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(56) Fremdragne publikationer:
WO-A1-2008/076356
WO-A1-2009/151529
WO-A2-2004/058149
US-A- 5 180 741
US-A1- 2006 161 005
US-A1- 2007 293 545
US-A1- 2008 009 534
US-A1- 2008 249 093

Fortsættes ...

US-B1- 6 511 975
EP-B1- 1 912 640
WO-A1-2009/080729
WO-A2-02/22557
Juan Rivera; Richard L Proia; Ana Olivera: "The alliance of sphingosine 1-phosphate and its receptors in immunity", Nature Reviews Immunology, vol. 8, October 2008 (2008-10), pages 753-763,
J. SONG et al.: "A novel sphingosine 1-phosphate agonist, 2-amino-2-propanediol hydrochloride (KRP-203), regulates chronic colitis in interleukine-10 gene -deficient mice", J. Pharmacology and Experimental Therapeutics, vol. 324, no. 1, 2008, pages 276-283,
Pérez-Jeldres et al, Frontiers in Pharmacology. March 2019 Vol 10. Article 212
https //www ecco-ibd eu/publications/congress-abstracts/abstrac ts-2016/item/p372-a-multicentre-double-bli nd-placebo- controlled-parallel-group-proof-of-concept -study-to-evaluate-the-efficacy-safety-and -tolerability-of-the-s1p- receptor-modulator-krp203-in-subjects-with -moderately- active-refractory-
Gonzalez-Cabrera et al. Mol. Pharmacol. 2008; 74(5) 1308-1318
Feagan B. et al. Abstract P-012. The American Journal of Gastroenterology (2018). vol. 113. page S3
Clinical trial number NCT02435992
Feagan et al. P661 Early histological improvement demonstrated with oral Ozanimod in patients with moderately to severely active Crohn?s disease in the STEPSTONE trial", European Crohn's and Colitis Organisation (2019)
https //www.crohnsstudies.com/home/new-clinical- trials/yellowstone-program/
Weisshof et al: Adv Ther(2018) 35: 1746-1762
Scott et al . British Journal of Pharmacology (2016) 173, 1778-1792
Baumgart et al (2007), vol 369. pages 1641-1657
Park et al. Biomol Ther 25(1). 80-90 (2017)
Keller et al. The American journal of Pathology, Vol, 170, No.1, Jan 2007
https://news.bms.com/press-release/corpora tefinancial-news/bristol-myers-squibb-anno unces-positive-topline results-pivotas
Zeposia (ozanimod) - An overview of Zeposia and why it is authorised in the EU
FDA approved label for ZeposiaÂ® (ozanimod)
https://news.bms.com/news/details/2020/Eur opean-Medicines-Agency-Validates-Bristol-M yers-Squibbs

DESCRIPTION

Description

FIELD OF THE INVENTION

[0001] The invention relates to compounds which are agonists of the sphingosine 1-phosphate receptor subtype 1, methods of their synthesis and discloses methods of their therapeutic and/or prophylactic use.

BACKGROUND

[0002] The $S1P_1/EDG_1$ receptor is a G-protein coupled receptor (GPCR) and is a member of the endothelial cell differentiation gene (EDG) receptor family. Endogenous ligands for EDG receptors include lysophospholipids, such as sphingosine-1-phosphate (S1P). Like all GPCRs, ligation of the receptor propagates second messenger signals via activation of G-proteins (alpha, beta and gamma).

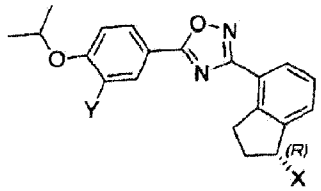
[0003] Development of small molecule $S1P_1$ agonists and antagonists has provided insight into some physiological roles of the $S1P_1$/S1P-receptor signaling system. Agonism of the $S1P_1$ receptor perturbs lymphocyte trafficking, sequestering them in lymph nodes and other secondary lymphoid tissue. This leads to rapid and reversible lymphopenia, and is probably due to receptor ligation on both lymphatic endothelial cells and lymphocytes themselves (Rosen et al, Immunol. Rev., 195:160-177,2003). A clinically valuable consequence of lymphocyte sequestration is exclusion of them from sights of inflammation and/or autoimmune reactivity in peripheral tissues.

[0004] Agonism of $S1P_1$ has also been reported to promote survival of oligodendrocyte progenitors (Miron et al, Ann. Neurol., 63:61-71, 2008). This activity, in conjunction with lymphocyte sequestration would be useful in treating inflammatory and autoimmune conditions of the central nervous system.

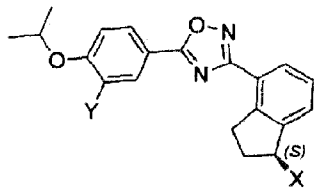
[0005] WO 2009/151529 A1 describes heterocyclic compounds adapted to act as agonists of SIP receptor subtype 1 (S1P1). WO 2004/058149 A2 describes 1-(amino)indanes and (1,2-dihydro-3-amino)-benzofurans, benzothiophenes and indoles as Edgl receptor agonists. Also these compounds are active at the S1P1 receptor and thus have immunosuppressive activities. US 5 180 741 A describes pyrenthrinoid esters carrying an indenyl nucleus and use of such compounds as pesticides.

SUMMARY OF THE INVENTION

[0006] The invention is directed to a compound of Formula I-R or Formula I-S, or a pharmaceutically acceptable salt thereof for use in a method for treating an inflammatory bowel disease (IBD) at a frequency and for a duration of time sufficient to provide a beneficial effect to the patient,



I-R



I-S

wherein

X is -$NHCH_2CH_2OH$, and

Y is -CN.

[0007] In certain embodiments, a pharmaceutical composition comprising a compound of the disclosure and a suitable excipient is provided.

[0008] In certain embodiments a method of use of a disclosed compound comprising preparation of a medicament is provided.

[0009] In certain combinations a pharmaceutical combination comprising a compound of the disclosure and a second medicament is provided. In various embodiments the second medicament is medically indicated for the treatment of multiple sclerosis, transplant rejection, acute respiratory distress syndrome or adult respiratory distress syndrome.

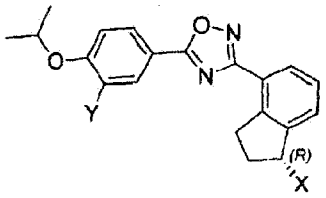
[0010] In certain embodiments, a method of activation or agonism of a sphingosine-1-phosphate receptor subtype 1 comprising contacting the receptor subtype 1 with a compound of claim 1 is provided. In various embodiments, the compound of claim 1 activates or agonizes the sphingosine-1-phosphate receptor subtype 1 to a greater degree than the compound activates or agonizes a sphingosin-1-phosphate receptor subtype 3.

[0011] In certain embodiments a method of treatment of a malcondition in a patient for which activation or agonism of an $S1P_1$ receptor is medically indicated, is provided. In various embodiment, selective activation or agonism of an $S1P_1$ receptor, such as with respect to an $S1P_3$ receptor, is medically indicated. In various embodiments, the malcondition comprises multiple sclerosis, transplant rejection, or acute respiratory distress syndrome.

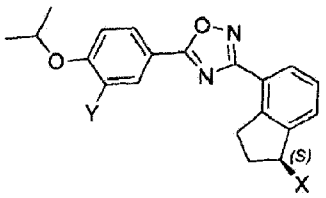
[0012] In certain embodiments, a method is provided for chiral synthesis of certain compounds including compounds of the disclosure. In certain other embodiments the disclosure provides certain intermediate compounds associated with such methods of chiral synthesis.

DETAILED DESCRIPTION OF THE INVENTION

[0013] Certain embodiments of the present disclosure comprise a compound having the structure of Formula **I-R** or **I-S** or a pharmaceutically acceptable salt, homolog, hydrate or solvate thereof:



I-R



I-S

[0014] X is defined as before. In certain embodiments, the compounds of the disclosure have the structure of Formula **I-R** or a pharmaceutically acceptable salt, ester, prodrug, homolog, hydrate or solvate thereof. In other embodiments, the compounds of the disclosure have the structure of Formula **I-S** or a pharmaceutically acceptable salt, ester, prodrug, homolog, hydrate or solvate thereof.

[0015] In certain embodiments the disclosure provides compounds which are substantially enantiomerically pure.

[0016] In certain embodiments the disclosure provides compounds which have an EC_{50} as an agonist of the wild type S1P receptor subtype 1 which is at least ten times smaller than the EC_{50} of such compound as an agonist of a mutant SIP receptor subtype 1 having a single

mutation with respect to wild type SIP receptor subtype 1 such that the 101st amino acid residue is changed from asparagine to alanine.

[0017] In certain embodiments the disclosure provides compounds which have an EC_{50} as an agonist of the wild type S1P receptor subtype 1 which is at least twenty times smaller than the EC_{50} of such compound as an agonist of a mutant SIP receptor subtype 1 having a single mutation with respect to wild type SIP receptor subtype 1 such that the 101st amino acid residue is changed from asparagine to alanine.

[0018] In certain embodiments the disclosure provides compounds which have a therapeutic index of at least 5 as measured in rats following 5 or 14 days of dosing of rats with the compound where the therapeutic index is calculated as a ratio of (i) the highest dose of such compound which achieves less than or equal to a ten percent increase in the ratio of lung to terminal body weight at the conclusion of such 5 or 14 days of dosing, to (ii) the dose of such compound achieving 50% lymphopenia in rats. In certain embodiments, such therapeutic index is at least 10 and in certain embodiments the therapeutic index is at least 20. In certain embodiments, the therapeutic index for a compound is at least five times greater than the therapeutic index for the enantiomer of such compound.

[0019] In certain embodiments the disclosure provides compounds which have a therapeutic index of at least 5 as measured in rats following 5 or 14 days of dosing of rats with the compound where the therapeutic index is calculated as a ratio of (i) the highest dose of such compound which achieves less than or equal to a ten percent increase in the ratio of lung to terminal body weight at the conclusion of such 5 or 14 days of dosing, to (ii) the dose of such compound achieving 50% lymphopenia in rats. In certain embodiments, such therapeutic index is at least 10 and in certain embodiments the therapeutic index is at least 20. In certain embodiments, the therapeutic index for a compound is greater than the therapeutic index for the enantiomer of such compound. In certain embodiments, the therapeutic index for a compound is at least 150% of the therapeutic index for the enantiomer of such compound.

[0020] In certain embodiments, a disclosed compound of Formula I is provided wherein the compound has at least one chiral center and is substantially enantiomerically pure.

[0021] In other embodiments, a pharmaceutical composition comprising a disclosed compound of Formula I and a suitable excipient is provided.

[0022] In other embodiments, a pharmaceutical combination comprising a disclosed compound and a second medicament is provided. In still other embodiments, a pharmaceutical combination comprising a disclosed compound and a second medicament is provided wherein the second medicament is medically indicated for the treatment of multiple sclerosis, transplant rejection, or adult respiratory distress syndrome.

[0023] In certain embodiments, a method of use of a disclosed compound for preparation of a

medicament is provided.

[0024] In certain embodiments, a method of activation or agonism of a sphingosine-1-phosphate receptor subtype 1 by contacting the receptor subtype 1 with an effective amount of a disclosed compound. In further embodiments, a method of activation or agonism of a sphingosine-1-phosphate receptor subtype 1 by contacting the receptor subtype 1 with an effective amount of a disclosed compound is provided, wherein the compound activates or agonizes the sphingosine-1-phosphatereceptor subtype 1 to a greater extent than the compound activates or agonizes a sphingosine-1-phosphate receptor subtype 3. In further embodiments, a method of activation or agonism of a sphingosine-1-phosphate receptor subtype 1 by contacting the receptor subtype 1 with an effective amount of a disclosed compound is provided, wherein the sphingosine-1-phosphate receptor subtype 1 is disposed within a living mammal.

[0025] In certain embodiments, a method is provided for treatment of a malcondition in a patient for which activation or agonism of an sphingosine-1-phosphate receptor subtype 1 is medically indicated, by administering an effective amount of a disclosed compound to the patient at a frequency and for a duration of time sufficient to provide a beneficial effect to the patient. In further embodiments, a method is provided for treatment of a malcondition in a patient for which activation or agonism of an sphingosine-1-phosphate receptor subtype 1 is medically indicated, by administering an effective amount of a disclosed compound to the patient at a frequency and for a duration of time sufficient to provide a beneficial effect to the patient, wherein selective activation or agonism of an SIP subtype 1 receptor with respect to other subtypes of SIP receptor is medically indicated. In yet further embodiments, a method is provided for treatment of a malcondition in a patient for which activation or agonism of an sphingosine-1-phosphate receptor subtype 1 is medically indicated, by administering an effective amount of a disclosed compound to the patient at a frequency and for a duration of time sufficient to provide a beneficial effect to the patient, wherein the malcondition comprises rejection of transplanted organs or tissue; graft-versus-host diseases brought about by transplantation; autoimmune syndromes including rheumatoid arthritis; acute respiratory distress syndrome; adult respiratory distress syndrome; influenza; cancer; systemic erythematosus; Hashimoto's thyroiditis; lymphocytic thyroiditis; multiple sclerosis; myasthenia gravis; type I and II diabetes; uveitis; posterior uveitis; uveitis associated with Behcet's disease; uveomeningitis syndrome; allergic encephalomyelitis; chronic allograft vasculopathy; post-infectious autoimmune diseases including rheumatic fever and post-infectious glomerulonephritis; inflammatory and hyperproliferative skin diseases; cutaneous manifestations of immunologically-mediated disorders; psoriasis; atopic dermatitis; osteomyelitis; contact dermatitis; eczematous dermatitis; seborrhoeic dermatitis; lichen planus; pemphigus; bullous pemphigoid; epidermolysis bullosa; urticaria; angioedema; vasculitis; erythema; cutaneous eosinophilia; acne; alopecia areata; keratoconjunctivitis; vernal conjunctivitis; keratitis; herpetic keratitis; dystrophia epithelialis corneae; corneal leukoma; ocular pemphigus; Mooren's ulcer; ulcerative keratitis; scleritis; Graves' ophthalmopathy; Vogt-Koyanagi-Harada syndrome; sarcoidosis; pollen allergies; reversible obstructive airway disease; bronchial asthma; allergic asthma; intrinsic asthma; extrinsic asthma; dust asthma;

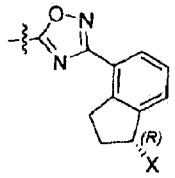
chronic or inveterate asthma; late asthma and airway hyper-responsiveness; bronchitis; gastric ulcers; ischemic bowel diseases; inflammatory bowel diseases; necrotizing enterocolitis; intestinal lesions associated with thermal burns; celiac diseases; proctitis; eosinophilic gastroenteritis; mastocytosis; Crohn's disease; ulcerative colitis; vascular damage caused by ischemic diseases and thrombosis; atherosclerosis; fatty heart; myocarditis; cardiac infarction; arteriosclerosis; aortitis syndrome; cachexia due to viral disease; vascular thrombosis; migraine; rhinitis; eczema; interstitial nephritis; IgA-induced nephropathy; Goodpasture's syndrome; hemolytic-uremic syndrome; diabetic nephropathy; glomerulosclerosis; glomerulonephritis; multiple myositis; Guillain-Barre syndrome; Meniere's disease; polyneuritis; multiple neuritis; mononeuritis; radiculopathy; hyperthyroidism; Basedow's disease; thyrotoxicosis; pure red cell aplasia; aplastic anemia; hypoplastic anemia; idiopathic thrombocytopenic purpura; autoimmune hemolytic anemia; agranulocytosis; perniclous anemia; megaloblastic anemia; anerythroplasia; osteoporosis; sarcoidosis; fibroid lung; idiopathic interstitial pneumonia; dermatomyositis; leukoderma vulgaris; ichthyosis vulgaris; photoallergic sensitivity; cutaneous T cell lymphoma; polyarteritis nodosa; Huntington's chorea; Sydenham's chorea; myocardosis; scleroderma; Wegener's granuloma; Sjogren's syndrome; adiposis; eosinophilic fascitis; lesions of gingiva, periodontium, alveolar bone, substantia ossea dentis; male pattern alopecia or alopecia senilis; muscular dystrophy; pyoderma; Sezary's syndrome; chronic adrenal insufficiency; Addison's disease; ischemia-reperfusion injury of organs which occurs upon preservation; endotoxin shock; pseudomembranous colitis; colitis caused by drug or radiation; ischemic acute renal insufficiency; chronic renal insufficiency; lung cancer; malignancy of lymphoid origin; acute or chronic lymphocytic; leukemias; lymphoma; psoriasis; inflammatory lung injury, pulmonary emphysema; cataracta; siderosis; retinitis pigmentosa; senile macular degeneration; vitreal scarring; inflammatory eye disease; corneal alkali burn; dermatitis erythema; ballous dermatitis; cement dermatitis; gingivitis; periodontitis; sepsis; pancreatitis; carcinogenesis; metastasis of carcinoma; hypobaropathy; autoimmune hepatitis; primary biliary cirrhosis; sclerosing cholangitis; partial liver resection; acute liver necrosis; cirrhosis; alcoholic cirrhosis; hepatic failure; fulminant hepatic failure; late-onset hepatic failure; "acute-on-chronic" liver failure. In yet further embodiments, the malcondition is one or more of rejection of transplanted organs or tissue; graft-versus-host diseases brought about by transplantation; autoimmune syndromes including rheumatoid arthritis, multiple sclerosis, myasthenia gravis; pollen allergies; type I diabetes; prevention of psoriasis; Crohn's disease; ulcerative colitis, acute respiratory distress syndrome; adult respiratory distress syndrome; influenza; post-infectious autoimmune diseases including rheumatic fever and post-infectious glomerulonephritis; and metastasis of carcinoma. In yet further empbodiments the malcondition is one of influenza, ulcerative colitis, multiple sclerosis, transplant rejection, acute respiratory distress syndrome or adult respiratory distress syndrome.

[0026] In certain embodiments, methods are provided for use of a disclosed compound for preparation of a medicament adapted for treatment of a disorder or a malcondition wherein activation or inhibition of a sphingosine-1-phosphate receptor subtype 1 is medically indicated.

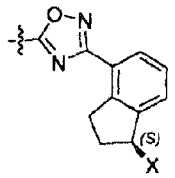
[0027] Described herein is a method for the chiral synthesis of a compound comprising an indane moiety having a chiral carbon in the five-membered ring of the indane moiety where the

compound is enantiomerically enriched with respect to the chiral carbon. In such embodiments, the method of the disclosure provides the steps of (i) providing a compound comprising an indane moiety where the ring carbon of the five-membered ring of the indane moiety where chiral substitution is desired is oxo substituted at such carbon; and (ii) reacting such compound with a chiral reagent selected from the group consisting of a Corey Bakshita Shibata-oxazaborolidine and a chiral sulfinamide of the form $RS(=O)NH_2$ where R is a bulky group [e.g. t-butyl]. In certain embodiments R is t-butyl, sec-butyl, isopropyl, cyclopropyl, adamantyl, C_{3-6} branched alkyl, or optionally bridged C_{3-8} cycloalkyl. In certain of such embodiments, the chiral reagent is a Corey Bakshita Shibata-oxazaborolidine and the compound comprising an indane moiety is enantiomerically enriched with respect to a carbon-oxygen bond on a ring carbon of the five-membered ring of the indane moiety. In further embodiments, the chiral reagent is (*R*)-(-)-(2)-methyl-CBS-oxazaborolidine or (*S*)-(-)-(2)-methyl-CBS-oxazaborolidine.

