biol. 2006;7(10):R100), the signal corresponding to nuclear YAP is derived. Nuclear YAP signal x is normalized to active control and neutral control treatment (DMSO), scoring median NC as 0% and median AC as +100% to calculate normalized nuclear YAP signal xn per well as [xn = ±100 (x - NC) / (AC - NC)]. The control-normalized data were used for automated curve fitting to derive curve parameters per compound, including EC_{50} values.

Example A13: In vivo treatment of mice

All animal studies were carried out in accordance with federal, state, local and institutional guidelines governing the use of laboratory animals in research. Male C57BL/6J mice (age 8-10 weeks) were purchased from Jackson Labs (Bar Harbor, ME). Example 46 was formulated for oral dosing at 3 mg/ml, and Example 261 at 1 mg/ml, in a suspension of methylcellulose:Tween80:Water (0.5:0.5:99). Single oral dose of either compound was at 10 mL/kg body weight. Blood and liver tissue were collected under deep anesthesia

(isoflurane) at either 2 h post-dose (for mRNA analysis) or 24 h post-dose (for immunohistochemistry).

Phospho-YAP (pYAP) HTRF in liver tissue

Frozen liver tissue was transferred into a Lysing Matrix tube (MP, cat# 6913-500) with a 1:50 solution of Protease inhibitor (Sigma-Aldrich, cat# P8340) in Phosphosafe extraction buffer (Novagen, cat# 71296-3) and homogenized using the FastPrep-24 (MP biomedicals). The mixture was kept on ice for 20min and then centrifuged at 14000rpm for 20min. The supernatant was transferred to a new tube. The protein concentration was measured using the BCA Protein Assay Kit (Pierce cat. 23227) following the manufacturer`s protocol. All samples were normalized to a final concentration of 5µg/µl in a total volume of 180µl of Phosphosafe and plated in a 96well plate for the HTRF assay. pYAP was assayed by HTRF using the Phospho-YAP (SER127) 50000 test kit (Cisbio, catalogue number 64YAPPEI). Mouse liver lysates in extraction buffer alone (Phosphosafe) or 1:1 mix of Phosphosafe with HTRF Lysis buffer (Cisbio) were assayed in duplicate wells of 384-well assay plates (12 µL/well). The same input quantity was used for each sample, based on total protein content (30-60ug per sample per well). Detection antibodies phospho-YAP d2 and phospho-YAP cryptate each diluted 1:40 in Detection buffer (Cisbio) were added (3 µL/well, final dilution 1:200 for each antibody) after which the plates were sealed and incubated overnight (18hrs) at room temperature protected from light. Plates were read on an EnVision (PerkinElmer) with emission wavelengths of 665nm & 620nm. The HTRF signal ratio was calculated as Signal(665nm)/Signal(620nm)) x 10000 as specified in the manufacturer's protocol. One HTRF signal value (mean of duplicate wells) was used for each lysate sample in treatment group plots.

Quantitative RT-PCR

Total liver RNA was isolated from tissue after storage in RNAlater reagent (Qiagen), using a TRIzol method according to the manufacturer's instructions (Life Technologies). Relative mRNA abundance was measured by quantitative RT-PCR using TaqMan gene expression assays and reagents according to standard protocols (Applied Biosystems). Mouse probes for YAP target genes were CTGF (Mm01192933_g1) and Cyr61 (Mm00487498_m1). Mouse GAPDH was used as an internal control (probe 4351309). Relative changes in

mRNA were calculated by the ΔΔCt method. Changes in gene expression with compound treatment are expressed as fold-change relative to the vehicle group.

Immunohistochemistry (IHC) for Ki67

Liver tissue was fixed in 10% neutral buffered formalin for 24-48 h. Processing and embedding in paraffin was by standard procedures. Sections were cut at 5 microns thickness and stained using a Ventana automated system. Primary antibody for mouse Ki67 was a rabbit IgG (Bethyl Labs). Ki67-positive hepatocytes were quantified by image analysis using the HALO platform. At least 3 sections per animal were analyzed, from the middle of the medial lobe.

Table 1E. Results of compound treatment in mouse

	pYAP (% of control)	CTGF mRNA (fold-change)	Cyr61 mRNA (fold-change)	Ki67 IHC (fold-change)
Example 46 30 mg/kg	64	2.1 (+/- 0.4)	3.5 (+/- 0.5)	3.3 (+/- 0.2)
Example 261 10 mg/kg	60	2.5 (+/- 0.2)	3.9 (+/- 0.6)	3.1 (+/- 0.5)

mRNAs for YAP target genes CTGF and Cyr61 were increased 2 h and proliferation indicated by Ki67 immunohistochemistry (IHC) was increased 24 h post single oral dose of LATS kinase inhibitors. Values are expressed as fold-change relative to the vehicle group with (mean +/- SEM).

Example B1: Human limbal epithelial cell isolation

Research-consented cadaveric human corneas were obtained from eye banks. Limbal rims were dissected and partially dissociated in a 1.2 mg/ml dispase solution for 2 hours at 37°C followed by 10 minutes in TrypLE (Life Technologies). Pieces of limbal crypts were then carefully cut out of the partially dissociated limbal rims and rinsed by centrifugation). Cells obtained in this manner were used in the Examples B2-B11 below.

Example B2: Exposure of cells to LATS inhibitors and measurement of intracellular YAP distribution

Cells obtained as described in Example B1 were plated in glass-bottom black wall 24-well dishes in limbal epithelium cell culture medium (DMEM F12 supplemented with 10% human serum and 1.3 mM calcium chloride) supplemented with LATS inhibitor compound example no. 133 or 49 at a concentration of 10 micromolar or supplemented in DMSO as a negative control. Cells were cultured under these conditions for 24 hours at 37°C in 5% CO2.

To measure the effect of the LATS inhibitors on the downstream target YAP, intracellular YAP distribution was analyzed by immunohistochemistry. Cell cultures were fixed with 4% PFA for 20 minutes, permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4°C. Primary antibody used was anti-YAP from Santa Cruz Biotechnology. Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Negative control was omitted primary antibody (data not shown). Fluorescence was observed using a Zeiss LSM 880 confocal microscope.

Only weak YAP immunostaining was observed in the nucleus of LSCs cultured without the LATS inhibitors (DMSO control). YAP immunostaining was stronger in the nucleus of LSCs exposed to the LATS inhibitor compound example no. 133 or 49 (data not shown).

Example B3 : Exposure of cells to LATS inhibitors and measurement of YAP phosphorylation

Cells obtained as described in Example B1 were detached from the culture dish with Accutase for 10 minutes at 37°C, cell suspensions were rinsed by centrifugation and plated in DMEM F12 supplemented with 10% human serum and 1.3 mM calcium chloride in 6-well plates (Corning) and cultured without LATS inhibitor compounds for 2-4 days.

The medium was then replaced by fresh limbal epithelium cell culture medium (DMEM F12 supplemented with 10% human serum and 1.3 mM calcium chloride) supplemented with LATS inhibitor compound example no. 133 or 49 at a concentration of 10 micromolar or

supplemented in DMSO as a negative control. Cells were cultured under these conditions for 1 hour at 37°C in 5% CO2.

To measure the effect of the LATS inhibitors on the downstream target YAP, the YAP phosphorylation levels were measured by western blot as follows. The cell pellets were obtained by trypsin dissociation and centrifugation and washed with PBS. The pellets were lysed with 30 microlitres of RIPA lysis buffer containing protease inhibitor cocktail (Life Technologies) for 30 minutes, with vortexing every 10 minutes. The cell debris were then pelleted at 4°C for 15 minutes at 14k rpm and the protein lysate was collected. Protein concentration was quantified using a micro BCA kit (Pierce). Fifteen micrograms of total protein was loaded in each well of 4-20% TGX gels (BioRad) and Western blotting was performed according to the manufacturer's instructions. Membranes were probed with phospho-YAP (ser127) (CST, 1:500) or total Yap (Abnova, 1:500) antibody and actin (Abcam) labelling was used as loading control. Membranes were stained with HRP-conjugated secondary antibodies, rinsed and imaged using a ChemiDoc system (Biorad) according to the manufacturer's instructions.

Western blot analysis (see Figure 1) showed that both compound example no. 133 and 49 caused a reduction in YAP phosphorylation levels in human LSCs.These results suggest that the LATS inhibitor compound example no. 133 and 49 can activate YAP signaling in human LSCs.

Example B4: Human limbal stem cell population expansion and immunohistochemical observation of cellular phenotype

Cells obtained as described in Example B1 were plated in 24-well plates (Corning) in limbal epithelium cell culture medium (DMEM F12 supplemented with 10% human serum and 1.3 mM calcium chloride) supplemented with LATS inhibitor compound example no. 133 or 49 at a concentration of 10 micromolar or supplemented in DMSO as a negative control. Cells were first cultured at 37°C in 5% CO2 for 6 days after isolation without passaging (Figures 2A, 2B and 2C).

To evaluate the ability of the compounds to enable LSC expansion after two passages, LSCs were passaged and cultured for two weeks in the presence of compound example 49

to enable expansion (Figure 2D). Limbal stem cells (LSCs) were passaged by treating cultures with Accutase for 10 minutes at 37°C, rinsing the cell suspension by centrifugation and plating cells in fresh LSC culture medium supplemented with LATS inhibitor compound example 49.

In order to observe that the expanded cell population expressed p63alpha, this was measured by immunohistochemistry as follows. Cell cultures were fixed with 4% PFA for 20 minutes, permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4°C. Primary antibody used was p63alpha from Cell Signalling. Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Cells were counter-stained with a human nuclear antigen antibody (Millipore) at a 1:500 dilution in order to label all cells in the culture and confirm their human identity. Negative control was omitted primary antibody (data not shown). Fluorescence was observed using a Zeiss LSM 880 confocal microscope.

Figure 2A shows that in the presence of growth medium and DMSO, only a few isolated cells attach to the culture dish and survive up to 6 days. Most cells expressed the human nuclear marker, but few expressed p63alpha. In contrast, in the presence of LATS inhibitors compound example no. 133 (Figure 2B) and compound example no. 49 (Figure 2C), the cells formed colonies and expressed p63alpha. This result indicated that the LATS inhibitors promote the expansion of the population of cells with the p63alpha-positive phenotype. Figure 2D: Passaging cells and culturing them in the presence of LATS inhibitor compound example no. 49 for two weeks enabled cell population expansion and the formation of confluent cultures expressing p63alpha.

Example B5: Human limbal stem cell population expansion and measurement thereof

Cells obtained as described in Example B1 were plated in 48-well plates (Corning) in XVIVO15 medium (Lonza) supplemented with LATS inhibitors (as listed in Table 2 and 3 below) at a concentration of 10 micromolar or supplemented in DMSO as a negative control. Cells were cultured at 37°C in 5% CO2.

For each compound, two sets of cultures were generated. A first set of cultures was fixed in 4% PFA for 20 minutes at room temperature after cells isolated from the cornea had attached to the cell culture dish (typically 24h after cell plating). A second set of cultures was fixed in 4% PFA for 20 minutes at room temperature after being cultured for two passages. Cells were passaged when they reached 90-100% confluence.

In order to observe that the expanded cell population expressed p63alpha, this was measured by immunohistochemistry as follows. The fixed cell cultures were permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4°C. Primary antibody used was p63alpha from Cell Signalling. Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Cell nuclei were then labeled in a solution of 0.5 micromolar of Sytox Orange (ThermoFisher) in PBS for 5 minutes at room temperature.

To evaluate the percentage of p63alpha-positive cells, the number of cells labeled by the anti-p63alpha antibody was counted and the total number of cells was determined by counting the number of nuclei stained by Sytox Orange. The proportion of p63alpha-positive cells was then determined by calculating the percentage of Sytox-orange-positive nuclei that also expressed p63alpha.

To evaluate cell expansion ratios, nuclei were counted using a Zeiss LSM 880 confocal microscope. The expansion factor was then determined by calculating the ratio of the expanded population of cells to population of seeded cells.

Results in the Tables below indicate that the LATS inhibitors enabled cell population expansion. In the presence of the LATS inhibitors, 57 to 97 percent of the cells express the p63alpha-positive phenotype.

Table 2

Compound Example No.	Expansion factor	Compound Example No.	Expansion factor
Ex. 47	2137	Ex. 290	1051

Ex. 12	2087	Ex. 65	1048
Ex. 49	2029	Ex. 287	991
Ex. 261	1717	Ex. 17	976
Ex. 62	1712	Ex.139	961
Ex. 14	1423	Ex. 11	705
Ex. 6	1275	Ex. 289	681
Ex. 288	1241	Ex. 33	39
Ex. 133	1205	DMSO	35
Ex. 66	1160		

Table 3

Compound Example No.	Percentage of p63a-positive cells	Compound Example No.	Percentage of p63a-positive cells
Ex. 47	97	Ex. 290	90
Ex. 12	95	Ex. 65	87
Ex. 49	92	Ex. 287	86
Ex. 261	93	Ex. 17	86
Ex. 62	95	Ex. 139	87
Ex. 14	93	Ex. 11	86
Ex. 6	93	Ex. 289	80
Ex. 288	95	Ex. 33	6
Ex. 133	89	DMSO	3
Ex. 66	89		

Example B6: siRNA knockdown of LATS1 and LATS2 in human limbal cells

Cells obtained as described in Example B1 were plated in 24-well plates (Corning) in XVIVO15 medium (Lonza). Cells were cultured at 37°C in 5% CO2. LATS1 and LATS2 were knocked down by transfection (lipofection, using RNAiMax, Thermofisher). Each well of the cell culture plate was transfected with 0.5 micrograms of pools of 4 siRNAs targeting each gene. siRNAs used in this study were Qiagen's LATS1 siRNA SI00067172 and LATS2 siRNA SI00106925. Scrambled siRNAs were used as negative controls according to the manufacturer's protocol (Qiagen).

In order to measure cell proliferation, EdU staining was performed according to the manufacturer's instructions (Life Technologies) 48 hours after transfection with LATS1 and LATS2 siRNAs or scrambled siRNA controls. Cell nuclei were labeled with Sytox Orange.

EdU and Sytox Orange fluorescence was observed using a Zeiss LSM 880 confocal microscope in order to measure the percentage of EdU-positive cell nuclei.

Figure 3 shows that the percentage of EdU-positive cells increased upon LATS1 and LATS2 knockdown, showing that knockdown of LATS leads to cell proliferation.

Example B7: Human limbal stem cell population expansion and immunohistochemical observation of markers DeltaN-p63 alpha/beta/gamma, ABCG2 and C/EBP delta

Cells obtained as described in Example B1 were plated in 48-well plates (Corning) in XVIVO15 medium (Lonza) supplemented with LATS inhibitors (Compound ex. 49 and compound ex. 133) at a concentration of 10 micromolar or supplemented in DMSO as a negative control. Cells were cultured at 37°C in 5% CO2 for 8 to 10 days.

In order to observe that the expanded cell population expresses markers normally expressed by limbal stem cells, the ability of the cells to express DeltaN-p63 alpha/beta/gamma, ABCG2 and C/EBP delta was measured by immunohistochemistry as follows. Cell cultures were fixed with 4% PFA for 20 minutes, permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4°C. Primary antibodies used were p63α (Cell Signaling Technology), ABCG2 (EMD Millipore), and C/EBPδ (Abcam), cytokeratin 15, cytokeratin 12 (Abcam). Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Cell nuclei were then labeled in a solution of 0.5 micromolar of Sytox Orange (ThermoFisher) in PBS for 5 minutes at room temperature. Samples were then observed using a Zeiss LSM 880 confocal microscope.

Results indicated (see Figure 4) that cells cultured in XVIVO medium supplemented with DMSO alone (no LATS inhibitor compound) do not typically express markers normally expressed by LSCs. In contrast, cells cultured in the presence of LATS inhibitors express markers normally expressed by LSCs.

Example B8 : Human limbal stem cell differentiation into corneal epithelial cells

Cells obtained as described in Example B1 were plated in 48-well plates (Corning) in XVIVO15 medium supplemented with LATS inhibitors compound example no. 49, 47, 12 or 261 at a concentration of 10 micromolar or supplemented in DMSO as a negative control. Cells were cultured at 37°C in 5% CO2 for 8 to 10 days.

Analysis was then conducted to determine whether a population of p63alpha-positive LSCs expanded in the presence of LATS inhibitors retained the ability to differentiate into keratin-12-positive corneal epithelial cells, which is required for corneal clarity to be restored in the patients that receive the LSC transplant.

To promote LSC differentiation into corneal epithelium cells, the culture medium was changed to DMEM (Invitrogen) without serum or LATS inhibitors and cells were cultured for 4-8 days. In order to observe LSC differentiation into corneal epithelium cells, the cell cultures were fixed with 4% PFA for 20 minutes, permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4°C. Primary antibodies used were p63α (Cell Signaling Technology) and cytokeratin 12 (Abcam). Samples were washed in PBS three times and donkey-raised secondary antibody conjugated to Alexa Fluor 488 or Alexa Fluor 647 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Samples were then observed using a Zeiss LSM 880 confocal microscope.

Results indicated that cultures maintained in the presence of LATS inhibitors and then induced to differentiate, contained areas where clusters of p63alpha-positive cells are contiguous to clusters of cells that are p63alpha-negative, but keratin-12-positive (Figure 5). Therefore limbal cells expanded in the presence of LATS inhibitors can differentiate into keratin-12-positive cells when under appropriate conditions.

Example B9: Delivery of LSC-biomatrix preparation to rabbit eyes

Rabbit model of limbal stem cell deficiency

Limbal stem cell deficiency (LSCD) was unilaterally created in the right eye of NZA rabbits by debridement of epithelial cells and ablation of limbal stem cells (LSC) with Whatman

paper soaked with a solution of 1M of sodium hydroxide. Limbal conjunctiva was treated with Mitomycin C to reduce corneal neovascularization. The left eye was left intact. The cell population expanded in LATS inhibitor compound example no. 12 was delivered as described below. After cell delivery, rabbits received analgesic treatment (Tramadol 10mg/kg PO BID in first 2 weeks, Meloxicam 0.3 mg/kg PO SID in first 2 weeks or as long as the animal showed signs of ocular discomfort), anti-inflammatory treatment (Ancef® (cefazolin) 50 mg sub-Tenon immediate post-procedure, Tobrex® ophthalmic solution t.i.d in 1st week and b.i.d thereafter, ampicillin 80mg/kg/day (40 mg BID) SQ for first week) and immunosuppression (Cyclosporine A (0.5%) top oc. t.i.d 1st week and b.i.d. thereafter, Gentocin®-durafilm® (Gentamicin sulfate and betamethasone, MERCK) top oc. t.i.d 1st week and b.i.d. thereafter, Cyclosporine A (5mg/kg/day) SQ in 1st week and then ½ dosage thereafter).

Biomatrix preparation.

Gelatin methacrylate (GelMA) was synthesized according to a previously published protocol. (Nichol, J. W. et al. Cell-laden microengineered gelatin methacrylate hydrogels. Biomaterials 31, 5536-5544, doi:10.1016/j.biomaterials.2010.03.064 (2010). In brief, 20 grams of porcine derived gelatin (Cat# G2500, Sigma) was dissolved overnight at 50°C in 200ml of PBS without calcium and magnesium (DPBS, Cat# 21-031, Corning). With strong agitation, methacrylic anhydride (Cat# 276685, Sigma) was added dropwise (approximately 1ml/min) into the gelatin solution to reach the concentration of 8% (vol/vol). The mixture was stirred at 60°C in an oil bath for 3 hours before adding 200ml of the DPBS and followed by thorough mixing for additional 15 minutes. The diluted mixture was purified via dialysis against Milli-Q water using dialysis tubings (15kDa MWCO, Spetrua/Por) for 1 week at 45°C to remove methacrylic acid. The purified samples were lyophilized and stored at -80°C until further use.

To prepare a 15% GelMA stock solution, 1.5 gram of the freeze-dried GelMA foam was dissolved in 10ml of pre-warmed DPBS at 37°C. After the GelMA foam was fully dissolved, the photoinitiator was introduced by adding into the GelMA solution 15mg of lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). 500 microlitre of 1N NaOH (Cat# BDH-7222-1, VWR) was added to the solution to adjust the pH to neutral before the solution was filtered using 0.22 micrometre sterile membranes (Millipore).The final filtrate was separated into 500 microlitre aliquots and stored at 4°C until further use.

LSC transplantation in vivo.

Cells obtained as described in Example B1 were plated in 6-well plates (Corning) in XVIVO15 medium supplemented with LATS inhibitor compound example no 12 at a concentration of 10 micromolar. Cells were cultured at 37°C in 5% CO2 for two passges (two weeks).

Cell suspensions for transplantation were prepared as follows. Cells were detached from the culture dish with Accutase for 10 minutes at 37°C. Cells were then rinsed by centrifugation and resuspended in 16 microlitres of XVIVO15 medium without LATS inhibitors.

A 5% GelMA solution was prepared by mixing 16 microlitres of the LSC suspension or saline control with 8 microlitres of the 15% GelMA stock solution and followed by adding all the mixture to the therapeutic contact lens (Lotrafilcon B, Alcon) on a supporting glass slide. The final solution contained 300 000 cells and 5% GelMA. The power of the UV LED light source (365nm, Hamamatsu) was adjusted to 15% with a UV intensity reading of 30mW/cm2. A brief UVA exposure of 25 seconds was used for the photopolymerization process to bioprint the human LSCs to the inner surface of the contact lens. After the polymerization process, the LSC loaded contact lens was applied to a dissected rabbit eye ex vivo or to the rabbit eye in vivo.

For experiments where the LSC-loaded contact lens was applied ex vivo, samples were observed under a Zeiss epifluorescence microscope within two hours.

For in vivo experiments, the LSC-loaded contact lens was applied to the right eye of the rabbit model of LSCD described above and tarsorrhaphy was performed to keep the contact lens on the ocular surface for 1 week. After one week, the contact lens was removed and rabbits were kept alive for an additional 4 weeks.

Rabbits were sacrificed five weeks after cell delivery, corneas were dissected and fixed in 4% PFA. Samples were embedded in paraffin and sectioned. Immunohistochemistry was performed to detect the presence of keratin-12, a marker of corneal epithelium cells and keratin-19, a marker of conjunctival cells. The anti-keratin 12 and 19 primary antibodies were from Abcam. Human mitochondrial protein was used to confirm the human origin of the cells. The anti-human mitochondrial protein primary antibody was from Novus. Samples

were then stained with HRP-conjugated secondary antibodies (ThermoFisher) and observed under a Zeiss microscope.

Results indicated that LSCs attached to a contact lens using GelMA polymerization can be delivered to the surface of the rabbit eye ex vivo (Figure 6).

Figure 7 shows that in a rabbit model of limbal stem cell deficiency, a LSC population expanded in the presence of the LATS inhibitor (compound ex. 12), attached to a contact lens using GelMA polymerization and delivered in vivo to the rabbit's corneal surface, lead to regeneration of the a keratin-12-positive corneal epithelium and prevented conjunctivalization by keratin-19-positive conjunctival cells in the transplanted eye (Figure 7A and B respectively). In contrast, non-transplanted rabbit eyes showed absence of keratin-12-positive corneal epithelium restoration. Instead, signs of conjunctivalization were observed, as showed by the presence of keratin-19 staining (Figure 7C and D respectively).

Figure 8 shows that the cells that restored the corneal epithelium of transplanted eyes were human, as demonstrated by the presence of human mitochondrial protein (Figure 8A). In contrast, the ocular surface of non-transplanted eyes did not exhibit human mitochondrial protein staining (Figure 8B).

Example B10: Delivery of LSC ex vivo using TISSEEL

To prepare TISSEEL (Baxter, cat# NDC 0944-4301-02) working solution, manufacturer's instructions were followed to reconstitute the supplied ingredients. Further dilution of the reconstituted fibrinogen solution can be done by adding sterile water to reach desired concentration of fibrinogen. Thrombin working solution was diluted 500 times with Dulbecco's phosphate-buffered saline (DPBS, GIBCO, Thermo Fisher Scientific, Waltham, MA) to achieve 1unit/ml solution.

LSC delivery to culture surface in vitro.

Cells obtained as described in Example B1 were plated in 6-well plates (Corning) in XVIVO15 medium supplemented with LATS inhibitor compound example no 12 at a concentration of 10 micromolar. Cells were cultured at 37°C in 5% CO_2 for two passages (two weeks).

Cell suspensions for transplantation were prepared as follows. Cells were detached from the culture dish with Accutase for 10 minutes at 37°C. Cells were then rinsed by centrifugation and resuspended in the thrombin working solution mentioned above and the final cell densities were adjusted by serial dilution to between 0.1 million per ml and 30 million per ml.

Immediately after mixing the cell suspension with fibrinogen solution, 10ul of the mixture were plated to the center of a collagen coated 24 well plate and incubated at room temperature for 5 minutes to solidify the fibrin. After replenishing each well with XVIVO15 medium without LATS inhibitors, all the samples were monitored for 2 weeks for fibrin degradation and cell repopulation. The results (Figure 9) suggested that TISSEEL was used successfully to deliver LSCs, and the culture area, similar size to human cornea, can be covered between 1 week and 2 weeks in the samples with 100k and 300k LSCs delivered initially.

LSC delivery to human cornea ex vivo.

Cells obtained as described in Example B1 were plated in 6-well plates (Corning) in XVIVO15 medium supplemented with LATS inhibitor compound example no 12 at a concentration of 10 micromolar. Cells were cultured at 37°C in 5% CO_2 for two passages (two weeks). One day before the delivery experiment, cells were labeled with CellTracker green CMFDA dye (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol.

Cell suspensions for transplantation were prepared as follows. Cells were detached from the culture dish with Accutase for 10 minutes at 37°C. Cells were then rinsed by centrifugation and resuspended in the thrombin working solution mentioned above to reach the final cell density of 30 million per ml.

Immediately after mixing the cell suspension with fibrinogen solution, 10ul of the mixture were plated to the center of a human cornea and incubated at room temperature for 5 minutes to solidify the fibrin. A LotraB contact lens (Air Optix Night and Day, Alcon) was used to cover the LSC-fibrin construct on the human cornea to mimic the real clinical procedure. Fluorescence images were taken by a Nikon fluorescence stereomicroscope. The data (Figure 10) demonstrated that TISSEEL was successfully used to deliver high

number of LSCs onto corneal surface *ex vivo* and the LSC-fibrin construct appears robust to tolerate further manipulation such as applying protective contact lens.

Example B11: Delivery of multiple cell populations to ocular surface into designated patterns via bioprinting

A customized bioprinter was built based on DOPsL technology as published in Advanced Materials (2015, DOI: 10.1002/adma.201202024).

Cell suspensions for bioprinting were prepared as follows. HEK-293 cells were pre-labelled with Celltracker green CMFDA dye or red CMTPX dye (Thermo Fisher Scientific, Waltham, MA) before harvest. 25µl of the cell suspension was mixed with 25µl of the 15% GelMA stock solution to reach a final cell density of 200 million cells/ml.

Rabbit eye balls were placed onto a petri dish filled with 1% low melting point agarose and the epithelium was carefully debrided by a surgical scalpel immediately before the bioprinting process. The cell-GelMA mixture was then applied to the top of the cornea with a Polydimethylsiloxane (PDMS) o-ring (Specialty Silicone Products, Inc., Ballston Spa, NY) placed on the limbus to prevent the cell-GelMA mixture dripping from the curved ocular surface.

A customized light pattern (i.e. half of the Yin-Yang pattern) was projected to the cell(green)-GelMA mixture on top of the rabbit eye for 4 seconds before the unpolymerized mixture was rinsed away. Another light pattern (i.e. the other half of the Yin-Yang pattern) was projected to the sample for 4 seconds after a new cell (red)-GelMA mixture was applied to the top of the same rabbit cornea. Several rinses with PBS were performed before imaging. See Figure 11.

Example B12 : Reducing Immune Rejection by CRISPR/Cas9-mediated deletion of the beta-2-microglobulin gene in LSCs

In the example below, HLA class I expression was eliminated from the LSC surface by CRISPR-mediated deletion of the beta-2-microglobulin gene.

Cell transfection: LSCs obtained as described in Example B1 were cultured in X-VIVO15 medium supplemented with the LATS inhibitor compound example no 48a to a confluency of

50-60% in a 35mm petri dish eight days after LSC isolation. LSCs were transfected with a mixture of tracrRNA-crRNA-Cas9 mRNA. To obtain the mixture for the size of a 35mm petri dish, 12.5 microlitre of 10 micromolar tracrRNA (Dharmacon, Cat # U-002000-20), 12.5 microlitre of 10 micromolar crRNA targeting human B2M (Dharmacon, Cat #CR-004366-01), 50 microlitre of 0.1 microgram/microlitre Cas9 mRNA (Dharmacon, Cat #CAS11195), and 15 microlitre of DharmaFECT Duo Transfection Reagent (Dharmacon, Cat #T-2010-02) were combined and incubated for 20 minutes at room temperature. The mixture was added drop wise to the culture dish in 2.5ml the medium (XVIVO15 supplemented with the LATS inhibitor compound example no 48a) (w/o antibiotics). In order to reduce mRNA cytotoxicity, 0.2 microgram/ml B18R (eBioscience, Cat #34-8185-81) was added to the medium. The transfection reagent alone represents the transfection negative control.

After 6 h incubation in 5% CO_2 at 37°C medium was replaced with fresh X-VIVO15 medium supplemented with compound example no 48a (w/o antibiotics) including compound and B18R. After 72 h in a 5% CO2 incubator cells were passaged 1:2 and expanded for additional 72 h in LSC medium (w/o antibiotics) including compound and B18R on synthemax coated petri dishes to obtain more cells for FACS sorting.

FACS analysis: LSCs were treated with Accutase (ThermoFisher, Cat # A1110501) for 20 minutes in 5% CO2 at 37°C. After scraping the cells, the reaction was stopped by using cell culture medium containing 10% Serum and transferred to a falcon tube for a centrifugation step (1000 rpm, 5 minutes). After aspirating the medium cells were resuspended in 200 microlitre FACS buffer (PBS/10%FBS).
To analyze the expression of B2M and HLA-ABC, 5 microlitre APC mouse anti-human β2-microglobulin antibody (Biolegend, Cat #316312) and 20 microlitre PE mouse anti-human HLA-ABC antibody (BD Bioscience, Cat # 560168) were added to the cell suspension and incubated for 30 minutes on ice. Cells were washed 3 times after antibody labelling with FACS buffer and resuspended in 500 microlitres in FACS buffer. Before FACS sorting, cells were filtered through a 70 micrometre filter and stored on ice until sorting.

In order to prevent cells sticking to the wall, collection tubes were filled with the serum for 30 minutes before the sort. Cells were sorted on a BD FACSAria II instrument into prepared collection tubes, using human serum enriched LSC medium including compound and B18R. FACS data were analyzed using BD FACSDiva software.

