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DESCRIPTION

TECHNICAL FIELD

[0001] The present invention relates to N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof, which are useful as inhibitors of ERK1 and ERK2. The present invention further relates to compositions containing such compounds, and such compounds for use in a method of treatment of a condition treatable by inhibiting ERK1/2.

BACKGROUND

[0002] ERK1 and ERK2 (collectively "ERK1/2") are related protein-serine/threonine kinases that participate in, amongst others, the Ras-Raf-MEK-ERK signal transduction pathway, which is sometimes denoted as the mitogen-activated protein kinase (MAPK) pathway. This pathway is thought to play a central role in regulating a number of fundamental cellular processes including one or more of cell proliferation, survival, adhesion, cycle progression, migration, differentiation, metabolism, and transcription. The activation of the MAPK pathway has been reported in numerous tumor types including lung, colon, pancreatic, renal, and ovarian cancers. Accordingly, substances that could reduce activation could be of interest for possible treatments.

[0003] ERK1/2 appear to be activated by MEK through phosphorylation of both a threonine and a tyrosine residue, namely at Tyr204/187 and Thr202/185. Once activated, ERK1/2 catalyze the phosphorylation of serine/threonine residues of more than 100 substrates and activate both cytosolic and nuclear proteins that are linked to cell growth, proliferation, survival, angiogenesis and differentiation, all hallmarks of the cancer phenotype. Thus it may be beneficial to target ERK to develop and use ERK1/2 inhibitors as a way to inhibit tumor growth.

[0004] Furthermore, an ERK inhibitor may have utility in combination with other MAPK inhibitors. Recently, researchers reported that dual inhibition of MEK and ERK by small molecule inhibitors was synergistic and acted to overcome acquired resistance to MEK inhibitors. See Hatzivassiliou et al., ERK Inhibition Overcomes Acquired Resistance to MEK Inhibition, *Mol. Cancer Ther.* 2012, 11, 1143-1154.

[0005] Small molecular ERK inhibitors have been reported in the literature including U.S. Patent No. 6,743,941, U.S. Patent No. 8,546,404, and Ren et al., Discovery of Highly Potent, Selective and Efficacious Small Molecule Inhibitors of ERK1/2, *J. Med. Chem.*, 2015, 58(4), 1976-1991. A small number of ERK inhibitors (e.g., BVD-523 and GDC-0994) are in early clinical development. However, no ERK inhibitor has been reported to advance into late stage

clinical trials. Therefore, there is a continuing need for the development of improved and efficacious ERK1/2 inhibitors for the treatment of cancer.

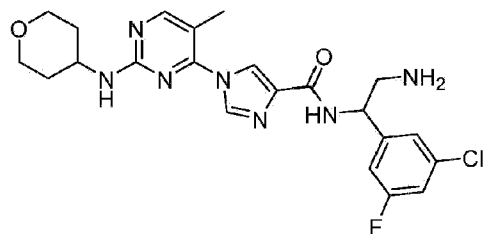
[0006] WO 2006/071644 A1 (06 July 2006) describes compounds that are useful as ERK2 inhibitors for treating or lessening the severity of a variety of disorders, including proliferate disorders such as cancer.

[0007] WO 2011/060295 A1 (19 May 2011) describes protein kinase inhibitors, pyrropyrimidine and pyrazolopyrimidine derivatives, and pharmaceutically acceptable salts thereof for treating cell proliferative disease and disorders such as cancer, autoimmune diseases, infection, cardiovascular disease and neurodegenerative disease and disorder.

[0008] WO 2002/064586 A2 (22 August 2002) describes compounds which are inhibitors of ERK2 and for treating diseases in mammals that are alleviated by a protein kinase inhibitor, particularly diseases such as cancer, inflammatory disorders, restenosis, diabetes, and cardiovascular disease.

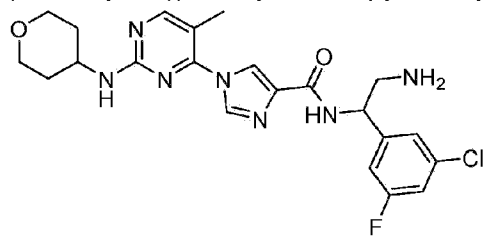
SUMMARY OF THE INVENTION

[0009] A first aspect of the invention is a compound that is N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:



or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof.

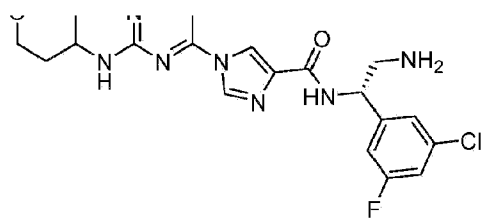
[0010] In one embodiment, the compound is N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:



or a pharmaceutically acceptable salt or stereoisomer thereof.

[0011] In one embodiment, the compound is (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:

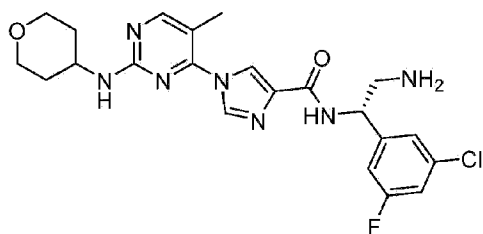




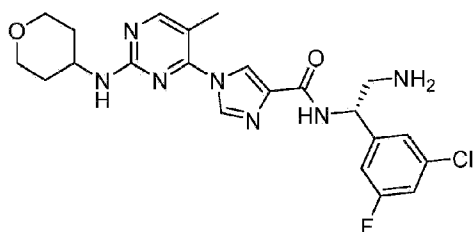
or a pharmaceutically acceptable salt thereof.

[0012] In one embodiment, the salt is a salt of an acid selected from acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, naphthalenesulfonic, benzenesulfonic, toluenesulfonic, trifluoroacetic, and camphorsulfonic.

[0013] In one embodiment, the compound is (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:

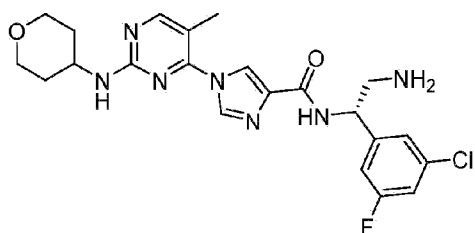


[0014] In one embodiment, the compound is (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:



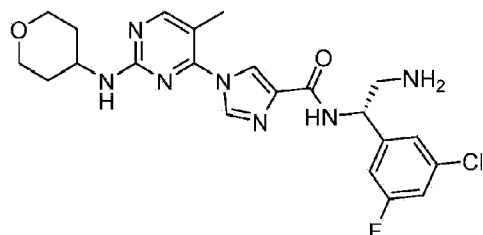
benzenesulfonic acid salt.

[0015] In one embodiment, the compound is (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:



hydrochloride salt.

[0016] In one embodiment, the compound is (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:



mandelic acid salt.

[0017] A second aspect of the invention is a composition comprising a compound of the first aspect, and at least one pharmaceutically acceptable carrier.

[0018] A third aspect of the present invention is a compound of the first aspect for use in a method of treatment of a condition treatable by inhibiting ERK1/2.

[0019] In one embodiment, the condition is associated with abnormal cellular proliferation.

[0020] In one embodiment, the condition is cancer of prostate, head, neck, eye, mouth, throat, esophagus, bronchus, larynx, pharynx, chest, bone, lung, colon, rectum, stomach, bladder, uterus, cervix, breast, ovaries, vagina, testicles, skin, thyroid, blood, lymph nodes, kidney, liver, intestines, pancreas, brain, central nervous system, adrenal gland, skin or a leukemia or lymphoma.

[0021] In one embodiment, the method comprises administration of the compound weekly.

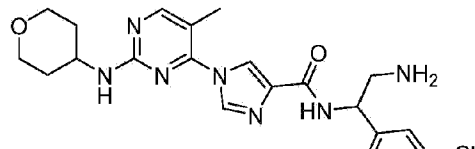
[0022] In one embodiment, the method comprises administration of the compound monthly.

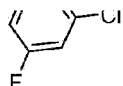
[0023] In one embodiment, the method comprises administration of equivalent dosages of the compound about every week.

[0024] In one embodiment, the method comprises administration of equivalent dosages of the compound about every two weeks.

DETAILED DESCRIPTION

[0025] The present invention provides novel inhibitors of ERK1 and ERK2, specifically, a compound that is N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide:





or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof.

[0026] The compounds of the invention include salts, solvates, hydrates, racemic forms, enantiomers, and mixtures thereof, to the extent practicable, unless otherwise stated or inconsistent from the context.

[0027] Representative "pharmaceutically acceptable salts" include, but are not limited to, watersoluble and water-insoluble salts. In one embodiment, the salt is of a base. The salt can be of a base selected from, e.g., alkali metal salt bases such as sodium, lithium, or potassium salt bases and organic bases, such as ammonium, mono-, di-, and trimethylammonium, mono-, di- and triethylammonium, mono-, di- and tripropylammonium, ethyldimethylammonium, benzyldimethylammonium, cyclohexylammonium, benzylammonium, dibenzylammonium, piperidinium, morpholinium, pyrrolidinium, piperazinium, 1-methylpiperidinium, 4-ethylmorpholinium, 1-isopropylpyrrolidinium, 1,4-dimethylpiperazinium, 1-n-butyl piperidinium, 2-methylpiperidinium, 1-ethyl-2-methylpiperidinium, mono-, di- and triethanolammonium, ethyl diethanolammonium, n-butylmonoethanolammonium, tris(hydroxymethyl)methylammonium, phenylmonoethanolammonium bases, among others. In another embodiment, the salt is of an acid. The salt can be of an acid selected from, e.g., acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, naphthalenesulfonic, benzenesulfonic, toluene sulfonic, trifluoroacetic, camphorsulfonic, among others. Optionally, a composition of the invention may contain both a pharmaceutically acceptable salt and the free base form of a compound of the invention.