[0028] In certain of such embodiments, the compound comprising an indane moiety having a chiral carbon in the five-membered ring of the indane moiety is a compound comprising an oxadiazole-indane moiety having a chiral carbon in the five-membered ring of the indane moiety of Formula **III-R** or **III-S:**

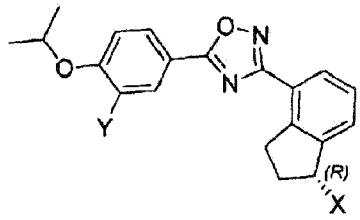


III-R

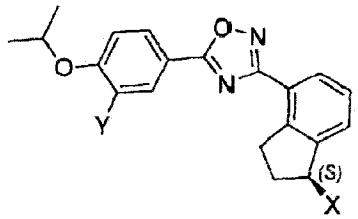


III-S

[0029] In certain embodiments, the disclosure provides a method for the chiral synthesis of the structure of Formula **I-R** or **I-S** or a pharmaceutically acceptable salt, ester, prodrug, homolog, hydrate or solvate thereof:



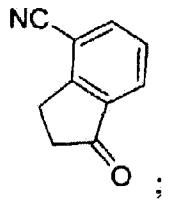
I-R



I-S

[0030] Where X and Y are as defined above and where the compound is enantiomerically enriched with respect to the chiral carbon. In such embodiments, the method of the disclosure provides the steps of

(i) providing the compound



;

and

(ii) reacting such compound with a chiral reagent selected from the group consisting of a Corey Bakshita Shibata-oxazaborolidine and a chiral sulfinamide of the form $RS(=O)NH_2$ where R is a bulky group [e.g. t-butyl, branched alkyl or cycloalkyl]; and

(iii) forming a chiral center at the indane moiety carbon previously bound to the oxo group by either reacting such compound with a suitable reducing agent along with the chiral reagent in step (ii) or reacting the result of the reaction of such compound with a suitable reducing agent.

[0031] In certain of such embodiments, the chiral reagent is a Corey Bakshita Shibata-oxazaborolidine and X is -OR'''. In further embodiments, the chiral reagent is (*R*)-(-)-(2)-methyl-CBS-oxazaborolidine or (*S*)-(-)-(2)-methyl-CBS-oxazaborolidine.

[0032] In certain of such embodiments the chiral reagent is $RS(=O)NH_2$ where R is branched alkyl or cycloalkyl and X is NR' R''. In further such embodiments, the chiral reagent is t-Bu-$S(=O)NH_2$.

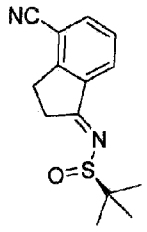
[0033] In certain of such embodiments a suitable reducing reagent includes a borohydride such as BH_3-DMS or $NaBH_4$.

[0034] Additional steps for the preparation of such compounds can be adapted from the synthetic methods disclosed herein including recrystallization and other processes for purification.

[0035] In certain of such embodiments the disclosure provides a method of synthesizing a chiral compound of the disclosure by (i) providing a compound comprising an indane moiety where the ring carbon of the five-membered ring of the indane moiety where chiral substitution is desired is oxo substituted at such carbon; (ii) reacting such compound with a chiral reagent

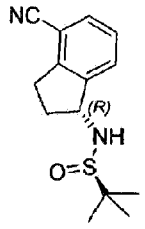
selected from the group consisting of a Corey Bakshita Shibata-oxazaborolidine and a chiral sulfinamide of the form $RS(=O)NH_2$ where R is a bulky group [e.g. t-butyl or other branched alkyl or cycloalkyl]; and (iii) forming a chiral center at the indane moiety carbon previously bound to the oxo group by either reacting such compound with a suitable reducing agent along with the chiral reagent in step (ii) or reacting the result of the reaction of such compound with a suitable reducing agent.

[0036] In certain embodiments, the compound comprising an indane moiety provided in step (i) is contacted with the chiral reagent to form in step (ii) Formula **VI**:

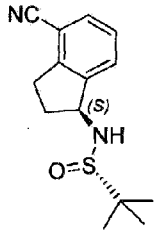


VI.

[0037] In certain embodiments, the compound of Formula **VII-R** or **VII-S** is formed in step (iii):



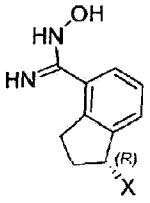
VII-R



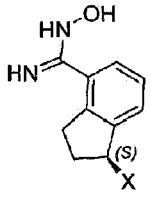
VII-S.

[0038] In certain embodiments, the compound comprising an indane moiety in step (i) has a cyano substituent on the 4-position of the indane ring.

[0039] In certain embodiments, the method further comprises the step of (iv) treating the compound with a chiral center at the indane moiety carbon resulting from step (iii) with a hydroxylamine or a hydroxylamine hydrochloride to convert the cyano substituent to a hydroxyamidine at the 4 position of the indane moiety having the Formula **IV-R** or **IV-S**:



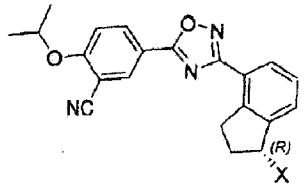
IV-R



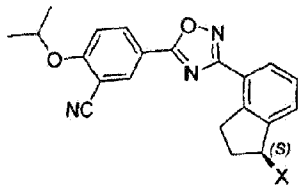
IV-S.

[0040] In further embodiments, step (iv) is carried out in the presence of a base.

[0041] In certain embodiments, the method further comprises the step of (v) contacting Formula **IV-R** or **IV-S** with substituted benzoic acid and a coupling reagent to form a compound of Formula **V-R** or **V-S:**



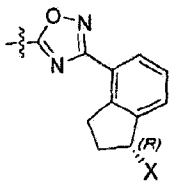
V-R



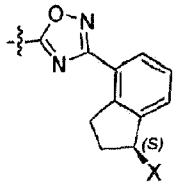
V-S.

[0042] In further embodiments, the coupling reagent used in step (v) is a mixture comprising hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). Other suitable coupling reagents, for example, HOAt, HATU, HBTU, HOOBt, can be used in the reaction of the disclosure.

[0043] In certain embodiments, the compound comprising an indane moiety having a chiral carbon in the five-membered ring of the indane moiety is a compound of the Formula **III-R** or **III-S**:



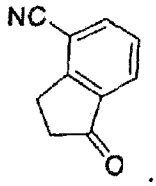
III-R



III-S.

[0044] In certain embodiments, the disclosure provides a compound comprising an indane moiety having a chiral carbon in the five-membered ring of the indane moiety of the disclosure.

[0045] In certain embodiments, the compound comprising an indane moiety where the ring carbon of the five-membered ring of the indane moiety where chiral substitution is desired is oxo substituted at such carbon is

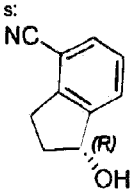


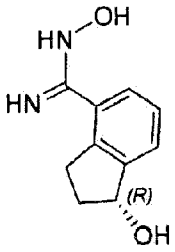
[0046] In certain of such embodiments, the disclosure provides a method for chiral synthesis of a chiral compound comprising an indane moiety having a chiral carbon in the five-membered ring of the indane moiety or a chiral compound comprising an oxadiazole-indane moiety having a chiral carbon in the five-membered ring of the indane moiety where the chiral compound has an enantiomeric enrichment of at least 75%, 85%, 90%, 95%, 98%, or 99%.

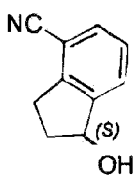
[0047] In certain of such embodiments, the disclosure provides a method for synthesis of a chiral compound of the disclosure having an enantiomeric enrichment of at least 75%, 85%, 90%, 95%, 98%, or 99%.

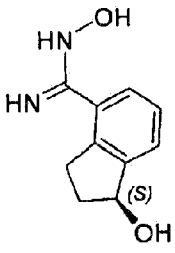
[0048] In certain embodiments, the disclosure provides compounds which can be intermediates in the herein described methods for chiral syntheses. In certain such embodiments, the disclosure provides one or more of the following compounds:

s:



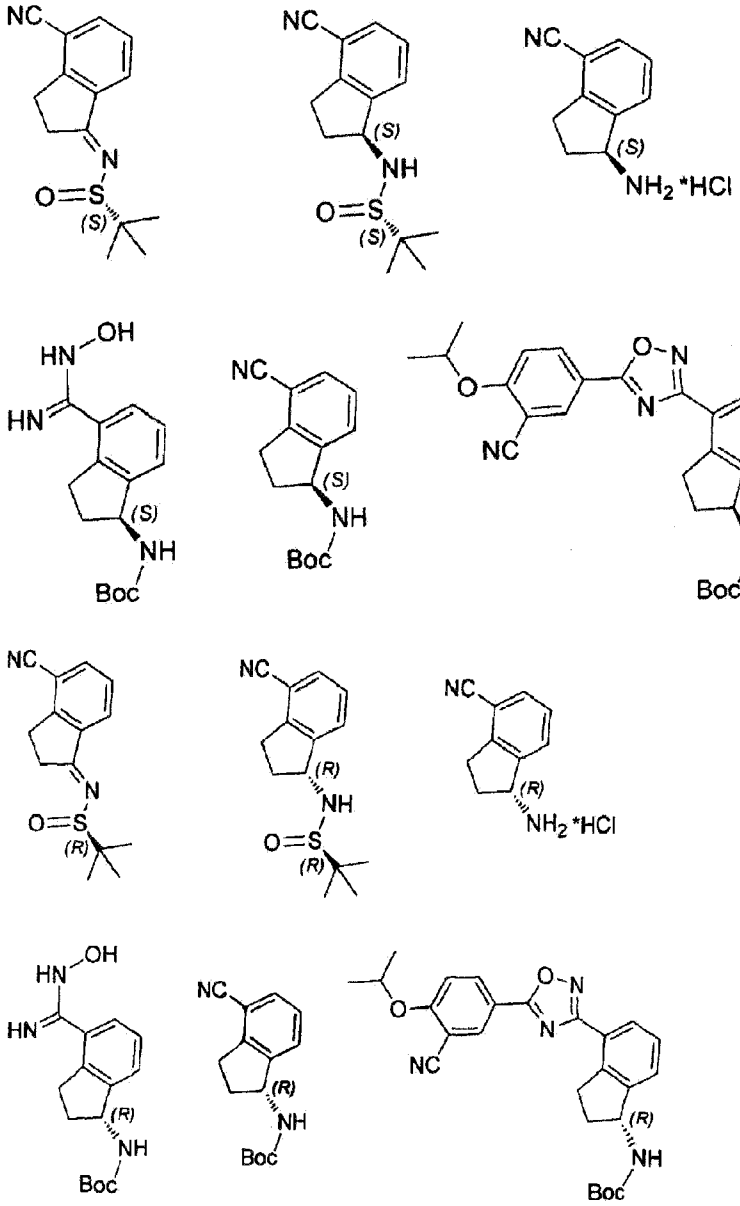






[0049] In certain other such embodiments, the disclosure provides one or more of the following

compounds:



[0050] In certain embodiments, a method for the synthesis of a compound comprising an indane moiety having a chiral carbon in the five-membered ring of the indane moiety where the compound is enantiomerically enriched with respect to the chiral carbon is provided. In certain embodiments, a method comprising a step of providing a compound of the structures described herein is provided.

[0051] Protecting groups can render chemical functionality inert to specific reaction conditions and can be appended to and removed from such functionality in a molecule without substantially damaging the remainder of the molecule. Practitioners in the art would be familiar with suitable protecting groups for use in the synthetic methods of the disclosure. See, *e.g.*, Greene and Wuts, Protective Groups in Organic Synthesis, 2nd ed., John Wiley & Sons, New York, 1991.

[0052] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0053] As used herein, "individual" (as in the subject of the treatment) means both mammals and non-mammals. Mammals include, for example, humans; non-human primates, e.g. apes and monkeys; cattle; horses; sheep; and goats. Non-mammals include, for example, fish and birds.

[0054] The term "$S1P_1$" as used herein refers to subtype 1 of a sphingosine-1-phosphate receptor, while other sphingosine-1-phosphate receptor subtypes are referred to in a corresponding manner, for example, sphingosine-1-phosphate receptor subtype 3 is referred to as "$S1P_3$".

[0055] A "receptor", as is well known in the art, is a biomolecular entity usually comprising a protein that specifically binds a structural class of ligands or a single native ligand in a living organism, the binding of which causes the receptor to transduce the binding signal into another kind of biological action, such as signaling a cell that a binding event has occurred, which causes the cell to alter its function in some manner. An example of transduction is receptor binding of a ligand causing alteration of the activity of a "G-protein" in the cytoplasm of a living cell. Any molecule, naturally occurring or not, that binds to a receptor and activates it for signal transduction, is referred to as an "agonist" or "activator." Any molecule, naturally occurring or not, that binds to a receptor, but does not cause signal transduction to occur, and which can block the binding of an agonist and its consequent signal transduction, is referred to as an "antagonist."

[0056] An "$S1P_1$ compound" or "$S1P_1$ agonist" or "$S1P_1$ activator" or "$S1P_1$ inhibitor" or "$S1P_1$ antagonist" as the terms are used herein refer to compounds that interact in some way with the S1P receptor subtype 1. They can be agonist or activators, or they can be antagonists or inhibitors. An "$S1P_1$ compound" of the disclosure can be selective for action on subtype 1 of the SIP receptor family; for example a compound of the disclosure can act at a lower concentration on subtype 1 of the SIP receptor family than on other subtypes of the SIP receptor family; more specifically, an "$S1P_1$ compound" of the disclosure can selectively act on subtype 1 receptors compared to its action on subtype 3, or "$S1P_3$" receptors.

[0057] In certain embodiments, compounds of the disclosure are orthostatic agonists. In certain other embodiments, compounds of the disclosure are allosteric agonists. Receptor agonists may be classified as either orthosteric or allosteric. An orthosteric agonist binds to a site in the receptor that significantly overlaps with the binding of the natural ligand and replicates the key interactions of the natural ligand with the receptor. An orthosteric agonist will activate the receptor by a molecular mechanism similar to that of the natural ligand, will be competitive for the natural ligand, and will be competitively antagonized by pharmacological agents that are competitive antagonists for the natural ligand. An allosteric agonist binds to a site in the receptor that makes some significant interactions that are partly or wholly non-

overlapping with the natural ligand. Allosteric agonists are true agonists and not allosteric potentiators. Consequently, they activate receptor signaling alone and without a requirement for a sub-maximal concentration of the natural ligand. Allosteric agonists may be identified when an antagonist known to be competitive for the orthosteric ligand shows non-competitive antagonism. The allosteric agonist site can also be mapped by receptor mutagenesis. The introduction of single point mutations in receptors that retain receptor activation by allosteric agonist, while diminishing or abolishing signaling induced by orthosteric agonist or vice versa provide formal evidence for differences in binding interactions. Orthosteric agonists may destabilize GPCR structure and conformation, while allosteric agonists may either stabilize or destabilize GPCR structure and conformation. Allosteric agonists, by virtue of their different interactions with receptor, may be pharmaceutically useful because the allosteric site may confer additional opportunities for agonist potency and selectivity within a related family of receptor subtypes that share a similar orthosteric ligand. In addition, the allosteric site may require very different physical and chemical properties of an agonist compared to the orthosteric ligand. These chemico-physical properties, which include hydrophobicity, aromaticity, charge distribution and solubility may also provide advantages in generating agonists of varying pharmacokinetic, oral bioavailability, distributional and metabolism profiles that facilitate the development of effective pharmaceutical substances.

[0058] "Substantially" as the term is used herein means completely or almost completely; for example, a composition that is "substantially free" of a component either has none of the component or contains such a trace amount that any relevant functional property of the composition is unaffected by the presence of the trace amount, or a compound is "substantially pure" is there are only negligible traces of impurities present.

[0059] Substantially enantiomerically pure means a level of enantiomeric enrichment of one enantiomer with respect to the other enantiomer of at least 90%, 95%, 98%, 99%, 99.5% or 99.9%.

[0060] "Treating" or "treatment" within the meaning herein refers to an alleviation of symptoms associated with a disorder or disease, or inhibition of further progression or worsening of those symptoms, or prevention or prophylaxis of the disease or disorder.

[0061] The expression "effective amount", when used to describe use of a compound of the disclosure in providing therapy to a patient suffering from a disorder or malcondition mediated by a sphingosine-1-phospate receptor of subtype 1 refers to the amount of a compound of the disclosure that is effective to bind to as an agonist or as an antagonist a $S1P_1$ receptor in the individual's tissues, wherein the $S1P_1$ is implicated in the disorder, wherein such binding occurs to an extent sufficient to produce a beneficial therapeutic effect on the patient. Similarly, as used herein, an "effective amount" or a "therapeutically effective amount" of a compound of the disclosure refers to an amount of the compound that alleviates, in whole or in part, symptoms associated with the disorder or condition, or halts or slows further progression or worsening of those symptoms, or prevents or provides prophylaxis for the disorder or condition. In particular, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods

of time necessary, to achieve the desired therapeutic result by acting as an agonist of sphingosine-1-phosphate receptor subtype 1 ($S1P_1$) activity. A therapeutically effective amount is also one in which any toxic or detrimental effects of compounds of the disclosure are outweighed by the therapeutically beneficial effects. For example, in the context of treating a malcondition mediated by activation of $S1P_1$, a therapeutically effective amount of an S1 P_1 agonist of the disclosure is an amount sufficient to control the malcondition, to mitigate the progress of the malcondition, or to relieve the symptoms of the malcondition. Examples of malconditions that can be so treated include multiple sclerosis, transplant rejection, adult respiratory distress syndrome.