Results presented in Figure 12 show that CRISPR-mediated deletion of B2M and subsequent elimination of HLA A, B and C occurred in 21 percent of the LSCs. As the XVIVO15 medium supplemented with the LATS inhibitor compound example no 48a enables efficient expansion of LSCs, the 21 percent B2M-negative/HLA A,B,C-negative population of LSCs could then be expanded to produce a cell preparation where 97 percent of the cells do not express HLA class-I drivers of immune rejection (Figure 13).

Example B13: Residual compound estimation study

Standard curve preparation

A dilution series of stock standards were made at concentrations of 100 micromolar, 10 micromolar, 1 micromolar, 500 nM, 100 nM, and 0 micromolar. Compound example no. 48a (10 mM in DMSO) stock was spiked into 50% acetonitrile 50% water to make the spike standards. The spiking standards were used to spike the blank media samples. 10 microlitres of spiking standard was added to 90 microlitres of media to create the spiked media standards. 10,000 nM, 1000 nM, 100 nM, 50 nM, 10 nM, and 0 nM media standards were used to generate the compound example no. 48a standard curve.

50 microlitres of each media sample was treated with 400 microlitres of the extraction solution. The extraction solution consists of acetonitrile/methanol (3:1). The standard curve samples were extracted using the same volumes and conditions as the unknown media samples. The extracted samples were centrifuged at 10,000 rpm for 5 min. After centrifugation, 200 microlitres of each sample supernatent was transferred to a clean 96-well plate. The extracted samples were analyzed by High Resolution LC-MS. Thermo Xcalibur Software and Quan Browser were used to generate the standard curve and calculate the concentration values. An external calibration method was used to calculate the concentration of compound example no. 48a in the culture media samples.

LC-MS analysis

High-resolution chromatography was performed using the Thermo Ultimate UPLC and a Kinetex 2.1 X 50 mM C18 RP column (2.6 micron particles). The mobile phases consisted of 5 mM Ammonium Acetate in H2O for Buffer A, and 0.1% Formic Acid in Acetonitrile for Buffer B. A standard binary linear gradient was used for Reverse Phase Chromatography. The Thermo Q Exactive mass spectrometer was used for this study. The UPLC effluent was

directed into the Q Exactive Mass Spectrometer which was equipped with an ESI ion source. The mass spectrometer was programmed to perform in High Resolution Full Scan mode and MS2 mode.

Test system

Cell preparation for the LSC washout: the washout experiment was performed in triplicates, where each sample contained 0.5 million cells. All samples (media only, media with cells, supplemented with compound example no. 48a and cells in media supplemented with compound example no. 48a) were cultured at 37 degree incubator for the duration of the experiment. The medium used for the experiment was X-VIVO 15.

The cells were cultured to passage 2 in X-VIVO-15 medium supplemented with 3 micromolar compound example no. 48a. When the culture reached 70% confluency, the the medium was removed by aspiration and replaced with fresh medium supplemented with 3 micromolar compound example no. 48a; the cells were cultured for an additional six days. On day seven, the following samples were generated:

1. 2 ml of “blank” medium (not supplemented with the compound)
2. 2 ml of medium cultured in the presence of the compound (pre-wash)
3. 2 ml of media for each wash sample 1-10
4. Cell pellets (post-wash)

The cells were collected by using the sell lifter (Costar, cat# 3008).

The media and cell pellet samples were analyzed and quantified using High Resolution LC-MS.

To estimate the residual levels of compound example no. 48a, cell pellets and supernatent were collected. The amount of compound example no. 48a was determined by LC-MS. Estimation of compound example no. 48a concentrations in the Pre-wash Media, Wash Media (Table 4) and the Cell Pellet are summarized (Table 5). Pre-wash Media samples have the highest levels of compound example no. 48a (approx. 9,830 nM to 10,837 nM range). Post-wash Pellet levels have low, but detectable levels of compound example no. 48a (nanomolar).

Table 4: Estimation of concentrations for compound example no. 48a in the Pre-wash Media and Wash Media

Sample	Volume (mL)	compound example no. 48a	compound example no.	compound example no.

		(nM) in Triplicate #1	48a (nM) in Triplicate #2	48a (nM) in Triplicate #3
Pre-wash Media	2	9829.547	10374.463	10837.706
Wash Media 1	2	461.271	401.058	354.547
Wash Media 2	2	31.933	38.721	18.400
Wash Media 3	2	BLQ	BLQ	BLQ
Wash Media 4	2	BLQ	BLQ	BLQ
Wash Media 5	2	BLQ	BLQ	BLQ
Wash Media 6	2	BLQ	BLQ	BLQ
Wash Media 7	2	BLQ	BLQ	BLQ
Wash Media 8	2	BLQ	BLQ	BLQ
Wash Media 9	2	BLQ	BLQ	BLQ
Wash Media 10	2	BLQ	BLQ	BLQ

BLQ = **Beneath Limit of Quantification**

*Table 5: Estimation of concentrations for **compound example no. 48a** in the Cell Pellet*

Sample	compound example no. 48a (nM)	compound example no. 48a (pg/cell)	Avg of compound example no. 48a (pg/cell)	# of cells in Pellet	Total compound example no. 48a (pg)
PostWash_Pellet 1	20.342	0.00068	0.00068	0.5×10^6	340
PostWash_Pellet 2	19.908	0.00066			
PostWash_Pellet 3	20.787	0.00069			

The amount of compound example no. 48a in the Pre-wash Media from three incubations ranges from 9,830-10,838 nM. The concentration of compound example no. 48a in the Post-wash Cell Pellet was in the range of 19.9-20.8 nM.

Residual amount of other compound examples after wash-out

For compound example numbers 12, 261, and 5, residual amounts in expanded LSCs were measured using the same method as for compound example no. 48a.

Table 6 Estimation of concentrations for compound ex. 12 in the Cell Pellet

Sample	compound ex. 12 (nM)	compound ex. 12 (pg/cell)	Avg of compound ex. 12 (pg/cell)	# of cells in Pellet	Total compound ex. 12 (pg)
PostWash_Pellet 1	7.370	0.000136	0.000178	1×10^6	178
PostWash_Pellet 2	14.443	0.000264			
PostWash_Pellet 3	7.319	0.000134			

Table 7: Estimation of concentrations for compound example no. 261 in the Cell Pellet

Sample	compound example no. 261 (nM)	compound example no. 261 (pg/cell)	Avg of compound example no. 261 (pg/cell)	# of cells in Pellet	Total compound example no. 261 (pg)
PostWash_Pellet 1	0.315	0.0000043	0.000083	1×10^6	83
PostWash_Pellet 2	6.941	0.000095			
PostWash_Pellet 3	11.008	0.00015			

Table 8 : Estimation of concentrations for compound example no. 5 in the Cell Pellet

Sample	compound example no. 5 (nM)	compound example no. 5 (pg/cell)	Avg of compound example no. 5 (pg/cell)	# of cells in Pellet	Total compound example no. 5 (pg)
PostWash_Pellet 1	21.733	0.00061	0.00083	0.5×10^6	415
PostWash_Pellet 2	13.454	0.00038			
PostWash_Pellet 3	46.529	0.0015			

Example C1: Human corneal endothelial cell isolation

Research-consented cadaveric human corneas were obtained from eye banks. The corneal endothelium cell (CEC) layer and Descemet's membrane (DM) were scored with a surgical-grade reverse Sinsky endothelial stripper. The DM-endothelium cell layer was carefully peeled off the corneal stroma and cells were dissociated from the DM using 1 mg/ml collagenase at 37^{0}C until cell detachment became apparent by microscopic observation (45 minutes to 3 hours). Cells obtained in this manner were used in the Examples C1-C17-below.

Example C2 : Exposure of cells to LATS inhibitors and measurement of intracellular YAP distribution

Cells obtained as described in Example C1 were plated in glass-bottom black wall 24-well dishes in corneal endothelial cell culture medium (human endothelial SF (serum free) medium (Invitrogen) with human serum) supplemented with LATS inhibitor compound example no. 133 or compound example no. 49 at a concentration of 10 micromolar or supplemented in DMSO as a negative control. Cells were cultured under these conditions for 24 hours at 37^{0}C in 5% CO2.

To measure the effect of the LATS inhibitors on the downstream target YAP, intracellular YAP distribution was analyzed by immunohistochemistry. Cell cultures were fixed with 4% PFA for 20 minutes, permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4^{0}C. Primary antibody used was anti-YAP from Santa Cruz Biotechnology. Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Negative control was omitted primary antibody (data not shown). Fluorescence was observed using a Zeiss LSM 880 confocal microscope.

YAP immunostaining is stronger in CECs exposed to cell proliferation medium with the LATS inhibitor compound example no. 133 or example 49 as indicated by immunofluorescence staining of YAP, as shown in Figure 14. These compounds therefore had an effect on the intracellular localisation of the downstream target YAP.

Example C3: Exposure of cells to LATS inhibitors and measurement of YAP phosphorylation

Cells obtained as described in Example C1 were detached from the culture dish with 1 mg/ml collagenase for 15 minutes at 37°C, cell suspensions were rinsed by centrifugation and plated in corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) in 6-well plates (Corning) and cultured for 2 to 4 days. The medium was then replaced by fresh corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) supplemented with LATS inhibitor, compound example no. 133 or example no 49 (diluted in DMSO) at a concentration of 10 micromolar or as a negative control DMSO alone without compound. Cells were cultured under these conditions for 1 hour at 37°C in 5% CO2.

Cell pellets were obtained by trypsin dissociation and centrifugation and washed with PBS. The pellets were lysed with 30 micromolar of RIPA lysis buffer containing protease inhibitor cocktail (Life Technologies) for 30 minutes, with vortexing every 10 minutes. The cell debris were then pelleted at 4°C for 15 minutes at 14k rpm and the protein lysate was collected. Protein concentration was quantified using a micro BCA kit (Pierce). Fifteen (15) micrograms of total protein was loaded in each well of 4-20% TGX gels (BioRad) and Western blotting was performed according to the manufacturer's instructions. Membranes were probed with phospho-YAP (ser127) (CST, 1:500) or total Yap (Abnova, 1:500) antibody and actin (Abcam) labelling was used as loading control. Membranes were stained with HRP-conjugated secondary antibodies, rinsed and imaged using a ChemiDoc system (Biorad) according to the manufacturer's instructions.

Western blot analysis showed that both compound example no. 133 and example no 49 caused a reduction in YAP phosphorylation levels in human CECs. A marked difference was observed after one hour of treatment with compound example no. 133 and example no 49 in human CECs, as shown by Western Blot in Figure 15a. Figure 15b shows by graphical representation the phosphorylated YAP levels normalized to beta-actin and Figure 15c shows phosphorylated YAP levels normalized to total YAP.

These results suggest that the LATS inhibitors compound example no 133 and example no 49 can activate YAP signaling in human CECs.

Example C4: Human corneal endothelial cell population expansion and measurement of cell density

Cells obtained as described in Example C1 were detached from the culture dish with 100 microlitres of Accutase (ThermoFisher) for 10 minutes at 37^{0}C, cell suspensions were rinsed by centrifugation and plated in corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) in 6-well plates (Corning) supplemented with LATS inhibitor compound example no. 133 or example no 49 (diluted in DMSO) at a concentration of 10 micromolar or as a negative control DMSO alone without compound. Cells were cultured at 37°C in 5% CO2.

To measure cell proliferation, an Incucyte machine was used following the manufacturer's instructions (Essen Biosciences) to perform real-time quantitative live-cell analysis of cell confluence every 3 hours for 10 days.

Figure 16 shows the percentage confluence of the cell population over time after exposure to LATS inhibitor or DMSO alone. Although human CECs are normally non-proliferative, the results show that both LATS inhibitors compound example no. 133 and example no 49 could activate proliferation in these cells.

Example C5: Human corneal endothelial cell population expansion and measurement of cell density

Cells obtained as described in Example C1 were plated in 48-well plates (Corning) in X-VIVO15 medium supplemented with LATS inhibitors as listed in Tables 9 and 10 (diluted in DMSO) at a concentration of 10 micromolar or as a negative control DMSO alone without compound. Cells were cultured at 37^{0}C in 5% CO2.

For each compound, two sets of cultures were generated. A first set of cultures was fixed in 4% PFA for 20 minutes at room temperature after cells isolated from the cornea had attached to the cell culture dish (typically 24h after cell plating). A second set of cultures was fixed in 4% PFA for 20 minutes at room temperature 10 days after the first one.

To measure cell density in all fixed cultures, the number of nuclei stained with Sytox Orange (ThermoFisher) were counted per surface area as follows: fixed cell cultures fixed were permeabilized in a solution of 0.3% Triton X-100 (Sigma-Aldrich). Cells were then labeled in a solution of 0.5 micromolar of Sytox Orange in PBS for 5 minutes at room temperature. Nuclei were counted under a Zeiss epifluorescence microscope. The expansion factor was then determined by calculating the ratio of the expanded population of cells to population of seeded cells.

The cell population expansion achieved with the tested compounds is shown by Tables 9 and 10 below.

Table 9: Fold Cell expansion

Compound example no.	Expansion factor	Compound example no.	Expansion factor
Ex. 12	521	Ex. 133	273
Ex. 261	461	Ex. 287	237
Ex. 47	449	Ex. 290	221
Ex. 48a	446	Ex. 65	203
Ex. 49	426	Ex. 17	187
Ex. 5	408	Ex. 139	107
Ex. 62	402	Ex. 289	84
Ex. 6	391	Ex. 11	79
Ex. 14	337	Ex. 48b	21
Ex. 288	302	Ex. 33	12
Ex. 66	280	DMSO	7

Table 10 : Endothelial Cell density in vitro (cells/mm^2 area)

Compound example number	Cell density: cells/mm^2	Compound example number	Cell density: cells/mm^2
Ex. 12	4226	Ex. 133	2028
Ex. 261	4308	Ex. 287	1893
Ex. 47	4294	Ex. 290	2071
Ex. 48a	4021	Ex. 65	1702
Ex. 49	3873	Ex.17	1628
Ex. 5	3911	Ex. 139	1179
Ex. 62	3301	Ex. 289	1421
Ex. 6	3378	Ex. 11	1121

Ex.14	3271	Ex. 48b	869
Ex. 288	2779	Ex. 33	25
Ex. 66	2503	DMSO	13

Example C6: siRNA knockdown of LATS1 and LATS2 in human corneal endothelial cells

Cells obtained as described in Example C1 were plated in 24-well plates (Corning) in X-VIVO15 medium (Lonza). Cells were cultured at 37^{0}C in 5% CO2. LATS1 and LATS2 were knocked down by transfection (lipofection, using RNAiMax, Thermofisher). Each well of the cell culture plate was transfected with 0.5 micrograms of pools of 4 siRNAs targeting each gene. siRNAs used in this study were Qiagen's LATS1 siRNA SI00067172 and LATS2 siRNA SI00106925. Scrambled siRNAs were used as negative controls according to the manufacturer's protocol (Qiagen).

In order to measure cell proliferation, EdU staining was performed 48 hours after transfection with LATS1 and LATS2 siRNAs or scrambled siRNA controls according to the manufacturer's instructions (Life Technologies). Cell nuclei were labeled with Sytox Orange. EdU and Sytox Orange fluorescence was observed using a Zeiss LSM 880 confocal microscope in order to measure the percentage of EdU-positive cell nuclei.

Figure 17 shows that the percentage of EdU-positive CEC increased upon LATS1 and LATS2 knockdown, showing that knockdown of LATS leads to CEC proliferation.

Example C7: Human corneal endothelial cell population expansion and immunohistochemical observation of cellular morphology

Cells obtained as described in Example C1 were plated in 24-well plates (Corning) in corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) supplemented with LATS inhibitor compound example no 49 (diluted in DMSO) at a concentration of 10 micromolar or supplemented with a negative control of DMSO without compound. Cells were cultured at 37^{0}C in 5% CO2 for 10 days.

In order to observe that the expanded cell population has the required cellular morphology for use in vivo, the ability of the cells to form tight junctions was measured by immunohistochemistry as follows. Cell cultures were fixed with 4% PFA for 20 minutes,

permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibody in the blocking solution for 12 hours at 4^{0}C. Primary antibody used was ZO-1 from Invitrogen. Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Cells were washed three times in PBS and nuclei (DNA) were stained with Sytox Orange (567nm, Life Technologies). Negative control was omitted primary antibody (data not shown). Fluorescence was observed using a Zeiss LSM 880 confocal microscope.

CECs proliferated in the presence of medium and DMSO control show signs of polymegatism characteristic of dysfunctional CECs (Figure 18A). CECs exposed to cell proliferation medium with the LATS inhibitor example no 49 retained a normal corneal endothelial cell morphology and the ability to form tight junctions as indicated by immunofluorescence staining of tight-junction marker Zonula Occludens-1 (ZO-1), as shown in Figure 18B. Both a normal corneal endothelial cell morphology and the ability to form tight junctions are crucial for the maintenance of corneal endothelium functions.

Example C8: Human corneal endothelial cell population expansion and measurement of markers Collagen 8a2, AQP1, SLC4A11, RPE65, CD31, and Na/K ATPase.

In order to verify that the expanded cell population expresses genes normally expressed by corneal endothelial cells in vivo, cells were cultured and RT-PCR analysis was then performed to measure the expression levels of Collagen 8a2, AQP1, SLC4A11, RPE65 and CD31. Immunohistochemical analysis was also performed to analyse the levels of Na/K ATPase and Collagen 8a2 as follows.

Cells obtained as described in Example C1 were plated in 24-well plates (Corning) in corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) supplemented with LATS inhibitor compound example no 49 (diluted in DMSO) at a concentration of 10 micromolar or supplemented with a negative control of DMSO without compound. Cells were cultured at 37^{0}C in 5% CO2 for 10 days. For the negative control, dermal fibroblasts (Lonza) were cultured in DMEM-F12 (LifeTechnologies) without LATS inhibitors.

To perform the RT-PCR analysis, total RNA was extracted using Trizol (Invitrogen), RNeasy Mini and QIA Shredder (Qiagen) according to manufacturer's protocol. RNA quality and quantity were measured using Nanodrop 100 (Thermo-Fisher Scientific) and Bioanalyzer 2100 (Agilent Technologies). cDNA was prepared by reverse transcription and relative mRNA expression was assessed by quantitative RT-PCR using an 7900HT system (Applied Biosystems). The following cycling parameters were used:
1) 50^{0}C for 2 minutes; 2) 95^{0}C for 10 minutes; 3) 95^{0}C for 15 seconds 4) 60^{0}C for 1 minute. Steps 3-4 were repeated 40 times.

Actin mRNA levels were measured and used as a endogenous controls to normalize gene expression levels and calculate delta Ct values according to the formula dCt = Ct for gene of interest - Ct for endogenous control. Primers were obtained from Applied Biosystems.

RT-PCR analysis as shown in Figure 19 indicates that a corneal endothelial cell population expanded with LATS inhibitor compound example no 49 express genes normally expressed by corneal endothelial cells in vivo, including Collagen 8a2, AQP1, SLC4A11. The cells do not express markers of other epithelia present in the eye, including RPE65 (a marker of retinal pigmented epithelium) and CD31 (a marker of vascular epithelium).

To check the expression of Na/K ATPase and Collagen 8a2 by immunohistochemistry the cell cultures were fixed with 4% PFA for 20 minutes, permeabilized and blocked in a blocking solution of 0.3% Triton X-100 (Sigma-Aldrich) and 3% donkey serum in PBS for 30 minutes. Cells were then labeled with primary antibodies in the blocking solution for 12 hours at 4^{0}C. Primary antibodies used were Na/K ATPase and Collagen 8a2 (Santa Cruz Biotechnology). Samples were washed in PBS three times and donkey-raised secondary antibody Alexa Fluor 488 or 647 (Molecular Probes) at 1:500 dilution were applied for 30 minutes at room temperature. Cells were washed three times in PBS and nuclei (DNA) were stained with Sytox Orange (567nm, Life Technologies). Negative controls were either omitted primary antibody or isotype control antibody (data not shown). Fluorescence was observed using a Zeiss LSM 880 confocal microscope.

Immunohistochemical analysis as shown in Figure 20 indicates that a corneal endothelial cell population expanded with LATS inhibitor compound example no 49 express genes

normally expressed by corneal endothelial cells in vivo, including Na/K ATPase (Figure 20a) and Collagen 8a2 (Figure 20b).

Example C9 : Human corneal endothelial cell population expansion and measurement of markers CD44, CD105, CD166 and CD73

In order to verify that the expanded cell population does not undergo endothelial to mesenchymal transition, CECs were cultured as follows and FACS analysis was then performed to analyse the levels of CD44, CD105, CD166 and CD73.

To generate mature CEC cultures, cells obtained as described in Example C1 were plated in X-VIVO15 supplemented with LATS inhibitor compound example no. 48a (diluted in DMSO) at a concentration of 3micromolar. Cells were cultured in 48 well plates (corning) at 37^{0}C in 5% CO2 for two weeks. Cells were passaged twice during this time by detaching cells with 1 mg/ml collagenase for 15 minutes at 37°C, rinsing cell suspensions by centrifugation and plating in fresh medium. These cells were then grown in XVIVO medium without a LATS inhibitor for a further 2 weeks.

To measure the levels of CD44, CD105, CD166 and CD73 by FACS the CECs were treated with Accutase (ThermoFisher, Cat # A1110501) for 20 minutes in 5% CO2 at 37°C. The reaction was stopped by using cell culture medium containing 10% Serum and transferred to a falcon tube for a centrifugation step (1000 rpm, 5 minutes). After aspirating the medium cells were resuspended in 200 microlitre FACS buffer (PBS/10%FBS).

Antibodies against CD44, CD105, CD166 and CD73 (BD Biosciences) were added to the cell suspension and incubated for 30 minutes on ice. Cells were washed 3 times after antibody labelling with FACS buffer and resuspended in 500 microlitre in FACS buffer. Before FACS sorting, cells were filtered through a 70 micrometre filter and stored on ice until sorting. Cells were sorted on a BD FACSAria II instrument. FACS data were analyzed using BD FACSDiva software.

FACS analysis as shown in Figure 21 indicates that the FACS marker expression profile of the cell population expanded in the presence of the LATS inhibitor is different from the FACS marker expression profile of cells that have undergone endothelial to mesenchymal transition. Specifically, cells cultured in the presence of the LATS inhibitor express lower levels of CD44, CD73, CD105 and CD166 compared to cells grown in the absence of the

LATS inhibitor. Importantly, CD73 is a marker of endothelial to mesenchymal transition, therefore these results indicate that CECs cultured in the presence of LATS inhibitors do not undergo endothelial to mesenchymal transition.

Example C10 : Cell Bioprinting : GelMA preparation

Synthesis of GelMA

Gelatin methacrylate (GelMA) with approximately 100% of Lys residues methacrylated was synthesized according to a previously published protocol (Nichol, J. W. et al. *Biomaterials*, **2010**, p.5536-5544). 20 grams of porcine derived gelatin (Cat# G2500, Sigma) was dissolved overnight at 50°C in 200ml of PBS without calcium and magnesium (DPBS, Cat# 21-031, Corning). With strong agitation, methacrylic anhydride (Cat# 276685, Sigma) was added dropwise (approximately 1ml/min) into the gelatin solution to reach the concentration of 8% (vol/vol). The mixture was stirred at 60°C in an oil bath for 3 hours before adding 200ml of the DPBS and followed by thorough mixing for an additional 15 minutes. The diluted mixture was purified via dialysis against Milli-Q water (used 15kDa MWCO Spectra/Por dialysis tubing) for 1 week at 45°C to remove methacrylic acid. The purified samples were lyophilized and stored at -80°C until further use.

^{1}H NMR (400 MHz, D_2O, 35°C) was used to determine the degree of methacrylation for a 15mg/mL solution of GelMA. The peak area ratio of Phe to methacrylamide was found to be 1.00:0.82 by comparing the area of the multiplet at 7.25-7.50ppm (assumed to be Phe protons, 5H per Phe in GelMA) to the sum of the areas of four singlets at 5.47ppm, 5.51ppm, 5.71ppm, and 5.76ppm (assumed to be vinyl protons, 2H per methacrylamide in GelMA). The molar Phe:Lys ratio in the gelatin used in this reaction was determined to be 1.00:2.06 by amino acid analysis. Therefore, the molar ratio of Phe:Lys:methacrylamide in GelMA is 1.00:2.06:2.05. Assuming that the primary site of gelatin methacrylation is at Lys residues, the 1H NMR analysis indicates the degree of methacrylation is ~100% (2.05/2.06x100% = 99.5%).

Stock solutions of GelMA and LAP were prepared in DPBS, pH-adjusted, sterile filtered, and stored at 4°C until further use as described below. The following protocol exemplifies the preparation of a 15% w/v GelMA and 0.15% w/v LAP stock, but other strengths were prepared following an identical procedure. To prepare a 15% w/v GelMA stock solution, 1.5

gram of the freeze-dried GelMA prepared as described above was dissolved in 10ml of pre-warmed DPBS at 37°C. After the GelMA was fully dissolved, the photoinitiator was introduced by adding into the GelMA solution 15mg of lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), resulting in a stock solution containing 15% w/v GelMA and 0.15% w/v LAP. LAP was synthesized using published procedure (Biomaterials 2009, 30, 6702-6707). 500 microlitres of 1N NaOH (Cat# BDH-7222-1, VWR) was added to the solution to adjust the pH to neutral before the solution was filtered using 0.22 micrometre sterile membranes (Millipore).The final filtrate was separated into 500ul aliquots and stored at 4°C until further use.

Example C11 : Bioprinters: design and operation

In order to develop a cell therapy where the location of cell delivery is precisely controlled, a bioprinting technology is used to precisely position CECs on the posterior side of the cornea.

Bioprinting experiments were performed on glass coverslips using HEK293 cells. Red-fluorescent protein-labeled HEK293 cells were cultured to confluence in 6-well culture plates in DMEM-F12 with 5% fetal bovine serum. Cells were then detached from the culture dish with 100 microlitre of TripLE (Invitrogen) for 10 minutes at 37°C, cell suspensions were rinsed by centrifugation and resuspended in DMEM-F12 at a concentration of 80 million cells per ml.

A first bioprinter was designed based on dynamic light projection (DLP) technology and the schematic of the system is illustrated in Figure 26. The system consists of five main components: 1. A UVA light source (365nm, S2000, EXPO); 2. A digital micromirror array device (DMD, DLP-07 XGA; Texas Instruments) to modulate light to generate light patterns; 3. An optical system to project the light pattern towards the sample; 4. An automated stage to move in all three axes in a synchronized manner with the corresponding light patterns generated by the DMD; 5. A computer which feeds the DMD chip image flows and controls all the components.

For bioprinting, a HEK293 cell/biomatrix mixture was prepared by mixing 50 microlitre of the 15% GelMA stock solution containing 0.15% LAP with the same volume of HEK293 cell suspension to reach a final cell density of 40 million per ml. The cell/GelMA mixture was then added to the center of a glass coverslip and polymerized in the shape of the letter “e”

by applying 365 nm UVA light mask for 30 seconds. Images were then acquired using a Zeiss LSM 880 confocal microscope.

Results showed that cell-laden constructs could be precisely bioprinted in the form of the letter “e” (actual results are shown on the bottom left hand side of Figure 26 : the “e”). This indicated that the bioprinting technology could enable precise control of the location of cell delivery.

In order to facilitate in vivo animal work, the core technology of the bioprinter described above was converted into a compact portable handheld design as follows. A light emitting head was composed of a high power LED emitter (365nm, LED Engin) and an aspherical lens to provide collimated light beam. The light emitting head was built in a customized aluminum case for better heat dissipation. A rechargeable battery, power switch and other electronics were accommodated in a 3D printed plastic handle, which was assembled together with the light emitting head via a pivot to enable more freedom to turn the head. 3D printed adaptors allowed attaching to the head light guides of different dimensions to control the size of the illumination area. Inspired by the DMD based system, this handheld device can use masks printed on transparency sheet attached to the front of the head to generate customized light pattern to control photopolymerization.

Example C12 : Bioprinting cell laden constructs on the posterior side of the human cornea ex vivo

In order to determine whether the portable handheld bioprinting device would enable the precise positioning of cells on the posterior side of the human cornea, bioprinting experiments were preformed ex vivo using HEK293 cells and human corneas obtained from eye banks. Red-fluorescent protein-labeled HEK293 cells were cultured to confluence in 6-well culture plates in DMEM-F12 with 5% fetal bovine serum. Cells were then detached from the culture dish with 100 microlitres of TripLE (Invitrogen) for 10 minutes at 37°C, cell suspensions were rinsed by centrifugation and resuspended in DMEM-F12 at a concentration of 80 million cells per ml.

For bioprinting, a HEK293 cell/biomatrix mixture was prepared by mixing 50 microlitre of the 15% GelMA stock solution containing 0.15% LAP with the same volume of HEK293 cell suspension to reach a final cell density of 40 million per ml.

The cell/GelMA mixture was then added to the center of a glass coverslip and polymerized in the shape of the letter “e” by applying 365 nm UVA light mask for 30 seconds. Images were then acquired using a Zeiss LSM 880 confocal microscope.

Human donor corneas obtained from eye banks were removed from their storage solution (Optisol) and rinsed briefly with DPBS. Under the dissecting microscope, the Descement membrane was carefully scraped before the corneas were further rinsed with DPBS. One cornea was then placed on a plastic lid of a 35mm petri dish with the inner surface facing up. A cell/biomatrix mixture was prepared freshly by mixing 50 microlitres of the 15% GelMA stock solution containing 0.15% LAP with the same volume of HEK-293-RFP cell suspension to reach the final cell density of 40 million per ml. The cell/GelMA mixture was then added to the center of the cornea and then a convex shaped plastic holder was applied to form a closed chamber mimicking the eye’s anterior chamber. The whole construct was then carefully flipped and the handheld bioprinting device was used to project a 5.5-mm diameter circular pattern of 365 nm UVA light through the cornea for 30 seconds. This pattern of UV light was expected to polymerize the cell/GelMA mixture in the form of a disk attached to the posterior side of the cornea. In order to determine whether this occurred, we separated the cornea from the lid, rinsed any unpolymerized and unattached material by pipetting DPBS on the posterior side of the cornea and images were acquired using a Zeiss LSM 880 confocal microscope.

Results show that after unpolymerized and unattached material was rinsed, a circular pattern of red-fluorescent protein-labelled cells was retained on the posterior side of the cornea (Figure 27). This confirmed that cells could be bioprinted on the posterior side of the cornea by using a handheld device that projects 365 nm UVA light through the cornea.