[0028] The compound of the invention may be a solvate. As used herein, "solvate" does not significantly alter the physiological activity or toxicity of the compounds, and as such may function as pharmacological equivalents to non-solvate compounds of the invention. The term "solvate" as used herein is a combination, physical association and/or solvation of a compound of the present invention with a solvent molecule. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances, the solvate can be isolated, such as when one or more solvent molecules are incorporated into the crystal lattice of a crystalline solid. Thus, "solvate" encompasses both solution-phase and isolatable solvates. A hydrate is a special form of solvate which includes water combined in a definite ratio as water of crystallization.

[0029] The compounds of the invention contain an asymmetric center and may thus exist as enantiomers. When bonds to the chiral center are depicted as straight lines in the formula of the invention, it is understood that both the (R) and (S) configurations, and hence both enantiomers and mixtures thereof, are embraced. The present invention includes all such possible stereoisomers, unless the specific stereochemistry is specifically indicated. It is well known in the art how to prepare substantially pure stereoisomer, such as by resolution of racemic forms or by synthesis from optically active starting materials. In one embodiment, the

compound of the invention is a substantially pure stereoisomer. "Substantially pure stereoisomer" refers to a stereoisomer form is at least 95% pure with respect to other stereoisomers of otherwise the same structure.

[0030] The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively.

[0031] As used herein, the term "about" means a variability of 10% from the reference given, unless otherwise specified.

[0032] A "patient" or "subject" is a mammal, e.g., a human or a veterinary patient or subject, e.g., mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon or gorilla.

[0033] The term "treating" or "treatment" is meant to encompass administering to a subject a compound of the present invention for the purposes of amelioration of one or more symptoms of a disease or disorder, including palliative care. A "therapeutically effective amount" refers to the minimum amount of the active compound which effects treatment.

[0034] Pharmaceutical compositions useful herein contain at least one or more of compounds of the invention or pharmaceutically acceptable salts, solvates, hydrates, or stereoisomers thereof in a pharmaceutically acceptable carrier optionally with other pharmaceutically inert or inactive ingredients. In another embodiment, a compound of the invention is present in a single composition. In a further embodiment, a compound of the invention is combined with one or more excipients and/or other therapeutic agents as described below.

[0035] The pharmaceutical compositions of the invention comprise an amount of at least one or more of compounds of the invention or pharmaceutically acceptable salts, solvates, hydrates, or stereoisomers thereof that is effective for treating a condition treatable by inhibiting ERK1/2 in a subject in need thereof. Specifically, the dosage of the compound of the invention to achieve a therapeutic effect will depend on the formulation, age, weight and sex of the patient and route of delivery. It is also contemplated that the treatment and dosage of the compound of the invention may be administered in unit dosage form and that one skilled in the art would adjust the unit dosage form accordingly to reflect the relative level of activity. The decision as to the particular dosage to be employed (and the number of times to be administered per day) is within the discretion of the ordinarily-skilled physician, and may be varied by titration of the dosage to the particular circumstances to produce the desired therapeutic effect. In one embodiment, the therapeutically effective amount is about 0.01 mg/kg to 10 mg/kg body weight. In another embodiment, the therapeutically effective amount is less than about 5 g/kg, about 500 mg/kg, about 400 mg/kg, about 300 mg/kg, about 200 mg/kg, about 100 mg/kg, about 50 mg/kg, about 25 mg/kg, about 10 mg/kg, about 1 mg/kg, about 0.5 mg/kg, about 0.25 mg/kg, about 0.1 mg/kg, about 100 µg/kg, about 75 µg/kg, about 50 µg/kg, about 25 µg/kg, about 10 µg/kg, or about 1 µg/kg. However, the therapeutically

effective amount of the compound of the invention can be determined by the attending physician and depends on the condition treated, the compound administered, the route of delivery, the age, weight, severity of the patient's symptoms and response pattern of the patient.

[0036] The therapeutically effective amounts may be provided on regular schedule, *i.e.*, daily, weekly, monthly, or yearly basis or on an irregular schedule with varying administration days, weeks, months, etc. Alternatively, the therapeutically effective amount to be administered may vary. In one embodiment, the therapeutically effective amount for the first dose is higher than the therapeutically effective amount for one or more of the subsequent doses. In another embodiment, the therapeutically effective amount for the first dose is lower than the therapeutically effective amount for one or more of the subsequent doses. Equivalent dosages may be administered over various time periods including, but not limited to, about every 2 hours, about every 6 hours, about every 8 hours, about every 12 hours, about every 24 hours, about every 36 hours, about every 48 hours, about every 72 hours, about every week, about every two weeks, about every three weeks, about every month, and about every two months. The number and frequency of dosages corresponding to a completed course of therapy will be determined according to the judgment of a health-care practitioner. The therapeutically effective amounts described herein refer to total amounts administered for a given time period; that is, if more than one compound of the invention or a pharmaceutically acceptable salt or solvate thereof is administered, the therapeutically effective amounts correspond to the total amount administered.

[0037] The pharmaceutical compositions containing a compound of the invention may be formulated neat or with one or more pharmaceutical carriers for administration. The amount of the pharmaceutical carrier(s) is determined by the solubility and chemical nature of the compound of the invention, chosen route of administration and standard pharmacological practice. The pharmaceutical carrier(s) may be solid or liquid and may incorporate both solid and liquid carriers. A variety of suitable liquid carriers is known and may be readily selected by one of skill in the art. Such carriers may include, *e.g.*, DMSO, saline, buffered saline, hydroxypropylcyclodextrin, and mixtures thereof. Similarly, a variety of solid carriers and excipients are known to those of skill in the art. The compounds of the invention may be administered by any route, taking into consideration the specific condition for which it has been selected. The compounds of the invention may be delivered orally, by injection, inhalation (including orally, intranasally and intratracheally), ocularly, transdermally, intravascularly, subcutaneously, intramuscularly, sublingually, intracranially, epidurally, rectally, and vaginally, among others.

[0038] Although the compound of the invention may be administered alone, it may also be administered in the presence of one or more pharmaceutical carriers that are physiologically compatible. The carriers may be in dry or liquid form and must be pharmaceutically acceptable. Liquid pharmaceutical compositions are typically sterile solutions or suspensions. When liquid carriers are utilized for parenteral administration, they are desirably sterile liquids. Liquid carriers are typically utilized in preparing solutions, suspensions, emulsions, syrups and

elixirs. In one embodiment, the compound of the invention is dissolved in a liquid carrier. In another embodiment, the compound of the invention is suspended in a liquid carrier. One of skill in the art of formulations would be able to select a suitable liquid carrier, depending on the route of administration. The compound of the invention may alternatively be formulated in a solid carrier. In one embodiment, the composition may be compacted into a unit dose form, *i.e.*, tablet or caplet. In another embodiment, the composition may be added to unit dose form, *i.e.*, a capsule. In a further embodiment, the composition may be formulated for administration as a powder. The solid carrier may perform a variety of functions, *i.e.*, may perform the functions of two or more of the excipients described below. For example, solid carrier may also act as a flavoring agent, lubricant, solubilizer, suspending agent, filler, glidant, compression aid, binder, disintegrant, or encapsulating material.

[0039] The composition may also be sub-divided to contain appropriate quantities of the compound of the invention. For example, the unit dosage can be packaged compositions, *e.g.*, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids.

[0040] Examples of excipients which may be combined with one or more compound of the invention include, without limitation, adjuvants, antioxidants, binders, buffers, coatings, coloring agents, compression aids, diluents, disintegrants, emulsifiers, emollients, encapsulating materials, fillers, flavoring agents, glidants, granulating agents, lubricants, metal chelators, osmo-regulators, pH adjustors, preservatives, solubilizers, sorbents, stabilizers, sweeteners, surfactants, suspending agents, syrups, thickening agents, or viscosity regulators. See, for example, the excipients described in the "Handbook of Pharmaceutical Excipients", 5th Edition, Eds.: Rowe, Sheskey, and Owen, APhA Publications (Washington, DC), December 14, 2005.

[0041] In one embodiment, the compositions may be utilized as inhalants. For this route of administration, compositions may be prepared as fluid unit doses using a compound of the invention and a vehicle for delivery by an atomizing spray pump or by dry powder for insufflation.

[0042] In another embodiment, the compositions may be utilized as aerosols, *i.e.*, oral or intranasal. For this route of administration, the compositions are formulated for use in a pressurized aerosol container together with a gaseous or liquefied propellant, *e.g.*, dichlorodifluoromethane, carbon dioxide, nitrogen, and propane. Also provided is the delivery of a metered dose in one or more actuations.

[0043] In another embodiment, the compositions may be administered by a sustained delivery device. "Sustained delivery" as used herein refers to delivery of a compound of the invention which is delayed or otherwise controlled. Those of skill in the art know suitable sustained delivery devices. For use in such sustained delivery devices, the compound of the invention is formulated as described herein.

[0044] In addition to the components described above for use in the composition and the compound of the invention, the compositions and kits described herein may contain one or

more medications or therapeutic agents. In one embodiment, the compositions and kits described herein may contain one or more medications or therapeutic agents which are used to treat cancers, including, e.g., cancers characterized by tumors, including solid tumors, and "liquid" or non-solid tumor cancers (e.g., lymphoma). In one embodiment, the medication is a chemotherapeutic. Examples of chemotherapeutics include those recited in the "Physician's Desk Reference", 64th Edition, Thomson Reuters, 2010. Therapeutically effective amounts of the additional medication(s) or therapeutic agents are well known to those skilled in the art. However, it is well within the attending physician to determine the amount of other medication to be delivered.