[0062] Diseases, disorders and conditions which may be treated by compounds of the disclosure include rejection of transplanted organs or tissue; graft-versus-host diseases brought about by transplantation; autoimmune syndromes including rheumatoid arthritis; acute respiratory distress syndrome; adult respiratory distress syndrome; influenza; cancer; systemic erythematosus; Hashimoto's thyroiditis; lymphocytic thyroiditis; multiple sclerosis; myasthenia gravis; type I and II diabetes; uveitis; posterior uveitis; uveitis associated with Behcet's disease; uveomeningitis syndrome; allergic encephalomyelitis; chronic allograft vasculopathy; post-infectious autoimmune diseases including rheumatic fever and post-infectious glomerulonephritis; inflammatory and hyperproliferative skin diseases; cutaneous manifestations of immunologically-mediated disorders; psoriasis; atopic dermatitis; osteomyelitis; contact dermatitis; eczematous dermatitis; seborrhoeic dermatitis; lichen planus; pemphigus; bullous pemphigoid; epidermolysis bullosa; urticaria; angioedema; vasculitis; erythema; cutaneous eosinophilia; acne; alopecia areata; keratoconjunctivitis; vernal conjunctivitis; keratitis; herpetic keratitis; dystrophia epithelialis corneae; corneal leukoma; ocular pemphigus; Mooren's ulcer; ulcerative keratitis; scleritis; Graves' ophthalmopathy; Vogt-Koyanagi-Harada syndrome; sarcoidosis; pollen allergies; reversible obstructive airway disease; bronchial asthma; allergic asthma; intrinsic asthma; extrinsic asthma; dust asthma; chronic or inveterate asthma; late asthma and airway hyper-responsiveness; bronchitis; gastric ulcers; ischemic bowel diseases; inflammatory bowel diseases; necrotizing enterocolitis; intestinal lesions associated with thermal burns; celiac diseases; proctitis; eosinophilic gastroenteritis; mastocytosis; Crohn's disease; ulcerative colitis; vascular damage caused by ischemic diseases and thrombosis; atherosclerosis; fatty heart; myocarditis; cardiac infarction; arteriosclerosis; aortitis syndrome; cachexia due to viral disease; vascular thrombosis; migraine; rhinitis; eczema; interstitial nephritis; IgA-induced nephropathy; Goodpasture's syndrome; hemolytic-uremic syndrome; diabetic nephropathy; glomerulosclerosis; glomerulonephritis; multiple myositis; Guillain-Barre syndrome; Meniere's disease; polyneuritis; multiple neuritis; mononeuritis; radiculopathy; hyperthyroidism; Basedow's disease; thyrotoxicosis; pure red cell aplasia; aplastic anemia; hypoplastic anemia; idiopathic thrombocytopenic purpura; autoimmune hemolytic anemia; agranulocytosis; pernicious anemia; megaloblastic anemia; anerythroplasia; osteoporosis; sarcoidosis; fibroid lung; idiopathic interstitial pneumonia; dermatomyositis; leukoderma vulgaris; ichthyosis vulgaris; photoallergic sensitivity; cutaneous T cell lymphoma; polyarteritis nodosa; Huntington's chorea; Sydenham's chorea; myocardosis; scleroderma; Wegener's granuloma; Sjogren's syndrome; adiposis; eosinophilic fascitis; lesions of gingiva, periodontium, alveolar bone, substantia ossea

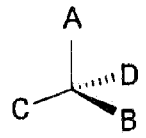
dentis; male pattern alopecia or alopecia senilis; muscular dystrophy; pyoderma; Sezary's syndrome; chronic adrenal insufficiency; Addison's disease; ischemia-reperfusion injury of organs which occurs upon preservation; endotoxin shock; pseudomembranous colitis; colitis caused by drug or radiation; ischemic acute renal insufficiency; chronic renal insufficiency; lung cancer; malignancy of lymphoid origin; acute or chronic lymphocytic; leukemias; lymphoma; psoriasis; inflammatory lung injury, pulmonary emphysema; cataracta; siderosis; retinitis pigmentosa; senile macular degeneration; vitreal scarring; inflammatory eye disease; corneal alkali burn; dermatitis erythema; ballous dermatitis; cement dermatitis; gingivitis; periodontitis; sepsis; pancreatitis; carcinogenesis; metastasis of carcinoma; hypobaropathy; autoimmune hepatitis; primary biliary cirrhosis; sclerosing cholangitis; partial liver resection; acute liver necrosis; cirrhosis; alcoholic cirrhosis; hepatic failure; fulminant hepatic failure; late-onset hepatic failure; "acute-on-chronic" liver failure. Particularly preferred diseases and conditions which may be treated with compounds of the disclosure comprise the group consisting of rejection of transplanted organs or tissue; graft-versus-host diseases brought about by transplantation; autoimmune syndromes including rheumatoid arthritis, multiple sclerosis, myasthenia gravis; pollen allergies; type I diabetes; prevention of pso-riasis; Crohn's disease; ulcerative colitis, acute respiratory distress syndrome; adult respiratory distress syndrome; influenza; post-infectious autoimmune diseases including rheumatic fever and post-infectious glomerulonephritis; and metastasis of carcinoma.

[0063] Furthermore, compounds of Formula **I-R or I-S** are also useful, in combination with one or several immunosuppressant agents, for the treatment of diseases, disorders and conditions associated with an activated immune system and selected from the list as above-mentioned. According to a preferred embodiment of the invention, said immunosuppressant agent is selected from the group comprising or consisting of cyclosporin, daclizumab, basiliximab, everolimus, tacrolimus (FK506), azathiopirene, leflunomide, 15-deoxyspergualin, or other immunosuppressant drugs

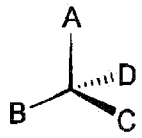
[0064] All chiral, diastereomeric, racemic forms of a structure are intended, unless a particular stereochemistry or isomeric form is specifically indicated. Compounds used in the present invention can include enriched or resolved optical isomers at any or all asymmetric atoms as are apparent from the depictions, at any degree of enrichment. Both racemic and diastereomeric mixtures, as well as the individual optical isomers can be synthesized so as to be substantially free of their enantiomeric or diastereomeric partners, and these are all within the scope of certain embodiments of the invention.

[0065] The isomers resulting from the presence of a chiral center comprise a pair of non-superimposable isomers that are called "enantiomers." Single enantiomers of a pure compound are optically active, *i.e.*, they are capable of rotating the plane of plane polarized light. Single enantiomers are designated according to the *Cahn-Ingold Prelog* system. Once the priority ranking of the four groups is determined, the molecule is oriented so that the lowest ranking group is pointed away from the viewer. Then, if the descending rank order of the other groups proceeds clockwise, the molecule is designated (*R*) and if the descending rank of the other groups proceeds counterclockwise, the molecule is designated (*S*). In the examples, the

Cahn-Ingold-Prelog ranking is A > B > C > D. The lowest ranking atom, D is oriented away from the viewer.



(*R*)-configuration



(*S*)-configuration

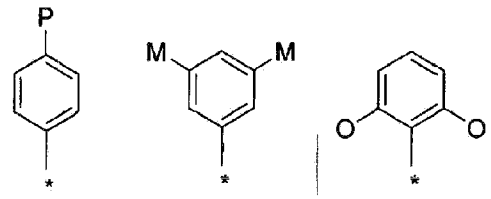
[0066] "Isolated optical isomer" means a compound which has been substantially purified from the corresponding optical isomer(s) of the same formula. Preferably, the isolated isomer is at least about 80%, more preferably at least 90% pure, even more preferably at least 98% pure, most preferably at least about 99% pure, by weight.

Rotational Isomerism

[0067] It is understood that due to chemical properties (*i.e.*, resonance lending some double bond character to the C-N bond) of restricted rotation about the amide bond linkage (as illustrated below) it is possible to observe separate rotamer species and even, under some circumstances, to isolate such species, example shown below. It is further understood that certain structural elements, including steric bulk or substituents on the amide nitrogen, may enhance the stability of a rotamer to the extent that a compound may be isolated as, and exist indefinitely, as a single stable rotamer. The present invention therefore includes any possible stable rotamers of compounds of the invention which are biologically active in the treatment of a disease, disorder or condition for which a compound of the invention may be effective as described herein.

Regioisomerism

[0068] The preferred compounds of the present invention have a particular spatial arrangement of substituents on the aromatic rings, which is related to the structure activity relationship demonstrated by the compound class. Often such substitution arrangement is denoted by a numbering system; however, numbering systems are often not consistent between different ring systems. In six-membered aromatic systems, the spatial arrangements are specified by the common nomenclature "para" for 1,4-substitution, "meta" for 1,3-substitution and "ortho" for 1,2-substitution as shown below.



"para" "meta" "ortho"

[0069] All structures encompassed within a claim are "chemically feasible", by which is meant that the structure depicted by any combination or subcombination of optional substituents meant to be recited by the claim is physically capable of existence with at least some stability as can be determined by the laws of structural chemistry and by experimentation. Structures that are not chemically feasible are not within a claimed set of compounds.

[0070] The terms "comprising," "including," "having," "composed of," are open-ended terms as used herein, and do not preclude the existence of additional elements or components. In a claim element, use of the forms "comprising," "including," "having," or "composed of" means that whatever element is comprised, had, included, or composes is not necessarily the only element encompassed by the subject of the clause that contains that word.

[0071] A "salt" as is well known in the art includes an organic compound such as a carboxylic acid, a sulfonic acid, or an amine, in ionic form, in combination with a counterion. For example, acids in their anionic form can form salts with cations such as metal cations, for example sodium, potassium, and the like; with ammonium salts such as NH_4^+ or the cations of various amines, including tetraalkyl ammonium salts such as tetramethylammonium and alkyl ammonium salts such as tromethamine salts, or other cations such as trimethylsulfonium, and the like. A "pharmaceutically acceptable" or "pharmacologically acceptable" salt is a salt formed from an ion that has been approved for human consumption and is generally non-toxic, such as a chloride salt or a sodium salt. A "zwitterion" is an internal salt such as can be formed in a molecule that has at least two ionizable groups, one forming an anion and the other a cation, which serve to balance each other. For example, amino acids such as glycine can exist in a zwitterionic form. A "zwitterion" is a salt within the meaning herein. The compounds of the present invention may take the form of salts. The term "salts" embraces addition salts of free acids or free bases which are compounds of the invention. Salts can be "pharmaceutically-acceptable salts." The term "pharmaceutically-acceptable salt" refers to salts which possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for example utility in process of synthesis, purification or formulation of compounds of the invention.

[0072] Suitable pharmaceutically-acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic,

mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β-hydroxybutyric, salicylic, galactaric and galacturonic acid. Examples of pharmaceutically unacceptable acid addition salts include, for example, perchlorates and tetrafluoroborates.

[0073] Suitable pharmaceutically acceptable base addition salts of compounds of the invention include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, *N,N'*-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Examples of pharmaceutically unacceptable base addition salts include lithium salts and cyanate salts. Although pharmaceutically unacceptable salts are not generally useful as medicaments, such salts may be useful, for example as intermediates in the synthesis of compounds, for example in their purification by recrystallization. All of these salts may be prepared by conventional means from the corresponding compound by reacting, for example, the appropriate acid or base with the compound. The term "pharmaceutically acceptable salts" refers to nontoxic inorganic or organic acid and/or base addition salts, see, for example, Lit et al., Salt Selection for Basic Drugs (1986), Int J. Pharm., 33, 201-217, incorporated by reference herein

[0074] Nonlimiting examples of potential salts of this invention include but are not limited to hydrochloride, citrate, glycolate, fumarate, malate, tartrate, mesylate, esylate, cinnamate, isethionate, sulfate, phosphate, diphosphate, nitrate, hydrobromide, hydroiodide, succinate, formate, acetate, dichloroacetate, lactate, *p*-toluenesulfonate, pamitate, pidolate, pamoate, salicylate, 4-aminosalicylate, benzoate, 4-acetamido benzoate, glutamate, aspartate, glycolate, adipate, alginate, ascorbate, besylate, camphorate, camphorsulfonate, camsylate, caprate, caproate, cyclamate, laurylsulfate, edisylate, gentisate, galactarate, gluceptate, gluconate, glucuronate, oxoglutarate, hippurate, lactobionate, malonate, maleate, mandalate, napsylate, napadisylate, oxalate, oleate, sebacate, stearate, succinate, thiocyanate, undecylenate, and xinafoate.

[0075] A "hydrate" is a compound that exists in a composition with water molecules. The composition can include water in stoichiometic quantities, such as a monohydrate or a dihydrate, or can include water in random amounts. As the term is used herein a "hydrate" refers to a solid form, i.e., a compound in water solution, while it may be hydrated, is not a hydrate as the term is used herein.

[0076] A "homolog" of a compound of the disclosure is a compound having one or more atoms of the compound replaced by an isotope of such atom. For example, homologs include compounds with deuterium in place of some hydrogen atoms of the compound such as compounds of the disclosure in which the methyl groups of the isopropoxy moiety of Formulas I-R and I-S are fully or partially deuterated (*e.g.*, $(D_3C)_2C$-O-). Isotopic substitutions which may be made in the formation of homologs of the disclosure include nonradioactive (stable) atoms

such as deuterium and carbon 13, as well as radioactive (unstable) atoms such as tritium, carbon 14, iodine 123, iodine 125, etc.

[0077] A "solvate" is a similar composition except that a solvent other that water replaces the water. For example, methanol or ethanol can form an "alcoholate", which can again be stoichiometic or non-stoichiometric. As the term is used herein a "solvate" refers to a solid form, i.e., a compound in solution in a solvent, while it may be solvated, is not a solvate as the term is used herein.

[0078] A "prodrug" as is well known in the art is a substance that can be administered to a patient where the substance is converted in vivo by the action of biochemicals within the patients body, such as enzymes, to the active pharmaceutical ingredient. Examples of prodrugs include esters of carboxylic acid groups, which can be hydrolyzed by endogenous esterases as are found in the bloodstream of humans and other mammals.

[0079] Any compound which can be converted *in vivo* to the active drug by chemical or biochemical transformations functions as a prodrug. Prodrugs of claimed compounds are covered under this disclosure.

[0080] Some examples of prodrugs within the scope of this disclosure include:

i. If the compound contains a hydroxyl group, the hydroxyl group may be modified to form an ester, carbonate, or carbamate. Examples include acetate, pivalate, methyl and ethyl carbonates, and dimethylcarbamate. The ester may also be derived from amino acids such as glycine, serine, or lysine.
ii. If the compound contains an amine group, the amine group may be modified to form an amide. Examples include acetamide or derivatization with amino acids such as glycine, serine, or lysine.

[0081] Certain compounds of the invention and their salts may exist in more than one crystal form and the present invention includes each crystal form and mixtures thereof. In addition, the compounds of the present invention can exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water to form hydrates or adducts with alcohols such as C_{1-4}-alkanols, and the like. Furthermore, compounds of this invention can be isolated in association with solvent molecules by crystallization from evaporation of an appropriate solvent. Such solvents include but are not limited to toluene, tetrahydrofuran, dioxane, dimethylformamide, acetonitrile, acetates such as methyl acetate, ethyl acetate, butyl acetate, isobutyl acetate, propyl- and isopropyl acetate, ethers such as diethyl ether and ethyl ether, alcohols such as methanol, ethanol, 1- or 2-butanol, 1- or 2-propanol, pentanol, and dimethylsulfoxide. In general, a depiction for the compound by structure or name is considered to embrace the compound in any form (e.g., by itself, as a hydrate, solvate, or otherwise in a mixture).

COMPOSITIONS AND COMBINATION TREATMENTS

[0082] The $S1P_1$ compounds, their pharmaceutically acceptable salts or hydrolyzable esters of the present invention may be combined with a pharmaceutically acceptable carrier to provide pharmaceutical compositions useful for treating the biological conditions or disorders noted herein in mammalian species, and more preferably, in humans. The particular carrier employed in these pharmaceutical compositions may vary depending upon the type of administration desired (e.g. intravenous, oral, topical, suppository, or parenteral).

[0083] In preparing the compositions in oral liquid dosage forms (e.g. suspensions, elixirs and solutions), typical pharmaceutical media, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like can be employed. Similarly, when preparing oral solid dosage forms (e.g. powders, tablets and capsules), carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like can be employed.

[0084] Another aspect of an embodiment of the disclosure provides compositions of the compounds of the disclosure, alone or in combination with another $S1P_1$ inhibitor or another type of therapeutic agent, or both. As set forth herein, compounds of the disclosure include stereoisomers, tautomers, solvates, hydrates, salts including pharmaceutically ac-ceptable salts, and mixtures thereof. Compositions containing a compound of the disclosure can be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy, 19th Ed., 1995, incorporated by reference herein. The compositions can appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

[0085] Typical compositions include a compound of the disclosure and a pharmaceutically acceptable excipient which can be a carrier or a diluent. For example, the active compound will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which can be in the form of an ampoule, capsule, sachet, paper, or other container. When the active compound is mixed with a carrier, or when the carrier serves as a diluent, it can be solid, semi-solid, or liquid material that acts as a vehicle, excipient, or medium for the active compound. The active compound can be adsorbed on a granular solid carrier, for example contained in a sachet. Some examples of suitable carriers are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatin, lactose, terra alba, sucrose, dextrin, magnesium carbonate, sugar, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone. Similarly, the carrier or diluent can include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

[0086] The formulations can be mixed with auxiliary agents which do not deleteriously react with the active compounds. Such additives can include wetting agents, emulsifying and suspending agents, salt for influencing osmotic pressure, buffers and/or coloring substances preserving agents, sweetening agents or flavoring agents. The compositions can also be sterilized if desired.

[0087] The route of administration can be any route which effectively transports the active compound of the invention which inhibits the enzymatic activity of the focal adhesion kinase to the appropriate or desired site of action, such as oral, nasal, pulmonary, buccal, subdermal, intradermal, transdermal or parenteral, e.g., rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment, the oral route being preferred.

[0088] For parenteral administration, the carrier will typically comprise sterile water, although other ingredients that aid solubility or serve as preservatives can also be included. Furthermore, injectable suspensions can also be prepared, in which case appropriate liquid carriers, suspending agents and the like can be employed.

[0089] For topical administration, the compounds of the present invention can be formulated using bland, moisturizing bases such as ointments or creams.

[0090] If a solid carrier is used for oral administration, the preparation can be tabletted, placed in a hard gelatin capsule in powder or pellet form or it can be in the form of a troche or lozenge. If a liquid carrier is used, the preparation can be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

[0091] Injectable dosage forms generally include aqueous suspensions or oil suspensions which can be prepared using a suitable dispersant or wetting agent and a suspending agent Injectable forms can be in solution phase or in the form of a suspension, which is prepared with a solvent or diluent. Acceptable solvents or vehicles include sterilized water, Ringer's solution, or an isotonic aqueous saline solution. Alternatively, sterile oils can be employed as solvents or suspending agents. Preferably, the oil or fatty acid is non-volatile, including natural or synthetic oils, fatty acids, mono-, di- or tri-glycerides.

[0092] For injection, the formulation can also be a powder suitable for reconstitution with an appropriate solution as described above. Examples of these include, but are not limited to, freeze dried, rotary dried or spray dried powders, amorphous powders, granules, precipitates, or particulates. For injection, the formulations can optionally contain stabilizers, pH modifiers, surfactants, bioavailability modifiers and combinations of these. The compounds can be formulated for parenteral administration by injection such as by bolus injection or continuous infusion. A unit dosage form for injection can be in ampoules or in multi-dose containers.

[0093] The formulations of the disclosure can be designed to provide quick, sustained, or

delayed release of the active ingredient after administration to the patient by employing procedures well known in the art. Thus, the formulations can also be formulated for controlled release or for slow release.

[0094] Compositions contemplated by the present invention can include, for example, micelles or liposomes, or some other encapsulated form, or can be administered in an extended release form to provide a prolonged storage and/or delivery effect. Therefore, the formulations can be compressed into pellets or cylinders and implanted intramuscularly or subcutaneously as depot injections. Such implants can employ known inert materials such as silicones and biodegradable polymers, e.g., polylactide-polyglycolide. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides).

[0095] For nasal administration, the preparation can contain a compound of the invention which inhibits the enzymatic activity of the focal adhesion kinase, dissolved or suspended in a liquid carrier, preferably an aqueous carrier, for aerosol application. The carrier can contain additives such as solubilizing agents, e.g., propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabens.

[0096] For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with the active compound dissolved in polyhydroxylated castor oil.