Example C13 : Bioprinting cell laden constructs on the posterior side of the rabbit cornea in vivo

CEC preparation

Cells obtained as described in Example C1 were detached from the culture dish with 100 microlitres of Accutase (ThermoFisher) for 10 minutes at 37^{0}C, cell suspensions were rinsed by centrifugation and plated in X-VIVO15 supplemented with LATS inhibitor compound example no. 48a (diluted in DMSO) at a concentration of 3 micromolar. Cells were cultured

in 6-well plates (corning) at 37^{0}C in 5% CO2 for two weeks. Cells were passaged twice during this time by detaching cells with 1 mg/ml collagenase for 15 minutes at 37°C, rinsing cell suspensions by centrifugation and plating in fresh medium. These cells were then grown in XVIVO medium without a LATS inhibitor for a further 2 weeks to create mature CECs.

In order to prepare CECs for bioprinting inside the rabbit eye, a cell/biomatrix mixture was generated by mixing 15 microlitres of the 15% GelMA stock solution containing 0.15% LAP with 30 microlitres of CEC suspension to reach the final cell density from 0.625 million per ml to 25 million per ml. The cell/GelMA mixture was then added to the center of the cornea.

Rabbit model of corneal endothelial cell deficiency

Corneal endothelial cell deficiency was unilaterally created in the right eye of NZA rabbits by scraping off the whole cornea endothelium using a silicon tip needle. The anterior chamber was rinsed using an aspiration cannula to remove floating debris. Forty microliters of freshly prepared cell/GelMA mixture was injected into the anterior chamber and followed by 365 nm UVA exposure for 30 seconds using the handheld bioprinter equipped with a 3mm light guide about 1cm away from the ocular surface. Gentle rinsing was performed using a small cannula to inject 500 microlitres of heparin supplemented balanced salt solution to remove unpolymerized material and floating cells. The left eye of each rabbit was left intact and served as a control. After cell delivery, rabbits received analgesic treatment (Tramadol 10mg/kg PO BID in first 2 weeks, Meloxicam 0.3 mg/kg PO SID in first 2 weeks or as long as the animal showed signs of ocular discomfort), anti-inflammatory treatment (Ancef® (cefazolin) 50 mg sub-Tenon immediate post-procedure, Tobrex® ophthalmic solution t.i.d in 1st week and b.i.d thereafter, ampicillin 80mg/kg/day (40 mg BID) SQ for first week) and immunosuppression (Cyclosporine A (0.5%) top oc. t.i.d 1st week and b.i.d. thereafter, Gentocin®-durafilm® (Gentamicin sulfate and betamethasone, MERCK) top oc. t.i.d 1st week and b.i.d. thereafter, Cyclosporine A (5mg/kg/day) SQ in 1st week and then ½ dosage thereafter).

Rabbits were sacrificed three weeks after cell delivery, corneas were dissected and fixed in 4% PFA. Immunohistochemistry was performed to detect the presence of human nuclear antigen (Millipore) to confirm the presence of human cells and ZO-1 (Invitrogen) to determine whether bioprinted CECs could form tight junctions observed in a normal corneal endothelioum. Images were then acquired using a Zeiss LSM 880 confocal microscope.

Results indicated that in experimental rabbits, the corneal endothelium structure can be detected using ZO-1 immunohistochemistry (Figure 28, panel A). In the right eye of a rabbit where the corneal endothelium was surgically removed and no CEC was bioprinted, the ZO-1 staining is absent, indicating an absence of normal corneal endothelium structure (Figure 28, panel B). In the right eye of a rabbit where the corneal endothelium was surgically removed and CEC were bioprinted, the ZO-1 staining is present, indicating that a corneal endothelium structure has been rebuilt (Figure 28, panel C).

Human nuclear antigen staining was used to confirm that the cells that rebuilt the corneal endothelium in Figure 28, panel C are the human CECs delivered by bioprinting. Figure 28, panels D and E show that human nuclear antigen immunostaining is absent in eyes that did not receive any human CECs. In contrast, human nuclear antigen-positive cells cover the imaged field in eyes where human CECs were bioprinted (Figure 28, panel F), indicating that the ZO-1-labeled corneal endothelium structure shown in Figure 28, panel C is composed of the human CECs bioprinted on the posterior side of the rabbit cornea.

Example C14: Bioprinting cell laden constructs of highly customizable shapes on the posterior side of the human cornea ex vivo

Human donor corneas obtained from eye banks were removed from their storage solution (Optisol) and rinsed briefly with DPBS. Under the dissecting microscope, the Descement membrane was carefully scraped before the corneas were further rinsed with DPBS. One cornea was then placed on a plastic lid of a 35mm petri dish with the inner surface facing up.

A cell/biomatrix mixture was prepared freshly by mixing 50 µl of the 15% GelMA stock solution containing 0.15% LAP with the same volume of HEK-293-RFP cell suspension to reach the final cell density of 40 million per ml. The cell/GelMA mixture was then added to the center of the cornea and then a convex shaped plastic holder was applied to form a closed chamber mimicking the eye's anterior chamber. The whole construct was then carefully flipped and the DLP bioprinting device mentioned in Example C11 was used to project customizable patterns of 365 nm UVA light through the cornea for 7.5 seconds. Any unpolymerized and unattached material was then rinsed by DPBS on the posterior side of the cornea and images were acquired using a Zeiss fluorescence stereomicroscope.

In order to demonstrate the reproducibility and customizability of our bioprinting technology, light patterns in the design of different letters, which can be easily recognized, were projected to the samples. Results (Figure 29) show that customizable cell laden constructs with high precision can be bioprinted to the posterior side of the cornea determined by the light patterns through the cornea.

Example C15: Residual compound estimation study

Standard curve preparation

A dilution series of stock standards were made at concentrations of 30 micromolar, 3 micromolar, 300 nM, 30 nM, 3 nM, 0.3 nM and 0 micromolar. Compound example 48a (10 mM in DMSO) stock was spiked into 50% acetonitrile 50% water to make the spike standards. The spiking standards were used to spike the blank media samples. 10 microlitre of spiking standard was added to 90 microlitres of media to create the spiked media standards. 3000 nM, 300 nM, 30 nM, 3 nM, 300 pM, 30 pM, and 0 nM media standards were used to generate the compound example no. 48a standard curve.

50 microlitre of each media sample was treated with 300 microlitre of the extraction solution. The extraction solution consists of acetonitrile/methanol (3:1). The standard curve samples were extracted using the same volumes and conditions as the unknown media samples. The extracted samples were centrifuged at 10,000 rpm for 5 min. After centrifugation, 200 microlitres of each sample supernatant was transferred to a clean 96-well plate. The extracted samples were analyzed by High Resolution LC-MS. Thermo Xcalibur Software and Quan Browser were used to generate the standard curve and calculate the concentration values. An external calibration method using linear log-log scaling was used to calculate the concentration of compound example no. 48a in the culture media samples.

LC-MS analysis

High-resolution chromatography was performed using the Thermo Ultimate UPLC and a Kinetex 2.1 X 50 mM C18 RP column (2.6 micron particles). The mobile phases consisted of 5 mM Ammonium Acetate in H_2O for Buffer A, and 0.1% Formic Acid in Acetonitrile for Buffer B. A standard binary linear gradient was used for Reverse Phase Chromatography. The Thermo Q Exactive mass spectrometer was used for this study. The UPLC effluent was directed into the Q Exactive Mass Spectrometer which was equipped with an ESI ion source. The mass spectrometer was programmed to perform in High Resolution Full Scan mode. Extracted Ion Chromatograms were used for chromatographic integration.

Test system

Cell preparation for the CEC washout: the washout experiment was performed in triplicates, where each sample contained 0.5 million cells. All samples (media only, media with cells, supplemented with compound example no. 48a and cells in media supplemented with compound example no. 48a) were cultured at 37 degree incubator for the duration of the experiment. The medium used for the experiment was X-VIVO 15 (Lonza , cat no. 04-744Q).

The cells were cultured to passage 2 in X-VIVO-15 medium supplemented with 3 micromolar of compound example no. 48a. Upon reaching confluency, the medium was removed by aspiration and replaced with fresh medium not supplemented with compound example no. 48a. The cells were cultured for an additional week in the absence of compound example no. 48a. After one week of culture in the absence of the compound, the medium was removed by aspiration, refreshed with the medium without the compound and cell culture was continued in the absence of the compound for another six days. On day 7, the following samples were generated:

5. 2 ml of “blank” medium (not supplemented with the compound)
6. 2 ml of medium cultured in the presence of the compound (pre-wash)
7. 2 ml of media for each wash sample 1-10
8. Cell pellets (post-wash)

The cells were collected by using the cell lifter (Costar, cat# 3008).

The media and cell pellet samples were analyzed and quantified using High Resolution LC-MS.

To estimate the residual levels of compound example no. 48a, cell pellets and supernatant were collected. The amount of compound example no. 48a was determined by LC-MS. Estimation of compound example no. 48a concentrations in the Pre-wash Media, Wash Media (Table 11) and the Cell Pellet are summarized (Table 12). Pre-wash Media samples have the highest levels of compound example no. 48a (approx. 35 nM to 122 nM range). Post-wash Pellet levels have low, but detectable levels of compound example no 48a (picomolar).

Table 11: Estimation of concentrations for compound example no. 48a in the Pre-wash Media and Wash Media

Sample	Volume (mL)	compound example no. 48a (nM)	compound example no. 48a (nM)	compound example no. 48a (nM)

		in Triplicate #1	in Triplicate #2	in Triplicate #3
Pre-wash Media	2	35.344	66.248	121.863
Wash Media 1	2	0.408	0.614	0.712
Wash Media 2	2	BLQ	0.003	0.002
Wash Media 3	2	BLQ	BLQ	BLQ
Wash Media 4	2	BLQ	BLQ	BLQ
Wash Media 5	2	BLQ	BLQ	BLQ
Wash Media 6	2	BLQ	BLQ	BLQ
Wash Media 7	2	BLQ	BLQ	BLQ
Wash Media 8	2	BLQ	BLQ	BLQ
Wash Media 9	2	BLQ	BLQ	BLQ
Wash Media 10	2	BLQ	BLQ	BLQ

Table 12 : Estimation of concentrations for compound example no. 48a in the Cell Pellet

Sample	compound example no. 48a (nM)	compound example no. 48a (pg/cell)	Avg of compound example no. 48a (pg/cell)	# of cells in Pellet	Total of compound example no. 48a (pg)
PostWash_Pellet 1	0.004	0.13×10^{-6}	1.5×10^{-6}	0.5×10^{6}	0.75
PostWash_Pellet 2	0.071	2.3×10^{-6}			
PostWash_Pellet 3	0.065	2.1×10^{-6}			

The amount of compound example no. 48a in the Pre-wash Media from three incubations ranges from 35.344-121.863 nM. The average amount of compound example no. 48a in the Cell Pellet was 0.75 picograms for 500,000 cells.

Example C16: Reducing Immune Rejection by AAV-mediated deletion of the beta-2-microglobulin gene in LSCs

AAV vector design:

The vector map shown in Figure 30 provides the design of the AAV vector used to express the CRISPR system in LSCs. The vector expressed Staphylococcus aureus Cas9 (Ran et al, Nature. 2015 Apr 9;520(7546):186-191) and a B2M-specific guide RNA inserted in the Aar I restriction site.

Cell transduction: LSCs obtained as described in Example B1 were cultured in X-VIVO15 medium supplemented with the LATS inhibitor compound example no 48a. LSC were transduced either in suspension immediately after isolation from human corneas or after isolation, attachment and expansion in the LATS inhibitor compound to a confluency of 50-60% in a 35mm petri dish eight days after LSC isolation. LSCs were transduced at MOIs of 10K, 50K, 100K or 200K. The vector particles were diluted in a buffer composed of 1xPBS + 0.001% Pluronic and pipetted into the culture dish. The viral particle storage buffer alone represented the transduction negative control. Cells were cultured for 72 hours in a 5% CO_2 incubator before FACS analysis and sorting was performed.

FACS analysis:

LSCs were treated with Accutase (ThermoFisher, Cat # A1110501) for 20 minutes in 5% CO_2 at 37°C. After scraping the cells, the reaction 5 was stopped by using cell culture medium containing 10% serum and transferred to a falcon tube for a centrifugation step (1000 rpm, 5 minutes). After aspirating the medium, cells were resuspended in 200 microlitre FACS buffer (PBS/10%FBS).

To analyze the expression of B2M and HLA-ABC, 5 microlitre APC mouse anti-human Beta2-microglobulin antibody (Biolegend, Cat #316312) and 20 microlitre PE mouse antihuman HLA-ABC antibody (BD Bioscience, Cat # 560168) were added to the cell suspension and incubated for 30 minutes on ice. Cells were washed 3 times after antibody labelling with FACS buffer and resuspended in 500 microlitres in FACS buffer.
Before FACS sorting, cells were filtered through a 70 micrometre filter and stored on ice until sorting. In order to prevent cells sticking to the wall, collection tubes were filled with the

serum for 30 minutes before the sort. Cells were sorted on a BD FACSAria II instrument into 20 prepared collection tubes, using human serum enriched LSC medium. FACS data were analyzed using BD FACSDiva software.

Results presented in Figure 31 show at an MOI of 200K, AAV-mediated expression of the CRISPR system enabled deletion of B2M and subsequent elimination of HLA A, B and C occurred in 24.9 percent of the LSCs when attached cells were transfected and 20.0 percent of LSCs when cells in suspension were transduced.

Example C17: Reducing Immune Rejection by AAV-mediated deletion of the beta-2-microglobulin gene in CECs

AAV vector design:

The vector map shown in Figure 30 provides the design of the AAV vector used to express the CRISPR system in CECs. The vector expressed Staphylococcus aureus Cas9 (Ran et al, Nature. 2015 Apr 9;520(7546):186-191) and a B2M-specific guide RNA inserted in the Aar I restriction site.

Cell transduction:

Cells obtained as described in Example C1 were detached from the culture dish with 100 microlitres of Accutase (ThermoFisher) for 10 minutes at 37°C, cell suspensions were rinsed by centrifugation and plated in corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) in 6-well plates (Corning) 10 supplemented with LATS inhibitor compound example no. 133 or example no 49 (diluted in DMSO) at a concentration of 10 micromolar or as a negative control DMSO alone without compound. Cells were cultured at 37°C in 5% CO_2.

CECs were cultured in corneal endothelial cell culture medium (human endothelial SF medium (Invitrogen) with human serum) in 6-well plates (Corning) supplemented with LATS inhibitor compound example no. 133 or example no 49 (diluted in DMSO) at a concentration of 10 micromolar. CEC were transduced after isolation, attachment and expansion in the LATS inhibitor compound to a confluency of 50-60% in a 6-well plate eight days after CEC isolation. CECs were transduced at MOIs of 0.1K, 1K, 5K, 10K, 20K or 40K. The vector particles were diluted in a buffer composed of 1xPBS + 0.001% Pluronic and pipetted into

the culture dish. The viral particle storage buffer alone represented the transduction negative control. Cells were cultured for 72 hours in a 5% CO_2 incubator before FACS analysis and sorting was performed.

FACS analysis:

CECs were treated with Accutase (ThermoFisher, Cat # A1110501) for 20 minutes in 5% CO_2 at 37°C. After scraping the cells, the reaction 5 was stopped by using cell culture medium containing 10% serum and transferred to a falcon tube for a centrifugation step (1000 rpm, 5 minutes). After aspirating the medium cells were resuspended in 200 microlitre FACS buffer (PBS/10%FBS).

To analyze the expression of B2M and HLA-ABC, 5 microlitre APC mouse anti-human Beta2-microglobulin antibody (Biolegend, Cat #316312) and 20 microlitre PE mouse antihuman HLA-ABC antibody (BD Bioscience, Cat # 560168) were added to the cell suspension and incubated for 30 minutes on ice. Cells were washed 3 times after antibody labelling with FACS buffer and resuspended in 500 microlitres in FACS buffer.

Before FACS sorting, cells were filtered through a 70 micrometre filter and stored on ice until sorting. In order to prevent cells sticking to the wall, collection tubes were filled with the serum for 30 minutes before the sort. Cells were sorted on a BD FACSAria II instrument into 20 prepared collection tubes, using human serum enriched CEC culture medium. FACS data were analyzed using BD FACSDiva software.

Results presented in Figure 32 show that AAV-mediated expression of the CRISPR system enabled deletion of B2M and subsequent elimination of HLA A, B and C. At an MOI of 40K, B2M deletion occurred in 17 percent of the CECs.

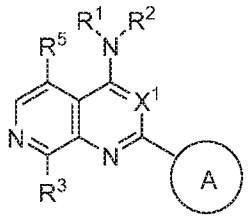
Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further

references cited therein. Unless indicated otherwise, each of the references cited herein is incorporated in its entirety by reference.

Claims to the invention are non-limiting and are provided below. Although particular embodiments and claims have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, or the scope of subject matter of claims of any corresponding future application. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the disclosure without departing from the spirit and scope of the disclosure as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other embodiments, advantages, and modifications are considered to be within the scope of the following claims. Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein.

Claims

1. A compound of Formula A2, or a salt, or stereoisomer thereof,


A2

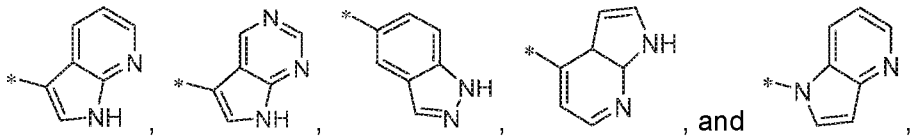
wherein

X^1 is CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule; and

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or, provided that when X^1 is CH, R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

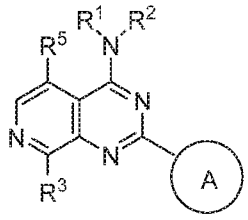
R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0;

with the proviso that:

(1) when X^1 is N, ring A is 4-primidinyl or 3-fluoro-4-primidinyl, R^1 is H or methyl, R^3 is H or Cl and R^5 is H; then R^2 is not C_{2-4}alkyl that is substituted with a substituent selected from –NH_2, C_{1-6}alkylamino or *t*-butyl-carbamoyl-amino and that is optionally further substituted with unsubstituted phenyl; and

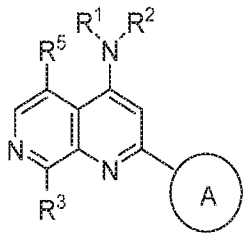
(2) when X^1 is N, ring A is indazol-5-yl, R^1, R^3 and R^5 are H; then R^2 is not C_4alkyl that is substituted with –NH_2.

2. The compound according to claim 1, wherein the compound is of Formula I:

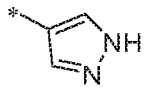
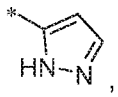
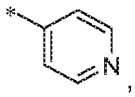
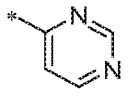
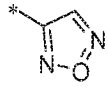


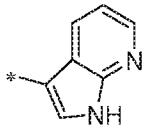
I.

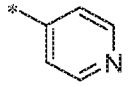
3. The compound according to claim 1, wherein the compound is of Formula II:



II.

4. The compound according to any one of claims 1 to 3, wherein ring A is selected from  ,  ,  ,  , and  which are are each unsubstituted or substituted by a substituent selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, and $-NH_2$;

or is  , which is unsubstituted or substituted by C_{1-6}alkyl.

5. The compound according to any one of claims 1 to 4, wherein ring A is  .

6. The compound according to any one of claims 1 to 5, wherein

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by A1 to A3 substituents independently selected from

(i) cyano;

(ii) C_2alkynyl;

(iii) C_{1-6}haloalkyl;

(iv) $-OR^6$, wherein R^6 is selected from hydrogen, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(v) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(vi) $-C(O)R^8$, wherein R^8 is R^0;

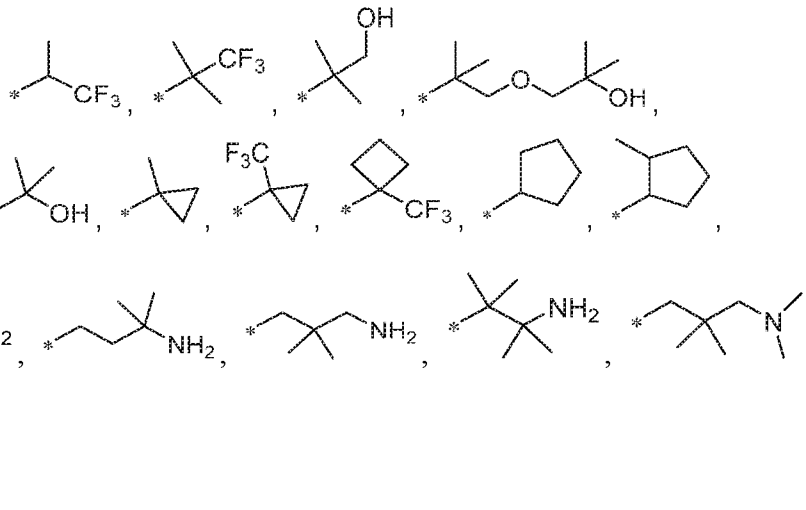
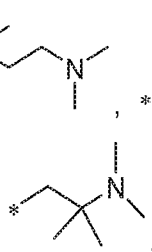
(vii) $-S(O)_2C_{1-4}$alkyl;

(viii) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by a substituent selected from C1-6alkyl, hydroxyC_{1-6}alkyl and R^0;

(ix) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N and O, and wherein the 6-membered heterocycloalkyl is unsubstituted or substituted by C1-6alkyl;

(x) phenyl that is unsubstituted or substituted by halogen; and

(b) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-R^0$ or $-C(O)R^0$.

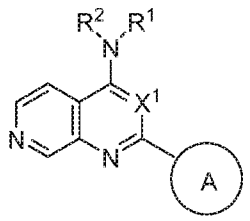
7. The compound according to any one of claims 1 to 6, wherein R^2 is selected from n-propyl, isopropyl, *t*-butyl,  , and  .

8. The compound according to claim 1 and claims 3 to 5, wherein

X^1 is CH; and

R^1 and R^2 are taken together with the nitrogen atom to which both are bound to form a 6-membered heterocycloalkyl that can include, as ring member, an additional heteroatom selected from N and O, wherein the 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from hydroxyl, $C_{1\text{-}6}$alkyl and $C_{1\text{-}6}$haloalkyl.

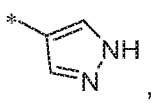
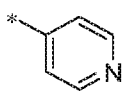
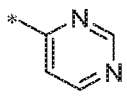
9. The compound according to claim 1, wherein the compound is of Formula A3:



A3

wherein

X^1 is CH or N;

Ring A is  ,  or  , each of which is unsubstituted or substituted by a substituent selected from halogen, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$haloalkyl, and $-NH_2$;

R^1 is hydrogen or unsubstituted C_{1-6}alkyl; and

R^2 is

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 substituents selected from

(i) C_{1-4}haloalkyl; and

(ii) $-OR^6$, wherein R^6 is selected from hydrogen and C_{1-6}alkyl that is unsubstituted or substituted by hydroxyl; or

(b) monocyclic C_{3-6}cycloalkyl that is unsubstituted or substituted by C_{1-6}alkyl or C_{1-6}haloalkyl and R^0.

10. The compound of Formula A2 according to claim 1, or a salt thereof, selected from: N-(2-cyclopropylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N,N-diethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-(1,1,1-trifluoropropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(propan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-butyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol; N-ethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-cyclohexylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(3-methyloxetan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; N-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methyl-4-phenylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-cyclopropyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methanesulfonyl-2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol; 3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)acetic acid; (1R,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-(1-methanesulfonyl-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (2S)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-

yl]amino}propanoic acid; 2-[(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)amino]acetic acid; (2R)-3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; methyl 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoate; (1S,2S)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentan-1-ol; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-(2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethoxy)ethan-1-ol; 2-(hydroxymethyl)-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propane-1,3-diol; 3-methyl-3-(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanamido)butanoic acid; 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-3-phenylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-{[4-(dimethylamino)oxan-4-yl]methyl}-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-(2-methanesulfonylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-(adamantan-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propanamide; 4,4,4-trifluoro-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]propane-2-sulfonamide; 2-(pyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(pyridin-4-yl)-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 4,4,4-trifluoro-2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopentyl)methanol; N-(3-methoxycyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (1R,2R)-1-N,2-N-dimethyl-1-N-[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]cyclohexane-1,2-diamine; methyl (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate; ethyl 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylate; 1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid; (1s,3s)-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutane-1-carboxylic acid; 2-(pyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; 2-(pyridin-4-yl)-N-[1-(trifluoromethyl)cyclobutyl]pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylbutan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(pyridin-4-yl)-N-[1-

(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; N-cyclopentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propan-1-ol; N-(butan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylbut-3-yn-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (1r,3s)-3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutan-1-ol; 2,3-dimethyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-2-ol; 2-(pyridin-4-yl)-N-(2,4,4-trimethylpentan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(pentan-3-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[1-(tert-butoxy)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4,4,4-trifluoro-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-pentyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butan-1-ol; N-[1-(1H-indol-3-yl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[1-(4-fluorophenyl)-2-methylpropan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-phenylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-[2-(4-fluorophenyl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3,3,3-trifluoro-2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanoic acid; 2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}ethan-1-ol; N-methyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 1-({[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}methyl)cyclopentan-1-ol; N,N-dimethyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(2-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-methoxyphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-phenyl-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(3-methylphenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 6-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}hexanoic acid; N-(3-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-fluorophenyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanoic acid; N-(1-phenylethyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; tert-butyl N-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propyl)carbamate; (1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; methyl 2-(1-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)acetate; N-(2-methylpropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butanenitrile; N-(6-

aminohexyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(4-aminobutyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propanenitrile; N-[2-methyl-1-(2-methylpiperidin-1-yl)propan-2-yl]-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine; N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-cyclopentyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-[4-(tert-butylamino)pyrido[3,4-d]pyrimidin-2-yl]pyridin-2-amine; 2-[1-(benzenesulfonyl)-2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl]-N-tert-butylpyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(3-methylpyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)pentan-2-ol; N-ethyl-2-(3-fluoropyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-methyl-1-[2-methyl-2-({2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-yl}amino)propoxy]propan-2-ol; 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-ethyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(1-methoxy-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol; 2-(3-chloropyridin-4-yl)-N-cyclopentylpyrido[3,4-d]pyrimidin-4-amine; 1-(2-{[2-(3-chloropyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2-methylpropoxy)-2-methylpropan-2-ol; N-methyl-2-(3-methylpyridin-4-yl)-N-(propan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-(4-methanesulfonyl-2-methylbutan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[3-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-[2-chloro-5-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-[3-(1H-1,2,3,4-tetrazol-5-yl)propyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-[(2R)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridin-2-amine; 2-(3-chloropyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; 2,4-

dimethyl-4-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile; 2-{2-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(1H-indazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3,5-dimethyl-1H-pyrazol-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1,1,1-trifluoro-2-methylpropan-2-yl)-2-[3-(trifluoromethyl)-1H-pyrazol-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(4-hydroxy-2,4-dimethylpentan-2-yl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-3-carbonitrile; 2-(3,5-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2,3-difluoropyridin-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1,3-thiazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-[2-(trifluoromethyl)pyridin-4-yl]pyrido[3,4-d]pyrimidin-4-amine; 4-{4-[(1-methylcyclopropyl)amino]pyrido[3,4-d]pyrimidin-2-yl}pyridine-2-carbonitrile; N-(1-methylcyclopropyl)-2-(1,2-oxazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(dimethyl-1,2-oxazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-{1H-pyrrolo[2,3-b]pyridin-3-yl}pyrido[3,4-d]pyrimidin-4-amine; N-propyl-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methylpyridin-4-yl)-N-(1,1,1-trifluoro-2-methylpropan-2-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclobutyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyrimidin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-{[2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}-2,4-dimethylpentan-2-ol; N-propyl-2-{7H-pyrrolo[2,3-d]pyrimidin-5-yl}pyrido[3,4-d]pyrimidin-4-amine; 2-(3-chloropyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(3-cyclopropyl-1H-pyrazol-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-(3-methylpyridin-4-yl)-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2-{1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl}-N-propylpyrido[3,4-d]pyrimidin-4-amine; 2,4-dimethyl-4-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}pentan-2-ol; N-[(1R)-1-phenylethyl]-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(5-methyl-1H-pyrazol-4-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-[(1R)-1-phenylethyl]pyrido[3,4-d]pyrimidin-4-amine; N-methyl-N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-ethyl-1H-pyrazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(pyridazin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1,3-oxazol-5-yl)pyrido[3,4-

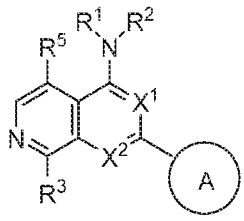
d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-imidazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-{1H-pyrrolo[3,2-b]pyridin-1-yl}pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-1,2,3-triazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1,2-oxazol-5-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(2H-1,2,3,4-tetrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; 2-(1-methyl-1H-pyrazol-5-yl)-N-(1-methylcyclobutyl)pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(1-methyl-1H-pyrazol-5-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; 2-(1H-pyrazol-4-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(trifluoromethyl)cyclopropyl]pyrido[3,4-d]pyrimidin-4-amine; 2-(3-methyl-1H-pyrazol-4-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclobutyl)methanol; (1-{[2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}cyclopropyl)methanol; 2-(1-methyl-1H-pyrazol-5-yl)-N-[1-(pyridin-4-yl)ethyl]pyrido[3,4-d]pyrimidin-4-amine; N-(1-methylcyclopropyl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-ethyl-1H-pyrazol-4-yl)-N-(2-methylpropyl)pyrido[3,4-d]pyrimidin-4-amine; 2-(1-methyl-1H-pyrazol-4-yl)-N-(1-methylcyclopropyl)pyrido[3,4-d]pyrimidin-4-amine; N-(1-amino-2-methylpropan-2-yl)-2-(1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-tert-butyl-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 2-(2-{[4-(tert-butylamino)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-5-yl]amino}ethoxy)ethan-1-ol; N-(4-methoxy-2-methylbutan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[2-methyl-1-(propan-2-yloxy)propan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[(2S)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-[(2R)-butan-2-yl]-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-(1-methoxy-2-methylpropan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-methyl-N-(propan-2-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butan-1-ol; N-tert-butyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2,2-dimethyl-1-[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]piperidin-4-ol; 2,4-dimethyl-4-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}pentan-2-ol; N-cyclopentyl-2-(pyridin-4-yl)-1,7-

naphthyridin-4-amine; dimethyl(3-methyl-3-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}butyl)amine; N,N-diethyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)-1,7-naphthyridin-4-yl]amino}propoxy)propan-2-ol; N-propyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; 2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine; N-tert-butyl-2-{1H-pyrrolo[2,3-b]pyridin-4-yl}-1,7-naphthyridin-4-amine; N-tert-butyl-2-(pyridazin-4-yl)-1,7-naphthyridin-4-amine; 2-(2-aminopyridin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine; N,N-diethyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine; (3-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-3-methylbutyl)dimethylamine; 2-(3-fluoropyridin-4-yl)-N-methyl-N-(propan-2-yl)-1,7-naphthyridin-4-amine; 2-(3-fluoropyridin-4-yl)-4-(piperidin-1-yl)-1,7-naphthyridine; 2-(3-fluoropyridin-4-yl)-4-(morpholin-4-yl)-1,7-naphthyridine; N-tert-butyl-2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-amine; 2-(3-fluoropyridin-4-yl)-N-(2-methylbutan-2-yl)-1,7-naphthyridin-4-amine; 2-{[2-(3-fluoropyridin-4-yl)-1,7-naphthyridin-4-yl]amino}-2-methylpropan-1-ol; 1-[2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-yl]-2,2-dimethylpiperidin-4-ol; 2-(3-fluoropyridin-4-yl)-N-[2-methyl-1-(morpholin-4-yl)propan-2-yl]-1,7-naphthyridin-4-amine; N-tert-butyl-2-(3-chloropyridin-4-yl)-1,7-naphthyridin-4-amine; N-((1R,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (S)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1S,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; (R)-N-(sec-butyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1S,2S)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; N-((1R,2R)-2-methylcyclopentyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine; 4-(4-methylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 4-(piperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 4-(2-methylpiperidin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 2-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclobutyl)-1,7-naphthyridin-4-amine; 2-methyl-N1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,2-diamine; N-(oxetan-3-yl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; N-(1-methylcyclopropyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 4-(3,3-dimethylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 2,2-dimethyl-4-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)morpholine; N-(1-methylcyclobutyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; 2,2-dimethyl-N1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,3-diamine; N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,2-diamine; 4-(2-methylpiperazin-1-yl)-2-(pyridin-4-yl)-1,7-naphthyridine; 2-methyl-N^1-(2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)propane-1,3-diamine; (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)-1,7-naphthyridine; N-(tert-butyl)-N-methyl-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; *N*-(1-

methylcyclobutyl)-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine; N^1,N^1,3-trimethyl-N^3-(2-(pyrimidin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; *tert*-butyl (2-methyl-1-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propan-2-yl)carbamate; *tert*-butyl (2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)ethyl)carbamate; 2-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine; N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)ethane-1,2-diamine; *N,N*,2-trimethyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide; N^1,3-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; *tert*-butyl (2,2-dimethyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propyl)carbamate; 2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; 3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butanamide; (*R*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine; 2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine; (*S*)-2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-*d*]pyrimidine; ethyl 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanoate; N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; 4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine; 2-methyl-2-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)propanamide; (*S*)-1,1,1-trifluoro-2-methyl-3-((2-(pyridin-4-yl)-1,7-naphthyridin-4-yl)amino)propan-2-ol; 2-(3-chloropyridin-4-yl)-N,N-diethyl-1,7-naphthyridin-4-amine; N-propyl-2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-d]pyrimidin-4-amine; *tert*-butyl (3-methyl-3-((2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)amino)butyl)carbamate; N^1,N^1,N^3,2,2-pentamethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine; N^1,N^1-diethyl-3-methyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^3-(2-(2-fluoropyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; N^3-(2-(3,5-dimethyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; N^1,N^1,3-trimethyl-N^3-(2-(3-(trifluoromethyl)-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine; N^3-(2-(2-aminopyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)-N^1,N^1,3-trimethylbutane-1,3-diamine; and 3-methyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine.