[0045] The compounds of the invention and/or other medication(s) or therapeutic agent(s) may be administered in a single composition. However, the present invention is not so limited. In other embodiments, the compounds of the invention may be administered in one or more separate formulations from other compounds of the invention, chemotherapeutic agents, or other agents as is desired.

[0046] Also provided herein are kits or packages of pharmaceutical formulations containing the compounds of the invention or compositions described herein. The kits may be organized to indicate a single formulation or combination of formulations to be taken at each desired time.

[0047] Suitably, the kit contains packaging or a container with the compound of the invention formulated for the desired delivery route. Suitably, the kit contains instructions on dosing and an insert regarding the active agent. Optionally, the kit may further contain instructions for monitoring circulating levels of product and materials for performing such assays including, e.g., reagents, well plates, containers, markers or labels. Such kits are readily packaged in a manner suitable for treatment of a desired indication. For example, the kit may also contain instructions for use of a spray pump or other delivery device. Other suitable components to include in such kits will be readily apparent to one of skill in the art, taking into consideration the desired indication and the delivery route.

[0048] The compounds of the invention or compositions described herein can be a single dose or for continuous or periodic discontinuous administration. For continuous administration, a package or kit can include the compound of the invention in each dosage unit (e.g., solution, lotion, tablet, pill, or other unit described above or utilized in drug delivery), and optionally instructions for administering the doses daily, weekly, or monthly, for a predetermined length of time or as prescribed. When the compound of the invention is to be delivered periodically in a discontinuous fashion, a package or kit can include placebos during periods when the compound of the invention is not delivered. When varying concentrations of a composition, of the components of the composition, or the relative ratios of the compounds of the invention or agents within a composition over time is desired, a package or kit may contain a sequence of dosage units which provide the desired variability.

[0049] A number of packages or kits are known in the art for dispensing pharmaceutical agents for periodic oral use. In one embodiment, the package has indicators for each period.

In another embodiment, the package is a labeled blister package, dial dispenser package, or bottle.

[0050] The packaging means of a kit may itself be geared for administration, such as an inhalant, syringe, pipette, eye dropper, or other such apparatus, from which the formulation may be applied to an affected area of the body, such as the lungs, injected into a subject, or even applied to and mixed with the other components of the kit.

[0051] The compositions of these kits also may be provided in dried or lyophilized forms. When reagents or components are provided as a dried form, reconstitution generally is by the addition of a suitable solvent. It is envisioned that the solvent also may be provided in another package.

[0052] The kits also will typically include a means for containing the vials in close confinement for commercial sale such as, e.g., injection or blow-molded plastic containers into which the desired vials are retained. Irrespective of the number or type of packages and as discussed above, the kits also may include, or be packaged with a separate instrument for assisting with the injection/administration or placement of the composition within the body of an animal. Such an instrument may be an inhalant, syringe, pipette, forceps, measuring spoon, eye dropper or any such medically approved delivery means.

[0053] In one embodiment, a kit is provided and contains a compound of the invention. The compound of the invention may be in the presence or absence of one or more of the carriers or excipients described above. The kit may optionally contain instructions for administering the medication and the compound of the invention to a subject having a disease characterized by the dysregulation of the RAS/RAF/MEK/ERK pathway.

[0054] In a further embodiment, a kit is provided and contains a compound of the invention in a second dosage unit, and one or more of the carriers or excipients described above in a third dosage unit. The kit may optionally contain instructions for administering the medication and the compound of the invention to a subject having a disease characterized by the dysregulation of the RAS/RAF/MEK/ERK pathway.

[0055] The compounds described herein are useful in regulating conditions which are associated with the RAS/RAF/MEK/ERK pathways. In one embodiment, such a disease is associated with abnormal cellular proliferation. The term "abnormal cellular proliferation" refers to the uncontrolled growth of cells which are naturally present in a mammalian body. In one embodiment, a disease which is characterized by abnormal cellular proliferation is cancer, including, without limitation, cancer of the prostate, head, neck, eye, mouth, throat, esophagus, bronchus, larynx, pharynx, chest, bone, lung, colon, rectum, stomach, bladder, uterus, cervix, breast, ovaries, vagina, testicles, skin, thyroid, blood, lymph nodes, kidney, liver, intestines, pancreas, brain, central nervous system, adrenal gland, skin or a leukemia or lymphoma. In one embodiment, the disease characterized by abnormal cellular proliferation is melanoma skin cancer or cancer of the lung, colon, breast or prostate. In another embodiment, the

abnormal cellular proliferation is associated with either solid tumor or hematological cancer.

[0056] The term "regulation" or variations thereof as used herein refers to the ability of a compound of the invention to inhibit one or more components of a biological pathway. In one embodiment, "regulation" refers to inhibition ERK1/2 activity. In yet another embodiment, regulation includes inhibition of the RAS/RAF/MEK/ERK pathway.

[0057] The activity of compounds of the invention was established in multiple *in vitro* and *in vivo* assays. For example, compounds of the invention were demonstrated to cause inhibition of ERK1 and ERK2 enzymatic activities in biochemical assays using a homogeneous time resolved fluorescence (HTRF) technique; representative data are provided in **Table 2**. Furthermore, compounds of the invention were found to be active in a cell-based mechanistic assay; that is, compounds of the invention were demonstrated to inhibit phosphorylation of RSK1(S380) (the downstream protein target of ERK1/2) by an enzyme-linked immunosorbent assay (ELISA) method. Representative data are provided in **Table 2**. The functional utility of compounds of the invention was demonstrated by their activity in *in vitro* tumor cell proliferation assays in a panel of tumor cell lines with mutations in the RAS/BRAF/MEK/ERK pathway; representative data are again provided in **Table 2**. The compounds of the invention exhibit ERK1/2 inhibitory activity, and therefore can be utilized in order to inhibit abnormal cell growth in which the RAS/RAF/MEK/ERK pathway plays a role. Thus, the compounds of the invention are effective in the treatment of disorders with which abnormal cell growth actions of RAS/RAF/MEK/ERK dysregulation are associated, such as cancer. One of skill in the art would recognize that there is an established link between activity in tumor cell proliferation assays *in vitro* and anti-tumor activity in the clinical setting. For example, the therapeutic utility of a variety of pharmaceutical agents, e.g., taxol (Silvestrini, Stem Cells, 1993, 11(6):528-535), taxotere (Bissery, Anti Cancer Drugs, 1995, 6(3):330) and topoisomerase inhibitors (Edelman, Cancer Chemother. Pharmacol., 1996, 37(5):385-39), has been demonstrated by using *in vitro* tumor proliferation assays.

[0058] Finally, compounds of the invention were demonstrated to inhibit *in vivo* tumor growth upon dosing the compounds in human tumor xenograft models, such as the A375 human melanoma xenograft model harboring B-RAF V600E mutation, the HT-29 human colon cancer xenograft model harboring B-RAF V600E mutation, the HCT116 human colon cancer xenograft model harboring KRAS mutation, the A549 human lung carcinoma xenograft model harboring KRAS mutation, and the BxPC3 human pancreatic carcinoma xenograft model. The compounds were also demonstrated to inhibit the level of phospho-RSK in the tumors in the A375 xenograft model, upon treatment with compounds; this indicates effective inhibition of the target proteins ERK1/2 *in vivo* by compounds of the invention. One of skill in the art would recognize that there is an established link between activity in human tumor xenograft models and anti-tumor activity in the clinical setting.

[0059] Compounds of the invention that have particularly promising utility can be identified by using the assays as described herein. For example, compounds of the invention that are found to exhibit IC₅₀ values less than 100 nM in the ERK1/2 biochemical assays, and IC₅₀ values less

than 500 nM in the phospho-RSK1 and cell proliferation assays, and causing 40% or greater tumor growth inhibition in one or more human tumor xenograft models, would be identified as particularly useful compounds.

[0060] Methods for regulating the RAS/RAF/MEK/ERK pathway are described herein (but do not form part of the invention) and include administering a therapeutically effective amount of a compound of the invention to a patient in need thereof.

[0061] Methods for inhibiting ERK1/2 are described herein (but do not form part of the invention) and include administering a therapeutically effective amount of a compound of the invention to a patient in need thereof.

[0062] Methods for treating a disease characterized by an abnormal cellular growth resulting from a dysregulated RAS/RAF/MEK/ERK pathway are described herein (but do not form part of the invention) and include administering of a therapeutically effective amount of a compound of the invention to a patient in need thereof.

[0063] Methods for treating a condition treatable by inhibiting ERK1/2 are described herein (but do not form part of the invention) and include administering a therapeutically effective amount of a compound of the invention to a patient in need thereof.

[0064] As described herein, "a therapeutically effective amount" of a compound when used for the treatment of a condition is an amount that at least slows the progression of the condition. "A therapeutically effective amount" of a compound when used for the treatment of cancer is an amount which may slow the progression of cancer, reduce the number of cancer cells in fluids (e.g., blood, peripheral cells or lymphatic fluids), reduce tumor size, inhibit metastasis, inhibit tumor growth and/or ameliorate one or more of the symptoms of the cancer. For cancer therapy, efficacy can be measured for example, by assessing the time to disease progression and/or determining the response rate.

PROCESS FOR PREPARING THE COMPOUNDS

[0065] Methods useful for preparing the compounds of the invention are set forth in the Examples. One of skill in the art will recognize that the processes can be adapted to produce other compounds and their pharmaceutically acceptable salts, solvates, hydrates, or stereoisomers of the compounds of the invention.