[0097] Dosage forms can be administered daily, or more than once a day, such as twice or thrice daily. Alternatively dosage forms can be administered less frequently than daily, such as every other day, or weekly, if found to be advisable by a prescribing physician.

[0098] An embodiment of the disclosure also encompasses prodrugs of a compound of the disclosure which on administration undergo chemical conversion by metabolic or other physiological processes before becoming active pharmacological substances. Conversion by metabolic or other physiological processes includes without limitation enzymatic (e.g, specific enzymatically catalyzed) and non-enzymatic (e.g., general or specific acid or base induced) chemical transformation of the prodrug into the active pharmacological substance. In general, such prodrugs will be functional derivatives of a compound of the disclosure which are readily convertible *in vivo* into a compound of the invention. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in Design of Prodrugs, ed. H. Bundgaard, Elsevier, 1985.

[0099] In another embodiment, there are provided methods of making a composition of a compound described herein including formulating a compound of the disclosure with a pharmaceutically acceptable carrier or diluent. In some embodiments, the pharmaceutically acceptable carrier or diluent is suitable for oral administration. In some such embodiments, the methods can further include the step of formulating the composition into a tablet or capsule. In other embodiments, the pharmaceutically acceptable carrier or diluent is suitable for parenteral administration. In some such em-bodiments, the methods further include the step of

lyophilizing the composition to form a lyophilized preparation.

[0100] The compounds of the invention can be used therapeutically in combination with i) one or more other $S1P_1$ inhibitors and/or ii) one or more other types of protein kinase inhibitors and/or one or more other types of therapeutic agents which can be administered orally in the same dosage form, in a separate oral dosage form (e.g., sequentially or non-sequentially) or by injection together or separately (e.g., sequentially or non-sequentially).

[0101] Accordingly, in another embodiment the disclosure provides combinations, comprising:

a) a compound of the disclosure as described herein; and
b) one or more compounds comprising:
    i) other compounds of the present disclosure,
    ii) other medicaments adapted for treatment of a malcondition for which activation of $S1P_1$ is medically indicated, for example multiple sclerosis, transplant rejection, or adult respiratory distress syndrome.

[0102] Combinations of the disclosure include mixtures of compounds from (a) and (b) in a single formulation and compounds from (a) and (b) as separate formulations. Some combinations of the invention disclosure can be packaged as separate formulations in a kit. In some embodiments, two or more compounds from (b) are formulated together while a compound of the disclosure is formulated separately.

[0103] The dosages and formulations for the other agents to be employed, where applicable, will be as set out in the latest edition of the *Physicians' Desk Reference*, incorporated herein by reference.

METHODS OF TREATMENT

[0104] In certain embodiments, the present invention encompasses orally bioavailable compounds that specifically agonize $S1P_1$ without binding ($S1P_2$, $S1P_3$ and $S1P_4$), or having significant specificity over ($S1P_5$), other EDG receptors. A selective $S1P_1$ agonist can be used to treat diseases with an autoimmune, hyperactive immune-response, angiogenesis or inflammatory components, but would not be limited to such conditions. Selective $S1P_1$ agonists have advantages over current therapies by increasing the therapeutic window because of reduced toxicity due to engagement of other EDG receptors.

[0105] In certain embodiments, the present invention encompasses compounds that bind with high affinity and specificity to the $S1P_1$ receptor in an agonist manner. Upon ligation of the $S1P_1$ receptor with agonist, signaling proceeds through $G_{\alpha i}$, inhibiting the generation of cAMP

by adenylate cyclase.

[0106] In certain embodiments, described herein is a method for activating or agonizing (i.e., to have an agonic effect, to act as an agonist) a sphingosine-1-phosphate receptor subtype, such as $S1P_1$ with a compound of the disclosure. The method involves contacting the receptor with a suitable concentration of a disclosed compound to bring about activation of the receptor. The contacting can take place *in vitro*, for example in carrying out an assay to determine the SIP receptor activation activity of a disclosed compound undergoing experimentation related to a submission for regulatory approval.

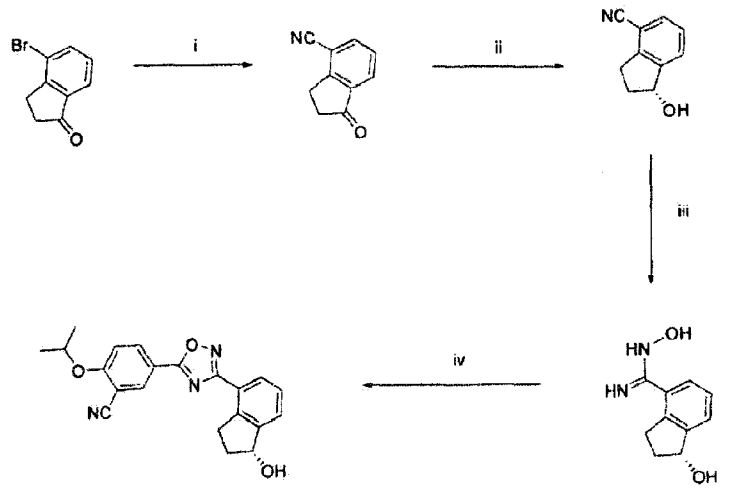
[0107] In certain embodiments, the method for activating an S1P receptor, such as $S1P_1$ can also be carried out *in vivo*, that is, within the living body of a mammal, such as a human patient or a test animal. The inventive compound can be supplied to the living organism via one of the routes as described above, e.g., orally, or can be provided locally within the body tissues, for example by injection of a tumor within the organism. In the presence of the inventive compound, activation of the receptor takes place, and the effect thereof can be studied.

[0108] An embodiment of the present disclosure provides a compound for use in treatment of a malcondition in a patient for which activation of an SIP receptor, such as $S1P_1$ is medically indicated, wherein the patient is administered the disclosed compound in a dosage, at a frequency, and for a duration to produce a beneficial effect on the patient The disclosed compound can be administered by any suitable means, examples of which are described above.

PREPARATION OF CERTAIN EMBODIMENTS

[0109]

Scheme 1:

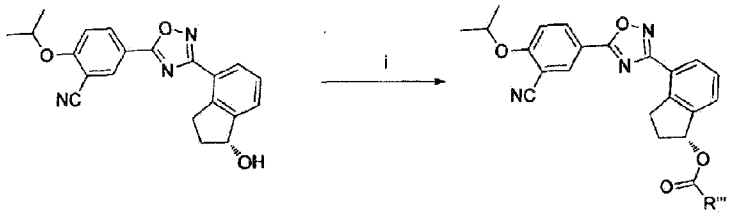


[0110] Reagents: (i) $Zn(CN)_2$, $Pd(PPh_3)_4$, NMP; (ii) (*S*)-(-)-2-methyl-CBS-oxazaborolidine, BH_3-

DMS, toluene; (iii) NH_2OH*HCl, Na_2CO_3 or TEA, EtOH; (iv) HOBt, EDC, substituted benzoic acid, DMF.

[0111] The (*S*)-enantiomer was prepared in the same manner outlined in *Scheme 1* using (*R*)-(+)-2-methyl-CBS-oxazaborolidine in step (ii). Racemic material can be prepared in the same manner outlined in *Scheme 1* using $NaBH_4$ in (ii).

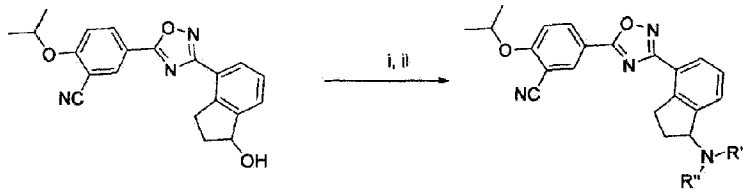
Scheme 2:



Reagents: (i) Pyridine, R'''-COCl, DCM.

[0112] The (*S*)-enantiomer and racemic material can be prepared in the same manner outlined in *Scheme* 2 using the appropriate starting materials.

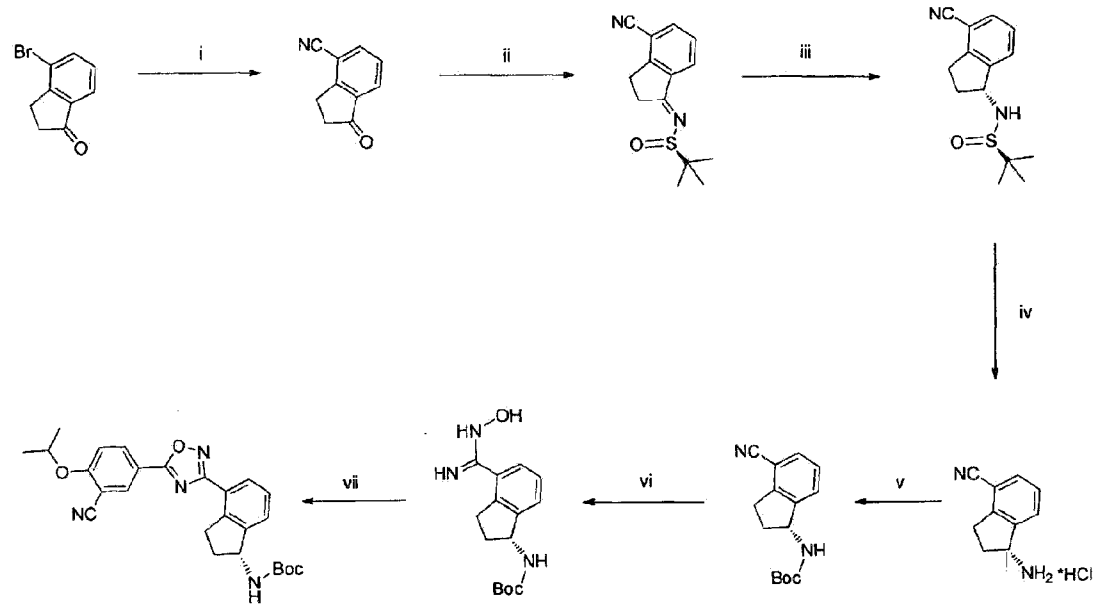
Scheme 3:

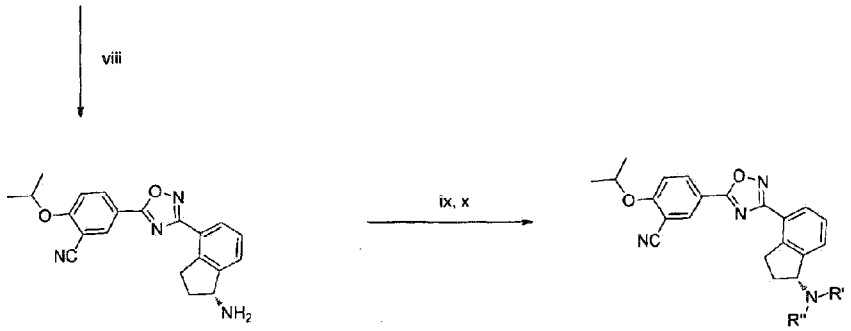


Reagents: (i) (a) MsCl, pyridine; (b) TsCl, pyridine; (c) NsCl, pyridine; (d) $SOCl_2$, DCM; (e) $SOCl_2$, pyridine, DCM; (f) NaN_3, PPh_3, CBr_4; (ii) (a) DIEA, DMA, HNR'R''; (b) DIEA, NaBr or NaI, DMA, HNR'R''.

[0113] Enantiomerically enriched material can be prepared in the same manner outlined in *Scheme* 3 using the (R)- or (*S*)-indanols.

Scheme 4:

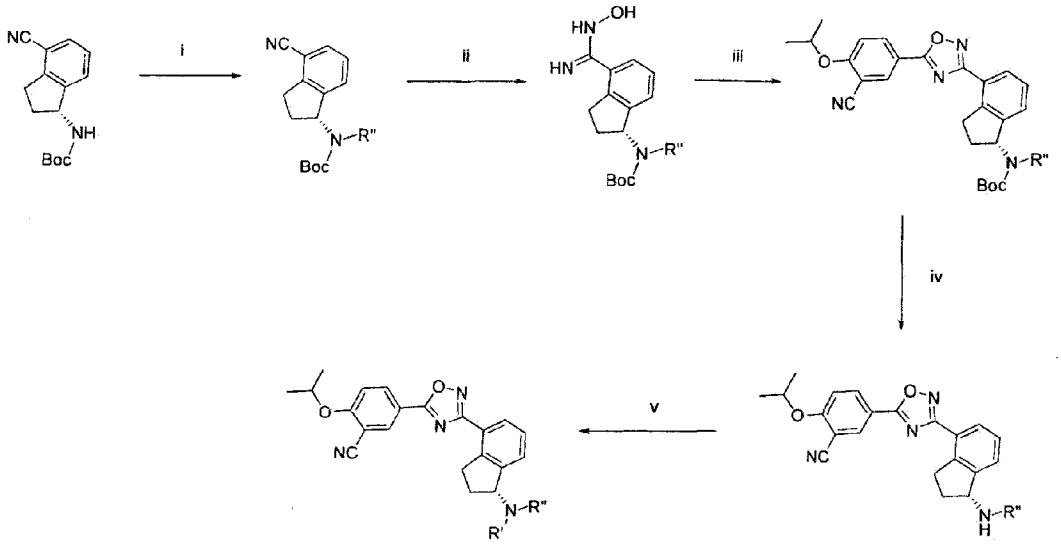




Reagents: (i) $Zn(CN)_2$, $Pd(PPh_3)_4$, NMP; (ii) (*R*)-2-methylpropane-2-sulfinamide, $Ti(OEt)_4$, toluene; (iii) $NaBH_4$, THF; (iv) 4M HCl in dioxane, MeOH; (v) Boc_2O, TEA, DCM; (vi) NH_2OH HCl, TEA, EtOH; (vii) HOBt, EDC, substituted benzoic acid, DMF (viii) 4M HCl in dioxane; (ix) (a) R'-LG or R''-LG, where LG represents a leaving group, K_2CO_3, CH_3CN; (b) R^1-CO_2H or R^2-CO_2H, HOBt, EDC, DMF or R^1-COCl or R^2-COCl, TEA, DCM; (c) R^1-SO_2Cl or R^3-SO_2Cl, TEA, DCM (d) R^2-CHO, HOAc, $NaBH_4$ or $NaCNBH_3$ or $Na(OAc)_3BH$, MeOH; (e) R^1-OCOCl or R^2-OCOCl, DIEA, DMF; (f) $HN(R^5R^5)$, CDI, TEA, DCM; (g) $H_2NSO_2NH_2$, Δ, dioxane; (h) dimethyloxirane, Δ, EtOH; (x) (a) If R' or R'' = H, then reactions (ix)(a-d) can be performed; (b) If R' or R'' contains an ester then (i) hydrolysis NaOH, EtOH or (ii) reduction $NaBH_4$, MeOH can be performed; (c) If R' or R'' contains an acid then couplings $HN(R^5R^5)$, HOBt, EDC, DMF can be performed; (d) If R' or R'' contains an appropriate activated alkene then Michael additions $HN(R^5R^5)$, DMF can be performed.

[0114] The (*S*)-enantiomer was prepared in the same manner outlined in *Scheme 4* using (*S*)-2-methylpropane-2-sulfinamide in step (ii).

Scheme 5:



Reagents: (i) NaH, DMF, and R''-halide; (ii) NH_2OH*HCl or Na_2CO_3, TEA, EtOH; (iii) HOBt, EDC, substituted benzoic acid, DMF; (iv) 4M HCl in dioxane; (v) (a) R'-LG, TEA, DCM; (b) R^1-SO_2Cl or R^3-SO_2Cl, TEA, DCM; (c) R^1-COCl or R^2-COCl, TEA, DCM or R^1-CO_2H or R^2-CO_2H,

HOBt, EDC, DMF or R^1-COCl or R^2-COCl, TEA, DCM; (d) R^2-CHO, HOAc, $NaBH_4$ or $NaCNBH_3$ or $Na(OAc)_3BH$, MeOH;

(a) If R' or R" contains an ester then (i) hydrolysis NaOH, EtOH or (ii) reduction $NaBH_4$, MeOH can be performed;

(b) If R' or R" contains an acid then couplings $H(R^5R^5)$, HOBt, EDC, DMF can be performed; (c) If R' or R" contains an appropriately activated alkene, then Michael additions $HN(R^5R^5)$DMF can be performed.

[0115] The (*S*)-enantiomer was prepared in the same manner outlined in *Scheme 5* from (*S*)-*tert*-butyl 4-cyano-2,3-dihydro-1*H*-inden-1-ylcarbamate.

EXAMPLES

[0116] Compounds 85 and 86 are of the invention. The remaining compounds are reference examples.

General Methods

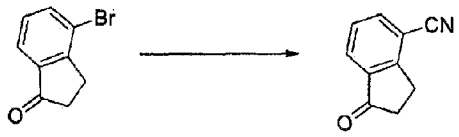
[0117] ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were obtained in solution of deuteriochloroform ($CDCl_3$), deuteriomethanol (CD_3OD) or dimethyl sulfoxide - D_6 (DMSO). NMR spectra were processed using Mestrec 5.3.0 and 6.0.1. ^{13}C NMR peaks that are bracketed are two rotomers of the same carbon. Mass spectra (LCMS) were obtained using an Agilent 1100/6110 HPLC system equipped with a Thompson ODS-A, 100A, 5 µ (50 X 4.6 mm) column using water with 0.1% formic acid as the mobile phase A, and acetonitrile with 0.1% formic acid as the mobile phase B. The gradient was 20-100% with mobile phase B over 2.5 min then held at 100% for 2.5 mins. The flow rate was 1 mL/min. Unless otherwise indicated, the LCMS data provided uses this method. For more hydrophobic compounds, the following gradient was used, denoted as *Method 1*: 40-95% over 0.5 min, hold at 95% for 8.5 min, then return to 40% over 2 min, with a flow rate of 1 mL/min. Final compounds were checked for purity using *Method* 2: 5% for 1 min, 5-95% over 9 min, then hold at 95% for 5 min, with a flow rate of 1 mL/min. Enantiomeric excess was determined by integration of peaks that were separated on a Chiralpak AD-H, 250 x 4.6 mm column, 5 µm particle size. Flow rate of 1 mL/min and an isocratic mobile phase. Unless otherwise indicated, the chiral data provided uses this method. Alternatively, chiral separations were performed under the following conditions, denoted as *Chiral Method 1*: Chiralpak AY-H, 250 x 4.6 mm column, 5 µm particle size. Flow rate of 1 mL/min and an isocratic mobile phase. *Chiral Method 2:* Chiralcel OZ-3, 250 x 4.6, 3 µm particle size at a flow rate of 0.75 ml/min. The pyridine, dichloromethane

(DCM), tetrahydrofuran (THF), and toluene used in the procedures were from Aldrich Sure-Seal bottles kept under nitrogen (N_2). All reactions were stirred magnetically and temperatures are external reaction temperatures. Chromatographies were carried out using a Combiflash Rf flash purification system (Teledyne Isco) equipped with Redisep (Teledyne Isco) silica gel (SiO_2) columns. Preparative HPLC purifications were done on Varian ProStar/PrepStar system using water containing 0.05% trifluoroacetic acid as mobile phase A, and acetonitrile with 0.05% trifluoroacetic acid as mobile phase B. The gradient was 10-80% with mobile phase B over 12 min, hold at 80% for 2 min, and then return to 10% over 2 min with flow rate of 22 mL/min. Other methods similar to this may have been employed. Fractions were collected using a Varian Prostar fraction collector and were evaporated using a Savant SpeedVac Plus vacuum pump. Compounds with salt-able centers were presumed to be the trifluoroacetic acid (TFA) salt. Microwave heating was performed using a Biotage Initiator microwave reactor equipped with Biotage microwave vessels. The following abbreviations are used: ethyl acetate (EA), triethylamine (TEA), diethyl amine (DEA), hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), isopropanol (IPA), dimethylformamide (DMF), dimethyl acetamide (DMA). Norit is activated charcoal.