11. A pharmaceutical composition comprising a compound of any one of claims 1 to 10 as an active ingredient and at least one pharmaceutically acceptable excipient.

12. A method of LATS inhibition in a cell population comprising corneal endothelial cells using a compound of Formula A1 or a salt thereof:



A1

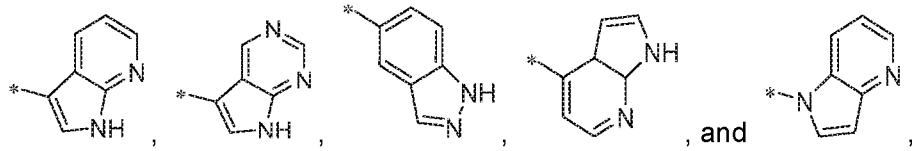
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, $-NH_2$, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

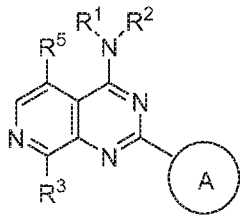
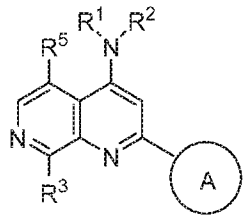
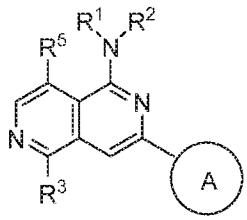
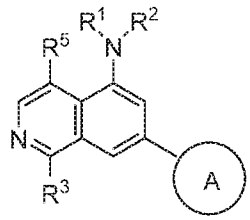
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional

heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

13. The method of LATS inhibition according to claim 12, wherein the compound is of a formula selected from Formulae I to IV:


I,

II,

III, and

IV.

14. The method of LATS inhibition in a cell population according to claim 12 or 13, wherein the compound, or a salt thereof, is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

15. The method of LATS inhibition according to claim 12, wherein the compound, or a salt thereof, is according to any one of claims 1 to 10.

16. A method of culturing cells comprising culturing a population of cells comprising corneal endothelial cells in the presence of a LATS inhibitor.

17. The method of culturing cells according to claim 16, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

18. A method of culturing cells comprising culturing a population of cells comprising corneal endothelial cells in the presence of a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

19. A method of cell population expansion, comprising the step of a) culturing a seeding population of cells comprising corneal endothelial cells in the presence of a LATS inhibitor to generate an expanded population of cells comprising corneal endothelial cells.

20. The method of cell population expansion according to claim 19 wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

21. A method of cell population expansion, comprising the step of a) culturing a seeding population of cells comprising corneal endothelial cells in the presence of a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 to generate an expanded population of cells comprising corneal endothelial cells.

22. The method of any one of claims 12 to 21, wherein said compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

23. The method of any one of claims 12 to 22, wherein in step a) the compound is present for one to two weeks and subsequently step b) is performed wherein the cells are cultured for a period in growth medium without supplementation with said compound, preferably wherein the period is one to two weeks.

24. The method of any one of claims 19 to 23, wherein the method produces greater than 10 fold expansion of the seeded amount of cells.

25. The method of any one of claims 19 to 24, wherein the method produces 15 fold to 600 fold, preferably 20 fold to 550 fold expansion of the seeded amount of cells.

26. The method of any one of claims 12 to 25, wherein the LATS inhibitor inhibits LATS1 and LATS2.

27. The method of any one of claims 12 to 26, wherein said method further comprises genetically modifying said corneal endothelial cells.

28. The method of claim 27, wherein said genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response.

29. The method of claim 27 or 28, wherein said genetically modifying comprises introducing into said cell a gene editing system which specifically taregts a gene associated with facilitating a host versus graft immune response.

30. The method of claim 29, wherein said gene editing system is selected from the group consisting of a CRISPR gene editing system, a TALEN gene editing system, a zinc finger nuclease gene editing system, a meganuclease gene editing system, AAV vector driven homologous recombination and lentiviral vectors-based genome editing technologies.

31. The method of any one of claims 28 to 30, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C.

32. The method of any one of claims 12 to 31, which comprises the further step after generation of an expanded population of cells of rinsing those cells to substantially remove the compound.

33. A cell population obtainable by the method of any one of claims 12 to 32.

34. A cell population obtained by the method of any one of claims 12 to 32.

35. A cell population comprising corneal endothelial cells or the cell population of claims 33 or 34, wherein one or more of said cells comprises a non-naturally occurring insertion or deletion of one or more nucleic acid residues of a gene associated with facilitating a host vs graft immune response, wherein insertion and/or deletion results in reduced or eliminated expression or function of said gene.

36. The cell population according to claim 35, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C.

37. The cell population according to claim 35 or 36, wherein said gene is B2M.

38. Growth promoting agent of corneal endothelial cells comprising a LATS inhibitor.

39. The growth promoting agent of corneal endothelial cells according to claim 38, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

40. Growth promoting agent of corneal endothelial cells comprising a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

41. A cell proliferation medium comprising a LATS inhibitor and a growth medium.

42. The cell proliferation medium according to claim 41, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

43. A cell proliferation medium comprising a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 and a growth medium.

44. The cell proliferation medium according to any one of claims 41 to 43, wherein the growth medium is selected from the group consisting of Dulbecco's Modified Eagle's Medium supplemented with Fetal Bovine Serum, human endothelial serum free medium with human serum, X-VIVO15 medium and mesenchymal stem cell-conditioned medium; preferably X-VIVO15 medium.

45. The cell proliferation medium according to any one of claims 41 to 44, additionally comprising corneal endothelial cells.

46. A cell preparation comprising a LATS inhibitor and corneal endothelial cells.

47. The cell preparation according to claim 46, wherein the LATS inhibitor is is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

48. A cell preparation comprising a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 and corneal endothelial cells.

49. The cell preparation according to any one of claims 46 to 48 which further comprises a growth medium, wherein the growth medium is selected from the group consisting of Dulbecco's Modified Eagle's Medium supplemented with Fetal Bovine Serum, human endothelial serum free medium with human serum, X-VIVO15 medium and mesenchymal stem cell-conditioned medium; preferably X-VIVO15 medium.

50. The pharmaceutical composition according to claim 11 or the cell preparation according to any one of claims 46 to 49, further comprising a preservation or cryopreservation solution.

51. The pharmaceutical composition or cell preparation according to claim 50, wherein the preservation or cryopreservation solution comprises a solution which is Optisol or PBS and the cryopreservation solution additionally comprises glycerol, dimethyl sulfoxide, propylene glycol or acetamide.

52. A kit comprising a composition suitable for ocular delivery and a LATS inhibitor.

53. A kit according to claim 52, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

54. A kit comprising a composition suitable for ocular delivery and a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

55. The kit according to any one of claims 52 to 54, wherein the composition suitable for ocular delivery is a localising agent or topical eye drops.

56. The kit according to any one of claims 52 to 55, further comprising corneal endothelial cells.

57. A cell delivery preparation, comprising a cell population according to any one of claims 33 to 37 or a cell preparation according to any one of claims 46 to 51 and a composition suitable for ocular delivery which is a localising agent.

58. The kit according to any one of claims 52 to 56 or the cell delivery preparation according to claim 57, wherein the composition suitable for ocular delivery is a localising agent which is a biomatrix.

59. The kit according to any one of claims 52 to 56 or 58 or the cell delivery preparation according to any one of claims 57 or 58, wherein the composition suitable for ocular delivery is a localising agent selected from the group consisting of fibrin, collagen, gelatin, cellulose, amniotic membrane, fibrin glue, polyethylene (glycol) diacrylate (PEGDA), GelMA, localising agents comprising a polymer, cross-linked polymer, or hydrogel comprising one or more of hyaluronic acid, polyethylene glycol, polypropylene glycol, polyethylene oxide, polypropylene oxide, poloxamer, polyvinyl alcohol, polyacrylic acid, polymethacrylic acid, polyvinyl pyrrolidone, poly(lactide-co-glycolide), alginate, gelatin, collagen, fibrinogen, cellulose, methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropylmethylcellulose, hydroxypropyl-guar, gellan gum, guar gum, xanthan gum and

carboxymethylcellulose, as well as derivatives thereof, co-polymers thereof, and combinations thereof.

60. The kit according to any one of claims 52 to 56 or 58 or 59 or the cell delivery preparation according to any one of claims 57 to 59, wherein the composition suitable for ocular delivery is a localising agent which is GelMa.

61. The kit according to any one of claims 52 to 56 or 58 to 60 or the cell delivery preparation according to any one of claims 57 to 60, wherein corneal endothelial cells are present in combination with the localising agent and the corneal endothelial cells are in a monolayer.

62. The kit according to any one of claims 52 to 56 or 58 to 61 or the cell delivery preparation according to any one of claims 57 to 61, wherein corneal endothelial cells are present in combination with the localising agent at a density greater than 500 cells per mm^2 (area).

63. The kit according to any one of claims 54 to 56 or 58 to 62 or the cell delivery preparation according to any one of claims 57 to 61, wherein corneal endothelial cells are present in combination with the localising agent at a density of 1 000 to 3 500 cells/mm^2 (area), preferably 2 000 to about 3 000 cells/mm^2 (area).

64. The pharmaceutical composition, growth promoting agent, cell proliferation medium, cell preparation or kit according to any one of claims 11 or 38 to 63, wherein the compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

65. The cell population according to any one of claims 33 to 37 or the cell preparation according to any one of claims 46 to 51, or the cell delivery preparation according to any one of claims 57 to 63, wherein the compound is present at only trace levels.

66. The growth promoting agent, cell proliferation medium, cell preparation, or the cell delivery preparation or kit according to any one of claims 38 to 65, wherein the LATS inhibitor inhibits LATS1 and LATS2.

67. The cell proliferation medium, cell preparation, the cell delivery preparation or kit according to any one of claims 41 to 60, wherein corneal endothelial cells are present and are in suspension.

68. A method of transplanting a population of corneal endothelial cells onto the cornea of a subject, said method comprising administering the population of cells of any one of claims 33 to 37 or 65 or cell delivery preparation of claim 57 to 63 or 65.

69. A method of transplanting a population of corneal endothelial cells onto the cornea of a subject, said method comprising expanding a population of corneal endothelial cells by culturing said population with cell proliferation medium according to any one of claims 41 to 44 or 65 or 66, rinsing the expanded population of cells to substantially remove the LATS inhibitor, and administering said cells onto the cornea of said subject.

70. The method of claim 69, wherein said isolated corneal endothelial cells are combined with a biomatrix prior to said administration.

71. The method of claim 70, wherein said isolated corneal endothelial cells are combined with a biomatrix which is GelMA prior to said administration.

72. A method of transplanting a population of corneal endothelial cells to the eye of a subject, comprising culturing a population of corneal endothelial cells in a cell proliferation medium that comprises a LATS inhibitor, combining the corneal endothelial cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject, and bioprinting the cells in the eye by guiding and fixing the cells on the cornea using a light source.

73. The method of any one of claims 70 to 71, wherein said isolated corneal endothelial cells are combined with a biomatrix which is GelMA and bioprinted onto the ocular surface by polymerising the GelMA by a light triggered reaction.

74. A method of prophylaxis or treatment of an ocular disease or disorder using a LATS inhibitor.

75. The method according to claim 74, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

76. A method of prophylaxis or treatment of an ocular disease or disorder using a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10.

77. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 76, comprising the method of LATS inhibition in a cell population according to any one of claims 12 to 15 or the method of cell population expansion of any one of claims 19 to 32.

78. A method of prophylaxis or treatment of an ocular disease or disorder comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 12 to 15 or cell delivery preparation according to any one of claims 57 to 63 or 65 to 67.

79. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 78, wherein the ocular disease or disorder is associated with decreased corneal endothelial cell density.

80. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 79, wherein the ocular disease or disorder is corneal endothelial dysfunction.

81. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 80, wherein the ocular disease or disorder is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy), corneal transplant failure, posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, X-linked endothelial corneal dystrophy, aniridia and corneal endothelitis.

82. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 81, wherein the ocular disease or disorder is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy) and corneal transplant failure.

83. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 82, wherein the method comprises the steps of the method of any one of claims 68 to 73.

84. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 74 to 83, wherein the cell population according to any one of claims 33 to 37 or cell delivery preparation according to any one of claims 57 to 63 or 65 or 66 is administered simultaneously or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin, and cefazolin.

85. A compound according to Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 for use in therapy or as a medicament.

86. A compound according to Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 for use in an ocular disease or disorder.

87. A LATS inhibitor for use in an ocular disease or disorder wherein the LATS inhibitor is a compound.

88. Use of a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 for the manufacture of a medicament.

89. Use of a compound of Formula A1 as defined in any one of claims 12 to 14 or the compound or a salt thereof as defined in any one of claims 1 to 10 for the manufacture of a medicament to treat an ocular disease or disorder.

90. A compound for use according to claim 85 or 86 or a LATS inhibitor for use according to claim 87, or use according to claim 88 or 89, comprising the method of LATS inhibition in a cell population according to claim 12 to 15 or the method of cell population expansion of any one of claims 19 to 32.

91. A compound for use according to any one of claims 85 or 86 or 90 or a LATS inhibitor for use according to any one of claims 87 or 90 or use according to any one of claims 88 to 90, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 33 to 37 or cell delivery preparation according to any one of claims 57 to 63 or 65 to 67.

92. A compound for use according to any one of claims 85 or 86 or 90 or 91 or a LATS inhibitor for use according to any one of claims 87 or 90 or 91 or use according to any one of claims 88 to 91 wherein the ocular disease or disorder is associated with decreased corneal endothelial cell density.

93. A compound for use according to any one of claims 85 or 86 or 90 to 92 or a LATS inhibitor for use according to any one of claims 87 or 90 to 92, or use according to any one of claims 88 to 92 wherein the ocular disease or disorder is corneal endothelial dysfunction.

94. A compound for use according to any one of claims 85 or 86 or 90 to 93 or a LATS inhibitor for use according to any one of claims 87 or 90 to 93 , or use according to any one of claims 88 to 93 wherein the ocular disease or disorder is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including pseudophakic bullous keratopathy and aphakic bullous keratopathy), corneal transplant failure, posterior polymorphous corneal dystrophy, congenital hereditary endothelial dystrophy, X-linked endothelial corneal dystrophy, aniridia and corneal endothelitis.

95. A compound for use according to any one of claims 85 or 86 or 90 to 94 or LATS inhibitor for use according to any one of claims 87 or 90 to 94, or use according to any one of claims 88 to 94 wherein the ocular disease or disorder is selected from the group consisting of Fuchs endothelial corneal dystrophy, bullous keratopathy (including

pseudophakic bullous keratopathy and aphakic bullous keratopathy) and corneal transplant failure.

96. A compound for use according to any one of claims 85 or 86 or 90 to 95 or LATS inhibitor for use according to any one of claims 87 or 90 to 95, or use according to any one of claims 88 to 95, wherein the method comprises the steps of the method of any one of claims 68 to 73.

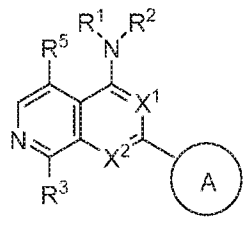
97. A compound for use according to any one of claims 85 or 86 or 90 to 96 or LATS inhibitor for use according to any one of claims 87 or 90 to 96, or use according to any one of claims 88 to 96, wherein the cell population according to any one of claims 33 to 37 or cell delivery preparation according to any one of claims 57 to 63 or 65 to 67 is administered simultaneously or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin and cefazolin.

98. YAP modulator for use in a method of transplanting a population of corneal endothelial cells onto the cornea of a subject, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 33 to 37 or cell delivery preparation according to any one of claims 57 to 63 or 65 to 67.

99. YAP modulator for use in a method of treating corneal endothelial dysfunction, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 33 to 37 or cell delivery preparation according to any one of claims 57 to 63 or 65 to 67.

100. YAP modulator of claim 98 or 99, wherein said modulator is a LATS inhibitor.

101. A method of LATS inhibition in a cell population comprising limbal stem cells using a compound of Formula A1 or a salt thereof:



A1

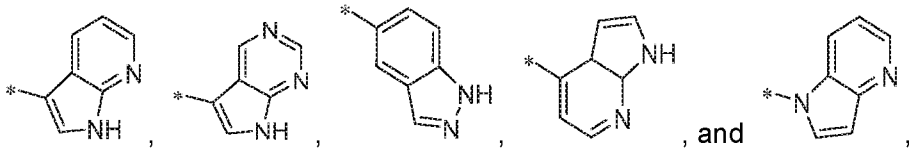
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(viii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(ix) $-C(O)R^8$, wherein R^8 is R^0 or $-NH$-C_{1-6}alkyl-$C(O)R^0$;

(x) $-S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

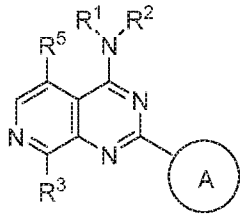
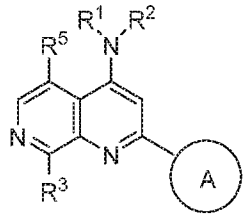
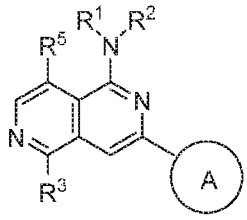
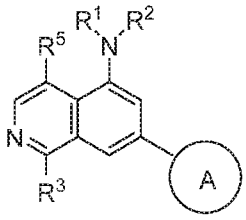
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl)

comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

102. The method of LATS inhibition according to claim 101, wherein the compound is of a formula selected from Formulae I to IV:


I,

II,

III, and

IV.

103. The method of LATS inhibition according to claim 101 or 102, wherein the compound, or a salt thereof, is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

104. The method of LATS inhibition according to claim 101, wherein the compound, or a salt thereof, is according to any one of claims 1 to 10.

105. A method of culturing cells comprising culturing a population of cells comprising limbal stem cells in the presence of a LATS inhibitor.

106. The method of culturing cells according to claim 105, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof, as defined in any one of claims 1 to 10.

107. A method of culturing cells comprising culturing a population of cells comprising limbal stem cells in the presence of a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

108. A method of cell population expansion, comprising the step of a) culturing a seeding population of cells comprising limbal stem cells in the presence of a LATS inhibitor to generate an expanded population of cells comprising limbal stem cells.

109. The method of cell population expansion according to claim 108, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

110. A method of cell population expansion, comprising the step of a) culturing a seeding population of cells comprising limbal stem cells in the presence of a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 to generate an expanded population of cells comprising limbal stem cells.

111. The method of any one of claims 101 to 110, wherein said compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

112. The method of any one of claims 101 to 111, wherein the compound is present for 12 to 16 days, preferably wherein the compound is present for 14 days.

113. The method of any one of claims 109 to 112, wherein the method produces greater than 30 fold expansion of the seeded amount of cells.

114. The method of any one of claims 109 to 113, wherein the method produces 100 fold to 2200 fold, preferably 600 fold to 2200 fold expansion of the seeded amount of cells.

115. The method of any one of claims 109 to 114, wherein the method produces a cell population with greater than 20% limbal stem cells, preferably wherein the method produces a cell population with greater than 50% limbal stem cells.

116. The method of any one of claims 109 to 114, wherein the method produces a cell population with greater than 20% expressing p63alpha, preferably wherein the method produces a cell population with greater than 50% expressing p63alpha.

117. The method of any one of claims 101 to 116, wherein the LATS inhibitor inhibits LATS1 and LATS2.

118. The method of any one of claims 101 to 117, wherein said method further comprises genetically modifying said limbal stem cells.

119. The method of claim 118, wherein said genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response.

120. The method of claim 118 or 119, wherein said genetically modifying comprises introducing into said cell a gene editing system which specifically targets a gene associated with facilitating a host versus graft immune response.

121. The method of claim 120, wherein said gene editing system is selected from the group consisting of a CRISPR gene editing system, a TALEN gene editing system, a zinc finger nuclease gene editing system, a meganuclease gene editing system, AAV vector driven homologous recombination and lentiviral vectors-based genome editing technologies.

122. The method of any one of claims 119 to 121, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C, preferably B2M.

123. The method of any one of claims 108 to 122, which further comprises step b) rinsing the expanded population of cells to substantially remove the compound according to the invention.

124. A cell population obtainable by the method of any one of claims 101 to 123.

125. A cell population obtained by the method of any one of claims 101 to 123.

126. An isolated cell population, wherein greater than 20% of cells are limbal stem cells, preferably wherein greater than 50% are limbal stem cells.

127. An isolated cell population, wherein greater than 70% of cells are limbal stem cells, preferably wherein greater than 90% are limbal stem cells.

128. An isolated cell population, wherein greater than 20% of cells are p63alpha positive, preferably wherein greater than 50% are p63alpha positive.

129. An isolated cell population, wherein greater than 70% of cells are p63alpha positive, preferably wherein greater than 90% are p63alpha positive.

130. A cell population comprising limbal stem cells or the cell population of any one of claims 124 to 129, wherein one or more of said cells comprises a non-naturally occurring insertion or deletion of one or more nucleic acid residues of a gene associated with facilitating a host vs graft immune response, wherein insertion and/or deletion results in reduced or eliminated expression or function of said gene.

131. A cell population according to claim 130, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C.

132. A cell population according to claim 130 or 131, wherein said gene is B2M.

133. Growth promoting agent of limbal stem cells comprising a LATS inhibitor.

134. The growth promoting agent of limbal stem cells according to claim 133, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

135. Growth promoting agent of limbal stem cells comprising a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

136. A cell proliferation medium comprising a LATS inhibitor and a growth medium.

137. The cell proliferation medium according to claim 136, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

138. A cell proliferation medium comprising a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 and a growth medium.

139. The cell proliferation medium according to any one of claims 136 to 138, wherein the growth medium is selected from the group consisting of Dulbecco's Modified Eagle's Medium supplemented with Fetal Bovine Serum, human endothelial serum free medium with human serum, X-VIVO15 medium and DMEM/F12 which is optionally supplemented with calcium chloride; preferably X-VIVO15 medium.

140. The cell proliferation medium according to any one of claims 136 to 139, additionally comprising limbal stem cells.

141. A cell preparation comprising a LATS inhibitor and limbal stem cells.

142. The cell preparation according to claim 141, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

143. A cell preparation comprising a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 and limbal stem cells.

144. The cell preparation according to any one of claims 141 to 143 which further comprises a growth medium, wherein the growth medium is selected from the group consisting of Dulbecco's Modified Eagle's Medium supplemented with Fetal Bovine Serum, human endothelial serum free medium with human serum, X-VIVO15 medium and

DMEM/F12 which is optionally supplemented with calcium chloride; preferably X-VIVO15 medium.

145. The pharmaceutical composition according to claim 11 or the cell preparation according to any one of claims 141 to 144, further comprising a preservation or cryopreservation solution.

146. The pharmaceutical composition or cell preparation according to claim 145, wherein the preservation or cryopreservation solution comprises a solution which is Optisol or PBS and the cryopreservation solution additionally comprises glycerol, dimethyl sulfoxide, propylene glycol or acetamide.

147. A kit comprising a composition suitable for ocular delivery and a LATS inhibitor.

148. A kit according to claim 147, wherein the LATS inhibitor is a compound or a salt thereof as defined in any one of claims 1 to 10.

149. A kit comprising a composition suitable for ocular delivery and compound or a salt thereof as defined in any one of claims 1 to 10.

150. The kit according to any one of claims 147 to 149, wherein the composition suitable for ocular delivery is a localising agent or topical eye drops.

151. The kit according to any one of claims 147 to 150, further comprising limbal stem cells.

152. A cell delivery preparation, comprising a cell population according to any one of claims 124 to 131 or a cell preparation according to any one of claims 141 to 146 and a composition suitable for ocular delivery which is a localising agent.

153. The kit according to any one of claims 147 to 151 or the cell delivery preparation according to claim 152, wherein the composition suitable for ocular delivery is a localising agent which is a biomatrix.

154. The kit according to any one of claims 147 to 151 or 153 or the cell delivery preparation according to any one of claims 152 or 153, wherein the composition suitable for ocular delivery is a localising agent selected from the group consisting of fibrin, collagen, gelatin, cellulose, amniotic membrane, fibrin glue, polyethylene (glycol) diacrylate (PEGDA), GelMA, localising agents comprising a polymer, cross-linked polymer, or hydrogel comprising one or more of hyaluronic acid, polyethylene glycol, polypropylene glycol, polyethylene oxide, polypropylene oxide, poloxamer, polyvinyl alcohol, polyacrylic acid, polymethacrylic acid, polyvinyl pyrrolidone, poly(lactide-co-glycolide), alginate, gelatin, collagen, fibrinogen, cellulose, methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropylmethylcellulose, hydroxypropyl-guar, gellan gum, guar gum, xanthan gum and carboxymethylcellulose, as well as derivatives thereof, co-polymers thereof, and combinations thereof.

155. The kit according to any one of claims 147 to 151 or 153 or 154 or the cell delivery preparation according to any one of claims 152 to 154, wherein the composition suitable for ocular delivery is a localising agent which is GelMa.

156. The kit according to any one of claims 147 to 155 or the cell delivery preparation according to any one of claims 152 to 155, wherein greater than 20% of the cells are limbal stem cells.

157. The kit according to any one of claims 147 to 155 or the cell delivery preparation according to any one of claims 152 to 155, wherein greater than 20% of the cells are p63alpha positive.

158. The kit according to any one of claims 147 to 157 or the cell delivery preparation according to any one of claims 152 to 157, wherein greater than 70% of the cells are p63alpha positive.

159. The pharmaceutical composition, growth promoting agent, cell proliferation medium, cell preparation or kit according to any one of claims 11 or 133 to 158, wherein the compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

160. The cell population according to any one of claims 124 to 131 or the cell preparation according to any one of claims 141 to 146, or the cell delivery preparation according to any one of claims 152 to 158, wherein the compound is present at only trace levels.

161. The growth promoting agent, cell proliferation medium, cell preparation, or the cell delivery preparation or kit according according to any one of claims 133 to 160, wherein the LATS inhibitor inhibits LATS1 and LATS2.

162. The cell proliferation medium, cell preparation, the cell delivery preparation or kit according to any one of claims 136 to 161, wherein limbal stem cells are present and are in suspension.

163. A method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject, said method comprising administering the population of cells of any one of claims 124 to 131 or 160 or cell delivery preparation of claim 152 to 158 or 160.

164. A method of transplanting a cell population comprising limbal stem cells onto the cornea of a subject, said method comprising expanding a cell population comprising limbal stem cells by culturing said population with cell proliferation medium according to any one of claims 136 to 139 or 159 or 161, preferably rinsing the expanded cell population to substantially remove the LATS inhibitor, and administering said cell population comprising limbal stem cells onto the cornea of said subject.

165. The method of claim 164, wherein said cell population comprising limbal stem cells is combined with a localising agent prior to said administration.

166. The method of claim 165, wherein said cell population comprising limbal stem cells is combined with a localising agent that is fibrin glue or a localising agent that is a biomatrix which is GelMA prior to said administration.

167. The method of any one of claims 164 to 166, wherein said cell population comprising limbal stem cells is combined with a carrier which is a contact lens.

168. The method of any one of claims 164 to 167, wherein said cell population comprising limbal stem cells is combined with a biomatrix which is GelMA and the GelMA is polymerized on a carrier wihich is a contact lens.

169. A method of prophylaxis or treatment of an ocular disease or disorder using a LATS inhibitor.

170. The method according to claim 169, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

171. A method of prophylaxis or treatment of an ocular disease or disorder using a compound of Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10.

172. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 169 to 171, comprising the method of LATS inhibition in a cell population according to any one of claims 101 to 104 or the method of cell population expansion of any one of claims 108 to 123.