[0066] In the following reactions described to prepare compounds described herein, it can be necessary to protect reactive functional groups, for example hydroxyl, amino, imino, thio or carboxyl groups, which are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups can be used in accordance with standard practices, for example, see Green et al., Protective Groups in Organic Chemistry, John Wiley & Sons, 1991.

[0067] The following abbreviations are used and have the indicated definitions: MHz is megahertz (frequency), m is multiplet, t is triplet, d is doublet, s is singlet, br is broad, CDCl₃ is deuterio chloroform, calcd is calculated, min is minutes, h is hours, g is grams, mmol is millimoles, mL is milliliters, N is Normal (concentration), M is molarity (concentration), μ M is micromolar, ee is enantiomeric excess, °C is degree centigrade, HPLC is High Performance Liquid Chromatography, LC-MS is Liquid Chromatography-Mass Spectroscopy, mp is melting point, NMR is Nuclear Magnetic Resonance, TLC is thin layer chromatography, THF is tetrahydrofuran, MeOH is methanol, DCM is dichloromethane, DMF is *N,N*-dimethyl formamide, DMSO is dimethyl sulfoxide, EtOH is ethyl alcohol, EtOAc is ethyl acetate, MeOH is methanol, RT is room temperature, HCl is hydrogen chloride or hydrochloric acid, TFA is trifluoroacetic acid, EtMgBr is ethyl magnesium bromide, *n*-BuLi is *n*-butyl lithium, NaHCO₃ is sodium bicarbonate, Na₂CO₃ is sodium carbonate, Na₂SO₄ is sodium sulfate, NMP is *N*-methyl-2-pyrrolidone, EDC or EDC.HCl is *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, TEA is triethylamine, DIPEA is diisopropylethylamine, HOBT is *N*-hydroxy-benzotriazole or *N*-hydroxy-benzotriazole hydrate, and T3P is propylphosphonic anhydride.

EXAMPLES

[0068] All reactions were carried out under dry nitrogen and or argon atmosphere unless otherwise specified. Unless otherwise stated, all the raw starting materials, solvents and reagents were purchased from commercial sources (e.g., Avocado Research Chemicals, Apollo Scientific Limited, Bepharma Ltd., Combi-Blocks Inc., Sigma Aldrich Chemicals Pvt. Ltd., Ultra Labs, Toronto Research Chemicals Inc., Chemical House, RFCL Limited, Spectro Chem Pvt. Ltd., Leonid Chemicals, Loba Chemie, Changzhou Yangyuan, NeoSynth., Rankem, etc.) and used as such without further purification or reagents can be synthesized by procedures known in the art. Typically, the progress of each reaction was monitored by TLC analysis.

[0069] Biotage Isolera[®] One and CombiFlash[®] (Teledyne Isco) Automated Flash Purification System were commonly used for the purification of crude products, using the eluent combination mentioned in the respective procedures. Flash Chromatography was performed using silica gel (60-100, 100-200 and 230-400 mesh) from ChemLabs, with nitrogen and/or compressed air to enable pressurized eluent flow. Preparative thin-layer chromatography (preparative TLC) was carried out using silica gel (GF 1500 μ M 20 × 20 cm and GF 2000 μ M 20 × 20 cm Prep-scored plates from Analtech, Inc. Delaware, USA). Analytical thin-layer chromatography (TLC) was carried out using pre-coated silica gel sheets (Merck 60 F₂₅₄). Visual detection was performed with ultraviolet light, *p*-anisaldehyde stain, ninhydrin stain, dinitrophenyl hydrazine stain, potassium permanganate stain, or iodine. Reactions at lower temperature were performed by using cold baths, e.g., H₂O/ice at 0°C, and acetone/dry ice at -78°C. Reactions under microwave conditions were conducted in a CEM Discover SP 909155 microwave oven. Melting points were determined by using a LabIndia MR-VIS visual melting

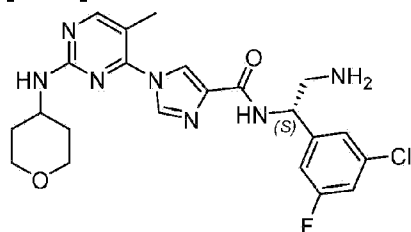
range apparatus. ^1H NMR spectra were recorded at 400 MHz with a Varian V400 spectrometer, Bruker 400 (unless otherwise noted) at ambient temperature, using tetramethylsilane as internal reference. The chemical shift values are quoted in δ (parts per million). Mass spectra of all the intermediates and final compounds were recorded using Acquity[®] UPLC-SQD (Waters) & Agilent 1290 Infinity[®] UHPLC with 6150 SQD machines. HPLC spectra were recorded using Agilent 1290 Infinity[®] UHPLC and Alliance (Waters) systems. LCMS spectra were recorded using Agilent 1200[®] LCMS, Agilent 1290[®] UHPLC-SQD with diode array detector (DAD) detection LC-MS instruments using a BEH C18 column and Zorbax[®] HD C18 column (50mm $\times$ 2.1mm $\times$ 1.7 μm) & (50mm $\times$ 2.1mm $\times$ 1.8 μm), a mobile phase of 0.01% of formic acid and acetonitrile or 0.01% of trifluoroacetic acid and acetonitrile, and a flow rate of 0.3 mL/min, a column temperature of 70 or 50 $^\circ\text{C}$, and a run time of 3 to 5 min. The purity of each of the final compounds was determined using Waters[®] PDA with SQD and Agilent[®] DAD with 6150 SQD instruments and the following conditions:

Condition 1: Column: BEH C18 (Waters); mobile phase: 0.01% acetic acid with acetonitrile & 0.01% acetic acid with methanol; gradient: (B/%T): 0/0, 1.2/100, 2.5/100, 2.8/0, 3.0/0; flow: 0.3 mL/min; temperature: 70 $^\circ\text{C}$; run time: 3.0 min.

Condition 2: Column: Zorbax[®] HD C18; mobile phase: 0.01% acetic acid with acetonitrile & 0.01% acetic acid with methanol; gradient: (B/%T): 0/0, 2.5/100, 4.5/100, 4.8/0, 5.0/0; flow: 0.3 mL/min; temperature: 50 $^\circ\text{C}$; run time: 5.0 min

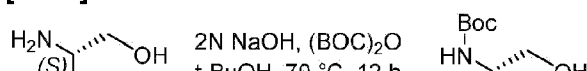
Example 24: (S)-N-(2-Amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide (Compound #275)

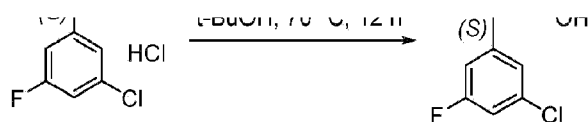
[0070]



Step 1: (S)-tert-Butyl (1-(3-chloro-5-fluorophenyl)-2-hydroxyethyl) carbamate

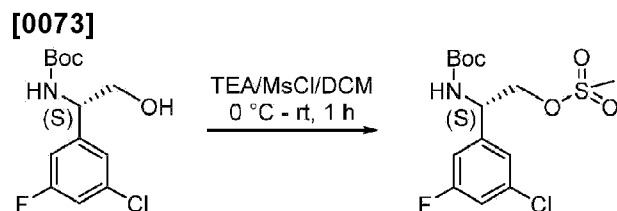
[0071]





[0072] To a stirred solution of (S)-2-amino-2-(3-chloro-5-fluorophenyl)ethanol hydrochloride (10 g, 44.44 mmol) in *t*-butanol (100 mL) was added 2N NaOH (2.22 g, 55.55 mmol, in 111 mL water) and di-*tert*-butyl dicarbonate (13.56 g, 62.22 mmol). The resulting mixture was stirred at 70° C for 12 h. The progress of the reaction was monitored by TLC. Then the reaction was quenched with water (2 × 100 mL) and extracted with ethyl acetate (2 × 100 mL), and the combined organic layers were washed with water (30 mL) followed by brine (30 mL), dried over sodium sulfate, filtered and evaporated under reduced pressure to provide 13 g of crude product. The crude product was combined with two additional crude product batches that were prepared in a similar manner, and the combined material was purified by gradient column chromatography using ethyl acetate in *n*-hexane as eluent to afford (S)-tert-butyl (1-(3-chloro-5-fluorophenyl)-2-hydroxyethyl)carbamate as an off white solid (94% yield). ¹HNMR (400 MHz, DMSO-*d*₆): δ 7.26 - 7.23 (m, 2H), 7.20 (s, 1H), 7.11 (d, *J* = 8 Hz, 1H), 4.83 (t, *J* = 4 Hz, 1 H), 4.52 - 4.50 (m, 1H), 3.50 - 3.43 (m, 2H), 1.34 (s, 9H). LC-MS calcd exact mass 289.74, found *m/z* 190.0 [M+H-Boc]⁺.