Experimental Procedures

1-oxo-2,3-dihydro-1H-indene-4-carbonitrile **(INT-1)**

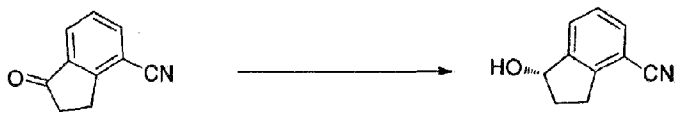
[0118]



[0119] To a stirred solution of 4-bromo-2,3-dihydro-1*H*-inden-1-one (100.0 g, 0.48 mol) in 150 mL of 1-methy-2-pyrrolidine (NMP) was added zinc cyanide (111.8 g, 0.95 mol) and tetrakis(triphenylphosphine)palladium [$Pd(PPh_3)_4$] (2.75 g, 0.024 mol). The solution was degassed with N_2 and the reaction mixture heated at 95°C for 7 h. Upon cooling, the reaction mixture was poured onto ice water (3.5 L). The compound and inorganic Zn salts precipitated. The solid was collected and partitioned between DCM (3 X 100 mL) and water. The organic layers were filtered to remove the Zn salts, and the filtrate was concentrated and crystallized from a 4:1 mixture of EtOH and MeOH (400 mL) to give 45.5 g (60 %) of 1-oxo-2,3-dihydro-1*H*-indene-4-carbonitrile **INT-1** as a light yellow solid. LCMS-ESI (m/z) calculated for $C_{10}H_7NO$: 157.2; found 158.1 $[M+H]^+$, t_R = 2.67 min. ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 - 7.90 (m, 1H), 7.86 (dd, *J*= 7.5, 1.1, 1H), 7.50 (t, *J*= 7.6, 1H), 3.40 - 3.19 (m, 2H), 2.90 - 2.61 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.70, 157.90, 138.38, 137.88, 128.44, 128.28, 116.31, 111.70, 36.01, 25.49.

(S)-1-hydroxy-2,3-dihydro-1H-indene-4-carbortitrile **(INT-2)**

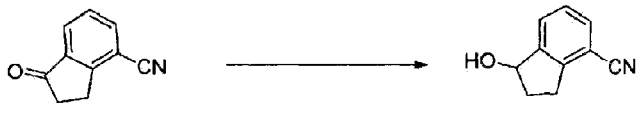
[0120]



[0121] To a 3-neck flask with an internal thermometer and an addition funnel was added (R)-(+)-2-methyl-CBS-oxazaborolidine solution in toluene (3.0 mL) and borane-dimethylsulfide (300 µL). The reaction was stirred at room temperature for 10 min then diluted with DCM (25 mL). Borane-dimethylsulfide (6.0 mL) was added and, after stirring for 5 min, the reaction was cooled to -20°C. 1-Oxo-2,3-dihydro-1H-indene-4-carbonitrile **INT-1** (4.7 g, 30 mmol) in DCM (25 mL) was added dropwise by addition funnel over 20 min while maintaining the reaction at -20 ± 5°C. The reaction was stirred for 1 h then quenched by the dropwise addition of MeOH (20 mL). After hydrogen evolution ceased, MeOH (30 mL) was added and removed by heating at atmospheric pressure. MeOH (50 mL) was added in two and removed by heating twice. All the solvent was evaporated to give a solid which was recrystallized from EA (9 mL) and hexane (22 mL). The compound was filtered and washed with 5:1 hexane/EA (30 mL) to provide 3.73 g (78%) of (*S*)-1-hydroxy-2,3-dihydro-*1H*-indene-4-carbonitrile **INT-2** as a white powder. LCMS-ESI (m/z) calculated for $C_{10}H_9NO$: 159.1; found 160.1 $[M+H]^+$, t_R = 2.39 min. ^1H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 5.28 (d, *J* = 4.1 Hz, 1H), 3.23 (ddd, *J* = 17.0, 8.7, 4.4 Hz, 1H), 3.04 - 2.90 (m, 1H), 2.64 - 2.51 (m, 1H), 2.00 (dddd, *J* = 13.4, 8.7, 7.1, 5.7 Hz, 1H), 1.91 (d, *J* = 5.4 Hz, 1H). Chiral HPLC: (*S*)-1-hydroxy-2,3-dihydro-*1H*-indene-4-carbonitrile was eluted in 20% IPA in hexane: >99.9% ee, t_R = 7.42 min. The (R)-enantiomer was obtained in an analogous fashion using (S) -(-)-2-methyl-CBS-oxazaborolidine. t_R for (R)-enantiomer = 6.79 min.

(+/-) 1-hydroxy-2,3-dihydro-1H-indene-4-carbonitrile

[0122]



[0123] To a stirred suspension of 1-oxo-2,3-dihydro-1H-indene-4-carbonitrile (1.2 g, 7.64 mmol) and silicagel (catalytic) in EtOH at 0°C was added $NaBH_4$ (237.2 mg, 7.64 mmol). The reaction was allowed to warm to room temperature and stirred for 2 h. The solvent was removed under reduced pressure, and the product was purified by chromatography (50%

EA/hexane) to afford 1.02 g (82.3%) of 1-hydroxy-2,3-dihydro-*1H*-indene-4-carbonitrile as white solid. LCMS-ESI (m/z) calculated for $C_{10}H_9NO$; 159.18; found 160.1 $[M+H]^+$, t_R = 2.39 min.

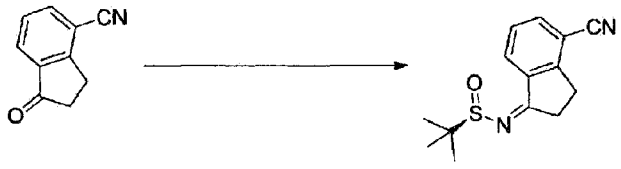
(S)-N,1-dihydroxy-2,3-dihydro-1H-indene-4-carboximidamide **(INT-3)**

[0124]

[0125] To hydroxylamine hydrochloride (0.87 g, 12.5 mmol) and sodium carbonate (1.32 g, 12.5 mmol) in EtOH (20 mL) was added (*S*)-1-hydroxy-2,3-dihydro-*1H*-indene-4-carbonitrile INT-2 (1.59 g, 10 mmol) in one portion and the solution was heated to reflux. After 16 h, the reaction was cooled and filtered to remove the solids. The EtOH was removed and the compound purified by chromatography (MeOH / DCM) to give 1.74 g (90%) of (*S*)-N,1-dihydroxy-2,3-dihydro-1*H*-indene-4-carboximidamide **INT-3** as a white foam. LCMS-ESI (m/z) calculated for $C_{10}H_{12}N_2O_2$: 192.1; found: 193.1 $[M+H]^+$, t_R = 0.56 min. ^1H NMR (400 MHz, MeOD) δ 10.30 (s, 1H), 9.97 (s, 1H), 7.72 - 7.58 (m, 1H), 7.46 - 7.37 (m, 2H), 5.22 (t, *J* = 6.5, 1H), 3.17 - 3.03 (m, 1H), 2.99 - 2.83 (m, 1H), 2.49 (dddd, *J* = 11.4, 8.0, 7.0, 4.4, 1H), 2.02 - 1.88 (m, 1H). (*R*)-N,1-dihydroxy-2,3-dihydro-1*H*-indene-4-carboximidamide is made in an analogous fashion from (*R*)-1-hydroxy-2,3-dihydro-1*H*-indene-4-carbonitrile.

(R)-N-(4-cyano-2,3-dihydro-1H-indene-1-ylidene)-2-methylpropane-2-sulfinamide **(INT-4)**

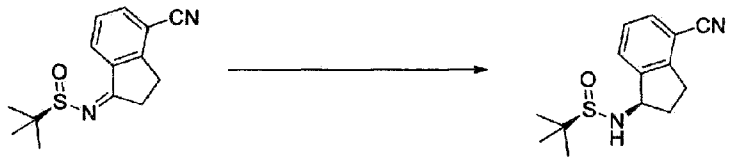
[0126]



[0127] To 1-oxo-2,3-dihydro-*1H*-indene-4-carbonitrile **INT-1** (42.5 g, 0.27 mol) and (R)-2-methylpropane-2-sulfinamide (36.0 g, 0.30 mol) in toluene (530 mL) was added titanium tetraethoxide (84.1 mL, 92.5 g, 0.40 mol) and the reaction mixture was heated at 60°C for 12 h under N_2. The crude (*R*)-N-(4-cyano-2,3-dihydro-1*H*-indene-1-ylidene)-2-methylpropane-2-sulfinamide INT-4 was used directly in the next experiment. LCMS-ESI (m/z) calculated for $C_{14}H_{16}N_2OS$: 260.3; found 261.1 $[M+H]^+$, t_R = 3.19 min.

(R)-N-((R)-4-cyano-2,3-dihydro-1H-inden-1-yl)-2-methylpropane-2-sulfinamide **(INT-5)**

[0128]



[0129] To a flask containing the crude suspension of (*R*)-N-(4-cyano-2,3-dihydro-*1H*-indene-1-ylidene)-2-methylpropane-2-sulfinamide **INT-4** under N_2 was added THF (1.0 L) and the reaction mixture cooled to -78°C. Sodium borohydride (40.9 g, 1.08 mol) was added portionwise over 30 mins. (The internal temperature did not rise during the addition). The reaction mixture was stirred at -78°C for 30 mins, half out of the bath for 30 mins, then warmed to 0°C over 1 h. The 0°C reaction mixture was placed in an ice bath and quenched with brine (100 mL) followed by saturated sodium potassium tartrate (420 mL) and the Ti salts precipitated. The reaction mixture was diluted with EA (1.5 L) and stirred at room temperature overnight. The organic layers were decanted and washed successively with saturated NH_4Cl, water, and brine. The organic layers were dried over $MgSO_4$ and filtered through a pad of $MgSO_4$. The filtrate was concentrated to produce 52.9 g of crude (*R*)-N-((*R*)-4-cyano-2,3-dihydro-1*H*-inden-1-yl)-2-methylpropane-2-sulfinamide **INT-5** as a brown oil, which was used directly in the next step. LCMS-ESI (m/z) calculated for $C_{14}H_{18}N_2OS$: 262.3; found 263.1 $[M+H]^+$, t_R = 2.99 min. ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.7, 1H), 7.56 (t, *J* = 6.8, 1H), 7.36 (t, *J* = 7.7, 1H), 4.97 (q, *J* = 7.5, 1H), 3.50 (d, *J* = 7.6, 1H), 3.22 (ddd, *J* = 16.9, 8.8, 3.9, 1H), 3.01 (dt, *J* = 22.4, 6.9, 1H), 2.70 - 2.53 (m, 1H), 2.15 - 1.95 (m, 1H), 1.33 - 1.20 (m, 9H).

(R)-1-amino-2, 3-dihydro-1H-indene-1-yl)-4-carbonitrile **(INT-6)**

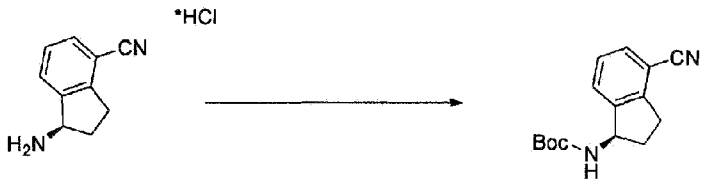
[0130]

[0131] To crude (*R*)-N-((*R*)-4-cyano-2,3-dihydro-1*H*-inden-1-yl)-2-methylpropane-2-sulfinamide **INT-5** (52.9 g, 0.20 mol) in MeOH (200 mL) was added 4N HCl in dioxane (152.0 mL, 0.60 mol) and the resulting yellow suspension was stirred at room temperature for 1.5 h. The crude reaction mixture was diluted with MeOH (500 mL) and filtered to remove some Ti by-products. The filtrate was concentrated and the resulting solid refluxed in acetonitrile (500

mL). The resulting white solid was collected to produce 13.0 g (31% over 3 steps) of the HCl salt of (*R*)-1-amino-2,3-dihydro-1*H*-indene-1-yl)-4-carbonitrile **INT-6**. LCMS-ESI (m/z) calculated for $C_{10}H_{10}N_2$: 158.2; found 142.0 $[M-NH_2]^+$, t_R = 0.84 min. ^1H NMR (400 MHz, DMSO) δ 8.61 (s, 3H), 7.96 (d, *J* = 7.7, 1H), 7.83 (d, *J* = 7.5, 1H), 7.52 (t, *J* = 7.7, 1H), 4.80 (s, 1H), 3.23 (ddd, *J* = 16.6, 8.7, 5.2, 1H), 3.05 (ddd, *J* = 16.6, 8.6, 6.3, 1H), 2.62 - 2.51 (m, 1H), 2.15 - 2.01 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 148.09, 141.15, 132.48, 130.32, 127.89, 117.27, 108.05, 54.36, 39.08, 29.64. The free base can be prepared by extraction with 1N $NaHCO_3$ and DCM. LCMS-ESI (m/z) calculated for $C_{10}H_{10}N_2$: 158.2; found 142.0 $[M-NH_2]^+$, t_R = 0.83 min. ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 - 7.38 (m, 2H), 7.23 *(dd, J* = 17.4, 9.8, 1H), 4.3 (t, *J* = 7.6, 1H), 3.11 (ddd, *J* = 16.8, 8.7, 3.2, 1H), 2.89 (dt, *J* = 16.9, 8.5, 1H), 2.53 (dddd, *J* = 12.8, 8.1, 7.3, 3.2, 1H), 1.70 (dtd, *J* = 12.8, 8.8, 8.0, 1H). ^{13}C NMR (101 MHz, DMSO) δ 150.16, 146.67, 130.19, 128.74, 127.38, 117.77, 107.42, 56.86, 38.86, 29.14. Chiral HPLC: (*R*)-1-amino-2,3-dihydro-*1H*-indene-1-yl)-4-carbonitrile was eluted using 5% EtOH in hexanes, plus 0.05% TEA: 95% ee, t_R = 23.02 min. The (*S*)-enantiomer **INT-7** was prepared in an analogous fashion using (*S*)-2-methylpropane-2-sulfinamide. t_R for (*S*)-enantiomer = 20.17 min.

(R)-tert-butyl 4-cyano-2,3-dihydro-1H-inden-1-ylcarbamate (INT-8)

[0132]



[0133] To (*R*)-1-amino-2,3-dihydro-*1H*-indene-1-yl)-4-carbonitrile HCl **INT-6** (11.6 g, 59.6 mmol) in DCM (100 mL) at 0°C was added TEA (12.0 mL, 131.0 mmol). To the resulting solution was added a solution of Boc anhydride (14.3 g, 65.6 mmol) in DCM (30 mL) and the reaction mixture stirred at room temperature for 1.5 h. The reaction mixture was washed with brine, and the organic layers were dried over $MgSO_4$ and filtered. Additional DCM was added to a total volume of 250 mL and Norit (4.5 g) was added. The product was refluxed for 15 mins and the hot mixture filtered through a pad of celite / silica. The filtrate was concentrated and recrystallized from EA (50 mL) and hexane (150 mL) to produce 12.93 g (84%) of (*R*)-*tert*-butyl 4-cyano-2,3-dihydro-*1H*-inden-1-ylcarbamate **INT-8** as an off-white solid. LCMS-ESI (m/z) calculated for $C_{15}H_{18}N_2O_2$: 258.3; found 281.1 $[M+Na]^+$, t_R = 3.45 min. Elemental Analysis determined for $C_{15}H_{18}N_2O_2$; C calculated = 69.74%; found = 69.98%. H calculated = 7.02%; found = 7.14%. N calculated = 10.84%; found = 10.89%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 - 7.49 (m, 2H), 7.34 (dt, *J* = 7.7, 3.8, 1H), 5.36 - 5.20 (m, 1H), 4.78 (d, *J* = 6.8, 1H), 3.20 (ddd, *J*

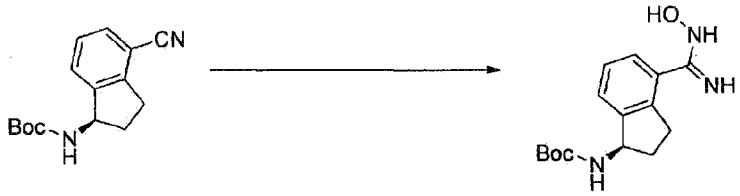
= 16.9, 8.9, 3.3, 1H), 3.02 (dt, *J* = 25.4, 8.4, 1H), 2.82 - 2.53 (m, 1H), 1.88 (dq, *J* = 13.2, 8.6, 1H), 1.55 - 1.44 (m, 9H). ^{13}C NMR (101 MHz, DMSO) δ 155.52, 146.68, 146.32, 130.89, 128.70, 127.63, 117.51, 107.76, 77.98, 55.09, 31.88, 29.11, 28.19. Chiral HPLC: (*R*)-tert-butyl 4-cyano-2,3-dihydro-1*H*-inden-1-ylcarbamate was eluted using 2.5% EtOH in hexanes: >99.9% ee, t_R = 19.36 min. The (*S*)-enantiomer **INT-9** was prepared in an analogous fashion using (*S*)-1-amino-2,3-dihydro-1*H*-indene-1-yl)-4-carbonitrile HCl. t_R for (*S*)-enantiomer = 28.98 min.

General Procedure 3. Preparation of Indane Amide Oximes

[0134] To (R)- or (*S*)-tert-butyl 4-cyano-2,3-dihydro-1*H*-inden-1-ylcarbamate (1 eq) in EtOH (0.56 M) was added hydroxylamine hydrochloride (3 eq) and TEA (3 eq) and the reaction mixture heated at 85°C for 1-2 h. The organic soluble amide oximes were isolated by removal of the solvent and partitioning between water and DCM. The water soluble amide oximes were chromatographed or used directly in the cyclization. Pure amide oximes can be obtained by recrystallization from alcoholic solvents.