173. A method of prophylaxis or treatment of an ocular disease or disorder comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 124 to 131 or cell delivery preparation according to any one of claims 152 to 162.

174. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 169 to 173, wherein the ocular disease or disorder is limbal stem cell deficiency.

175. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 169 to 174, wherein the ocular disease or disorder is limbal stem cell deficiency which arises due an injury or disorder selected from the group consisting of chemical burns, thermal burns, radiation injury, aniridia, sclerocornea, multiple endocrine neoplasia, Stevens Johnson syndrome, ocular cicatricial pemphigoid, collagen vascular

diseases, chronic non-auto-immune inflammatory disorders arising from contact lens use, dry eye disease, rosacea, staph marginal, keratitis (including bacterial, fungal & viral keratitis), pterygia or neoplasm, limbal stem cell deficiency arising after multiple eye surgeries, excision of pterygia or neoplasm or cryotherapy; and limbal stem cell deficiency arising as a result of medication toxicity from a medication selected from the group consisting of preservatives (thimerosal, benzalkonium), topical anesthetics, pilocarpine, beta blockers, mitomycin, 5-fluorouracil, silver nitrate, and oral medications causing Stevens Johnson syndrome.

176. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 169 to 175, wherein the ocular disease or disorder is limbal stem cell deficiency which arises due an injury or disorder selected from the group consisting of chemical burns, aniridia, Stevens Johnson Syndrome and contact lens use.

177. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 169 to 176, wherein the method comprises the steps of the method of any one of claims 163 to 168.

178. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 169 to 177, wherein the cell population according to any one of claims 124 to 131 or cell delivery preparation according to any one of claims 152 to 158 or 160 to 162 is administered simulataneusly or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin, and cefazolin.

179. A compound according to Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 for use in therapy or as a medicament.

180. A compound according to Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 for use in an ocular disease or disorder.

181. A LATS inhibitor for use in an ocular disease or disorder wherein the LATS inhibitor is a compound.

182. Use of a compound according to Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 in the manufacture of a medicament.

183. Use of a compound according to Formula A1 as defined in any one of claims 101 to 103 or the compound or a salt thereof as defined in any one of claims 1 to 10 in the manufacture of a medicament to treat an ocular disease or disorder.

184. A compound for use according to claim 179 or 180 or a LATS inhibitor for use according to claim 181, or use according to claim 182 or 183, comprising the method of LATS inhibition in a cell population according to claim 101 to 104 or the method of cell population expansion of any one of claims 108 to 123.

185. A compound for use according to any one of claims 179 to 180 or 184 or a LATS inhibitor for use according to any one of claims 181 or 184 or use according to any one of claims 182 to 184, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 124 to 131 or cell delivery preparation according to any one of claims 152 to 162.

186. A compound for use according to any one of claims 180, 184, 185 or a LATS inhibitor for use according to any one of claims 181, 184, 185 or use according to any one of claims 183 to 185 wherein the ocular disease or disorder is limbal stem cell deficiency.

187. A compound for use according to any one of claims 180, 184 to 186 or a LATS inhibitor for use according to any one of claims 181, 184 to 186 or use according to any one of claims 183, 184 to 186 wherein the ocular disease or disorder is limbal stem cell deficiency which arises due an injury or disorder selected from the group consisting of chemical burns, thermal burns, radiation injury, aniridia, sclerocornea, multiple endocrine neoplasia, Stevens Johnson syndrome, ocular cicatricial pemphigoid, collagen vascular diseases; chronic non-auto-immune inflammatory disorders arising from contact lens use, dry eye disease, rosacea, staph marginal, keratitis (including bacterial, fungal & viral keratitis), pterygia or neoplasm, limbal stem cell deficiency arising after multiple eye surgeries, excision of pterygia or neoplasm or cryotherapy; and limbal stem cell deficiency

arising as a result of medication toxicity from a medication selected from the group consisting of preservatives (thimerosal, benzalkonium), topical anesthetics, pilocarpine, beta blockers, mitomycin, 5-fluorouracil, silver nitrate, and oral medications causing Stevens Johnson syndrome.

188. A compound for use according to any one of claims 180, 184 to 187 or a LATS inhibitor for use according to any one of claims 181, 184 to 187 or use according to any one of claims 183, 184 to 187, wherein the ocular disease or disorder is limbal stem cell deficiency which arises due an injury or disorder selected from the group consisting of chemical burns, aniridia, Stevens Johnson Syndrome and contact lens use.

189. A compound for use according to any one of claims 180, 184 to 188 or a LATS inhibitor for use according to any one of claims 181, 184 to 188 or use according to any one of claims 183, 184 to 188, wherein the method comprises the steps of the method of any one of claims 163 to 168.

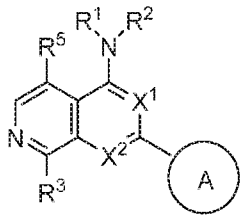
190. A compound for use according to any one of claims 180, 184 to 189 or a LATS inhibitor for use according to any one of claims 181, 184 to 189 or use according to any one of claims 183, 184 to 189, wherein the cell population according to any one of claims 124 to 131 or cell delivery preparation according to any one of claims 152 to 162 is administered simulataneusly or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin and cefazolin.

191. YAP modulator for use in a method of transplanting a population of cells comprising limbal stem cells onto the cornea of a subject, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 124 to 131 or cell delivery preparation according to any one of claims 152 to 162.

192. YAP modulator for use in a method of treating limbal stem cell deficiency, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 124 to 131 or cell delivery preparation according to any one of claims 152 to 162.

193. YAP modulator of claim 191 or 192, wherein said modulator is a LATS inhibitor.

194. A method of promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutical effective amount of a compound of Formula A1, or a pharmaceutically acceptable salt thereof:



A1

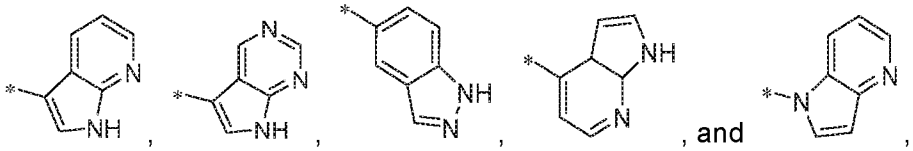
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, $-NH_2$, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii)–OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

(viii) –$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;

(ix) –$C(O)R^8$, wherein R^8 is R^0 or –NH-C_{1-6}alkyl-$C(O)R^0$;

(x) –$S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii)6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) -$S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

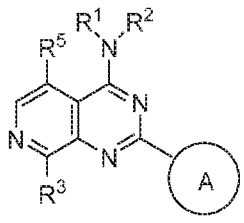
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional

heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

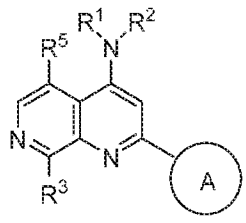
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

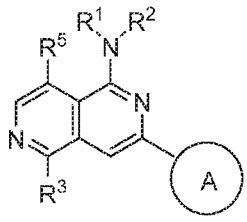
195. The method of claim 194, wherein the compound is of a formula selected from Formulae I to IV:



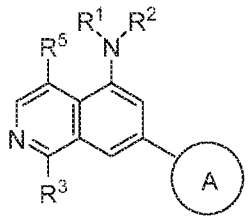
I,



II,



III, and



IV.

196. The method according to claim 194, wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from:

2-methyl-1-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)propan-2-ol;

dimethyl(3-methyl-3-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}butyl)amine;

N-(1-amino-2-methylpropan-2-yl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

8-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

8-methyl-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-(tert-butyl)-5-chloro-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

5-chloro-N-(1-methylcyclopropyl)-2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-amine;

N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;

N-tert-butyl-2-(3-methyl-1H-pyrazol-4-yl)-1,7-naphthyridin-4-amine;
N-tert-butyl-2-(pyrimidin-4-yl)-1,7-naphthyridin-4-amine;
2-(2-aminopyrimidin-4-yl)-N-tert-butyl-1,7-naphthyridin-4-amine;
N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;
N^1,N^1,3-trimethyl-N^3-(2-(3-methyl-1H-pyrazol-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-1,3-diamine;
2,2-dimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;
2,3-dimethyl-N^2-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)butane-2,3-diamine;
N^1,N^1,2,2-tetramethyl-N^3-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,3-diamine;
4-(4-(tert-butylamino)pyrido[3,4-*d*]pyrimidin-2-yl)-1,2,5-oxadiazol-3-amine; and
N^2,N^2,2-trimethyl-N^1-(2-(pyridin-4-yl)pyrido[3,4-*d*]pyrimidin-4-yl)propane-1,2-diamine.

197. The method according to claim 194, wherein the compound, or a pharmaceutically acceptable salt thereof, is according to any one of claims 1 to 10.

198. The method according to any one of claims 194 to 197, wherein promoting liver regeneration and liver regrowth comprises treating insufficient liver regrowth following transplantation of marginal grafts; supporting enhanced regrowth of the remnant liver mass following extensive hepatectomy; regeneration of patients' of livers following acute liver failure from viral hepatitis, drug -induced liver injury, autoimmune hepatitis, ischemic- and congestive liver disease; and treating chronic liver injury and underlying liver fibrosis, from non-alcoholic steatohepatitis, alcoholic steatohepatitis, chronic viral hepatitis B and C, hemochromatosis, alpha-1 anti-trypsin deficiency, Wilson's disease and drug-induced liver fibrosis.

199. A method for promoting liver regeneration and liver regrowth comprising administering to a subject in need thereof a therapeutically effective amount of an agent capable of inhibiting the activity of LATS kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation.

200. The method of Claim 199, wherein the agent is a compound according to any one of claims 1 to 10.

201. A method for expanding a population of liver cells *ex vivo* which comprises contacting the liver cells with a compound according to any one of claims 1 to 10.

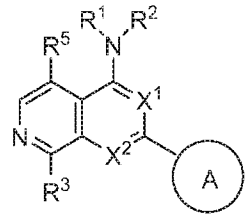
202. A method according to claim 201, wherein said further comprises gene editing said liver cells.

203. A method according to claim 201 or claim 202, wherein the liver cells are liver progenitor cells.

204. Liver cells obtained by the method according to claim 201 or claim 202.

205. Liver cells according to claim 204 wherein the liver cells are liver progenitor cells.

206. A method of promoting wound healing comprising administering to a subject in need thereof a therapeutical effective amount of a compound of Formula A1, or a pharmaceutically acceptable salt thereof:



A1

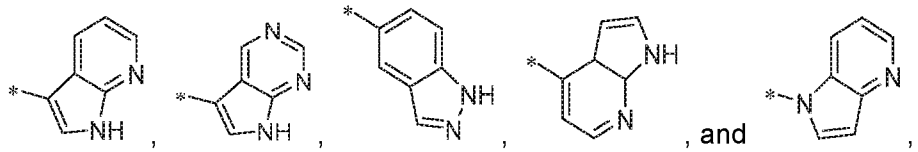
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from

 , and ,

wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, -NH_2, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(a) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(i) halogen;

(ii) cyano;

(iii) oxo;

(iv) C_2alkenyl;

(v) C_2alkynyl;

(vi) C_{1-6}haloalkyl;

(vii) –OR^6, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

(viii) –$NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, -$C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by –$C(O)R^0$;

(ix) –$C(O)R^8$, wherein R^8 is R^0 or –NH-C_{1-6}alkyl-$C(O)R^0$;

(x) –$S(O)_2C_{1-6}$alkyl;

(xi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xiii) phenyl that is unsubstituted or substituted by halogen;

(xiv) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xv) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(b) $-S(O)_2C_{1-6}$alkyl;

(c) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(d) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$; and

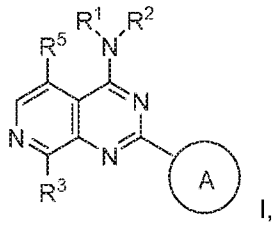
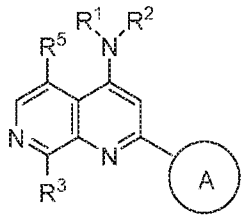
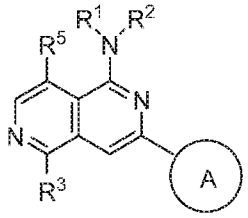
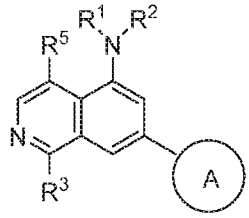
(e) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, $-C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

207. The method of claim 206, wherein the compound is of a formula selected from Formulae I to IV:


I,

II,

III, and

IV.

208. The method according to claim 206, wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from 2-(2-methyl-2-{[2-(pyridin-4-yl)pyrido[3,4-d]pyrimidin-4-yl]amino}propoxy)ethan-1-ol, 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

209. The method according to claim 206, wherein the compound, or a pharmaceutically acceptable salt thereof, is according to any one of claims 1 to 10.

210. The method according to any one of claims 206 to 209, wherein promoting wound healing comprising treating or ameliorating the symptomology of burns, acute skin ulcers and chronic skin ulcers.

211. The method according to claim 210, wherein the chronic skin ulcer is selected from diabetic ulcers, pressure ulcers and vascular ulcers.

212. The method according to claim 210, wherein the chronic skin ulcers is selected from venous leg ulcers, diabetic foot ulcers and bed sores.

213. A method of promoting wound healing comprising administering to a subject in need thereof a therapeutically effective amount of an agent capable of inhibiting the

activity of LATS kinases; thereby inducing YAP translocation and driving downstream gene expression for cell proliferation.

214. The method according to Claim 213, wherein the agent is a compound according to any one of claims 1 to 10.

215. A cell population comprising gene edited cells, wherein the gene edited cells have been cultured in a medium comprising a compound according to any one of claims 1 to 10.

216. The cell population of claim 215, wherein the edited gene is a gene associated with facilitating a host vs graft immune response, wherein the gene editing results in reduced or eliminated expression or function of said gene.

217. The cell population according to claim 216, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C.

218. The cell population according to claim 216 or 217, wherein said gene is B2M.

219. The cell population according to any of claims 215 to 218, wherein the gene edited cells are limbal stem cells or corneal endothelial cells.

220. A method for expanding a population of cells *ex vivo* which comprises contacting the cells with a compound according to any one of claims 1 to 10.

221. A method according to claim 220, wherein said further comprises gene editing said cells.

222. A method according to claim 220 or claim 221, wherein the cells are ocular cells, epithelial cells, neural stem cells, mesenchymal stem cells, basal stem cells of the lungs, embryonic stem cells, adult stem cells, induced pluripotent stem cells or liver cells.

223. A method according to any one of claims 220 to 222, wherein the cells are ocular cells, liver cells or epithelial skin cells.

224. A method according to any one of claims 220 to 223, wherein the cells are ocular cells, preferably cornelial endothelial cells or limbal stem cells.

225. Cells obtained by the method according to any one of claims 220 to 224.

226. A method for ex vivo cell therapy which comprises contacting the cells with a compound according to any one of claims 1 to 10.

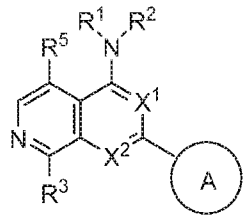
227. A method for ex vivo cell therapy according to claim 226, in which the cells have been gene edited.

228. A method for ex vivo cell therapy according to claim 226 or 227, wherein the cell therapy is selected from the group consisting of stem cell transplantation, tissue regeneration, cellular immunotherapy and gene therapy.

229. The method for ex vivo cell therapy according to any one of claims 226 to 228, wherein stem cell transplantation is selected from the group consisting of hematopoietic stem cell transplantation, autologous stem cell transplantation, cord blood stem cell transplantation and limbal stem cell transplantation.

230. The method for ex vivo cell therapy according to claim 228 or 229, wherein stem cell transplantation is limbal stem cell transplantation.

231. A method of LATS inhibition in a cell population using a compound of Formula A1 or a salt thereof:



A1

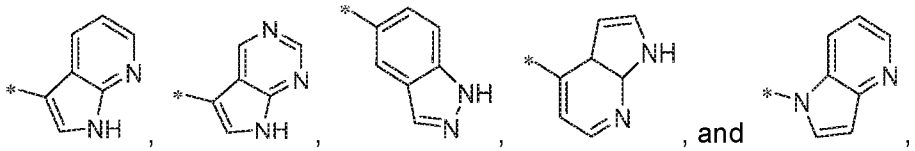
wherein

X^1 and X^2 are each independently CH or N;

Ring A is

(a) a 5- or 6-membered monocyclic heteroaryl that is linked to the remainder of the molecule through a carbon ring member and comprises, as ring member, 1 to 4 heteroatoms that are independently selected from N, O and S, provided that at least one of the heteroatom ring member is an unsubstituted nitrogen (-N=) positioned at the 3- or the 4-position relative to the linking carbon ring member of the 5-membered heteroaryl or at the para ring position of the 6-membered heteroaryl; or

(b) a 9-membered fused bicyclic heteroaryl that is selected from



wherein "*" represents the point of attachment of ring A to the remainder of the molecule;

wherein ring A is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, cyano, C_{1-6}alkyl, C_{1-6}haloalkyl, -NH_2, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, C_{3-6}cycloalkyl, and phenylsulfonyl;

R^0 is hydroxyl or C_{1-6}alkoxy;

R^1 is hydrogen or C_{1-6}alkyl;

R^2 is selected from

(f) C_{1-8}alkyl that is unsubstituted or substituted by 1 to 3 substituents independently selected from

(xvi) halogen;

(xvii) cyano;

(xviii) oxo;

(xix) C_2alkenyl;

(xx) C_2alkynyl;

(xxi) C_{1-6}haloalkyl;

(xxii) $-OR^6$, wherein R^6 is selected from hydrogen, C_{1-6}alkyl that is unsubstituted or substituted by R^0 or $-C(O)R^0$;

(xxiii) $-NR^{7a}R^{7b}$, wherein R^{7a} is hydrogen or C_{1-6}alkyl, and R^{7b} is selected from hydrogen, $-C(O)R^0$, C_{1-6}alkyl that is unsubstituted or substituted by $-C(O)R^0$;

(xxiv) $-C(O)R^8$, wherein R^8 is R^0 or $-NH-C_{1-6}$alkyl-$C(O)R^0$;

(xxv) $-S(O)_2C_{1-6}$alkyl;

(xxvi) monocyclic C_{3-6}cycloalkyl or polycyclic C_{7-10}cycloalkyl that are each unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-}

$_6$alkyl, hydroxyC_{1-6}alkyl, C_{1-6}haloalkyl, R^0, -NH_2, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xxvii) 6-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms independently selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from hydroxyl, halogen, C_{1-6}alkyl, C_{1-6}alkylamino, and di-(C_{1-6}alkyl)amino;

(xxviii) phenyl that is unsubstituted or substituted by halogen;

(xxix) 5- or 6-membered monocyclic heteroaryl comprising, as ring members, 1 to 4 heteroatoms independently selected from N and O; and

(xxx) 9- or 10-membered fused bicyclic heteroaryl comprising, as ring member, 1 to 2 heteroatoms independently selected from N and O;

(g) -$S(O)_2C_{1-6}$alkyl;

(h) phenyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from halogen, C_{1-6}alkyl and R^0;

(i) C_{3-6}cycloalkyl that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$; and

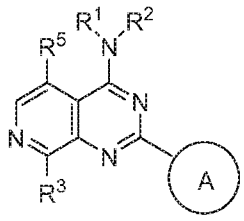
(j) 4-membered heterocycloalkyl comprising, as ring members, 1 to 2 heteroatoms selected from N, O and S and that is unsubstituted or substituted by 1 to 2 substituents independently selected from C_{1-6}haloalkyl, R^0, C_{1-6}alkylamino, di-(C_{1-6}alkyl)amino, -$C(O)R^0$, and C_{1-6}alkyl that is unsubstituted or substituted by R^0 or –$C(O)R^0$;

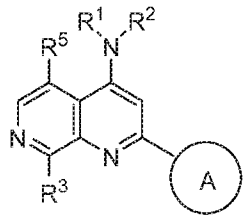
or R^1 and R^2 can be taken together with the nitrogen atom to which both are bound to form a 4- to 6-membered heterocycloalkyl that can include, as ring members, 1 to 2 additional heteroatoms independently selected from N, O, and S, wherein the 4- to 6-membered heterocycloalkyl formed by R^1 and R^2 taken together with the nitrogen atom to which both are bound is unsubstituted or substituted by 1 to 3 substituents independently selected from halogen, C_{1-6}alkyl, C_{1-6}haloalkyl, and R^0;

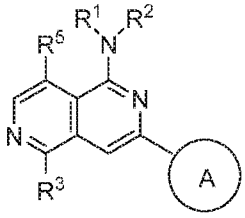
R^3 is selected from hydrogen, halogen and C_{1-6}alkyl; and

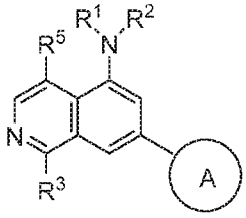
R^5 is selected from hydrogen, halogen and –NH-(3- to 8-membered heteroalkyl), wherein the 3- to 8-membered heteroC_{3-8}alkyl of the –NH-(3- to 8-membered heteroalkyl) comprises 1 to 2 oxygen atoms as chain members and is unsubstituted or substituted by R^0.

232. The method of LATS inhibition according to claim 231, wherein the compound is of a formula selected from Formulae I to IV:


I,


II,


III, and


IV.

233. The method of LATS inhibition in a cell population according to claim 231 or 232, wherein the compound, or a salt thereof, is selected from 3-(pyridin-4-yl)-N-(1-(trifluoromethyl)cyclopropyl)-2,6-naphthyridin-1-amine; N-(1-methylcyclopropyl)-7-(pyridin-4-yl)isoquinolin-5-amine; 2-(pyridin-4-yl)-4-(3-(trifluoromethyl)piperazin-1-yl)pyrido[3,4-d]pyrimidine; N-(tert-butyl)-2-(pyridin-4-yl)-1,7-naphthyridin-4-amine; and N-methyl-2-(pyridin-4-yl)-N-[(2S)-1,1,1-trifluoropropan-2-yl]pyrido[3,4-d]pyrimidin-4-amine.

234. The method of LATS inhibition according to claim 231, wherein the compound, or a salt thereof, is according to any one of claims 1 to 10.

235. A method of culturing cells comprising culturing a population of cells comprising ocular cells in the presence of a LATS inhibitor.

236. The method of culturing cells according to claim 235, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

237. A method of culturing cells comprising culturing a population of cells comprising ocular cells in the presence of a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

238. A method of cell population expansion, comprising the step of a) culturing a seeding population of cells comprising ocular cells in the presence of a LATS inhibitor to generate an expanded population of cells comprising ocular cells.

239. The method of cell population expansion according to claim 238 wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

240. A method of cell population expansion, comprising the step of a) culturing a seeding population of cells comprising ocular cells in the presence of a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 to generate an expanded population of cells comprising ocular cells.

241. The method of any one of claims 231 to 240, wherein said compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

242. The method of any one of claims 231 to 241, wherein the LATS inhibitor inhibits LATS1 and LATS2.

243. The method of any one of claims 231 to 242, wherein said method further comprises genetically modifying said ocular cells.

244. The method of claim 243, wherein said genetically modifying comprises reducing or eliminating the expression and/or function of a gene associated with facilitating a host versus graft immune response.

245. The method of claim 243 or 244, wherein said genetically modifying comprises introducing into said cell a gene editing system which specifically taregts a gene associated with facilitating a host versus graft immune response.

246. The method of claim 245, wherein said gene editing system is selected from the group consisting of a CRISPR gene editing system, a TALEN gene editing system, a zinc

finger nuclease gene editing system, a meganuclease gene editing system, AAV vector driven homologous recombination and lentiviral vectors-based genome editing technologies.

247. The method of any one of claims 244 to 246, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C.

248. The method of any one of claims 231 to 247, which comprises the further step after generation of an expanded population of cells of rinsing those cells to substantially remove the compound.

249. A cell population obtainable by the method of any one of claims 231 to 248.

250. A cell population obtained by the method of any one of claims 231 to 248.

251. A cell population comprising ocular cells or the cell population of claims 249 or 250, wherein one or more of said cells comprises a non-naturally occurring insertion or deletion of one or more nucleic acid residues of a gene associated with facilitating a host vs graft immune response, wherein insertion and/or deletion results in reduced or eliminated expression or function of said gene.

252. The cell population according to claim 251, wherein said gene is selected from the group consisting of B2M, HLA-A, HLA-B and HLA-C.

253. The cell population according to claim 251 or 252, wherein said gene is B2M.

254. Growth promoting agent of ocular cells comprising a LATS inhibitor.

255. The growth promoting agent of ocular cells according to claim 254, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

256. Growth promoting agent of ocular cells comprising a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

257. A cell proliferation medium comprising a LATS inhibitor and a growth medium.

258. The cell proliferation medium according to claim 257, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

259. A cell proliferation medium comprising a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 and a growth medium.

260. The cell proliferation medium according to any one of claims 257 to 259, wherein the growth medium is selected from the group consisting of Dulbecco's Modified Eagle's Medium supplemented with Fetal Bovine Serum, human endothelial serum free medium with human serum, X-VIVO15 medium and mesenchymal stem cell-conditioned medium; preferably X-VIVO15 medium.

261. The cell proliferation medium according to any one of claims 257 to 260, additionally comprising ocular cells.

262. A cell preparation comprising a LATS inhibitor and ocular cells.

263. The cell preparation according to claim 262, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

264. A cell preparation comprising a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 and ocular cells.

265. The cell preparation according to any one of claims 262 to 264 which further comprises a growth medium, wherein the growth medium is selected from the group consisting of Dulbecco's Modified Eagle's Medium supplemented with Fetal Bovine Serum,

human endothelial serum free medium with human serum, X-VIVO15 medium and mesenchymal stem cell-conditioned medium; preferably X-VIVO15 medium.

266. The pharmaceutical composition according to claim 11 or the cell preparation according to any one of claims 262 to 265, further comprising a preservation or cryopreservation solution.

267. The pharmaceutical composition or cell preparation according to claim 266, wherein the preservation or cryopreservation solution comprises a solution which is Optisol or PBS and the cryopreservation solution additionally comprises glycerol, dimethyl sulfoxide, propylene glycol or acetamide.

268. A kit comprising a composition suitable for ocular delivery and a LATS inhibitor.

269. A kit according to claim 268, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

270. A kit comprising a composition suitable for ocular delivery and a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

271. The kit according to any one of claims 268 to 270, wherein the composition suitable for ocular delivery is a localising agent or topical eye drops.

272. The kit according to any one of claims 268 to 271, further comprising ocular cells.

273. A cell delivery preparation, comprising a cell population according to any one of claims 249 to 253 or a cell preparation according to any one of claims 262 to 267 and a composition suitable for ocular delivery which is a localising agent.

274. The kit according to any one of claims 268 to 272 or the cell delivery preparation according to claim 273, wherein the composition suitable for ocular delivery is a localising agent which is a biomatrix.

275. The kit according to any one of claims 268 to 272 or 274 or the cell delivery preparation according to any one of claims 273 or 274, wherein the composition suitable for ocular delivery is a localising agent selected from the group consisting of fibrin, collagen, gelatin, cellulose, amniotic membrane, fibrin glue, polyethylene (glycol) diacrylate (PEGDA), GelMA, localising agents comprising a polymer, cross-linked polymer, or hydrogel comprising one or more of hyaluronic acid, polyethylene glycol, polypropylene glycol, polyethylene oxide, polypropylene oxide, poloxamer, polyvinyl alcohol, polyacrylic acid, polymethacrylic acid, polyvinyl pyrrolidone, poly(lactide-co-glycolide), alginate, gelatin, collagen, fibrinogen, cellulose, methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropylmethylcellulose, hydroxypropyl-guar, gellan gum, guar gum, xanthan gum and carboxymethylcellulose, as well as derivatives thereof, co-polymers thereof, and combinations thereof.

276. The kit according to any one of claims 268 to 272 or 274 or 275 or the cell delivery preparation according to any one of claims 273 to 275, wherein the composition suitable for ocular delivery is a localising agent which is GelMa.

277. The pharmaceutical composition, growth promoting agent, cell proliferation medium, cell preparation or kit according to any one of claims 11 or 254 to 276, wherein the compound is present in a concentration of 0.5 to 100 micromolar, preferably 0.5 to 25 micromolar, more preferably 1 to 20 micromolar, particularly preferably of about 3 to 10 micromolar.

278. The cell population according to any one of claims 249 to 253 or the cell preparation according to any one of claims 262 to 267, or the cell delivery preparation according to any one of claims 273 to 276, wherein the compound is present at only trace levels.

279. The growth promoting agent, cell proliferation medium, cell preparation, or the cell delivery preparation or kit according to any one of claims 254 to 278, wherein the LATS inhibitor inhibits LATS1 and LATS2.

280. The cell proliferation medium, cell preparation, the cell delivery preparation or kit according to any one of claims 257 to 276, wherein ocular cells are present and are in suspension.

281. A method of transplanting a population of corneal endothelial cells onto the cornea of a subject, said method comprising administering the population of cells of any one of claims 33 to 37 or 65 or cell delivery preparation of claim 57 to 63 or 65.

282. A method of transplanting a population of ocular cells to a subject, said method comprising expanding a population of ocular cells by culturing said population with cell proliferation medium according to any one of claims 257 to 260 or 278 or 279, rinsing the expanded population of cells to substantially remove the LATS inhibitor, and administering said cells to said subject.

283. The method of claim 282, wherein said isolated ocular cells are combined with a biomatrix prior to said administration.

284. The method of claim 283, wherein said isolated ocular cells are combined with a biomatrix which is GelMA prior to said administration.

285. A method of transplanting a population of ocular cells to the eye of a subject, comprising culturing a population of ocular cells in a cell proliferation medium that comprises a LATS inhibitor, combining ocular cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject, and bioprinting the cells in the eye by guiding and fixing the cells on the cornea using a light source.

286. The method of any one of claims 283 to 284, wherein said isolated ocular cells are combined with a biomatrix which is GelMA and bioprinted onto the ocular surface by polymerising the GelMA by a light triggered reaction.

287. A method of prophylaxis or treatment of an ocular disease or disorder using a LATS inhibitor.

288. The method according to claim 287, wherein the LATS inhibitor is a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

289. A method of prophylaxis or treatment of an ocular disease or disorder using a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10.

290. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 287 to 289, comprising the method of LATS inhibition in a cell population according to any one of claims 231 to 234 or the method of cell population expansion of any one of claims 238 to 248.

291. A method of prophylaxis or treatment of an ocular disease or disorder comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 231 to 234 or cell delivery preparation according to any one of claims 273 to 276 or 278 to 280.

292. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 287 to 291, wherein the method comprises the steps of the method of any one of claims 281 to 286.