Step 2: (S)-2-((tert-Butoxycarbonyl)amino)-2-(3-chloro-5-fluorophenyl)ethylmethanesulfonate

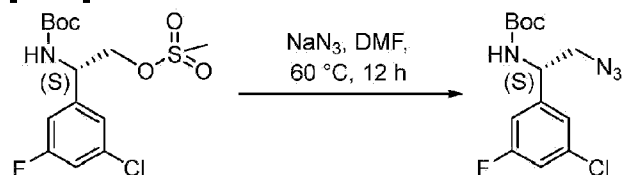


[0074] To a stirred solution of (S)-tert-butyl (1-(3-chloro-5-fluorophenyl)-2-hydroxyethyl)carbamate (12 g, 41.52 mmol) in dichloromethane (100 mL) at 0° C was added triethylamine (6.93 mL, 49.83 mmol) and the mixture was stirred for 10 min at 0° C. Then methane sulfonyl chloride (3.73 mL, 45.674 mmol) was added, and the mixture was stirred at room temperature for 1 h. The progress of the reaction was monitored by TLC. The reaction was quenched with water (100 mL) and extracted with dichloromethane (3 × 100 mL), and the combined organic layers were washed with saturated ammonium chloride solution (100 mL) and brine (50 mL). The organic layer was dried over sodium sulfate, filtered and evaporated under reduced pressure to afford (S)-2-((tert-butoxycarbonyl)amino)-2-(3-chloro-5-fluorophenyl)ethylmethanesulfonate (15.25 g) as a light yellow solid, which was used for the next step without further purification. ¹HNMR (400 MHz, DMSO-*d*₆): δ 7.68 (d, *J* = 8.4 Hz, 1H), 7.36 (s, 1H), 7.33 (s, 1H), 7.29 (d, *J* = 9.6 Hz, 1H), 4.28 - 4.19 (m, 2H), 3.15 (s, 3H), 1.36 (s, 9H). LC-MS calcd

exact mass 367.07, found m/z 268.0 $[M+H-Boc]^+$.

Step 3: (S)-tert-Butyl (2-azido-1-(3-chloro-5-fluorophenyl)ethyl)carbamate

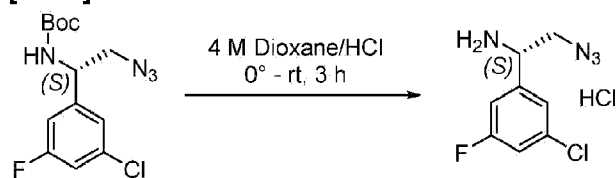
[0075]



[0076] To a stirred solution of (S)-2-((tert-butoxycarbonyl)amino)-2-(3-chloro-5-fluorophenyl)ethyl methanesulfonate (15.25 g, 41.55 mmol) in N,N-dimethylformamide (100 mL) at room temperature was added sodium azide (5.4 g, 83.11 mmol). The reaction mixture was heated at 60° C for 12 h. The progress of the reaction was monitored by TLC, then the reaction mixture was cooled to room temperature, diluted with water (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (100 mL) followed by brine (100 mL), and dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product was combined with two additional crude product batches that were prepared in a similar manner, and the combined material was purified by gradient column chromatography using ethyl acetate in n-hexane as eluent to afford (S)-tert-butyl (2-azido-1-(3-chloro-5-fluorophenyl)ethyl)carbamate as an off white solid (83% yield). ¹HNMR (400 MHz, DMSO-d₆): δ 7.66 (d, *J* = 8.0 Hz, 1H), 7.31 (bs, 2H), 7.21 (d, *J* = 12.0 Hz, 1H), 4.77-4.75 (m, 1H), 4.44 (d, *J* = 8.0 Hz, 2H), 1.36 (s, 9H). LC-MS calcd exact mass 314.09, found m/z 259 $[M+H-tBu]^+$.

Step 4: (S)-2-Azido-1-(3-chloro-5-fluorophenyl)ethanamine hydrochloride

[0077]

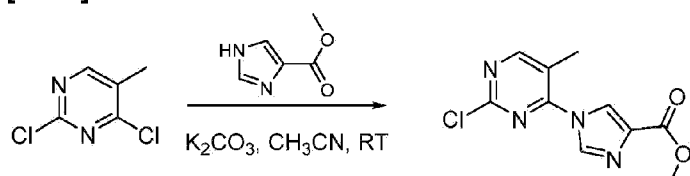


[0078] To a stirred solution of (S)-tert-butyl (2-azido-1-(3-chloro-5-fluorophenyl)ethyl)carbamate (10 g, 31.85 mmol) in 1,4-dioxane (100 mL) was added drop wise 4M HCl in 1,4-dioxane (100 mL) at 0° C. The reaction mixture was stirred at room temperature for 3 h. Excess solvent was evaporated under reduced pressure to obtain a solid residue. The solid was washed with pentane (2 × 50 mL) and dried to give (S)-2-azido-1-(3-chloro-5-

fluorophenyl) ethanamine hydrochloride (7.87 g, 98.8 %) as an off-white solid. ^1H NMR (400 MHz, DMSO- d_6): δ 8.94 (br s, 3H), 7.56 (s, 1H), 7.49 (d, J = 4.8 Hz, 2H), 4.55 (t, J = 6.4 Hz, 1H), 3.92 - 3.81 (m, 2H). LC-MS calcd exact mass 214.04, found m/z 215.1 $[\text{M}+\text{H}]^+$.

Step 5a: Methyl 1-(2-chloro-5-methylpyrimidin-4-yl)-1H-imidazole-4-carboxylate

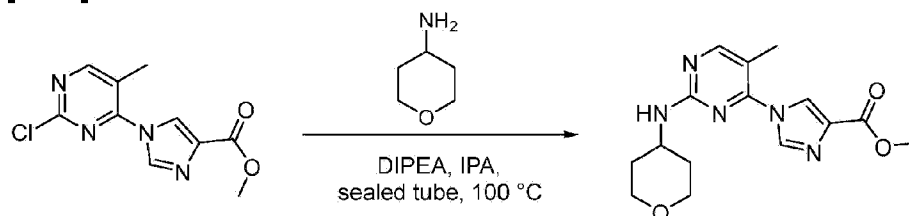
[0079]



[0080] To a stirred solution of methyl 1H-imidazole-4-carboxylate (2.32 g, 18.4 mmol) in acetonitrile (75 mL) was added potassium carbonate (5.08 g, 36.8 mmol). The reaction mixture was stirred at RT for 5 min, then 2,4-dichloro-5-methylpyrimidine (2 g, 18.4 mmol) was added. The resulting mixture was stirred at RT overnight. The mixture was quenched with water (50 mL) and extracted with ethyl acetate (3×150 mL). The combined organic layer was dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was purified by gradient column chromatography using ethyl acetate in n-hexane as eluent to afford methyl 1-(2-chloro-5-methylpyrimidin-4-yl)-1H-imidazole-4-carboxylate as an off-white solid (53.7%). ^1H NMR (400 MHz, CDCl_3): δ 8.63 (s, 1H), 8.25 (d, J = 5.2 Hz, 2H), 3.95 (s, 3H), 2.52 (s, 3H). LC-MS calcd exact mass 252.04, found m/z 253.1 $[\text{M}+\text{H}]^+$.

Step 5b: Methyl 1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxylate

[0081]

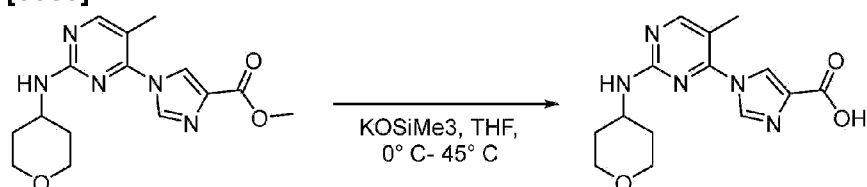


[0082] To a solution of 1-(2-chloro-5-methylpyrimidin-4-yl)-1H-imidazole-4-carboxylate (0.27 g, 1.07 mmol) in isopropanol (5 mL) was added DIPEA (0.58 mL, 3.21 mmol) and tetrahydro-2H-pyran-4-amine (0.16 mL, 1.60 mmol). The resulting mixture was stirred in a sealed glass tube at 100 °C for 20 h. The reaction mixture was quenched with water (10 mL), and extracted with ethyl acetate (50 mL). The organic layer was dried over sodium sulfate, filtered and

evaporated under reduced pressure. The residue was purified by gradient column chromatography using methanol in DCM as eluent to afford methyl 1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxylate as an off-white solid (0.25 g, 76%). ¹HNMR (400 MHz, DMSO-d₆): δ 8.35 (s, 1H), 8.29 (s, 1H), 8.26 (s, 1H), 7.38 (d, *J*=7.6 Hz, 1H), 3.85-3.82 (m, 3H), 3.77 (s, 3H), 3.37 (t, *J*=10 Hz, 2H), 2.15 (s, 3H), 1.80 (d, *J*=10.4 Hz, 2H), 1.50-1.46 (m, 2H). LC-MS calcd exact mass 317.15, found *m/z* 318.4 [M+H]⁺.

Step 5c: 1-(5-Methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxylic acid

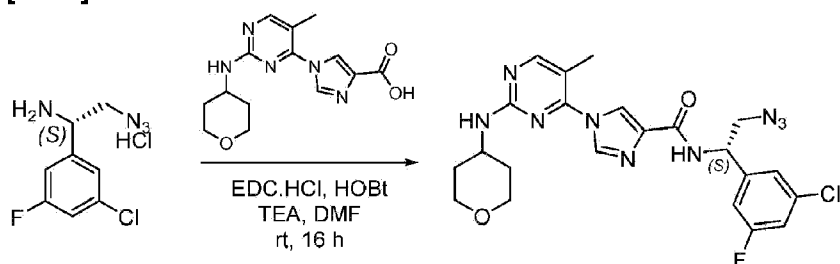
[0083]



[0084] To a stirred solution of methyl 1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxylate (1.5 g, 4.731 mmol) in tetrahydrofuran (30 mL) was added potassium trimethylsilanolate (1.82 g, 14.18 mmol) at 0 °C. The reaction mixture was stirred at 45 °C for 1.5 h. Then the reaction mixture was quenched with water (25 mL), and washed with ethyl acetate (2 × 10 mL). The aqueous layer was adjusted to pH ~5-6 by addition of 4N HCl solution. The aqueous layer was then extracted with ethyl acetate (3 × 60 mL), and the combined organic layer was concentrated under reduced pressure, to afford 1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxylic acid, as an off-white solid, (1.2 g, 84%). ¹HNMR (400 MHz, DMSO-d₆): δ 12.47 (br s, 1H), 8.33 (s, 1H), 8.23 (br s, 1H), 8.20 (s, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 3.88 (br s, 1H), 3.83 (d, *J* = 11.6 Hz, 2H), 3.36 (t, *J* = 10.8 Hz, 2H), 2.15 (s, 3H), 1.80 (d, *J* = 10.4 Hz, 2H), 1.52 - 1.42 (m, 2H). LC-MS calcd exact mass 303.13, found *m/z* 304.4 [M+H]⁺.