(R)-tert-butyl 4-(N-hydroxycarbamimidoyl)-2,3-dihydro-1H-inden-1-ylcarbamate **(INT-10)**

[0135]



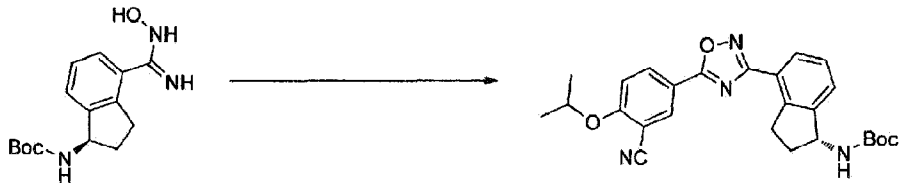
[0136] Prepared using *General Procedure 3.* To (*R*)-tert-butyl 4-cyano-2,3-dihydro-*1H*-inden-1-ylcarbamate **INT-8** (15.0 g, 58.2 mmol) in EtOH (100 mL) was added hydroxylamine hydrochloride (12.1 g, 174.2 mmol) and TEA (17.6 mL, 174.2 mmol) and the reaction mixture heated at 85°C for 2 h. The solvents were removed and the resulting white solid was partitioned between water and DCM. The organic layers were dried over Na_2SO_4, concentrated, and recrystallized from isopropanol (50 mL) to afford 14.4 g (85%) of *(R)-tert*-butyl 4-(N-hydroxycarbamimidoyl)-2,3-dihydro-*1H*-inden-1-ylcarbamate **INT-10** as white crystalline solid. LCMS-ESI (m/z) calculated for $C_{15}H_{21}N_3O_3$: 291.4; found 292.1 $[M+H]^+$, t_R = 2.04 min. ^{1}H NMR (400 MHz, DMSO) δ 9.53 (s, 1H), 7.38 - 7.32 (m, 1H), 7.32 - 7.12 (m, 3H), 5.68 (s, 2H), 4.97 (q, *J* = 8.5, 1H), 3.07 (ddd, *J* = 16.6, 8.7, 2.6, 1H), 2.86 (dt, *J* = 16.8, 8.4, 1H), 2.30 (ddd, *J* = 12.6, 7.6, 3.6, 1H), 1.75 (dq, *J* = 12.3, 9.0, 1H), 1.44 (s, 9H).

General Procedure 4. Cyclization to Indane Oxadiazole Amines

[0137] A solution of the appropriate acid (1 eq), HOBt (1.3 eq), and EDC (1.3 eq) in DMF (0.08 M in acid) was stirred at room temperature under an atmosphere of N_2. After the complete formation of the HOBt-acid complex (1-3 h), the (*R*)- or (*S*)-amide oxime (1.1 eq) was added to the mixture. After complete formation of the coupled intermediate (ca. 0.5-2 h), the mixture was heated to 75-95°C until the cyclization was complete (8-12 h). The reaction mixture was diluted with saturated $NaHCO_3$ and extracted with EA. The combined organic extracts were dried, concentrated, and either purified by chromatography (EA/hexanes) or taken on directly. The oxadiazole was treated with HCl (5N in dioxane, 5 eq) at 50-60°C for 0.5-6 h. The reaction mixture could be extracted (DCM /$NaHCO_3$), or the resulting HCl salt concentrated, suspended in Et_2O, and collected. Pure indane amines can be obtained by recrystallization from alcoholic solvents or by chromatography.

(R)-tert-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-inden-1-ylcarbamate **(INT-12)**

[0138]



[0139] Prepared using *General Procedure* 4. To a solution of 3-cyano-4-isopropoxybenzoic acid (7.74 g, 37.7 mmol) in DMF (50 mL) was added HOBt (6.02 g, 44.6 mmol) and EDC (8.53 g, 44.6 mmol) at room temperature. The reaction was stirred for 2 h until complete formation of the HOBt-acid complex. (*R*)-tert-butyl 4-(N-hydroxycarbamimidoyl)-2,3-dihydro-*1H*-inden-1-ylcarbamate **INT-10** (10.0 g, 34.3 mmol) was added and the reaction mixture stirred at room temperature for 2 h until the formation of **INT-11,** (*R*)-tert-butyl 4-(*N*-(3-cyano-4-isopropoxybenzolyloxy) carbamimidoyl)-2,3-dihydro-*1H*-inden-1-ylcarbamate. The mixture was partitioned between EA and $NaHCO_3$ and the organic layer was collected and dried over $MgSO_4$. **INT-11** (16.3 g, 34.0 mmol) was re-dissolved in DMF (50 mL) and the mixture was heated to 95°C for 12 hrs. The reaction was diluted with $NaHCO_3$ (200 mL) and extracted with EA (3 X 50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to produce 12.8 g (81%) of (*R*)-tert-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1*H*-inden-1-ylcarbamate INT-12 as a light brown solid and used without further purification in the next step. LCMS-ESI (m/z) calculated for $C_{26}H_{28}N_4O_4$: 460.5; found 483.2 $[M+Na]^+$, t_R = 4.25 min. ^1H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 2.1, 1H), 8.34 (dd, *J* = 8.9, 2.2, 1H), 8.09 (d, *J* = 7.6, 1H), 7.51 (d, *J* = 7.5, 1H), 7.39 (t, *J* = 7.6, 1H), 7.12 (d, *J* = 9.0, 1H), 5.28 (d, *J* = 8.2, 1H), 4.80 (hept, *J* = 6.0, 1H), 3.47 (ddd, *J* = 17.4, 8.9, 3.5, 1H), 3.27 - 3.03 (m, 1H), 2.68 (d, *J* = 8.7, 1H), 1.87 (td, *J* = 16.7, 8.5, 1H), 1.53 - 1.43 (m, 15H). ^{13}C

NMR(101 MHz, $CDCl_3$) δ 173.00,168.82, 162.70,155.68, 145.31,142.96, 134.05, 133.83, 128.25, 127.21, 126.79, 123.09, 116.78, 115.24, 113.52, 103.87, 79.52, 72.70, 55.72, 33.86, 31.47, 28.39, 21.70. Chiral HPLC: (*R*)-tert-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1*H*-inden-1-ylcarbamate was eluted using 20% *i*-PrOH in hexanes: >99.9% ee, t_R = 13.33 min. The (*S*)-enantiomer **INT-13** was prepared in an analogous fashion using (*S*)-tert-butyl 4-cyano-2,3-dihydro-*1H*-inden-1-ylcarbamate using *General Procedures 3* and 4 (t_R for (*S*)-enantiomer = 16.31 min).

***(R)-5-(3-(1-amino-2,3-dihydro-1H-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile hydrochloride* (Compound 49)**

[0140]

[0141] To *(R)-tert-butyl* 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-ylcar-bamate(12.8 g, 27.8 mmol) in dioxane (200 mL) was added 4N HCl in dioxane (69 mL). The solution was heated to 55°C for 1 h, and product precipitated. Dioxane was removed and the resulting solid suspended in ether and collected. The material was recrystallized from MeOH (200 mL) to produce 8.11 g (81%) of (*R*)-5-(3-(1-amino-2,3-dihydro-*1H*-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile **49** as the HCl salt. LCMS-ESI (m/z): calcd for: $C_{21}H_{20}N_4O_2$: 360.4; found 383.2 $[M+Na]^+$, t_R = 2.49 min. Elemental Analysis and NMR spectra determined for $C_{21}H_{21}N_4O_2Cl$ * 0.5 H_2O; C calculated = 62.14%; found = 62.25%. H calculated = 5.46%; found = 5.30%. N calculated = 13.80%; found = 13.84%. Cl calculated = 8.73%; found = 8.34%. ^1H NMR (400 MHz, DMSO) δ 8.71 (s, 3H), 8.49 (d, *J* = 2.3, 1H), 8.39 (dd, *J* = 9.0, 2.3, 1H), 8.11 (d, *J* = 7.6, 1H), 7.91 (d, *J* = 7.6, 1H), 7.5 5 (t, *J* = 8.5, 2H), 4.97 (hept, *J* = 6.1, 1H), 4.80 (s, 1H), 3.47 (ddd, *J* = 17.4, 8.7, 5.3, 1H), 3.23 (ddd, *J* = 17.4, 8.6, 6.4, 1H), 2.55 (ddd, *J* = 13.7, 8.3, 3.2, 1H), 2.22 - 1.97 (m, 1H), 1.38 (d, *J* = 6.0, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.28, 167.98, 162.53, 143.69, 141.29, 134.59, 133.80, 128.93, 128.11, 127.55, 122.72, 115.87, 115.24, 114.91, 102.46, 72.54, 54.38, 31.51, 29.91, 21.47. Chiral HPLC of the free base: (*R*)-5-(3-(1-amino-2,3-dihydro-1*H*-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxy benzonitrile was eluted using 15% *i*-PrOH in hexanes plus 0.3% DEA: > 99.9% ee, t_R = 30.80 min. (*S*)- 5-(3-(1-amino-2,3-dihydro-1*H*-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxy-benzonitrile **50** was prepared in an analogous fashion from (*S*)-*tert*-butyl 4-cyano-2,3-dihydro-1*H*-inden-1-ylcarbamate: >99.9% ee, t_R for (*S*)-enantiomer = 28.58 min.

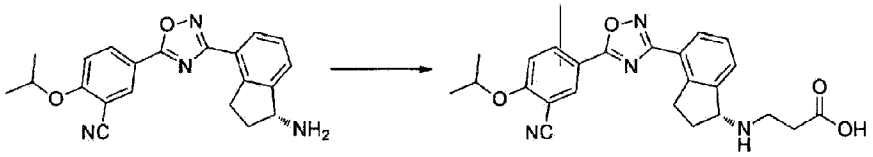
General Procedure 6. Preparation of Indane Acids

[0142] To the solution of (*R*)- or (*S*)-indane amine (1 eq) in CH_3CN (0.1 M) was added K_2CO_3 (3 eq) and the bromo methyl esters (1 eq) or mesylate methyl esters (1 eq). The reaction was heated to 80°C for 30 min or until the reaction was complete. The solvent was evaporated, and the residues partitioned between EA and water. The organic layer was collected, dried over $MgSO_4$, and purified by chromatography (MeOH/DCM with 0.025% TEA) to give the indane methyl ester as white solid. The indane methyl ester was dissolved in EtOH (0.03 M) and NaOH aqueous (11.8 M) was added. The reaction mixture was stirred for 4 h at 40°C. The crude material was purified by preparative HPLC.

[0143] Compounds **61** and **62** were prepared using *General Procedure 6.*

(R)-3-((4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-inden-1-yl)amino)propanoic acid **(Compound 62) (comparative)**

[0144]



[0145] Prepared using *General Procedure 6.* To the solution of (*R*)-5-(3-(1-amino-2,3-dihydro-1*H*-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile **49** (90.0 mg, 0.25 mmol) and K_2CO_3 (103.5 mg, 0.75 mmol) was added methyl 3-bromopropanoate (41.8 mg, 0.25 mmol). The reaction was heated to 80°C for 30 min and repeated four time at 80°C for 30 min with additional methyl 3-bromopropanoate (41.8 mg, 0.25 mmol) added each time. The solvent was evaporated, and the residues partitioned between EA and water. The organic layer was collected, dried over $MgSO_4$, and purified by chromatography (MeOH/ DCM with 0.025% TEA) to give 71 mg (63%) of (R)-methyl 3-((4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1*H*-inden-1-yl)amino)propanoate as a solid. LCMS-ESI (m/z) calculated for $C_{25}H_{26}N_4O_4$: 446.5; found 447.2 $[M+H]^+$, t_R = 2.61 min. ^1H NMR (400 MHz, CDCl3)□δ 8.40 (d, *J* = 2.1, 1H), 8.31 (dd, *J* = 8.9, 2.2, 1H), 8.04 (d, *J* = 7.6, 1H), 7.49 (d, *J* = 7.5, 1H), 7.3 5 (t, *J* = 7.6, 1H), 7.09 (d, *J* = 9.0, 1 H), 4.77 (dt, *J* = 12.2, 6.1, 1H), 4.31 (t, *J* = 6.8, 1H), 3.73 - 3.58 (m, 3H), 3.43 (ddd, *J* = 17.4, 8.7, 4.6, 1H), 3.24 - 3.08 (m, 1H), 3.04 - 2.85 (m, 2H), 2.56 (t, *J* = 6.5, 2H), 2.47 (dtd, *J* = 12.8, 8.4, 4.7, 1H), 1.99 - 1.82 (m, 1H), 1.54 - 1.32 (m, 6H).

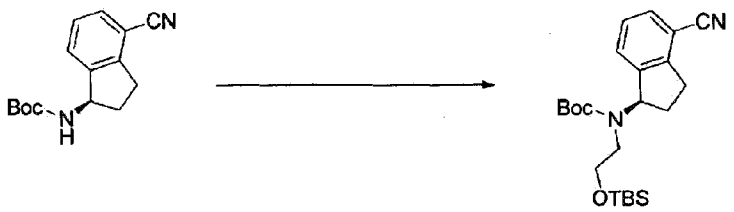
[0146] To (*R*)-methyl 3-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1*H*-inden-1-ylamino) propanoate (71.0 mg, 0.16 mmol) in EtOH (5 ml) was added aqueous NaOH (1.9 mL, 1M). The solution was stirred at 40°C for 4 h. The reaction mixture was poured onto ice (10 mL) and neutralized to pH 7 with 1M HCl. The solution was partitioned between

DCM and H_2O. The organic layer was collected, dried under vacuum, and purified by preparative HPLC to give 29.7 mg (31%) of (*R*)-3-((4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1*H*-inden-1-yl)amino)propanoic acid 62. LCMS-ESI (m/z): calcd for: $C_{24}H_{24}N_4O_4$, 432.5; $[M+H]^+$ found 433.20, t_R = 2.51 min. ^1H NMR (400 MHz, MeOD) δ 8.46 (d, *J* = 2.1, 1H), 8.45 - 8.40 (m, 1H), 8.29 - 8.23 (m, 1H), 7.82 - 7.73 (m, 1H), 7.60 - 7.52 (m, 1H), 7.45 (d, *J* = 9.0, 1H), 5.06 - 4.92 (m, 2H), 3.69 - 3.52 (m, 1H), 3.51 - 3.37 (m, 1H), 3.26 (s, 2H), 2.75 - 2.58 (m, 1H), 2.56 - 2.46 (m, 2H), 2.44 - 2.29 (m, 1H), 1.46 (d, *J* = 6.0, 6H).

[0147] Compounds **85** and **86** were prepared using *General Procedures 9, 3*, and 4 sequentially.

(R)-tert-butyl 2-(tert-butyldimethylsilyloxy)ethyl(4-cyano-2,3-dihydro-1H-inden-1-yl)carbamate **(INT-16)**

[0148]

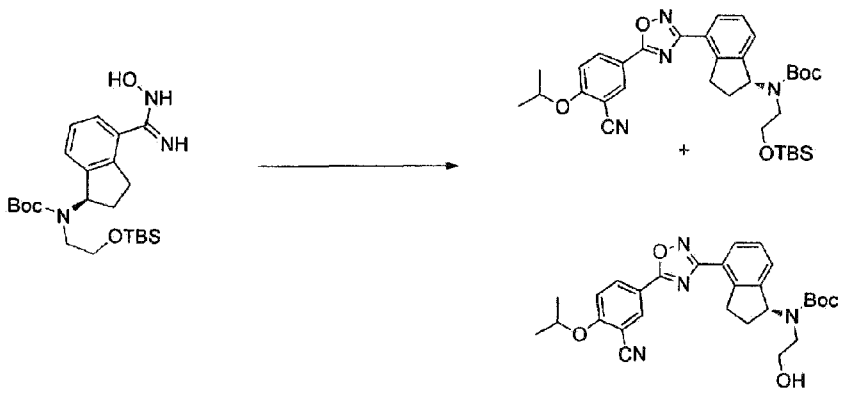


[0149] Prepared using *General Procedure 9*. To a flame-dried flask under N_2 was added (R)-*tert-butyl* 4-cyano-2,3-dihydro-1*H*-inden-1-ylcarbamate **INT-8** (8.3 g, 32.1 mmol) in anhydrous DMF (240 mL). The reaction mixture was cooled to 0°C and sodium hydride (3.8 g, 60% in oil, 160.6 mmol) was added portionwise. After stirring at 0°C for 2.75 h, (2-bromoethoxy)(*tert*-butyl)dimethylsilane (16.9 mL, 70.7 mmol) was added. The ice bath was removed after 5 mins and the reaction mixture was allowed to warm to room temperature. After 1.5 h, the reaction mixture was quenched by the slow addition of sat. $NaHCO_3$ at 0°C. Once gas evolution was complete the reaction was extracted with EA. The organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated. The product was purified by chromatography (EA/hexanes) to provide 10.76 g (80%) of (*R*)-tert-butyl 2-(tert-butyldimethylsilyloxy)ethyl(4-cyano-2,3-dihydro-*1H*-inden-1-yl)carbamate **INT-16** as a colorless oil. LCMS-ESI (m/z) calculated for $C_{23}H_{36}N_2O_3Si$: 416.6; found 317.2 $[M\text{-}Boc]^+$ and 439.0 $[M+Na]^+$, t_R = 4.04 min (Method 1). ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.6, 1H), 7.38- 7.32 (m, 1H), 7.33 - 7.18 (m, 1H), 5.69 (s, 0.5 H), 5.19 (s, 0.5 H), 3.70 (ddd, *J* = 48.8, 26.6, 22.9, 1.5 H), 3.50 - 3.37 (m, 1H), 3.17 (ddd, *J* = 16.7, 9.4, 2.2, 2H), 2.93 (m, 1.5 H), 2.45 (s, 1H), 2.21 (dd, *J* = 24.5, 14.5, 1H), 1.56 - 1.37 (bs, 4.5H), 1.22 (bs, 4.5H), 0.87 - 0.74 (m, 9H), -0.04 (dd, *J* = 26.6, 8.2, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.03, 146.55, 145.54, 131.16, 130.76, [128.11, 127.03], 117.58, 109.20, 79.88, [63.93, 61.88], [61.44, 60.34], [49.73, 46.76], 30.30, 29.70, 28.44, 28.12, [25.87, 25.62], -5.43. (*S*)-tert-butyl 2-(tert-butyldimethylsilyloxy)ethyl(4-cyano-2,3-dihydro-1H-inden-1-

yl)carbamate **INT-17** is prepared in an analogous fashion using **INT-9.**

(R)-tert-butyl 2-(tert-butyldimethylsilyloxy)ethyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-inden-1-yl)carbamate and (R)-tert-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-in-den-1-yl)(2-hydroxethyl) carbamate (comparative compound)

[0150]