293. The method of prophylaxis or treatment of an ocular disease or disorder according to any one of claims 287 to 291, wherein the cell population according to any one of claims 249 to 253 or cell delivery preparation according to any one of claims 273 to 276 or 278 or 279 is administered simultaneously or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin, and cefazolin.

294. A compound according to Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 for use in therapy or as a medicament.

295. A compound according to Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 for use in an ocular disease or disorder.

296. A LATS inhibitor for use in an ocular disease or disorder wherein the LATS inhibitor is a compound.

297. Use of a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 for the manufacture of a medicament.

298. Use of a compound of Formula A1 as defined in any one of claims 231 to 233 or the compound or a salt thereof as defined in any one of claims 1 to 10 for the manufacture of a medicament to treat an ocular disease or disorder.

299. A compound for use according to claim 294 or 295 or a LATS inhibitor for use according to claim 296, or use according to claim 297 or 298, comprising the method of LATS inhibition in a cell population according to claim 231 to 235 or the method of cell population expansion of any one of claims 238 to 248.

300. A compound for use according to any one of claims 294 or 295 or 299 or a LATS inhibitor for use according to any one of claims 298 or 299 or use according to any one of claims 297 to 299, comprising administering to a subject in need thereof of a therapeutically effective amount of a cell population according to any one of claims 249 to 253 or cell delivery preparation according to any one of claims 273 to 276 or 278 to 280.

301. A compound for use according to any one of claims 294 or 295 or 299 or 300 or LATS inhibitor for use according to any one of claims 296 or 299, or use according to any one of claims 297 to 300, wherein the method comprises the steps of the method of any one of claims 281 to 286.

302. A compound for use according to any one of claims 294 or 295 or 299 or 300 or LATS inhibitor for use according to any one of claims 296 or 299 to 301, or use according to any one of claims 295 to 301, wherein the cell population according to any one of claims 249 to 253 or cell delivery preparation according to any one of claims 273 to 276 or 278 to

280 is administered simultaneously or sequentially with an agent or agents selected from the group consisting of dexamethasone, cyclosporine, tobramycin and cefazolin.

303. A method of transplanting a population of isolated cells to the eye of a subject, comprising combining the cells with a biomatrix to form a cell/biomatrix mixture, injecting the mixture into the eye of the subject, and bioprinting the cells in the eye by guiding and fixing the cells in the eye using a light source.

304. The method of claim 303, wherein the isolated cells are combined with a biomatrix which is GelMA and bioprinted onto the cornea by polymerising the GelMA by a light triggered reaction.

305. The method of claim 303 or claim 304, wherein the light source produces a light having a wavelength in the 350 nm to 700 nm range.

306. The method of any one of claims 303 to 305, wherein the wavelength is 350 nm to 420 nm.

307. The method of any one of claims 303 to 306, wherein the wavelength is 365 nm.

308. The method of any one of claims 303 to 307, wherein the isolated cells are corneal endothelial cells.

309. A method of transplanting a population of isolated cells to the eye of a subject, comprising combining the cells with a biomatrix to form a cell/biomatrix mixture, applying the mixture onto the eye of the subject, and bioprinting the cells on the eye by guiding and fixing the cells on the eye using a light source.

310. The method of claim 309, wherein the isolated cells are combined with a biomatrix which is GelMA and bioprinted onto the ocular surface by polymerising the GelMA by a light triggered reaction.

311. The method of claim 309 or 310, wherein the light source produces a light having a wavelength in the 350 nm to 700 nm range.

312. The method of any one of claims 309 to 311, wherein the wavelength is 350 nm to 420 nm.

313. The method of any one of claims 309 to 312, wherein the wavelength is 365 nm.

314. The method of any one of claims 309 to 313, wherein the isolated cells are limbal stem cells.

Fig. 1

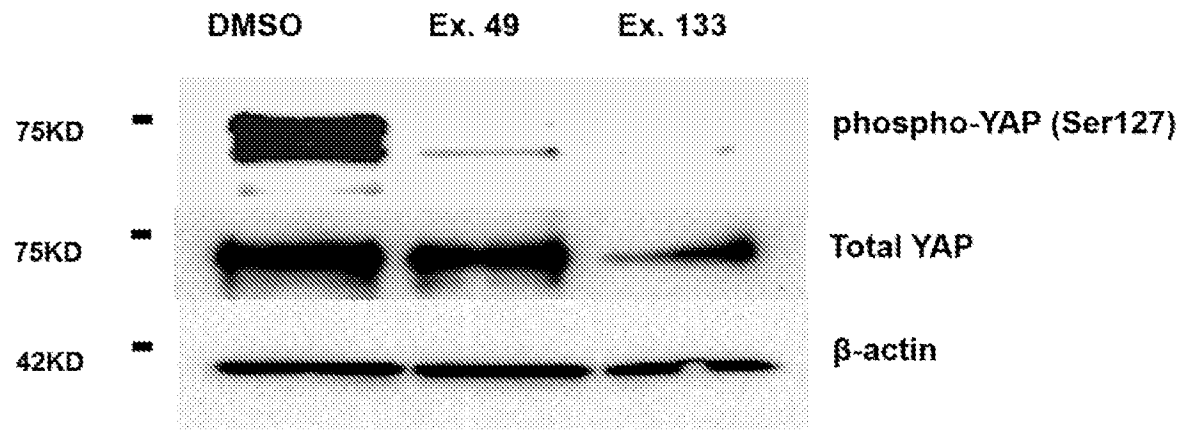

DMSO
Ex. 49
Ex. 133
75KD
75KD
42KD
phospho-YAP (Ser127)
Total YAP
β-actin

Fig. 2A

Fig. 2B

Fig. 2C

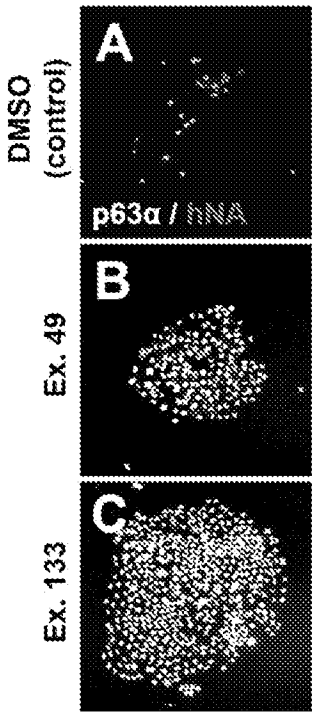


Fig. 2D

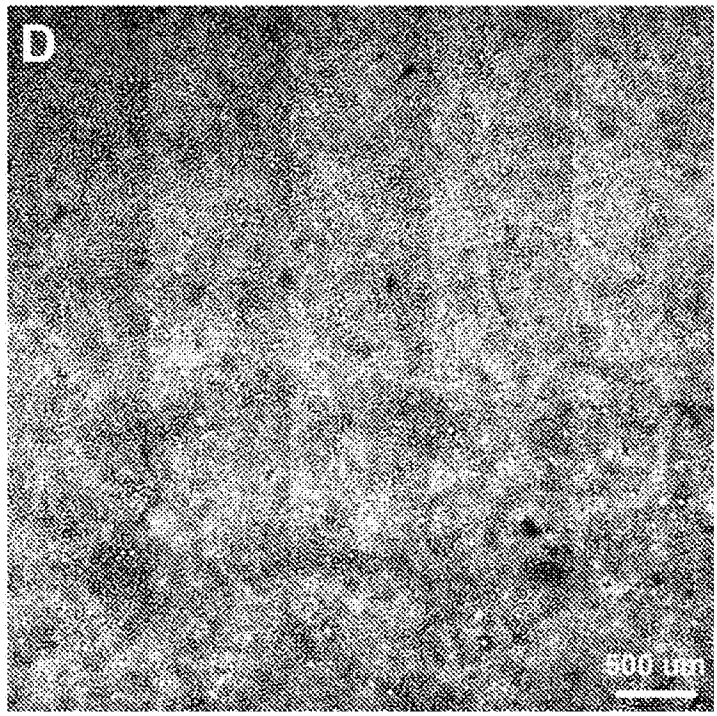


Fig. 3

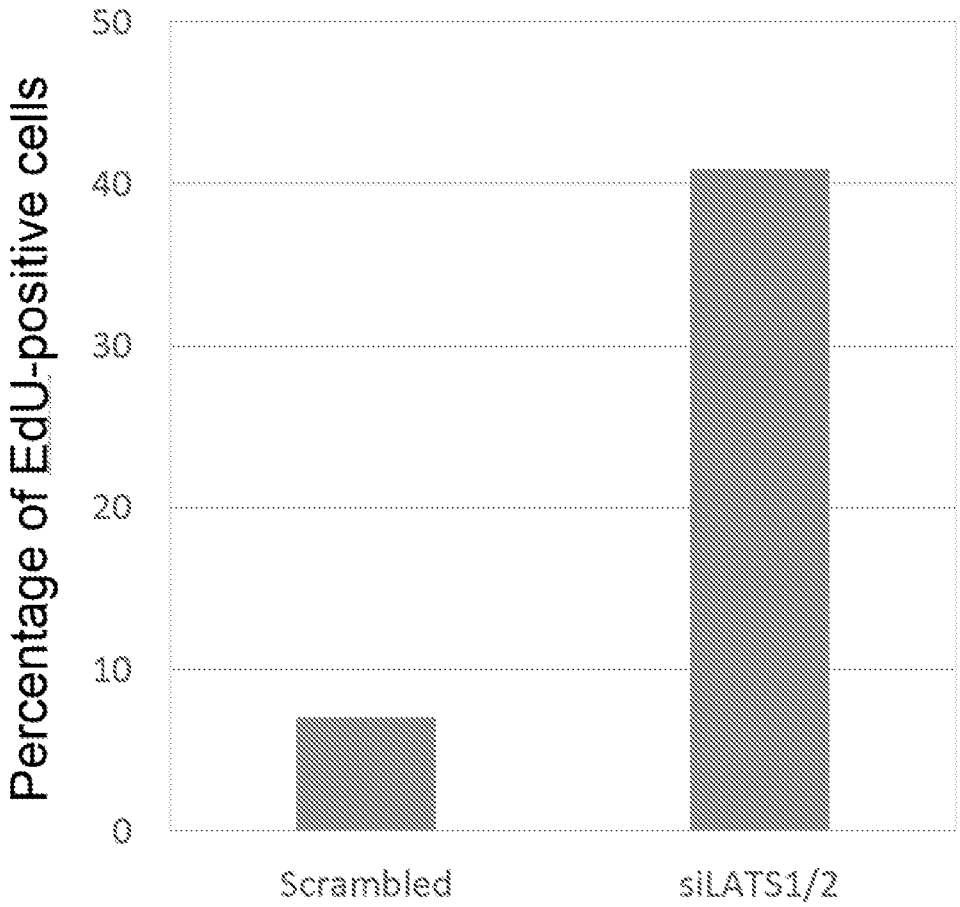

Percentage of EdU-positive cells
50
40
30
20
10
0
Scrambled
siLATS1/2

Fig. 4

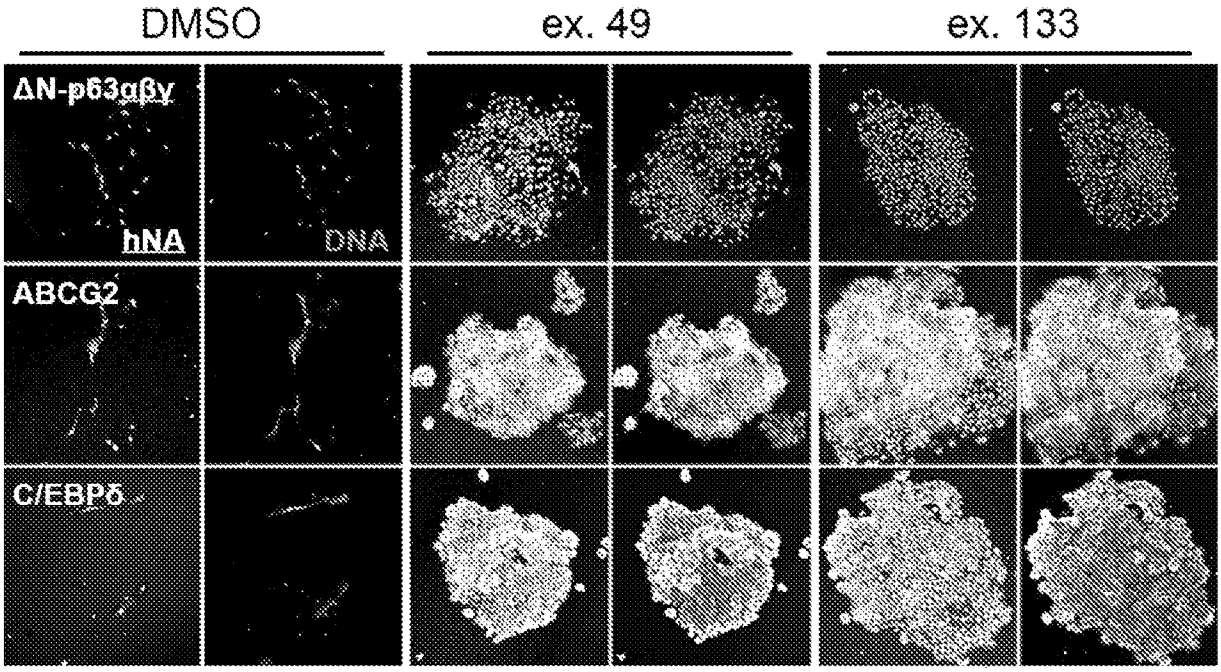


Fig. 5A Fig. 5B Fig. 5C Fig. 5D

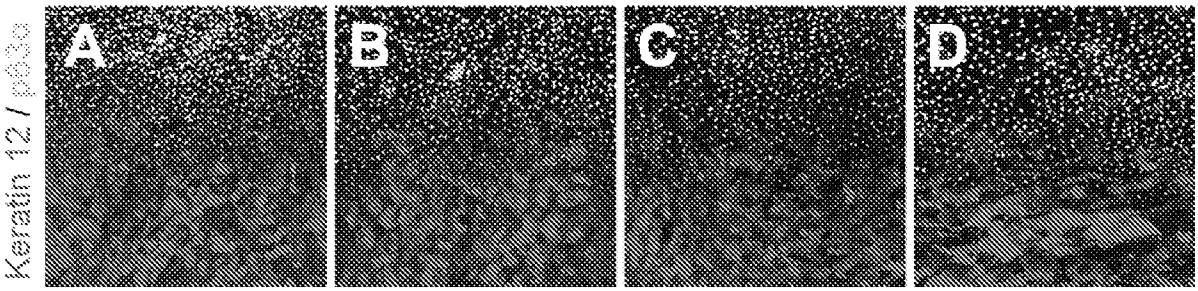


Fig. 6

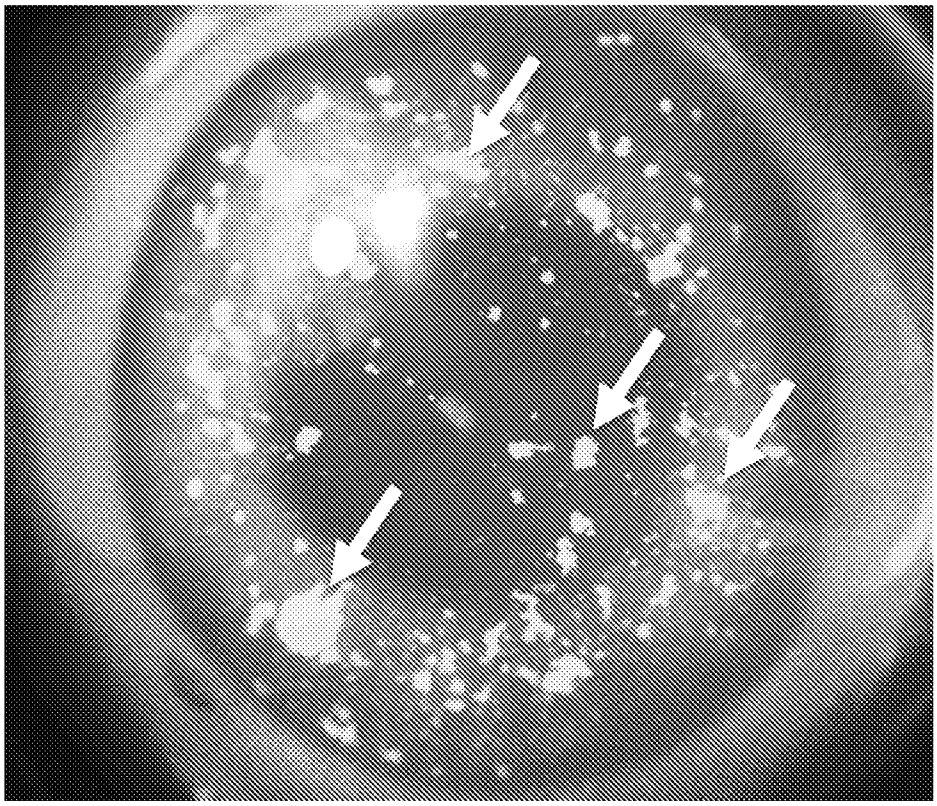


Fig. 7A

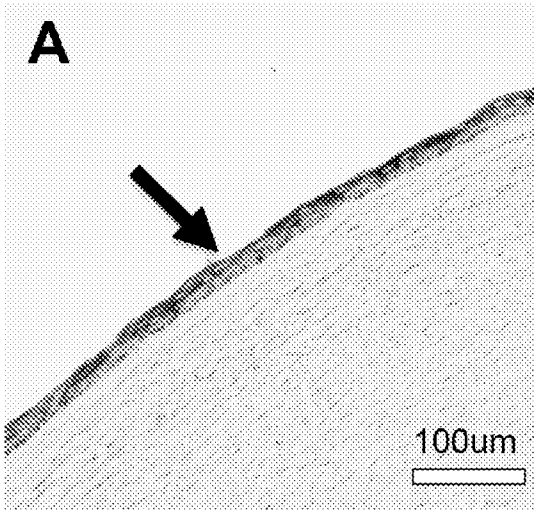
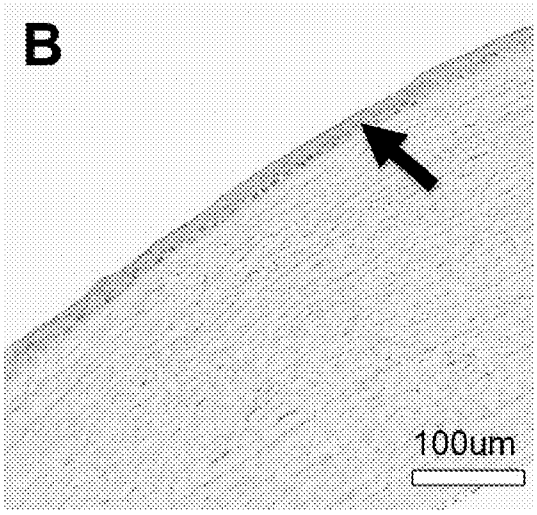


Fig. 7B



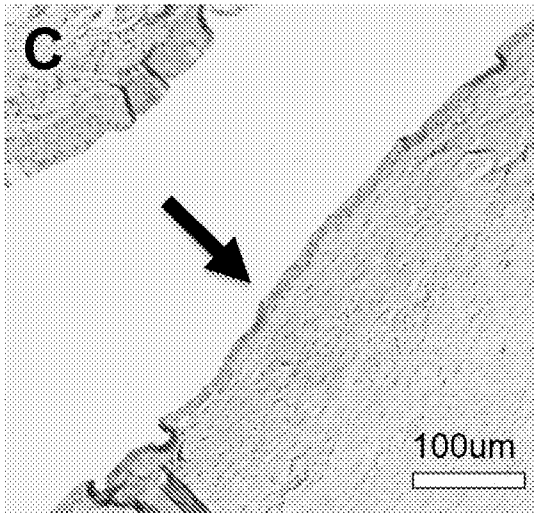


Fig. 7C

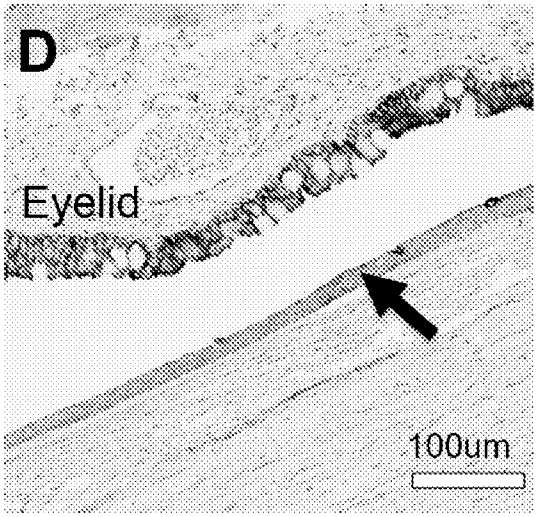


Fig. 7D

Fig. 8A

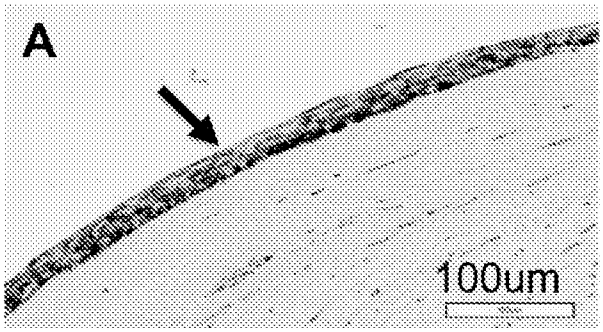


Fig. 8B

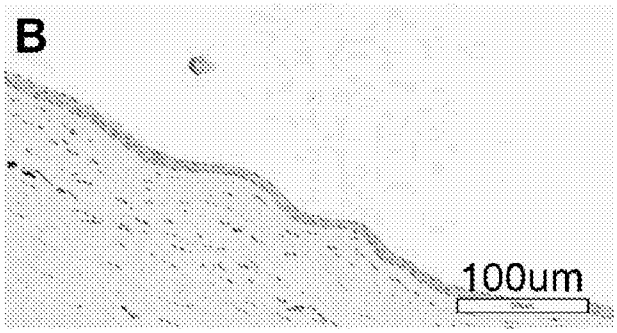


Fig. 9

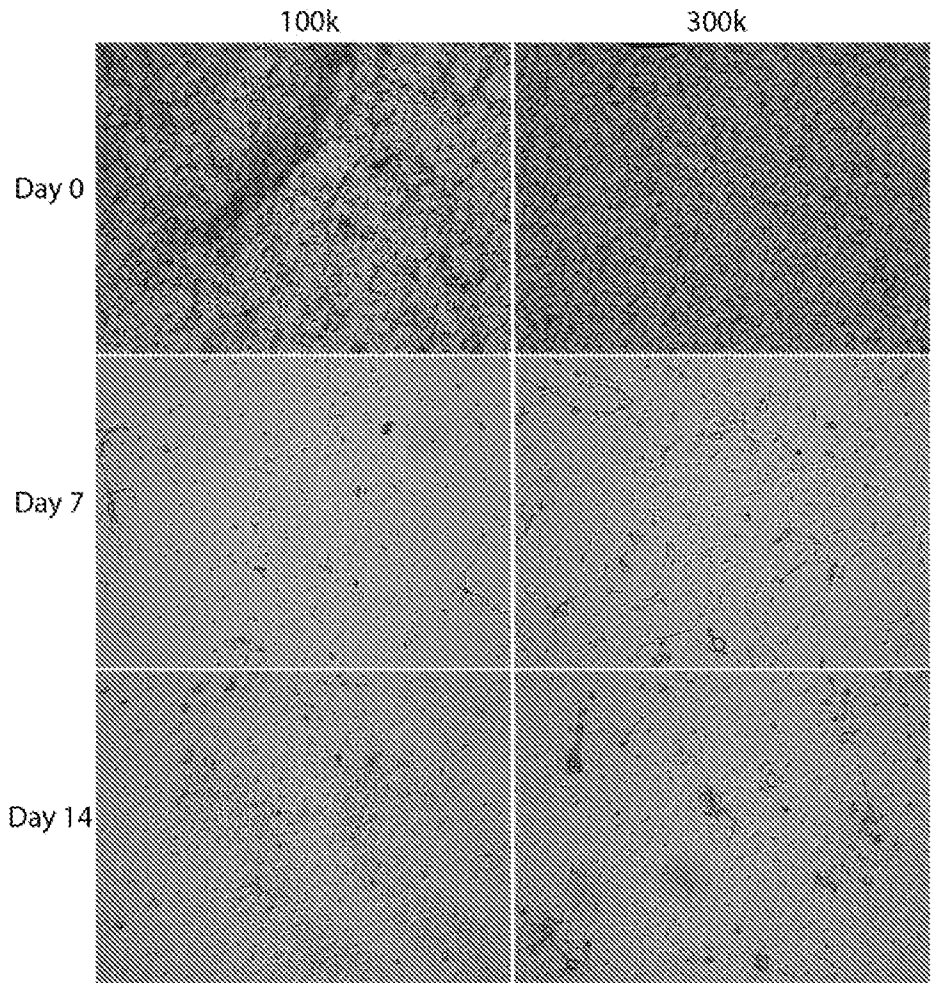


Fig. 10

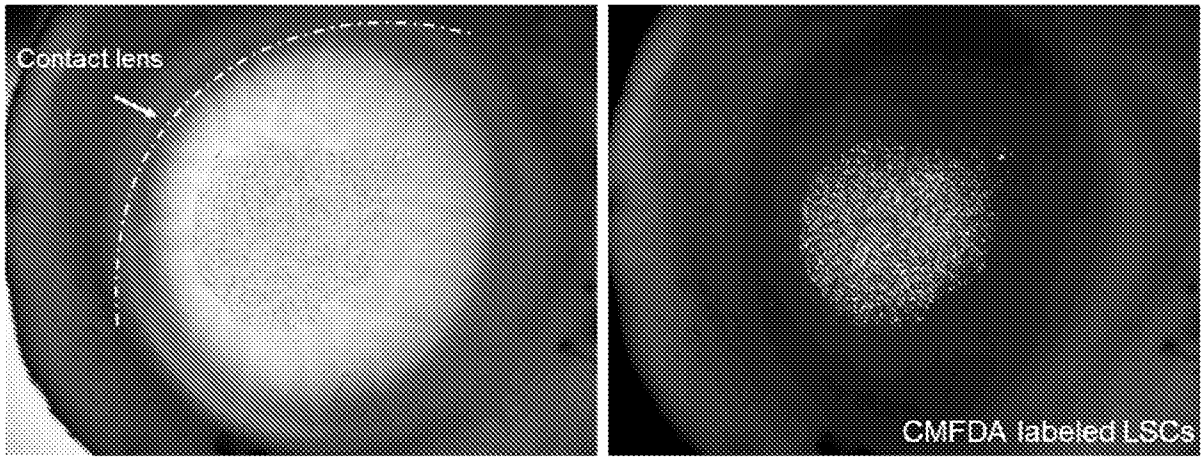


Fig. 11

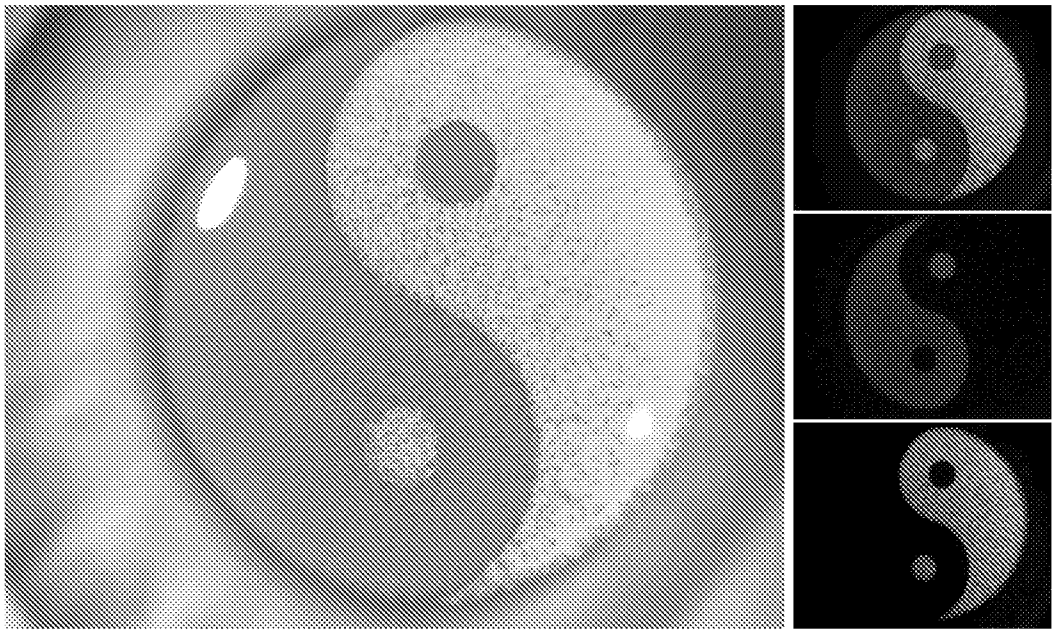


Fig. 12

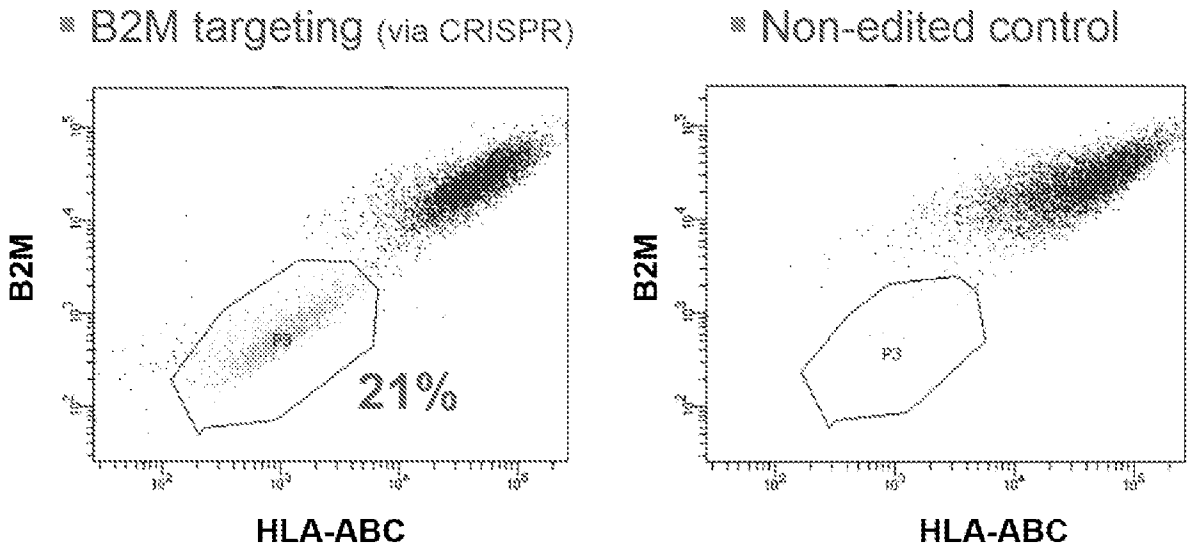

B2M targeting (via CRISPR)
Non-edited control
B2M
HLA-ABC
P3
21%
B2M
HLA-ABC
P3

Fig. 13

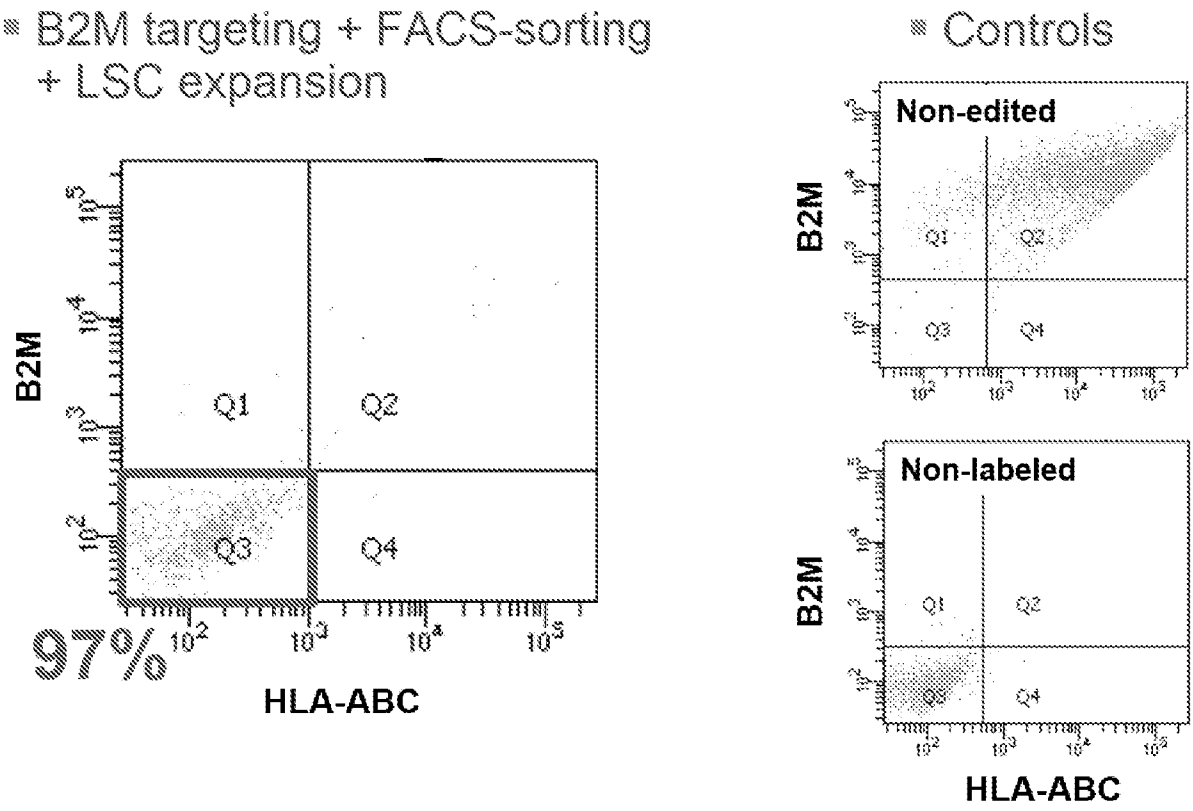

B2M targeting + FACS-sorting + LSC expansion
Controls
B2M
HLA-ABC
Q1
Q2
Q3
Q4
97%
Non-edited
Non-labeled