Step 5d: (S)-N-(2-Azido-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide

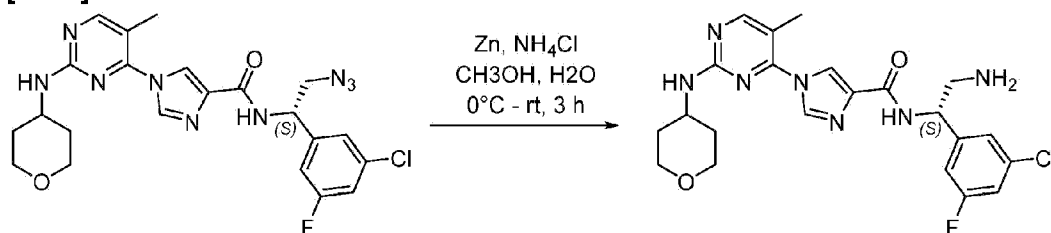
[0085]



[0086] To a stirred solution of 1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxylic acid (12.2 g, 40.26 mmol) in *N,N*-dimethylformamide (120 mL) was added triethylamine (16.8 mL, 120.79 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (15.44 g, 80.53 mmol), hydroxybenzotriazole (3.08 g, 20.13 mmol) and (*S*)-2-azido-1-(3-chloro-5-fluorophenyl)ethanamine hydrochloride (8.01 g, 32.21 mmol) under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 16 h. The progress of the reaction was monitored by TLC (8% methanol in dichloromethane). The reaction mixture was diluted with water (2 × 100 mL) and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with saturated ammonium chloride solution (1 × 200 mL), followed by saturated sodium bicarbonate solution (1 × 200 mL) and brine (1 × 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give the desired crude product. The crude product was combined with two additional crude product batches that were prepared in a similar manner, and the combined material was purified by gradient column chromatography using methanol in dichloromethane as eluent to afford (*S*)-*N*-(2-azido-1-(3-chloro-5-fluorophenyl) ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide (69%) as an off-white solid. ¹HNMR (400 MHz, DMSO-*d*₆): δ 8.90 (d, *J* = 8.8 Hz, 1H), 8.34 (s, 1H), 8.28 (s, 1H), 8.11 (s, 1H), 7.42 (s, 1H), 7.34 - 7.31 (m, 3H), 5.29 - 5.23 (m, 1H), 3.86 (br s, 1H), 3.85 - 3.84 (m, 3H), 3.66 - 3.62 (m, 1H), 3.36 (t, *J* = 10.8 Hz, 2H), 2.17 (s, 3H), 1.80 (d, *J* = 10.8 Hz, 2H), 1.51 - 1.44 (m, 2H). LCMS calcd exact mass 499.16, found *m/z* 500.1 [*M*+*H*]⁺.

Step 6: (*S*)-*N*-(2-Amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide

[0087]



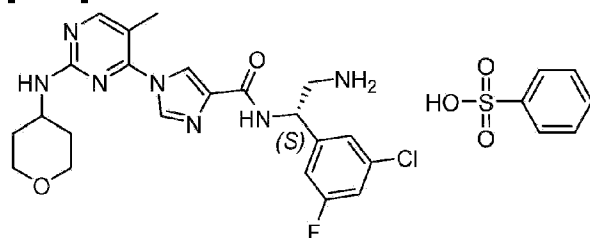
[0088] To a stirred solution of (*S*)-*N*-(2-azido-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide (9.0 g, 18.04 mmol) in methanol (100 mL) was added zinc dust (5.89 g, 90.18 mmol), followed by ammonium chloride (4.823 g, 90.18 mmol) in water (20 mL) at 0° C, then the mixture was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC (8% methanol in dichloromethane). The reaction mixture was quenched with saturated sodium bicarbonate solution (100 mL) and methanol (100 mL), then filtered through celite, washing with methanol. The filtrate was evaporated and diluted with 50 mL sodium bicarbonate, and

extracted with DCM (3 × 100 mL). The combined organic layers were dried over sodium sulfate, filtered and evaporated to give the crude product. The crude product was combined with two additional crude product batches that were prepared in a similar manner, and the combined material was purified by gradient column chromatography using methanol in dichloromethane with 0.1% isopropylamine as eluent, to afford (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide (18.5 g, 55 %) as an off-white solid. ¹HNMR (400 MHz, DMSO-d₆): δ

8.58 (d, *J* = 8 Hz, 1H), 8.34 (s, 1H), 8.27 (s, 1H), 8.08 (s, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.28 - 7.25 (m, 2H), 7.19 (d, *J* = 9.2 Hz, 1H), 4.93 - 4.90 (m, 1H), 3.90 (br s, 1H), 3.83 (d, *J* = 11.6 Hz, 2H), 3.36 (t, *J* = 10.8 Hz, 2H), 2.95 - 2.95 (m, 1H), 2.91 - 2.88 (m, 1H), 2.17 (s, 3H), 1.98 - 1.9 (br s, 2H), 1.80 (d, *J* = 12 Hz, 2H), 1.50 - 1.46 (m, 2H), LCMS calcd exact mass 473.17, found *m/z* 474.2 [M+H]⁺. HPLC purity: 99.79%, Chiral HPLC purity: 99.92%.

Example 30: (S)-N-(2-Amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide benzenesulfonic acid salt (Compound #302)

[0089]

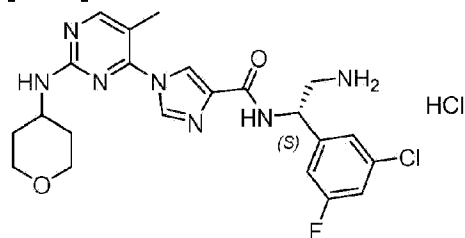


[0090] To a stirred solution of (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide (0.2 g, 0.422 mmol) in 1,4-dioxane (10 mL) was slowly added benzenesulfonic acid (0.066 g, 0.422 mmol) at 0°C. The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was evaporated, washed with diethyl ether and dried to afford (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide benzenesulfonic acid salt as an off-white solid (0.22 g, 83%). ¹HNMR (400 MHz, DMSO-d₆): δ

8.92 (d, *J* = 8.8 Hz, 1H), 8.35 (s, 1H), 8.30 (s, 1H), 8.13 (s, 1H), 7.83 (br s, 3H), 7.57 (d, *J* = 6.4 Hz, 2H), 7.37 (s, 3H), 7.28 (d, *J* = 6.4 Hz, 4H), 5.32 (d, *J* = 4.4 Hz, 1H), 3.83 (d, *J* = 11.6 Hz, 3H), 3.41 - 3.27 (m, 3H), 3.18 - 3.14 (m, 1H), 2.16 (s, 3H), 1.80 (d, *J* = 12 Hz, 2H), 1.52 - 1.44 (m, 2H). LC-MS calcd exact mass 473.17, found *m/z* 474.2 [M+H]⁺. HPLC purity 99.85%, Melting point: 161-162°C.

Example 33: (S)-N-(2-Amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide hydrochloride salt (Compound #305)

[0091]

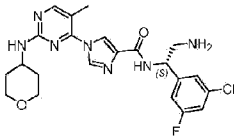
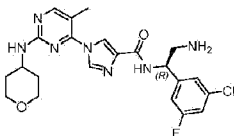
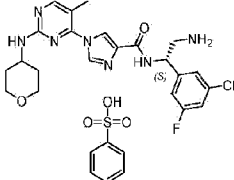
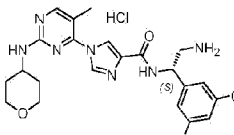


[0092] To a stirred solution of (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide (0.05 g, 0.105 mmol) in 1,4-dioxane (3 mL) was slowly added 4M HCl in dioxane (0.02 mL, 0.105 mmol) at 0°C. The reaction mixture was stirred for 1.0 h at room temperature. The reaction mixture was evaporated, washed with diethyl ether and dried to afford (S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide hydrochloride salt as an off white solid (0.05 g, 94%). ¹HNMR (400 MHz, DMSO-d₆): δ 8.95 (d, *J* = 8.8 Hz, 1H), 8.35 (s, 1H), 8.30 (s, 1H), 8.15 (s, 1H), 8.00 (br s, 3H), 7.37 (br s, 3H), 7.28 (d, *J* = 9.6 Hz, 1H), 5.33 (t, 1H), 3.85 - 3.82 (m, 3H), 3.37 - 3.33 (m, 3H), 3.25 (t, 1H), 2.16 (s, 3H), 1.80 (d, *J* = 11.6 Hz, 2H), 1.52 - 1.44 (m, 2H). LC-MS calcd exact mass 473.17, found *m/z* 474.2 for [M+H]⁺. HPLC purity 99.86%, Melting point: 210 - 211°C.

[0093] The following Table 1 provides data obtained and utilized in the characterization of the prepared compounds.