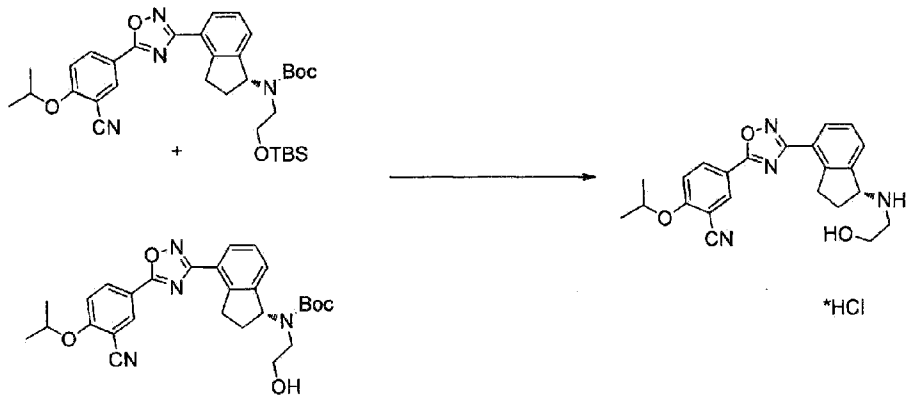


[0151] Prepared using *General Procedure 4.* To a solution of 3-cyano-4-isopropoxybenzoic acid (4.5 g, 21.9 mmol) in anhydrous DMF (100 mL) was added HOBt (5.4 g, 40.0 mmol) and EDC (5.6 g, 29.6 mmol). After 1 h, *(R)-tert-butyl* 2-(*tert*-butyldimethylsilyloxy)ethyl (4-(*N*-hydroxycarbamimidoyl)-2,3-dihydro-*1H*-inden-1-yl)carbamate **INT-18** (11.8 g, 26.3 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. LCMS analysis showed complete conversion to the intermediate, *(R)-tert-butyl* 2-(*tert*-butyldimethylsilyloxy) ethyl (4-(*N*-(3-cyano-4-isopropoxybenzoyloxy) carbamimidoyl)-2,3-dihydro-*1H*-inden-1-yl)carbamate **INT-20.** The reaction mixture was then heated to 80°C for 12 h. The reaction mixture was cooled to room temperature and diluted with EA (250 mL). $NaHCO_3$ (250 mL) and water (350 mL) were added until all the solids dissolved. The mixture was extracted with EA and the organic layers washed successively with water and brine. The organic layers were dried over $MgSO_4$ and concentrated to produce 15.3 g of a mixture of *(R)-tert-butyl* 2-(*tert*-butyldimethylsilyloxy)ethyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)- 2,3-dihydro-*1H*-inden-1-yl) carbamate **INT-21,** and the corresponding material without the TBS protecting group, *(R)-tert-butyl* 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl) (2-hydroxyethyl) carbamate **INT-22.** The mixture was a brown oil, which could used directly without further purification or purified by chromatography (EA/hexane). **INT-21:** LCMS-ESI (m/z) calculated for $C_{34}H_{46}N_4O_5Si$: 618.8; found 519.2 $[M\text{-Boc}]^+$ and 641.3 $[M+Na]^+$, t_R = 7.30 min (Method 1). ^1H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 2.1, 1H), 8.34 (dd, *J* = 8.9, 2.2, 1H), 8.07 (d, *J* = 8.1, 1H), 7.46 - 7.26 (m, 2H), 7.12 (d, *J* = 9.0, 1H), 5.85 (s, 0.5H), 5.37 (s, 0.5H), 4.80 (dt, *J* = 12.2, 6.1, 1H), 3.92 - 3.32 (m, 3.5 H), 3.17 (s, 2H), 2.95 (s, 0.5 H), 2.62 - 2.39 (m, 1H), 2.38 - 2.05 (m, 1H), 1.53 (s, 4.5H), 1.48 (d, *J* = 6.1, 6H), 1.33 -

1.27 (m, 4.5H), 0.94 - 0.77 (m, 9H), 0.01 (d, *J* = 20.9, 6H). ^{13}C NMR (101 MHz, DMSO) δ 173.02, 169.00, 162.75, [156.22,155.52], [145.18, 144.12], [143.39, 142.76],134.16, 133.89, 128.20, [128.01, 127.85], [127.04, 126.90], 126.43, 123.31, 116.93, 115.30, 113.55, 103.96, [79.95, 79.68], 72.73, 67.61, 63.42, [61.91, 61.77], 60.99, 46.11, 31.78, [30.47, 29.87], [28.55, 28.26], 25.93, 21.75, 18.30, 0.00, -5.37. **INT-22:** LCMS-ESI calculated for $C_{28}H_{32}N_4O_5$: 504.6; found 527.2 $[M+Na]^+$, t_R = 2.65 min (Method 1). ^1H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 2.1, 1H), 8.27 (dd, *J* = 8.9, 2.2, 1H), 8.03 (d, *J* = 7.2, 1H), 7.35 - 7.26 (m, 2H), 7.06 (d, *J* = 9.0, 1H), 5.44 (s, 1H), 4.73 (dt, *J* = 12.2, 6.1, 1H), 3.64 (s, 2H), 3.44 (ddd, *J* = 17.5, 9.5, 3.2, 2H), 3.11 (dt, *J* = 17.4, 8.6, 3H), 2.54 - 2.38 (m, 1H), 2.04 (td, *J* = 17.6, 8.8, 1H), 1.50 -1.24 (m, 15H). (*S*)-*tert*-butyl 2-(*tert*-butyldimethylsilyloxy)ethyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl)carbamate **INT-23** and *(S)-tert*-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl)(2-hydroxyethyl) carbamate **INT-24** were made in an analogous fashion.

***(R)-5-(3-(1-(2-hydroxyethylamino)-2,3-dihydro-1H-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile* (Compound 85) (comparative)**

[0152]

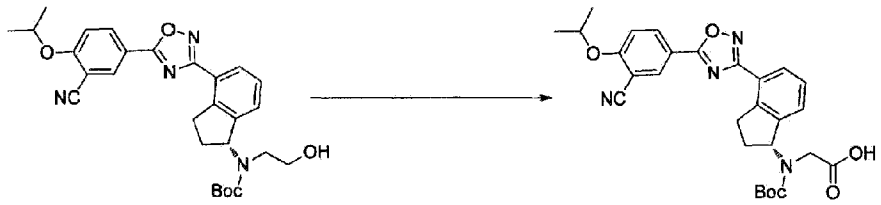


[0153] To a solution of *(R)-tert*-butyl 2-(*tert*-butyldimethylsilyloxy)ethyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxa-diazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl)carbamate INT-21 and *(R)-tert*-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl) (2-hydroxethyl) carbamate **INT-22** (13.9 g, 27.5 mmol) in dioxane (70 mL) at 0°C was added 4N HCl in dioxane (68.8 g, 275.4 mmol). The reaction mixture was warmed to room temperature and then heated to 50°C for 1 h. The resulting suspension was cooled to room temperature and Et_2O (75 mL) was added. The precipitate was collected by filtration, washed with Et_2O and dried to produce 10.5 g of an off-white solid. The HCl salt was recrystallized from MeOH (165 mL) to produce 5.98 g (56% overall yield from (*R*)-*tert*-butyl 2-(tert-butyldimethylsilyloxy)ethyl(4-cyano-2,3-dihydro-*1H*-inden-1-yl)carbamate) of (*R*)-5-(3-(1-(2-hydroxyethylamino)-2,3-dihydro-*1H*-in-den-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile

85 as a white solid. LCMS-ESI (m/z) calculated for $C_{23}H_{24}N_4O_3$: 404.5; found 405.4 $[M+H]^+$, t_R = 2.44 min. ^1H NMR (400 MHz, DMSO) δ 9.25 (s, 2H), 8.53 (d, *J* = 2.3, 1H), 8.42 (dd, J = 9.0, 2.3, 1H), 8.17 (d, *J*= 7.7, 1H), 7.97 (d, *J* = 7.6, 1H), 7.63 - 7.50 (m, 2H), 5.28 (t, *J* = 5.0, 1H), 4.99 (hept, *J* = 6.1, 1H), 4.92 (s, 1H), 3.72 (q, *J* = 5.2, 2H), 3.57 - 3.43 (m, 1H), 3.27 (ddd, J = 17.6, 9.1, 5.0, 1H), 3.15-2.85 (m, *J* = 24.2, 2H), 2.53 (dtd, *J* = 9.0, 5.5, 5.3, 3.6, 1H), 2.30 (ddd, *J* = 13.4, 8.9, 4.6, 1H), 1.39 (d, *J* = 6.0, 6H). ^{13}C NMR (101 MHz, DMSO) δ 173.25, 167.86, 162.47, 144.56, 139.13, 134.53, 133.77, 129.30, 128.93, 127.45, 122.83, 115.79, 115.15, 114.84, 102.40, 72.46, 61.04, 56.51, 46.38, 31.53, 27.74, 21.37. Elemental analysis for $C_{23}H_{25}N_4O_3Cl$: C calc. = 62.65%; found = 62.73%; H calc. = 5.71%; found = 5.60%; N calc. = 12.71%; found = 12.64%; Cl calc. = 8.04%; found = 8.16%. Chiral HPLC of the free base: (*R*)-5-(3-(1-(2-hydroxyethylamino)-2,3-dihydro-*1H*-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxy - benzo-nitrile was eluted using 10% *i*-PrOH in hexanes plus 0.3% DEA: >99.9% ee, t_R = 37.72 min. (*S*)-5-(3-(1-(2-hydroxyethylamino)-2,3-dihydro-*1H*-inden-4-yl)-1,2,4-oxadiazol-5-yl) -2-isopropoxy benzonitrile **86** was obtained in analogous fashion from *(S)-tert*-butyl 2-(*tert*-butyldimethylsilyloxy)ethyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2, 3-dihydro-*1H*-inden-1-yl)carbamate **INT-23** and (*S*)-*tert*-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl) (2-hydroxyethyl) carbamate **INT-24:** >99.9% ee, t_R for (*S*)-enantiomer = 35.86 min.

(R)-2-(tert-butoxycarbonyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-inden-1-yl)amino)acetic acid **(INT-25)**

[0154]



[0155] *(R)-tert*-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl) (2-hydroxethyl) carbamate **INT-22** (4.8 g, 9.5 mmol) was dissolved in CH_3CN (48 mL) and 0.67 M pH 6.7 sodium phosphate buffer (38 mL). To the reaction mixture was added TEMPO (0.10 g, 0.67 mmol) and the reaction was heated to 35°C. Sodium chlorite (1.72 g, 19 mmol) in water (9.5 mL) and sodium hypochlorite (0.28 mL, 0.19 mmol) in water (5.70 mL) were simultaneously added dropwise from separate addition funnels over 1 hour. After addition, the reaction was heated to 35°C for an additional hour. The reaction was cooled to room temperature, water (80 mL) was added, and the pH of the reaction mixture was adjusted to 8.5 with 2.0 N NaOH (12 mL). The reaction was quenched by pouring into an ice cold solution of sodium sulfite (2.9 g in 50 mL of water) and the temperature was maintained below 20°C. After stirring for 30 min at room temperature, Et_2O (50 mL) was added and the organic

layer was separated and discarded. The aqueous layer was acidified with 1.0 N HCl (55 mL) to pH 3.0 and extracted with EA (3 x 100 mL). The organic layer was dried over $MgSO_4$ and filtered to give 4.9 g (>99%) of (*R*)-2-(*tert*-butoxycarbonyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl)amino)acetic acid **INT-25** as a white foam. LCMS-ESI (m/z) calculated for $C_{28}H_{30}N_4O_6$: 518.2; found 541.2 $[M+Na]^+$, t_R = 3.97 min. ^1H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 2.2 Hz, 1H), 8.24 (dd, *J* = 8.9, 2.2 Hz, 1H), 8.08 - 7.94 (m, *J* = 6.9 Hz, 1H), 7.41 - 7.22 (m, 2H), 7.03 (d, *J* = 9.1 Hz, 1H), 5.85 (t, *J* = 7.9 Hz, 0.6H), 5.51 (t, *J* = 7.8 Hz, 0.4H), 4.70 (hept, *J* = 6.2 Hz, 1H), 3.88 (d, *J* = 17.1 Hz, 0.4H), 3.69 (d, *J* = 18.0 Hz, 0.6H), 3.56 (d, *J* = 17.2 Hz, 0.4H), 3.43 (d, *J* = 18.0 Hz, 0.6H), 3.40 - 3.25 (m, 1H), 3.07 (dt, *J* = 17.3, 8.5 Hz, 1H), 2.53 - 2.38 (m, 1H), 1.93 -1.77 (m, 1H), 1.39 (s, 9H), 1.38 (d, *J* = 6.1 Hz, 6H).

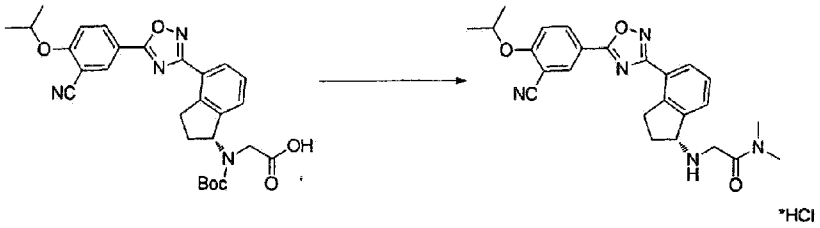
General Procedure 10. Amide Formation (comparative)

[0156] To the boc-protected (R)- or (*S*)-indane aminoacid (1 equivalent) in DMF (2 M) was added HOBt (3 eq) and EDC (3 eq) and the reaction was stirred at room temperature for 30 min. The amine (3 eq) was added and the reaction was stirred at room temperature for 2 h until complete. The Boc protected product was precipitated out of water or extracted (DCM /5 % MeOH) and dried over $MgSO_4$. The solid was dissolved in 4M HCl in dioxane and the mixture was heated to 50°C. After 1 h, the solvent was removed under reduced pressure and the solid residue was purified by recrystallization or preparative HPLC.

[0157] Compound **90** was prepared using *General Procedure 10.*

(R)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-inden-1-ylamino)-N,N-dimethylacetamide hydrochloride **(Compound 90)**

[0158]

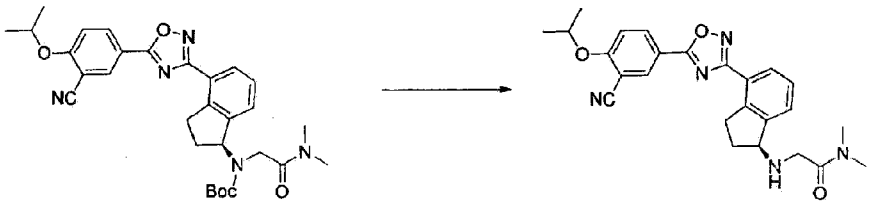


[0159] Prepared using *General Procedure 10.* To 4.9 g (9.5 mmol) of (*R*)-2-(*tert*-butoxycarbonyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl)am ino)acetic acid **INT-25** in DMF (20 mL) was added HOBt (4.4 g, 28.5 mmol) and EDC (5.5 g, 28.5 mmol) and the reaction mixture was stirred at room temperature for 30 min. Dimethylamine (2.0N in THF, 14.25 mL, 28.5 mmol) was added and the reaction was stirred at

room temperature for 2 h. The reaction mixture was poured into water (300 mL) and the precipitate was filtered. The solid was thoroughly washed with water (200 mL). The solid was dissolved in DCM with 5 % MeOH, dried over $MgSO_4$ and filtered. 4M HCl in dioxane was added and the mixture was heated to 50°C. After 1 h, the solvent was removed under reduced pressure and the solid residue was recrystallized from 120 mL MeOH / 120 mL Et_2O / 70 mL hexane/ 10 mL of IPA mixture to provide 3.37 g (74%) of (R)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden- 1-ylami-no) -N,N-dimethylacetamide hydrochloride **90** as a white powder. LCMS-ESI (m/z) calculated for $C_{25}H_{27}N_5O_3$: 445.5; found 446.2 $[M+H]^+$, t_R = 2.52 min. Elemental analysis of $C_{25}H_{28}N_5O_3Cl$ * H_2O: C calc. = 60.05%; found = 59.68%; H calc. = 6.05%; found = 6.45%; N calc. = 14.01%; found = 13.91%; Cl calc. = 7.09; found = 6.98%. ^1H NMR (400 MHz, DMSO) δ 9.44 (s, 2H), 8.53 (d, *J* = 2.3 Hz, 1H), 8.41 (dd, *J* = 9.0, 2.3 Hz, 1H), 8.16 (d, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.62 - 7.52 (m, 2H), 5.05 - 4.92 (m, 1H), 4.88 (dd, *J* = 7.0, 4.2 Hz, 1H), 4.11 (d, *J* = 16.1 Hz, 1H), 4.02 (d, *J* = 16.0 Hz, 1H), 3.51 (ddd, *J* = 17.2, 8.2, 6.6 Hz, 1H), 3.25 (ddd, *J* = 17.4, 8.8, 5.0 Hz, 1H), 2.97 (s, 3H), 2.91 (s, 3H), 2.60 - 2.51 (m, 1H), 2.33 (dq, *J* = 9.0, 4.9 Hz, 1H), 1.39 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 173.33, 167.95, 164.97, 162.56, 144.68, 139.16, 134.61,133.85, 129.43, 128.70, 127.63, 122.90, 115.87, 115.24, 114.92, 102.48, 72.54, 61.28, 44.84, 35.77, 34.98, 31.52, 27.68, 21.45. Chiral HPLC of the free base: (*R*)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-ylamino) -N,N-dimethylacetamide was eluted using 15% *i*-PrOH in hexanes plus 0.3% DEA: 98.5% ee, t_R = 41.19 min. (*S*)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-ylamino)-N,N-dimethyl-acetamide 91 can be obtained in an analogous fashion from (*S*)-2-(tert-butoxycarbonyl(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl)amino)acetic acid. t_R for (S)-enantiomer = 34.35 min. An alternative route is described below.

***(S)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-1H-inden-1-ylamino)-N,N-dimethylacetamide* (Compound 91) (comparative)**

[0160]

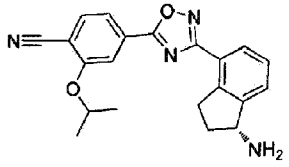


[0161] To a solution of the crude (*S*)-*tert*-butyl 4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-yl(2-(dimethylamino)-2-oxoethyl) carbamate (2.36 g, 4.33 mmol) in dioxane (5 mL) was added 4 N HCl in dioxane (10 mL). The solution was stirred at room temperature for 2 h. The reaction mixture was concentrated and then suspended in

Et_2O. The resulting solid was filtered and dried to obtain 2.3 g (78.4%) of the HCl salt of (*S*)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-ylamino) -N,N-dimethyl acetamide 91 which was 95% pure. The material can be further recrystallized from isopropanol. LCMS-ESI (m/z) calculated for $C_{25}H_{27}N_5O_3$: 445.51; found 446.2 $[M+H]^+$, t_R = 2.55 min. ^1H NMR and ^{13}C for $C_{25}H_{28}N_5O_3Cl$: (400 MHz, DMSO) δ 9.46 (s, 2H), 8.53 (d, *J* = 2.3, 1H), 8.42 (dd, *J* = 9.0, 2.3, 1H), 8.17 (d, *J* = 7.6, 1H), 7.97 (d, *J* = 7.6, 1H), 7.67 - 7.51 (m, 2H), 4.99 (hept, *J* = 6.1, 1H), 4.90 (s, 1H), 4.12 (d, *J* = 16.0, 1H), 4.04 (d, *J* = 16.0, 1H), 3.59 - 3.44 (m, 1H), 3.30 - 3.11 (m, 1H), 2.97 (s, 3H), 2.91 (s, 3H), 2.60-2.51 (m, 1H), 2.34 (s, 1H), 1.39 (d, *J* = 6.0, 6H). ^{13}C NMR (101 MHz, DMSO) δ 173.30, 167.95, 164.93, 162.54, 144.69, 139.17, 134.61, 133.83, 129.39, 128.77, 127.58, 122.86, 115.87, 115.23, 114.92, 102.47, 72.54, 61.26, 44.73, 35.77, 34.99, 31.54, 27.61, 21.45. Chiral HPLC of the free base: (*S*)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-ylamino)- N,N -dimethyl-acetamide was eluted using 15% isopropanol in hexanes, plus 0.3% DEA: > 99.9% ee, t_R = 34.35 min. (R)-2-(4-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-2,3-dihydro-*1H*-inden-1-ylamino)-N,N-dimethyl acetamide **90** can be obtained in an analogous fashion from (*R*)-*tert*-butyl *4*-cyano-2,3-dihydro-*1H*-inden-1-ylcarbamate. t_R for (*R*)-enantiomer = 41.19 min.