Fig. 14

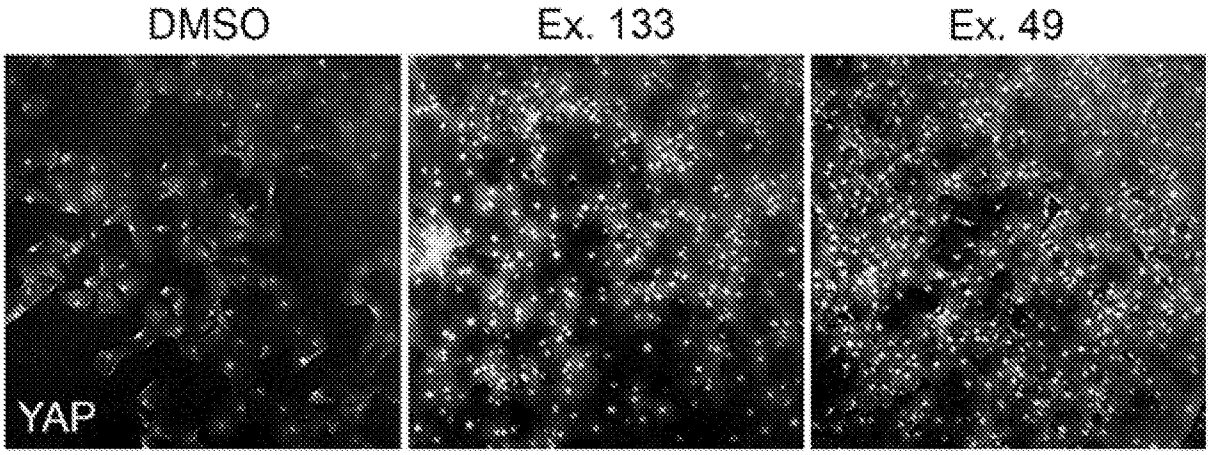


Fig 15A

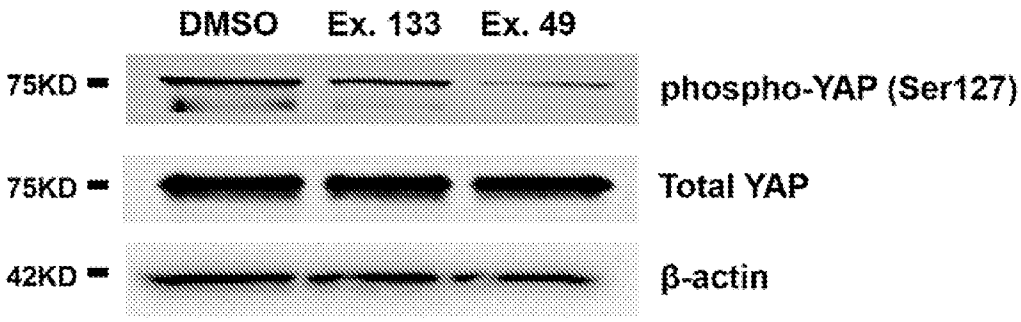

DMSO
Ex. 133
Ex. 49
75KD
phospho-YAP (Ser127)
75KD
Total YAP
42KD
β-actin

Fig 15B

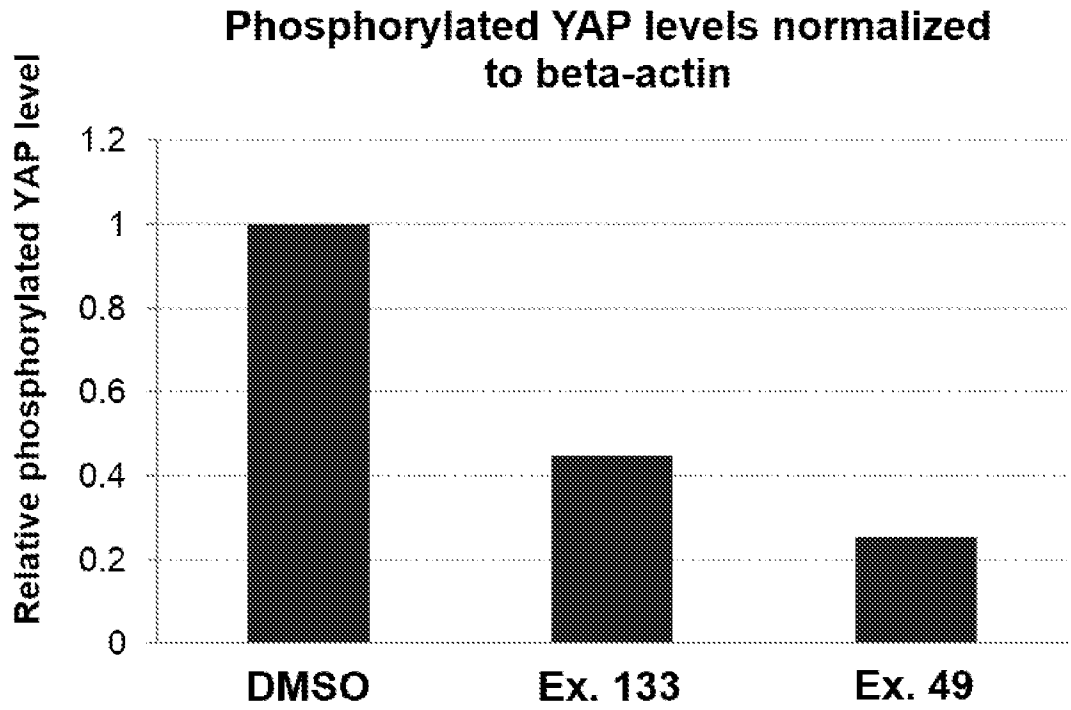

Phosphorylated YAP levels normalized to beta-actin
Relative phosphorylated YAP level
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
Ex. 133
Ex. 49

Fig. 15C

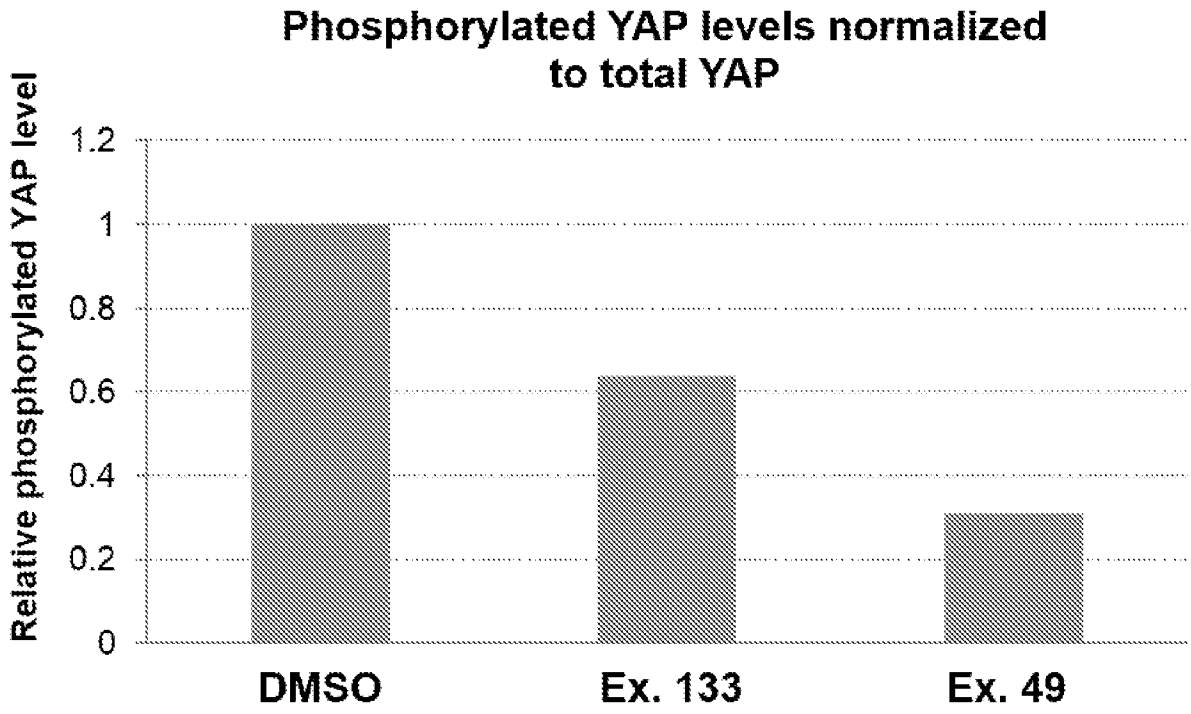

Phosphorylated YAP levels normalized to total YAP
Relative phosphorylated YAP level
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
Ex. 133
Ex. 49

Fig. 16

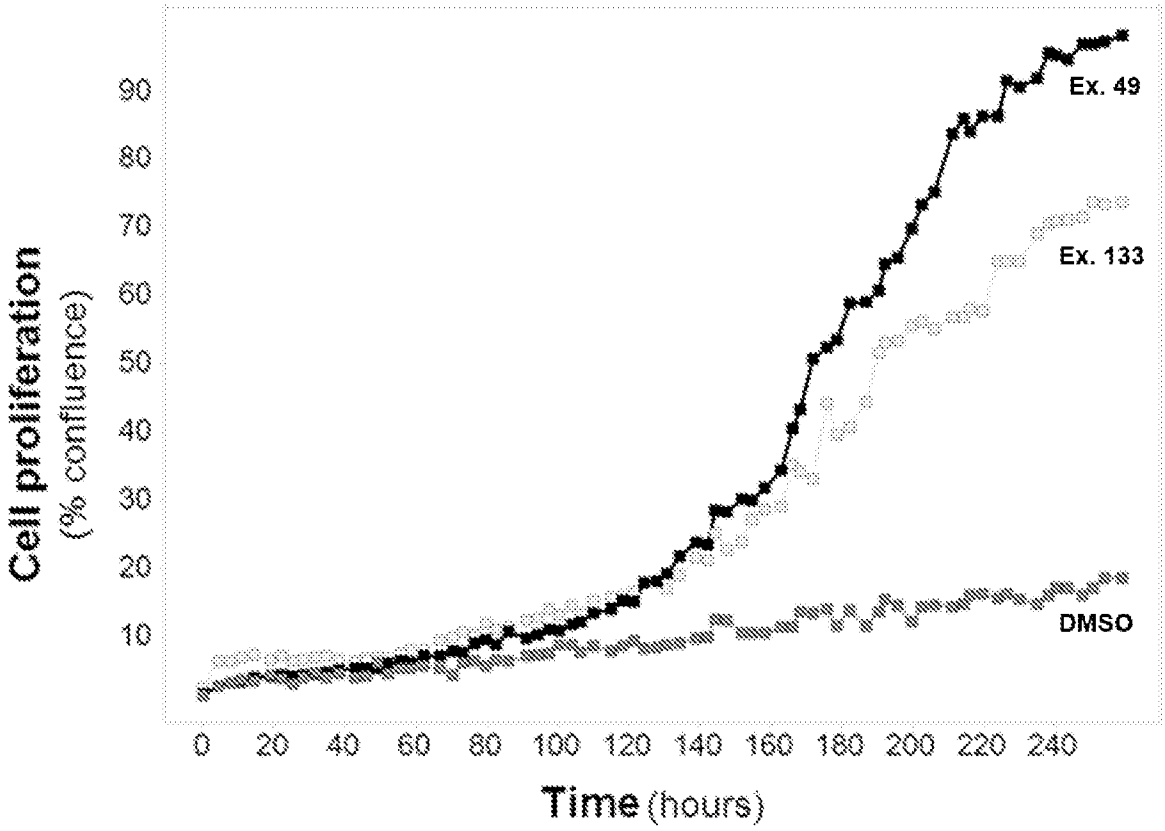

Cell proliferation
(% confluence)
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
120
140
160
180
200
220
240
Time (hours)
Ex. 49
Ex. 133
DMSO

Fig. 17

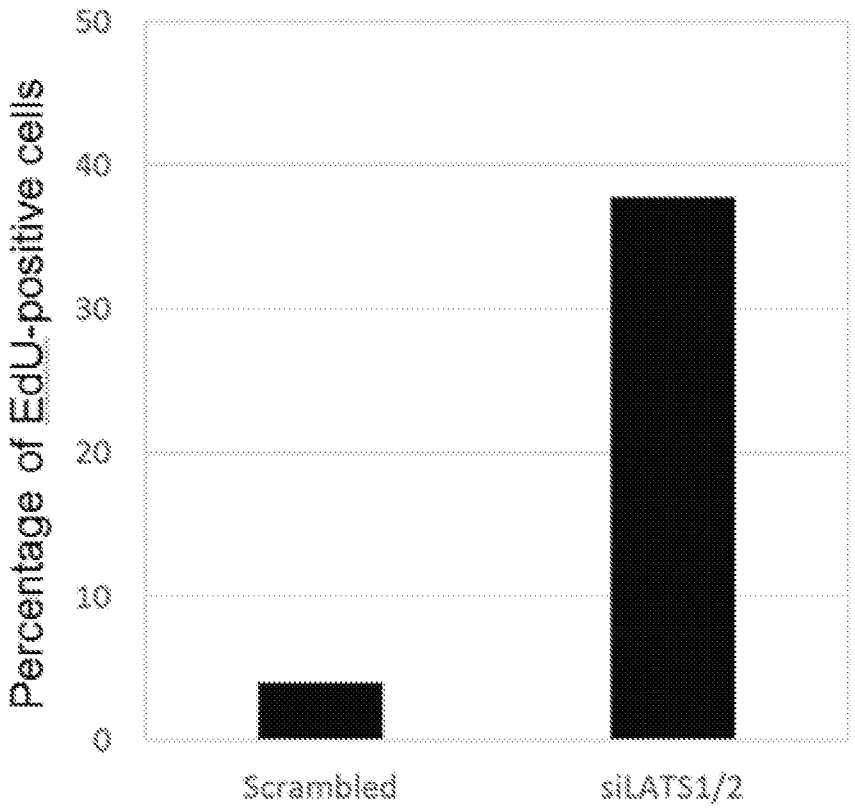

Percentage of EdU-positive cells
50
40
30
20
10
0
Scrambled
siLATS1/2

Fig. 18A

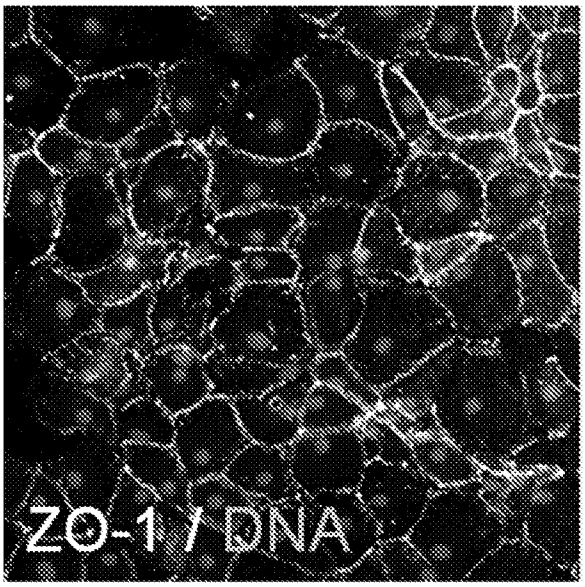


Fig. 18B

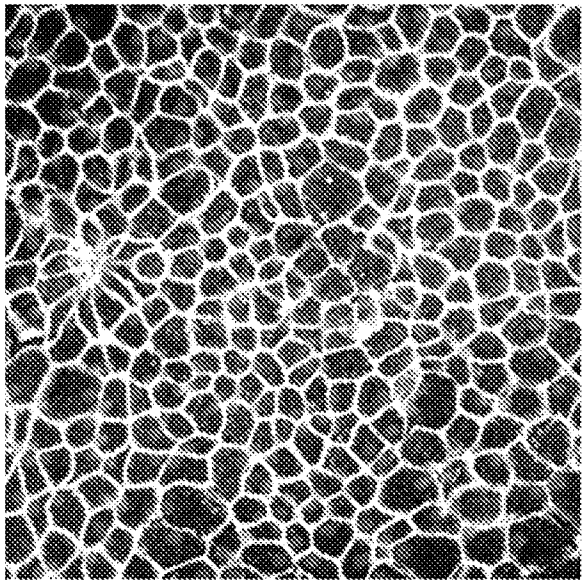


Fig. 19

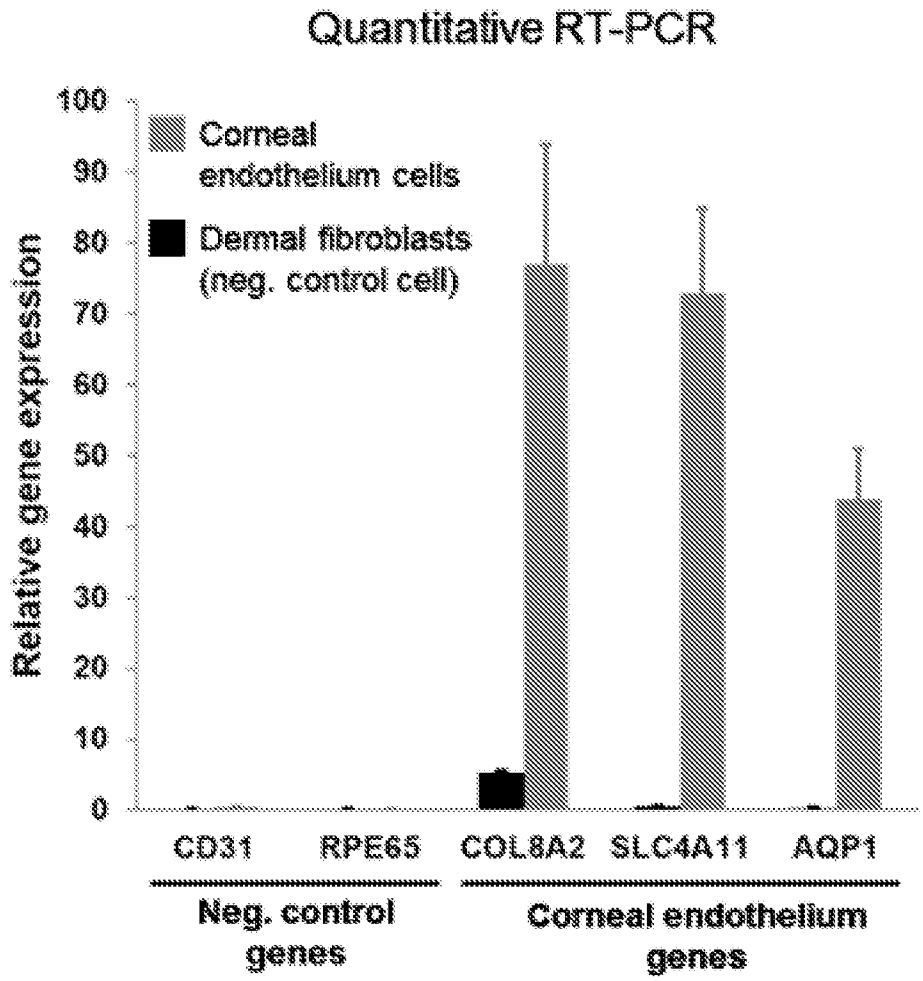

Quantitative RT-PCR
Relative gene expression
100
90
80
70
60
50
40
30
20
10
0
Corneal endothelium cells
Dermal fibroblasts (neg. control cell)
CD31
RPE65
COL8A2
SLC4A11
AQP1
Neg. control genes
Corneal endothelium genes