Table 1

Cmpd#	Structure	Name	NMR data
236		N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)-pyrimidin-4-yl)-1H-imidazole-4-carboxamide	¹ HNMR (400 MHz, DMSO-d ₆): δ 8.57 (d, <i>J</i> = 7.2 Hz, 1H), 8.33 (s, 1H), 8.27 (s, 1H), 8.08 (s, 1H), 7.34 (br s, 1H), 7.27 (br s, 1H), 7.18 (d, <i>J</i> = 10 Hz, 1H), 4.91 (s, 1H), 3.84 - 3.81 (m, 3H), 3.38 - 3.27 (m, 2H), 2.93 (s, 1H), 2.87 (s, 1H), 2.30 (s, 3H), 1.83 - 1.78 (m, 2H), 1.49 (br s, 2H)

Cmpd#	Structure	Name	NMR data
275		(S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide	¹ HNMR (400 MHz, DMSO-d ₆): δ 8.72 (d, <i>J</i> = 8.0 Hz, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 8.07 (s, 1H), 7.35 (d, <i>J</i> = 7.2 Hz, 1H), 7.27 - 7.24 (m, 2H), 7.17 (d, <i>J</i> = 9.2 Hz, 1H), 7.0 (bs, 1H), 5.15 - 5.05 (m, 1H), 3.95 - 3.81 (m, 3H), 3.38 - 3.35 (m, 4H), 2.16 (s, 3H), 1.81 - 1.78 (m, 2H), 1.51 - 1.41 (m, 2H), 1.30 (s, 9H).
276		(R)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide	¹ HNMR (400 MHz, DMSO-d ₆): δ 8.72 (d, <i>J</i> = 8.0 Hz, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 8.07 (s, 1H), 7.35 (d, <i>J</i> = 7.2 Hz, 1H), 7.27 - 7.24 (m, 2H), 7.17 (d, <i>J</i> = 9.2 Hz, 1H), 7.0 (bs, 1H), 5.15 - 5.05 (m, 1H), 3.95 - 3.81 (m, 3H), 3.38 - 3.35 (m, 4H), 2.16 (s, 3H), 1.81 - 1.78 (m, 2H), 1.51 - 1.41 (m, 2H), 1.30 (s, 9H).
302		(S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide benzene sulfonic acid salt	¹ HNMR (400 MHz, DMSO-d ₆): δ 8.92 (d, <i>J</i> = 8.8 Hz, 1H), 8.35 (s, 1H), 8.30 (s, 1H), 8.13 (s, 1H), 7.83 (br s, 3H), 7.57 (d, <i>J</i> = 6.4 Hz, 2H), 7.37 (s, 3H), 7.28 (d, <i>J</i> = 6.4 Hz, 4H), 5.32 (d, <i>J</i> = 4.4 Hz, 1H), 3.83 (d, <i>J</i> = 11.6 Hz, 3H), 3.41 - 3.27 (m, 3H), 3.18 - 3.14 (m, 1H), 2.16 (s, 3H), 1.80 (d, <i>J</i> = 12 Hz, 2H), 1.52 - 1.44 (m, 2H).
305		(S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide hydrochloride salt	¹ HNMR (400 MHz, DMSO-d ₆): δ 8.95 (d, <i>J</i> = 8.8 Hz, 1H), 8.35 (s, 1H), 8.30 (s, 1H), 8.15 (s, 1H), 8.00 (br s, 3H), 7.37 (br s, 3H), 7.28 (d, <i>J</i> = 9.6 Hz, 1H), 5.34 - 5.31 (m, 1H), 3.85 - 3.82 (m, 3H), 3.37 - 3.33 (m, 3H), 3.26 - 3.23 (m, 1H), 2.16 (s, 3H), 1.80 (d, <i>J</i> = 11.6 Hz, 2H).

Cmpd#	Structure	Name	NMR data
			1.52 - 1.44 (m, 2H)

Example 34. BIOLOGICAL ASSAYS

ERK1 and ERK2 HTRF (Biochemical) Assays

[0094] These assays employed a homogeneous time resolved fluorescence (HTRF) technique. The compounds were serially diluted by half-log with concentrations ranging from 0.0005 to 10 μ M in the assay buffer (50 mM Tris pH=7.5, 1 mM EGTA, 2 mM DTT, 10 mM MgCl₂, 0.1% Tween-20) and 20 μ L of substrate-ATP mix [1 μ M Biotin - LC - Myelin Basic Protein (MBP) derivatized Peptide (Anaspec)-24 μ M ATP (Sigma)] was added to each well of the assay plate. Then 10 μ L of enzyme mix [25 nM ERK1 or ERK2 (Jubilant Biosys) in assay buffer] was added to each well. The plate was incubated at room temperature for 60 min with shaking. The HTRF mix [625 nM LANCE[®] Ultra Europium-anti-phospho-MBP (Perkin Elmer) and 2 nM Phycolink[®] Streptavidin-Allophycocyanin (SA-APC) (Prozyme) in HTRF buffer (50 mM Tris-HCl pH=7.5, 100 mM NaCl, 0.1% BSA, 0.05% Tween20, 0.5 mM EDTA)] was prepared and 75 μ L of this mix was added to the HTRF plate. After incubation for 60 min at room temperature, 10 μ L of the reaction mixture was transferred to the HTRF assay plate and incubated for 45 min at room temperature with shaking. Plate was read using Pherastar in HTRF mode (excitation 337 nm, emission 665 & 620 nm). The IC₅₀ values (half maximal inhibitory concentration values) were subsequently determined using a sigmoidal dose-response curve (variable slope) in GraphPad Prism[®] 5 software. Compounds of the invention caused inhibition of ERK1 and ERK2 as determined in these assays. Representative data are provided in **Table 2**.

Cell Proliferation (Alamar Blue) Assay

[0095] HT-29 (colorectal carcinoma, B-RafV600E), HCT116 (colorectal carcinoma, K Ras G13D), A375 (melanoma, B-RafV600E) and SK-Mel2 (melanoma, NRAS Q61R) cells (obtained from ATCC, USA) were seeded (5000 cells/well) in 96-well tissue culture plate and incubated at 37 °C / 5% CO₂ for 16-24 hours. The cells were then treated with compounds, at concentrations typically from 0.0005 to 10 μ M prepared in 3-fold serial dilutions. The plates were then incubated for 72 h at 37 °C / 5% CO₂ in a moist environment. Then Alamar Blue[™] reagent (final concentration IX) was added to each well and incubated for 1-3 h at 37 °C / 5% CO₂. The plates were read on fluorescence reader at 540 nm excitation and 590 nm emission wavelengths. The IC₅₀ values were subsequently determined using a sigmoidal dose-response

curve (variable slope) using GraphPad Prism[®] 5 software. Compounds of the invention caused inhibition of HT-29, HCT116, A375 and SK-Mel2 cell proliferation as determined in these assays. Representative data for the HT-29 and HCT116 cell proliferation assays are provided in **Table 2**.

Phospho-RSK1(S380) ELISA Assay

[0096] HT-29 cells (colorectal carcinoma, B-RafV600E); obtained from ATCC, USA) were seeded (60,000 cells/well) in a 96-well plate and incubated at 37 °C / 5% CO₂ overnight and then treated with desired compound dilutions for 2 h. Medium was removed and cells were rinsed once with ice-cold IX PBS, then 0.070 mL ice-cold IX cell lysis buffer containing 1 mM PMSF was added to each well and the plate was incubated on a shaker for 2 h and 30 min at 4 °C. The plate was then centrifuged for 20 min (x4000 rpm) at 4 °C and the supernatant was transferred to a new plate. Cell lysates were diluted with sample diluent at a ratio of 1:1. The ELISA was then carried out following the manufacturer's protocol (PathScan[®] phospho-RSK1(Ser380) Sandwich ELISA Kit, Cell Signaling Technologies). The plate was read at 450 nm within 30 min after adding STOP solution. The IC₅₀ values were subsequently determined using a sigmoidal dose-response curve (variable slope) in GraphPad Prism[®] 5 software. Compounds of the invention inhibited phosphorylation of RSK1(S380) (the downstream target of ERK1/2) as determined in this assay. Representative data are provided in **Table 2**.

In vivo Studies in Tumor Xenograft Models

Tumor Cell Implantation and Randomization of Animals

[0097] Foxn1 nu/nu strain of female mice (obtained from Charles River Laboratories, USA), 8-10 weeks of age, body weight range 23-25 g, were used for the tumor xenograft efficacy studies. Human cancer cell lines (such as melanoma A375, colorectal HT29, pancreatic BxPC3, colorectal HCT116, and lung A549) were first grown in vitro, and then about five million (5×10⁶) of these cells in 100 µL of serum free medium were mixed with an equal amount of matrigel, and the entire mixture was injected subcutaneously at the right flank region of mice. The tumors were measured with Vernier calipers periodically after the first week of injection. When the tumor volume reached 120-150 mm³ (about 3-4 weeks after injection) the animals were randomized into different groups so that their tumor volume was approximately the same in all groups.

Determination of *in vivo* Efficacy of Tumor Growth Inhibition

[0098] For PO dosing, the compounds were prepared in a formulation containing 0.5% Methyl cellulose and 0.01% Tween 80. For IV, SC, or IP dosing, the compounds were prepared in 6% solutol - ethanol (1:1), 6% DMSO and 88% saline. Animals were dosed with compounds prepared in specific formulations via PO, IP or SC route either QD or BID at the required doses. Tumors size and body weights were measured twice or thrice in a week. Tumors were harvested at the end of the study after euthanizing the animals according to approved protocols. From the harvested tumor one part was snap frozen and submitted for PK studies, and the other part was homogenized and the lysates were tested for target inhibition using western blotting. Before the tumor was harvested, blood (~200 µL) was collected by ocular bleeding for PK studies.

[0099] Changes in tumor volume (Δ volumes) for each treated (T) and control (C) group were calculated by subtracting the mean tumor volume on the first day of treatment (starting day) from the mean tumor volume on the specified observation day. These values were used to calculate a percentage growth (% T/C) using the formula:

$$\% T/C = (\Delta T / \Delta C) \times 100,$$

where $\Delta T > 0$, or

$$\% T/C = (\Delta T / \Delta T_i) \times 100,$$

where $\Delta T < 0$ and T_i is the mean tumor volume at the start of the experiment. Percentage tumor growth inhibition was calculated as $[100 - \% T/C]$. Percentage body weight change was calculated as $[(\text{Body weight on specified observation day} - \text{Body weight on starting day}) / \text{Body weight on starting day}] \times 100$.

Results

[0100] Compounds of the invention were active in these in vivo tumor xenograft studies.

[0101] In a human colon cancer xenograft model (HCT116; harboring KRAS mutation), the compound of Example 275 caused approximately 90-100% tumor growth inhibition when dosed orally at 50 mg/kg BID for 24 days. There was no significant body weight loss observed at this dose in this study.

[0102] In a human lung carcinoma xenograft model (A549; harboring KRAS mutation), the compound of Example 302 caused about 65 to 82% tumor growth inhibition when dosed orally at 50 mg/kg BID for 20 days. There was no significant body weight loss observed at this dose in this study.

Table 2: Biochemical, Mechanistic and Proliferation Cell-based Assay Results

Cmpd #	Biochemical Assay ¹		Mechanistic Cell Assay ²	Cell Proliferation Assay ²	
	ERK1	ERK2	RSK1 Phos HT29	HT29	HCT116
236	A	A	A	A	A

Cmpd #	Biochemical Assay ¹		Mechanistic Cell Assay ²	Cell Proliferation Assay ²	
	ERK1	ERK2	RSK1 Phos HT29	HT29	HCT116
275	A	A	A	A	A
276	A	A	B	C	D
302	A	A	NA	NA	NA
305	A	A	NA	NA	NA
¹ Biochemical Assay: IC ₅₀ values; A: ≤50 nM, B: >50 to 100 nM, C: >100 to 500 nM, D: >500 nM to 2.5 μM, E: >2.5 μM, NA: Data not available ² Mechanistic Cell Assay and Cell Proliferation Assays: IC ₅₀ values; A: ≤100 nM, B: >100-250 nM, C: >250-500 nM, D: >500 nM-2.5 μM, E: >2.5 μM; NA: Data not available.					

[0103] In one embodiment, the compound of the invention is:

(S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide;

or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof.

[0104] In one embodiment, the compound is a substantially pure stereoisomer.

[0105] In one embodiment, a composition of the invention comprises:

(S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide;

or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof, and a pharmaceutically acceptable carrier.

[0106] In one embodiment, the composition further comprises an additional therapeutic agent.

[0107] Also described herein (but not forming part of the invention) is a method of treating a condition treatable by inhibiting ERK1/2 comprising administration to an individual in need a composition comprising a therapeutically effective amount of at least one compound of the invention as to at least slow the progression of the condition.

[0108] The condition may be cancer of prostate, head, neck, eye, mouth, throat, esophagus, bronchus, larynx, pharynx, chest, bone, lung, colon, rectum, stomach, bladder, uterus, cervix,

breast, ovaries, vagina, testicles, skin, thyroid, blood, lymph nodes, kidney, liver, intestines, pancreas, brain, central nervous system, adrenal gland, skin or a leukemia or lymphoma.

[0109] The method may comprise administration to an individual in need a composition comprising a therapeutically effective amount of:

(S)-N-(2-amino-1-(3-chloro-5-fluorophenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazole-4-carboxamide;

or a pharmaceutically acceptable salt, solvate, hydrate, or stereoisomer thereof.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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- [US6743941B \[0005\]](#)
- [US8546404B \[0005\]](#)
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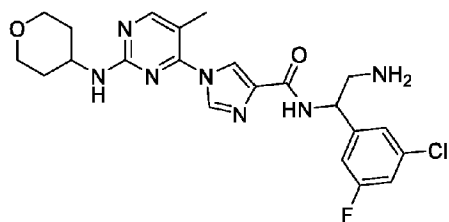
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- **REN et al.** Discovery of Highly Potent, Selective and Efficacious Small Molecule Inhibitors of ERK1/2. *J. Med. Chem.*, 2015, vol. 58, 41976-1991 [\[0005\]](#)

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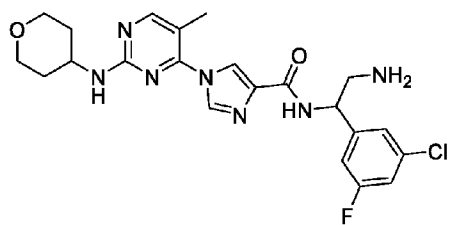
Patentkrav

1. Forbindelse, der er N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:



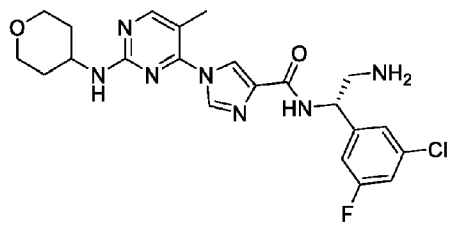
eller et farmaceutisk acceptabelt salt, solvat, hydrat eller stereoisomer deraf.

2. Forbindelse ifølge krav 1, hvor forbindelsen er N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:



eller et farmaceutisk acceptabelt salt eller stereoisomer deraf.

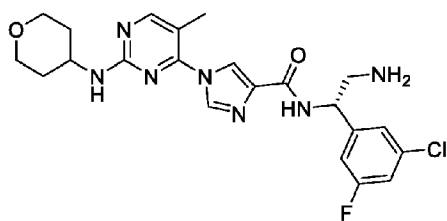
3. Forbindelse ifølge krav 2, hvor forbindelsen er (S)-N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:



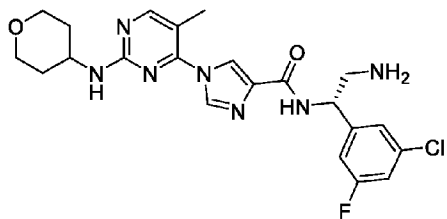
eller et farmaceutisk acceptabelt salt deraf.

4. Forbindelse ifølge et af kravene 1-3, hvor saltet er et salt af en syre udvalgt blandt eddikesyre, propionsyre, mælkesyre, citronsyre, vinsyre, ravsyre, fumarisyre, maleinsyre, malonsyre, mandelsyre, æblesyre, phthalsyre, saltsyre, hydrobromsyre, phosphorsyre, salpetersyre, svovlsyre, methansulfonsyre, naftalensulfonsyre, benzenesulfonsyre, toluenesulfonsyre, trifluoreddikesyre og camphersulfonsyre.

5. Forbindelse ifølge krav 3, hvor forbindelsen er (S)-N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:

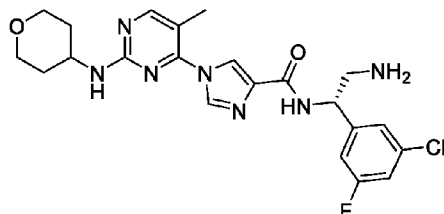


6. Forbindelse ifølge krav 4, hvor forbindelsen er (S)-N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:



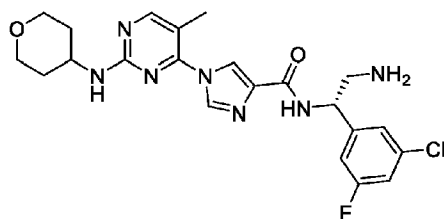
benzenesulfonsyresalt.

7. Forbindelse ifølge krav 4, hvor forbindelsen er (S)-N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:



hydrochloridsalt.

- 5 **8.** Forbindelse ifølge krav 4, hvor forbindelsen er (S)-N-(2-amino-1-(3-chlor-5-fluorphenyl)ethyl)-1-(5-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrimidin-4-yl)-1H-imidazol-4-carboxamid:



10 mandelsyresalt.

9. Sammensætning omfattende en forbindelse ifølge et af kravene 1-8 og mindst en farmaceutisk acceptabel bærer.

- 15 **10.** Forbindelse ifølge et af kravene 1-8, til anvendelse i en fremgangsmåde til behandling af en tilstand, der kan behandles ved at inhibere ERK1/2.

11. Forbindelse til anvendelse ifølge krav 10, hvor tilstanden er associeret med abnorm cellulær proliferation.

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12. Forbindelse til anvendelse ifølge krav 10, hvor tilstanden er cancer i prostata, hoved, hals, øje, mund, hals, spiserør, bronkie, strubehoved, svælg, brystkasse, knogle, lunge, colon, rektum, mave, blære, livmoder, livmoderhals, bryst, ovarier, vagina, testikler, hud, skjoldbruskkirtel, blod, lymfekirtler, nyre, lever, tarme, bugspytkirtel, hjerne, centralnervesystem, binyre, hud eller en

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leukæmi eller lymfom.

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13. Forbindelse til anvendelse ifølge et af kravene 10-12, hvor fremgangsmåden omfatter ugentlig indgivelse af forbindelsen.

14. Forbindelse til anvendelse ifølge et af kravene 10-12, hvor fremgangsmåden omfatter månedlig indgivelse af forbindelsen.

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15. Forbindelse til anvendelse ifølge et af kravene 10-12, hvor fremgangsmåden omfatter indgivelse af ækvivalente doseringer af forbindelsen omtrent hver uge.

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16. Forbindelse til anvendelse ifølge et af kravene 10-12, hvor fremgangsmåden omfatter indgivelse af ækvivalente doseringer af forbindelsen omtrent hver anden uge.