Fig. 20A

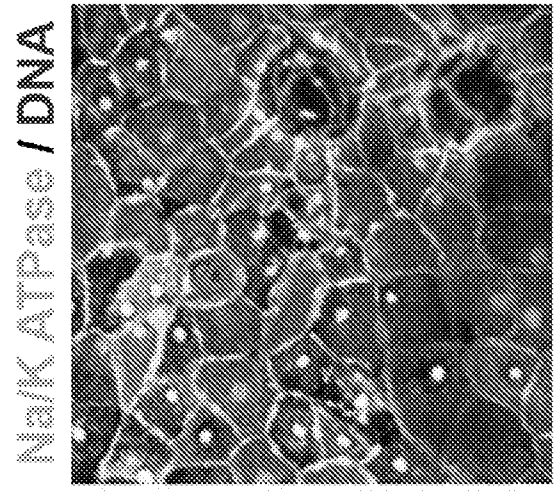


Fig. 20B

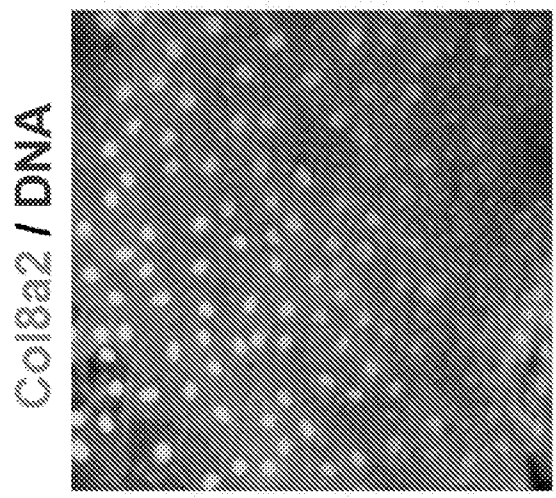


Fig. 21A

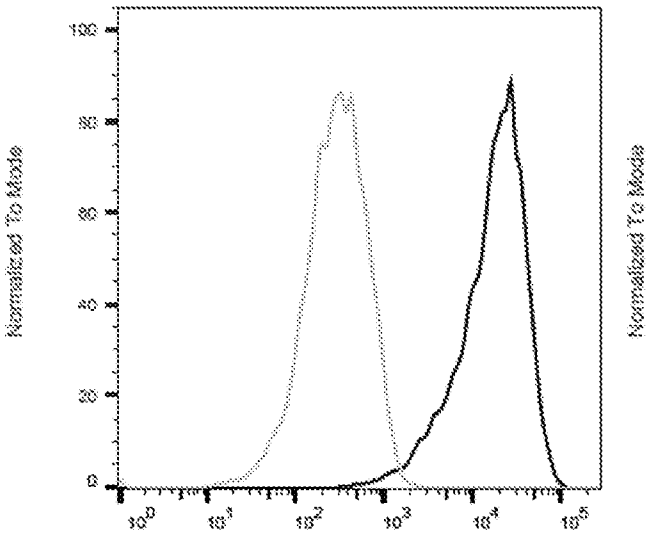


Fig. 21B

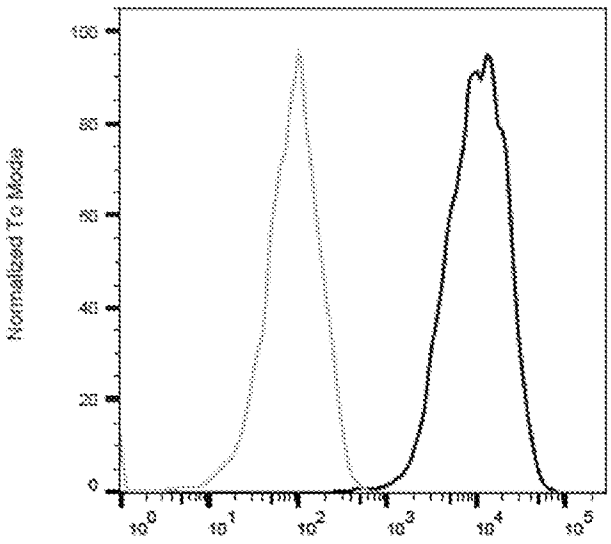


Fig. 21C

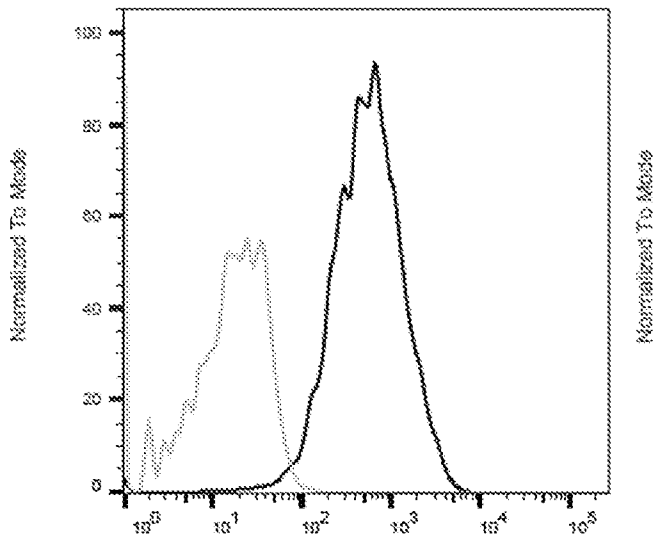


Fig. 21D

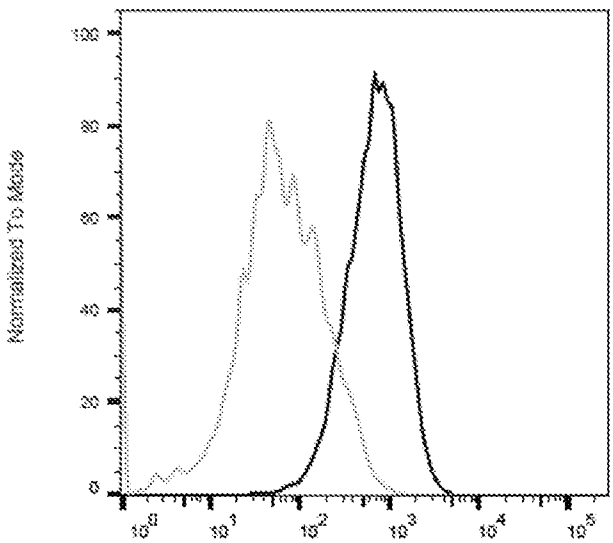


Fig. 22A

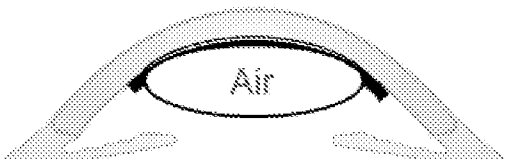


Fig. 22B

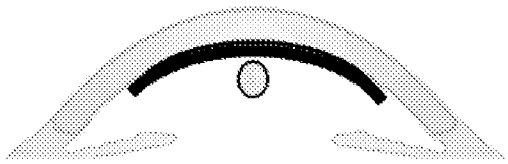


Fig. 22C

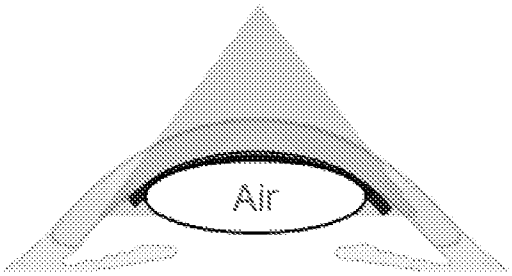


Fig. 22D

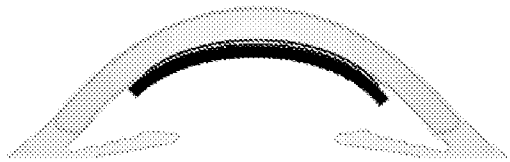


Fig. 23A

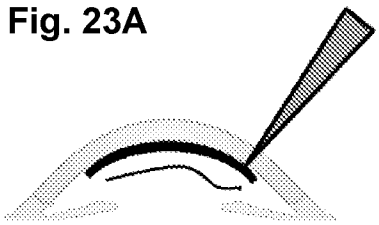


Fig. 23B

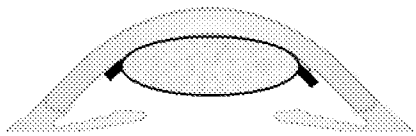


Fig. 23C

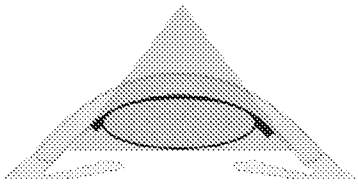


Fig. 23D

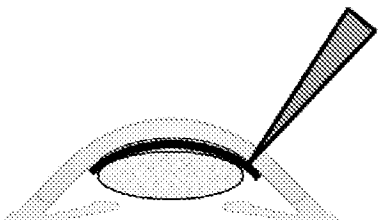


Fig. 23E

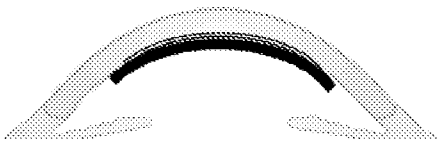


Fig. 24A

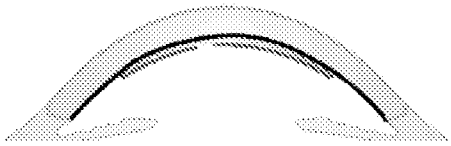


Fig. 24B

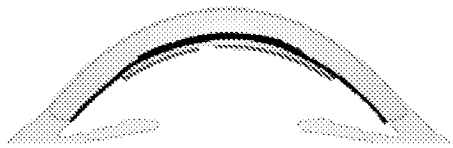


Fig. 24C

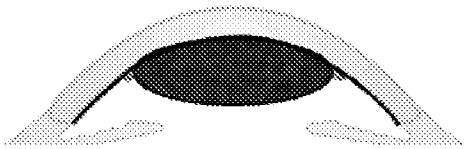


Fig. 24D

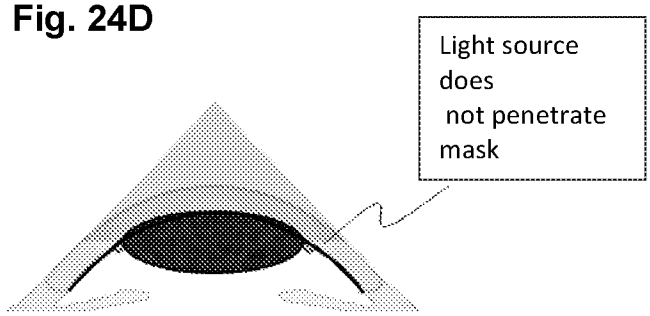


Fig. 24E

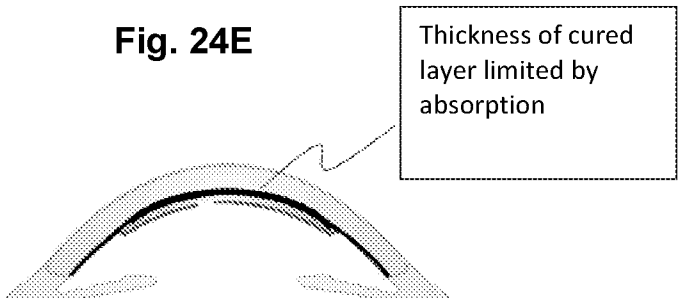


Fig. 25A

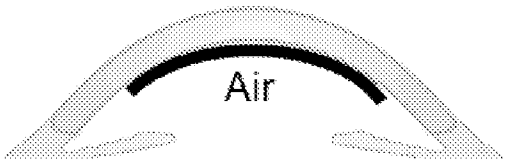


Fig. 25B



Fig. 25C

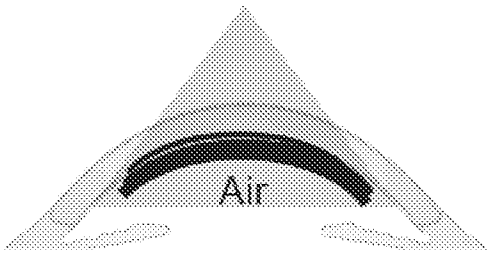


Fig. 25D

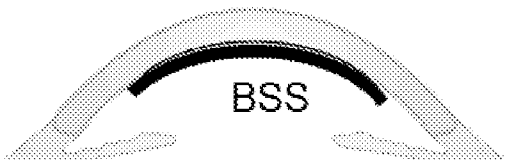


Fig. 26

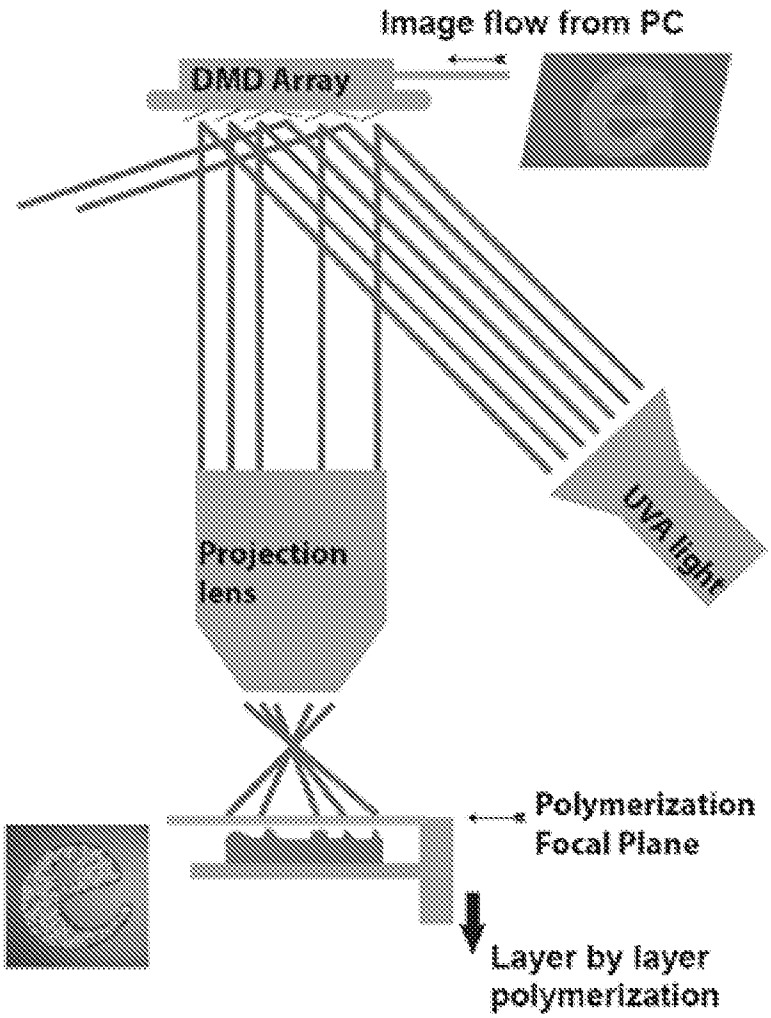

Image flow from PC
DMD Array
UVA light
Projection lens
Polymerization Focal Plane
Layer by layer polymerization

Fig. 27

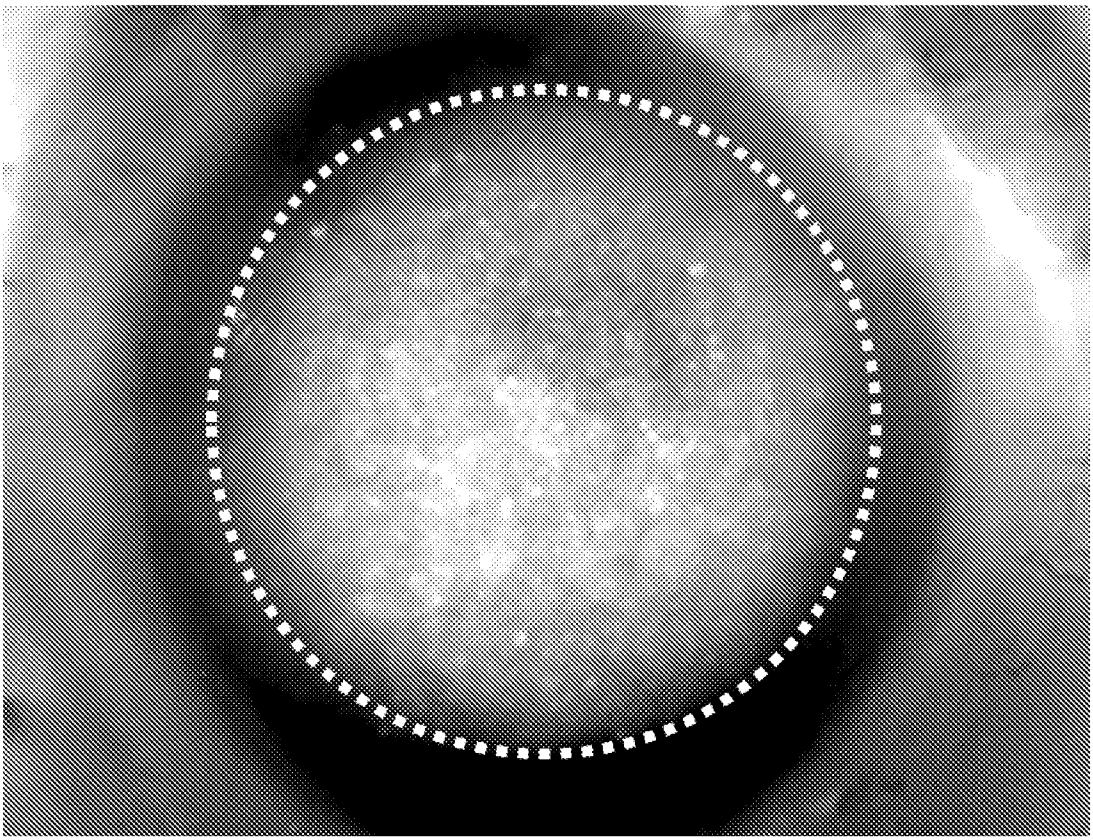


Fig. 28A Fig. 28B Fig. 28C

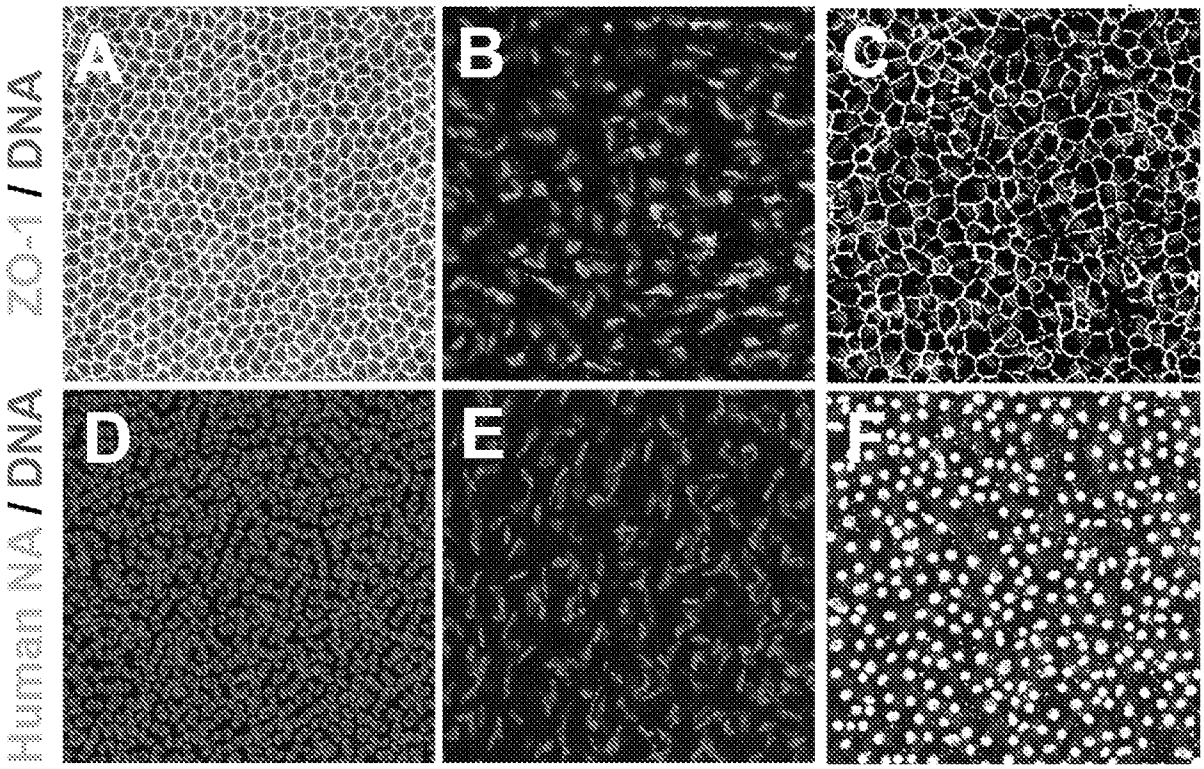


Fig. 28D Fig. 28E Fig. 28F

Fig. 29

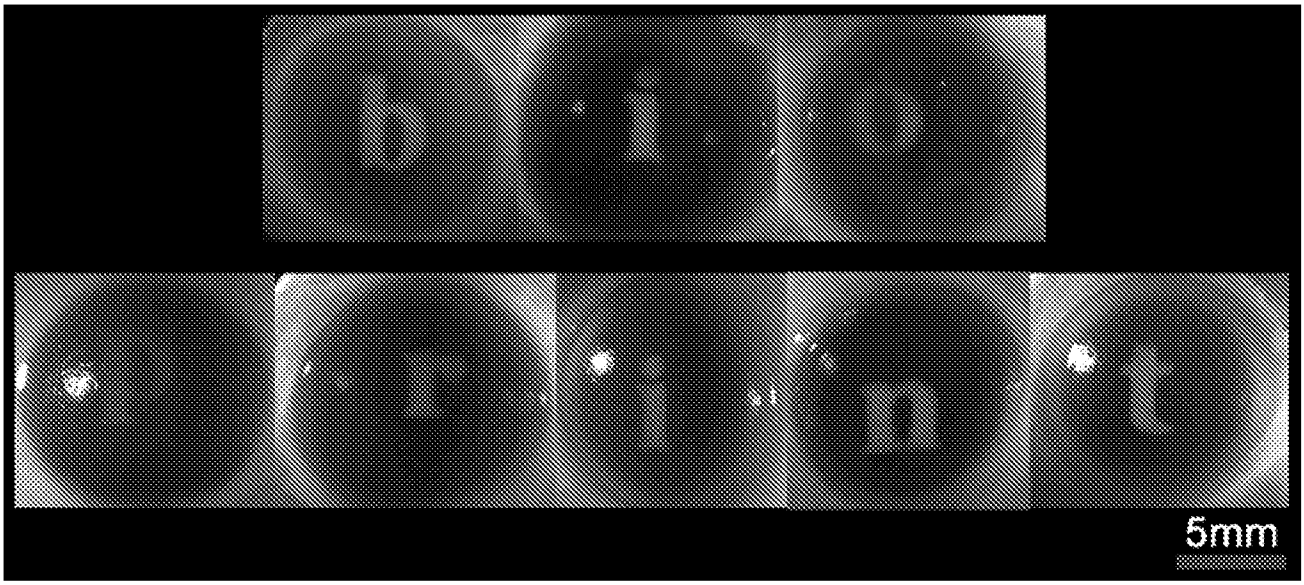


Fig. 30

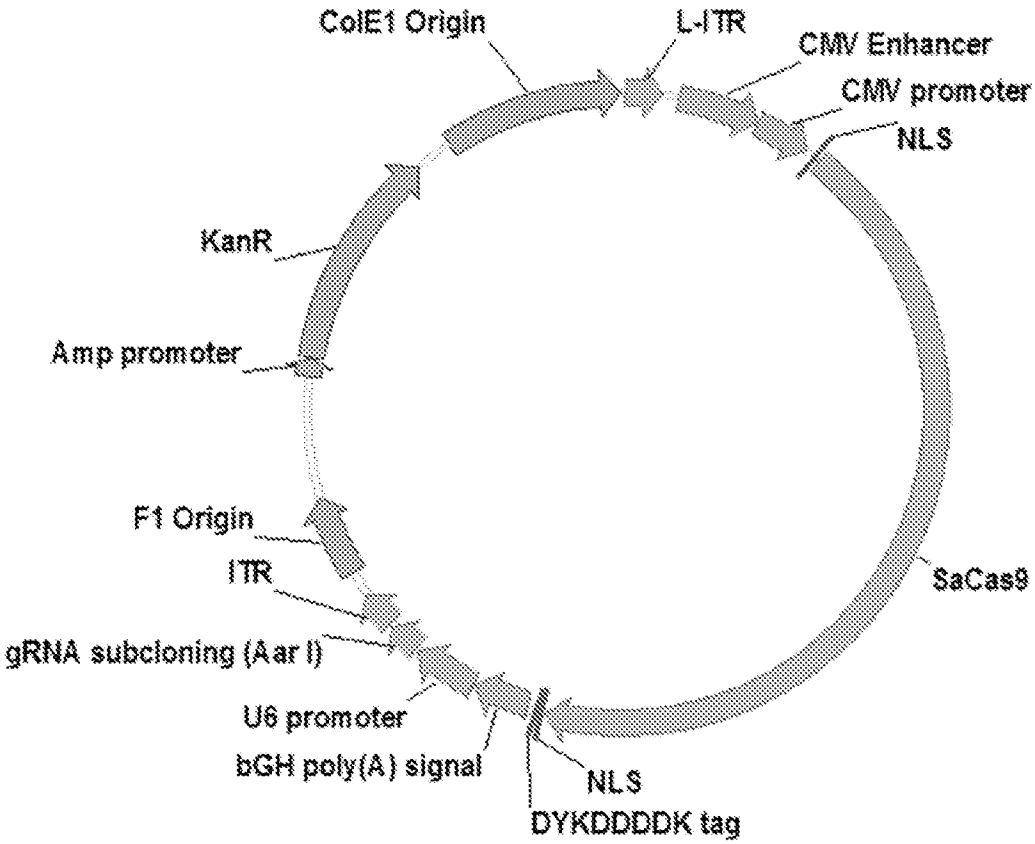

ColE1 Origin
L-ITR
CMV Enhancer
CMV promoter
NLS
KanR
Amp promoter
F1 Origin
ITR
gRNA subcloning (Aar I)
U6 promoter
bGH poly(A) signal
NLS
DYKDDDDK tag
SaCas9

Fig. 31

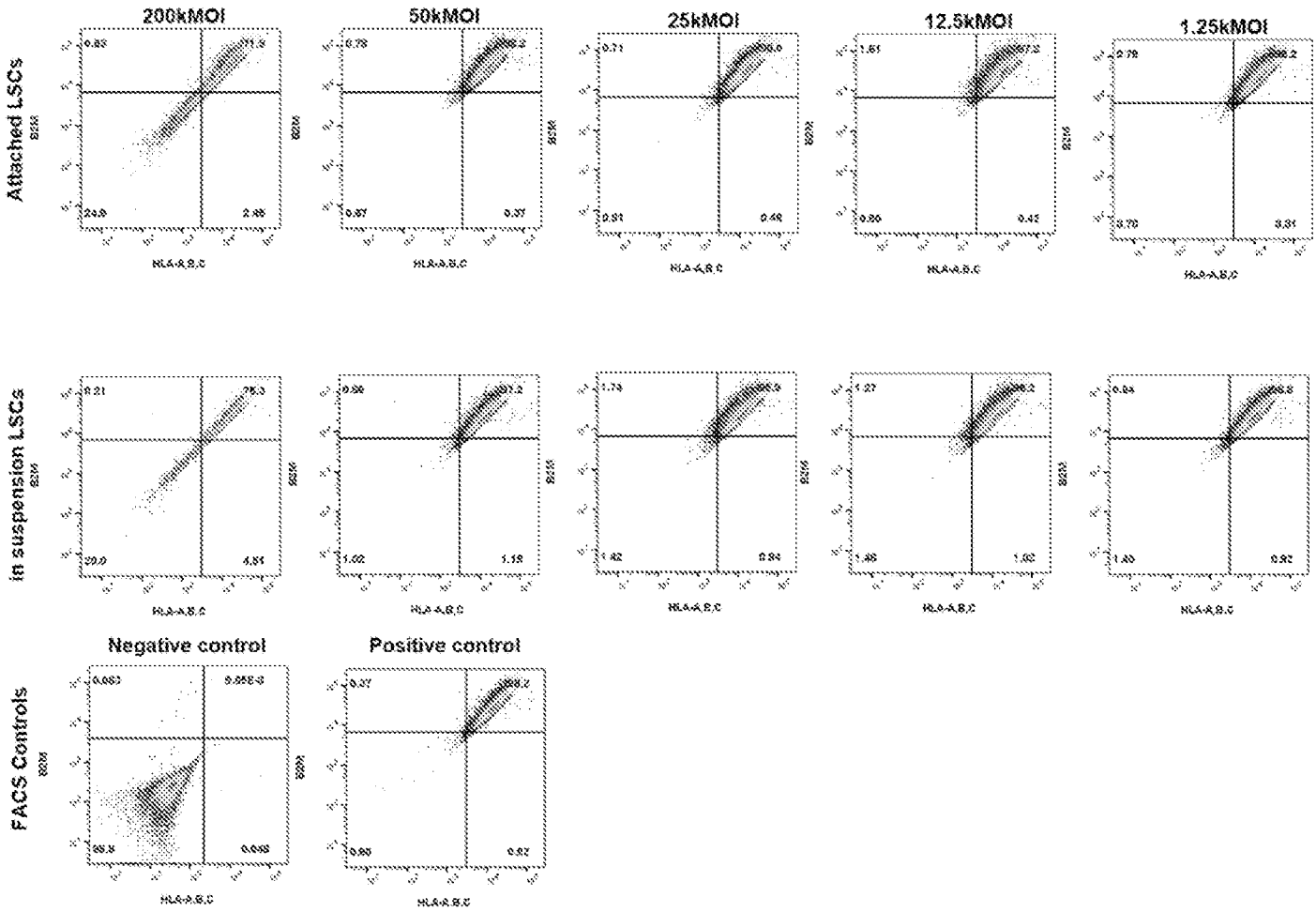

200kMOI
50kMOI
25kMOI
12.5kMOI
1.25kMOI
Attached LSCs
in suspension LSCs
FACS Controls
Negative control
Positive control

Fig. 32

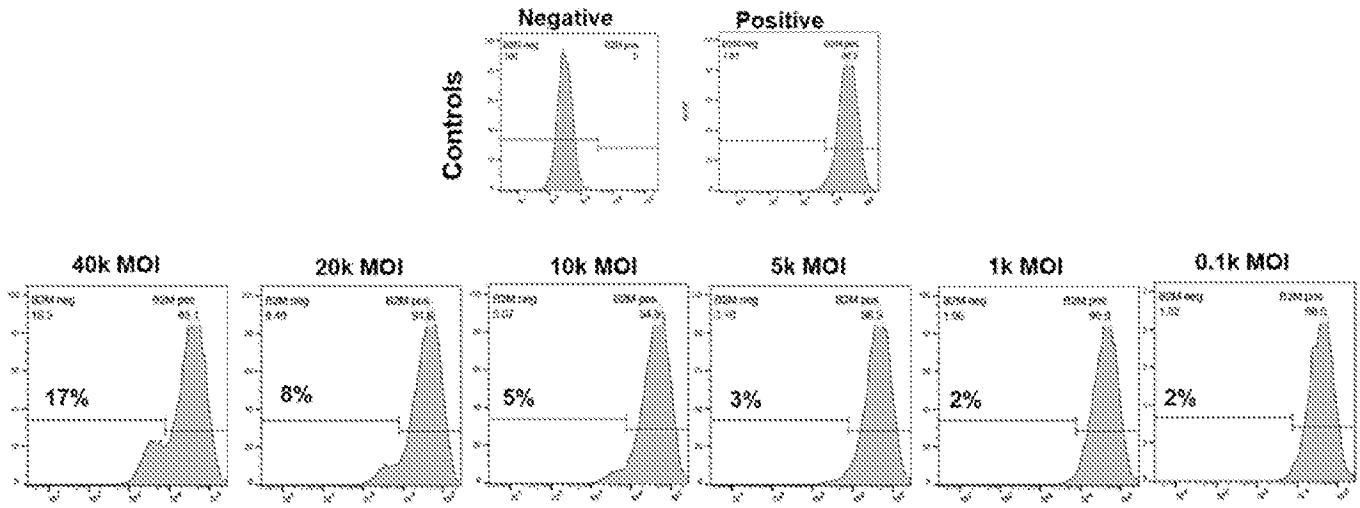

Controls
Negative
Positive
40k MOI
20k MOI
10k MOI
5k MOI
1k MOI
0.1k MOI
17%
8%
5%
3%
2%
2%

Fig. 33A

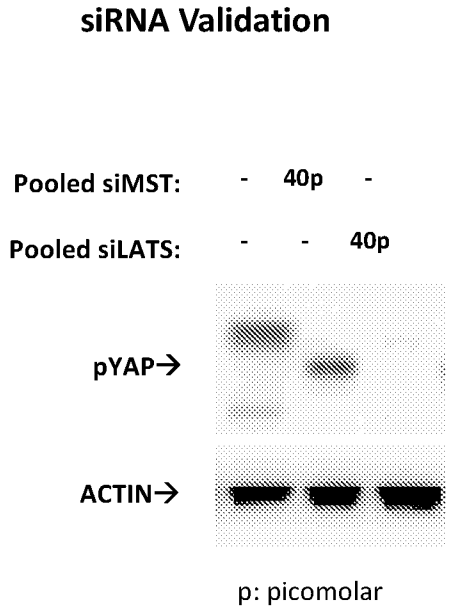

siRNA Validation
Pooled siMST: - 40p -
Pooled siLATS: - - 40p
pYAP→
ACTIN→
p: picomolar

Fig. 33B

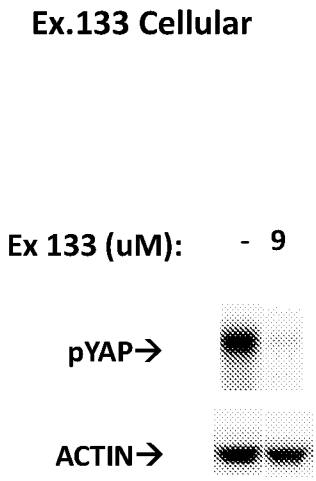


Fig. 33C

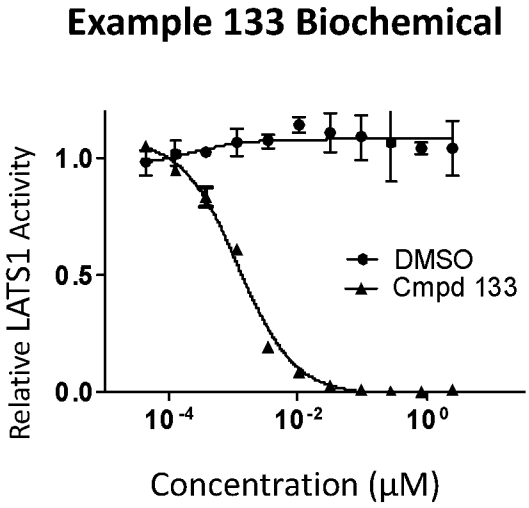

Example 133 Biochemical
Relative LATS1 Activity
1.0
0.5
0.0
DMSO
Cmpd 133
10 -4
10 -2
10 0
Concentration (µM)

Fig. 34

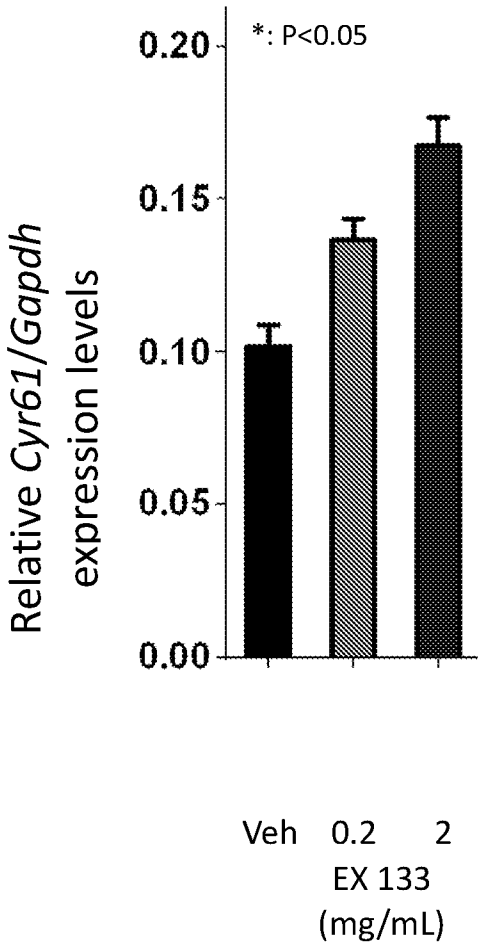

*: P<0.05
0.20
0.15
0.10
0.05
0.00
Relative Cyr61/Gapdh expression levels
Veh
0.2
2
EX 133
(mg/mL)

Fig. 35A

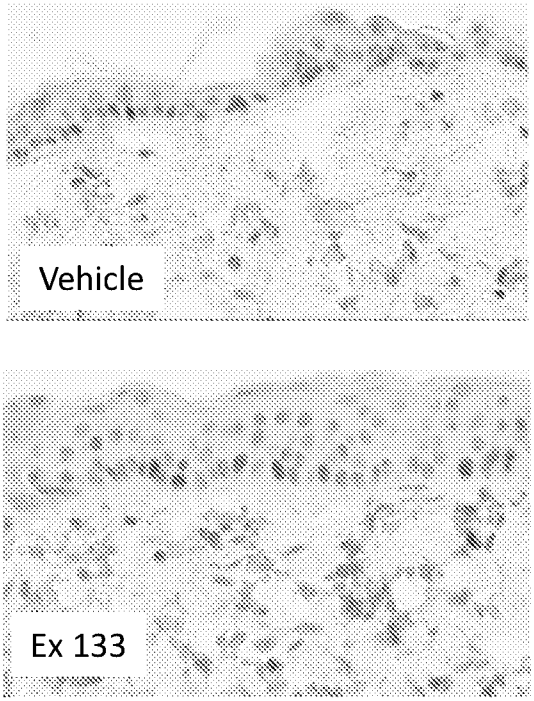


Fig. 35B