***(R)-4-(3-(1-amino-2,3-dihydro-1H-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxy benzonitrile* (Compound 98)**

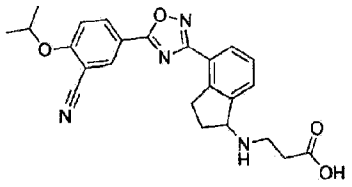
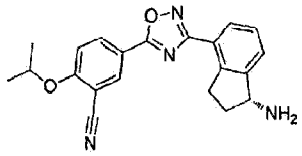
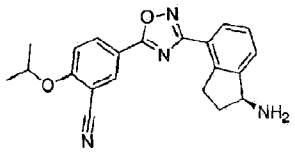
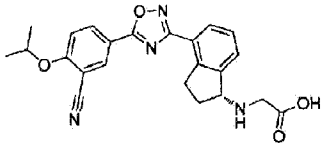
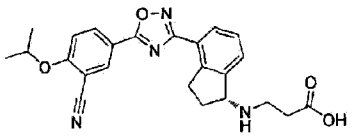
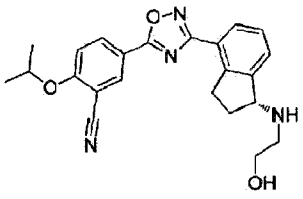
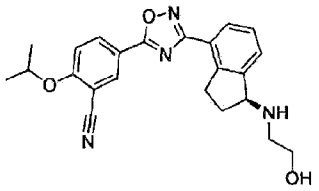
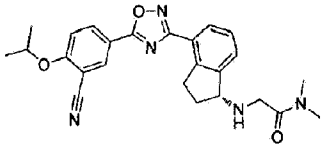
[0162]

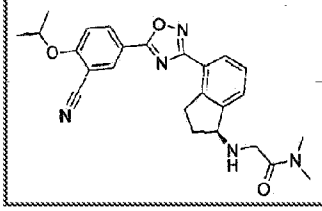


[0163] Prepared from 4-cyano-3-isopropoxybenzoic acid using *General Procedure 4.* LCMS-ESI (m/z) calculated for $C_{21}H_{20}N_4O_2$: 360.4; found 344.1 $[M-NH_2]^+$, t_R = 2.52 min.

[0164] Selected compounds and their corresponding analytical data is shown in *Table 1*, where the LCMS data was collected using Method 2 (see General Methods). The enantiomeric purity was determined for key intermediates and selected final compounds and is presumed from the synthesis for the remaining compounds.

TABLE 1

STRUCTURE	COMPOUND NUMBER	LCMS RETENTION TIME (min)	CHIRALITY OF INDANE CARBON
	**38**	6.51	*Racemic mixture*
	**49**	6.29	*R*
	**50**	6.42	*S*
	**61**	6.63	*R*
	**62**	6.56	*R*
	**85**	6.30	*R*
	**86**	6.41	*S*
	**90**	6.58	*R*

STRUCTURE	COMPOUND NUMBER	LCMS RETENTION TIME (min)	CHIRALITY OF INDANE CARBON
	**91**	6.56	*S*

Biological Assays

Assay Procedures

Generation of $S1P_1$-mediated inhibition of cAMP reporter assay

[0165] A mammalian expression plasmid containing $S1P_1$/EDG1 cloned into pcDNA3.1 was purchased from Missouri S&T cDNA Resource Centre. The nucleotide and amino acid sequence of human $S1P_1$/EDG1 are published in H1a and Maciag (J Biol Chem, 265(1990), 9308-9313). $S1P_1$/pcDNA3.1 was transfected into the CRE-bla CHO K1 (Invitrogen) cell line, and stable single cell clones were selected using standard techniques. Expression of functional $S1P_1$/EDG1 receptor was confirmed by cell surface FACS with a $S1P_1$ antibody (R&D Systems, clone 218713) and S1P-mediated inhibition of Forskolin induced cAMP.

$S1P_1$ CRE-bla CHOK1 reporter assay - characterization of $S1P_1$ agonists

[0166] Cells were seeded into 384-well black wall/clear bottom plates at 10^4 cells/well/19.5 µl assay media (DMEM-phenol free, 0.5% charcoal/dextran stripped serum, 2 mM glutamine, 0.1 mM NEAA, 1 mM Na-Pyruvate, 25 mM Hepes) and incubated for 18 hrs at 37°C in 5% CO_2. Dose response curves (10-point) were generated in 10 mM Hepes, 0.1% Pluronic F127, in the presence of Forskolin. Cells were treated with 0.5 µl compound in the presence of 2 µM Forskolin for 4 hrs at 37°C. The FRET-based β-lactamase fluorescent substrate (LiveBLAzer™-FRET B/G Loading Kit CC4-AM; Invitrogen) was prepared according to manufacturer's directions, and incubated with cells for 2 hrs at room temperature. Plates were read at Ex:410/Em:458 and Ex:410/Em:522, and the response ratio determined. Data was analyzed by non-linear regression to determine the EC50 for inhibition of Forskolin induced cAMP.

Specificity over other S1P receptors

[0167] To assess compound specificity on other S1P receptors the following cell lines were used: S1P2 CRE-bla CHOK1, $S1P_3$-Gα15 NFAT-bla HEK293T (Invitrogen), $S1P_4$-bla TANGO U2OS (Invitrogen), $S1P_5$-bla TANGO U2OS (Invitrogen). The same assay set up for $S1P_1$ was used but without Forskolin. $S1P_4$ and $S1P_5$ assays were performed in FreeStyle Expression medium (Invitrogen). $S1P_5$ cells were incubated for 48 hrs in prior to treatment with compound.

Reported $S1P_1$ Activity

[0168] Activity data for selected $S1P_1$ agonists is displayed in *Table 2.* The activity range is denoted as follows: ++++ denotes agonist activity <0.05 nM. +++ denotes agonist activity between 0.05 to 0.50 nM, and ++ denotes agonist activity between 0.50-5.00 nM, and + denotes agonist activity > 5.00 nM. N/A denotes not available.

TABLE 2

COMPOUND NUMBER	$S1P_1$ ACTIVITY	COMPOUND NUMBER	$S1P_1$ ACTIVITY
49	+++		
50	+++		
61	++++		
85	+++		
86	+++		
90	+++		
91	+++		

$S1P_1$ mutagenesis

[0169] Quick-change mutagenesis with PfuTurbo DNA polymerase (Stratagene) was conducted using $S1P_1$/pcDNA3.1 (Missouri S&T cDNA Resource Centre) as the template. Primers were as follows:

	Primer sequence
R120A/E121A Forward	CCAGTGGTTTCTGGCGGCAGGGAGTATGTTTGT GGCC
R120A/E121A Reverse	GGCCACAAACATACTCCCTGCCGCCAGAAACC ACTGG

	Primer sequence
N101A Forward	CTACACAGCTGCCCTGCTCTTGTCTGGGGC
N101A Reverse	GCCCCAGACAAGAGCAGGGCAGCTGTGTAG

[0170] PCR conditions were 15 cycles with the following parameters: 95°C 30 sec, 58°C 30 sec, 68°C for 60 sec. All constructs were sequence verified.

Phosphorylated-ERK1/2 In Cell Western

[0171] CHOK1 cells were transfected using Fugene (Roche). Stably expressing mixed pools were selected with 2 mg/ml G418. Expression of functional $S1P_1$/EDG1 receptor was confirmed by cell surface FACS with a $S1P_1$ antibody (R&D Systems, clone 218713). Stable pools were seeded at40,000 cells/well in a clear bottom 96-well tray, and incubated at 37°C in 5% CO_2 for 18 hrs. Cells were serum-starved in FreeStyle 293 medium (Invitrogen) for 4-6 h, then incubated for 5 min with a dose response of compound, in duplicate. Cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 in PBS (4x 5 min washes) and blocked for 1 h in Odyssey Blocking Buffer (LI-COR). All incubations were at room temperature. Cells were incubated for 18 h at 4°C in Rabbit anti-Phospho-ERK1/2 (Cell Signaling #4377) and Mouse anti-ERK1/2 (Cell Signaling #9107) both diluted 1:800 in Odyssey Blocking Buffer. Plates were washed with 0.1% Tween-20 in PBS and then incubated with Odyssey Blocking Buffer containing IRDye 680-labeled goat anti-rabbit antibody (#926-32221; diluted 1/500) and IRDye 800CW-labeled goat anti-mouse antibody (#926-32210; diluted 1/1000). Plates were washed with 0.1% Tween-20 in PBS, all liquid was removed from the wells and the plates were scanned using a LICOR Odyssey scanner. The phospho-ERK1/2 signal was normalized to the ERK1/2 signal. Data was analyzed by non-linear regression using GraphPad Prism to determine the EC_{50} of binding.

[0172] Results of the mutagenesis analysis are shown in *Table 3.*

Table 3

$S1P_1$ Variant	**Fold change in EC_{50} compared to wild type $S1P_1$**	
	Compound 50	**Compound 38**
R120A/E121A	2	11
N101A	32	2

Conclusions from $S1P_1$ mutagenesis analysis

[0173] Included in this invention are $S1P_1$ agonists that potentially bind to the $S1P_1$ receptor at different sites. For example, compounds **50** and **38** are both $S1P_1$ agonists that induce phosphorylation of ERK1/2 (*Table 3*). Mutation of $S1P_1$ to produce $S1P_1$ R120A/E121A has no influence on the binding of compound **50,** but diminishes binding of compound **38.** In contrast, mutation of $S1P_1$ to produce N101A had no effect on binding of compound **38** but reduces the binding of compound **50.** Finally, mutation of W269L abolishes binding of both compounds.

In Vivo Assays

Determination of absolute oral bioavailability in rats.

[0174] All pharmacokinetic studies were conducted in non-fasted female Sprague-Dawely rats (Simonsen Laboratories or Harlan Laboratories). Rats were housed in an ALAAC accredited facility and the research was approved by the facilities Institutional Animal Care and Use Committee (IACUC). The animals were acclimated to the laboratory for at least 48 h prior to initiation of experiments.

[0175] Compounds were formulated in 5% DMSO/5%Tween20 and 90% purified water (intravenous infusion) or 5%DM-SO/5%Tween20 and 90% 0.1N HCL (oral gavage).. The concentration of the dosing solutions was verified by HPLC-UV. For intravenous dosing, compounds were administered by an infusion pump into the jugular vein over one minute to manually restrained animals (n=4 rats/compound). The intravenous doses were 0.8 for a 1:1 mixture (racemic) of **85** and **86,** and 0.3 and 0.3 mg/kg for compounds **49** and **50**, respectively. Oral dosing was by gavage using a standard stainless steel gavage needle (n=2-4 rats/compound).. The oral solution doses were 0.3, 2 and 2 mg/kg for compounds **85, 49** and **50,** respectively.. For both routes of administration, blood was collected at eight time-points after dosing with the final sample drawn 24 h post dose. Aliquots of the blood samples were transferred to polypropylene 96-well plate and frozen at -20°C until analysis.

[0176] After thawing the blood samples at room temperature, 5µL of DMSO was added to each well. Proteins were precipitated by adding 150 µL acetonitrile containing 200 nM internal standard (4-hydroxy-3-(alpha-iminobenzyl)-1-methyl-6-phenylpyrindin-2-(1*H*)-one) and 0.1% formic acid. Plates were mixed for 1 min on a plate shaker to facilitate protein precipitation and then centrifuged at 3,000 rpm for 10 min to pellet protein. The supernatant was transferred to a clean plate and centrifuged at 3,000 rpm for 10 min to pellet any remaining solid material prior to LC/MS/MS analysis. Calibration curve standards were prepared by spiking 5µL compound stock in DMSO into freshly collected EDTA rat blood. An eight point standard curve spanning a range of 5 nM to 10,000 nM was included with each bio-analytical run. The standards were processed identically to the rat pharmacokinetic samples.

[0177] Concentrations in the rat pharmacokinetic samples were determined using a standardized HPLC-LC/MS/MS method relative to the eight point standard curve. The system consisted of a Leap CTC Pal injector, Agilent 1200 HPLC with binary pump coupled with an Applied Biosystems 3200 QTrap. Compounds were chromatographed on a Phenomenex Synergy Fusion RP 20x2mm 2um Mercury Cartridge with Security Guard. A gradient method was used with mobile phase A consisting of 0.1% formic acid in water and mobile phase B consisting of 0.1% formic acid in acetonitrile at flow rates varying from 0.7 to 0.8 mL/min. Ions were generated in positive ionization mode using an electrospray ionization (ESI) interface. Multiple reaction monitoring (MRM) methods were developed specific to each compound. The heated nebulizer was set at 325°C with a nebulizer current of 4.8 μA. Collision energies used to generate daughter ions ranged between 29 and 39 V. Peak area ratios obtained from MRM of the mass transitions specific for each compound were used for quantification. The limit of quantification of the method was typically 5 nM. Data were collected and analyzed using Analyst software version 1.4.2.

[0178] Blood concentration versus time data were analyzed using non-compartmental methods (WinNonlin version 5.2; model 200 for oral dosing and model 202 for intravenous infusion). Absolute oral bioavailability (%) was calculated using the following expression: (Oral AUC × IV Dose)/(IV AUC × Oral Dose)× 100.

Lymphopenia

[0179] *In mice:* Female C57BL6 mice (Simonsen Laboratories, Gilroy CA) were housed in an ALAAC accredited facility and the research was approved by the facilities Institutional Animal Care and Use Committee (IACUC). The animals were acclimated to the laboratory for at least 5 days prior to initiation of experiments. Mice (n=3/compound/time-point) were dosed by oral gavage with 1 mg/kg compound formulated in a vehicle consisting of 5%DMSO/5%Tween 20 and 90% 0.1N HCl. Control mice were dosed PO with the vehicle. Terminal whole blood samples were collected from isoflurane anesthetized mice by cardiac puncture into EDTA. Whole blood was incubated with rat anti-mouse CD16/CD32 (Mouse BD Fc Block, #553141), PE-Rat anti-mouse CD45R/B220 (BD #553089), APC-Cy7-Rat anti-mouse CD8a (BD #557654), and Alexa Fluor647-Rat anti-mouse CD4 (BD #557681) for 30 min on ice. Red blood cells were lysed using BD Pharm Lyse Lysing buffer (#555899) and white blood cells were analyzed by FACS. Lymphopenia was expressed as the % of white blood cells that were CD4 or CD8 positive T cells. The overall lymphopenia response over 24 h was estimated by calculating the area under the effect curve (AUEC) using the linear trapezoidal rule.

[0180] In rats: Female rats (Simonsen Laboratories, Gilroy CA) were housed in an ALAAC accredited facility and the research was approved by the facilities Institutional Animal Care and Use Committee (IACUC). The animals were acclimated to the laboratory for at least 5 days prior to initiation of experiments. Rats (n=3/compound/time-point) were dosed by oral gavage with 1 mg/kg compound formulated in a vehicle consisting of 5%DMSO/5%Tween 20 and 90%

0.1N HCL. Control rats were dosed PO with the vehicle. Whole blood was collected from isoflurane anesthetized rats via the retro-orbital sinus and terminal samples were collected by cardiac puncture into EDTA. Whole blood was incubated with mouse anti-rat CD32 (BD #550271), PE-mouse anti-rat CD45R/B220 (BD #554881), PECy5-mouse anti-rat CD4 (BD #554839), and APC-mouse anti-rat CD8a (eBioscience #17-0084) for 30 minutes on ice. Red blood cells were lysed using BD Pharm Lyse Lysing buffer (#555899) and white blood cells were analyzed with a BD FACSArray. Lymphopenia was expressed as the % of white blood cells that were CD4 or CD8 positive T cells. The overall lymphopenia response over 24 h was estimated by calculating the area under the effect curve (AUEC) using the linear trapezoidal rule.

Evaluation of Therapeutic Index in Rats

[0181] All studies were conducted in non-fasted male and female Sprague-Dawely rats (Simonsen Laboratories). Rats were housed in an AAALAC accredited facility and the research was approved by the facilities Institutional Animal Care and Use Committee (IACUC). The animals were acclimated to the laboratory for at least 5 days prior to initiation of experiments.

[0182] The compounds listed in Table 6 were formulated as suspensions in a vehicle consisting of 0.5% carboxymethyl cellulose (Acros Organics) in purified water (pH adjusted to ~2.2 with hydrochloric acid). The same formulation was used in the rat lymphopenia and toxicology studies described below. The concentration of each compound in suspension was verified to be within ± 10% of the target concentration by HPLC-UV.

[0183] Prior to the conduct of toxicology studies, the effect of three to five daily doses of each compound on peripheral T-cell counts of female rats was determined (see lymphopenia measurements in rats above). In these lymphopenia studies, blood samples were collected onto EDTA at intervals after the final study dose. The collection times were not identical for each study, however, all studies included a sample collected 24 hours after the final dose.. The lymphopenia data was used as a biomarker to select equally pharmacologically active doses for the subsequent toxicology study. The low dose for the toxicology study was the dose of each compound that resulted in a 50% reduction of T-cell count 24 h after the final dose in the lymphopenia study relative to vehicle treated rats. The high dose in the toxicology study represented a ≥20-fold increment over the low dose.

[0184] In the toxicology studies, three male and three female rats per group were assigned to dosing groups using body weight based randomization. A control group in each study received vehicle.. All animals were dosed orally by gavage on 5 or 14-consecutive days at a dose volume of 5 mL/kg/day. The animals were observed daily for any manifestations of adverse effect. Twenty-four hours after the final study dose, the rats were anesthetized with isoflurane and a terminal blood sample was taken by intra-cardiac puncture for hematology and clinical chemistry evaluation (IDEXX Laboratories, Sacramento, CA). The lungs with trachea were collected, weighed, and then prepared for histology by perfusion with 10% neutral buffered

formalin via the trachea. The internally fixed lungs were then preserved in 10% neutral buffered formalin and submitted for histological examination (IDEXX).

[0185] The dose of each compound resulting in a 10% increase in the lung to terminal body weight ratio was estimated for each compound by linear interpolation. The therapeutic index was estimated as the ratio of the dose producing 10% lung weight increase to the dose producing 50% T-Cell depletion.

Description of the TNBS Crohn's Colitis Model in Rats

[0186] Male Sprague-Dawley rats (180-200 g) were acclimatized for seven days and then assigned to 8 rats per group so that each group had approximately the same mean weight. Twenty-four hours prior to disease initiation, rats are deprived of food. Rats were anaesthetized and weighed, then 80 mg/kg TNBS solution (50% TNBS: 50% 200 proof ethanol) was instilled into colon via a 20g feeding needle inserted into the anus. The rats were maintained in head down position until recovery from anesthesia. Daily oral dosing was initiated 2 h post TNBS-instillation for six days. Prednisolone served as a positive control and was administered orally daily at 10 mg/kg. Body weights were monitored daily and 24 h after the last dose, all groups are terminated. The colon was removed, flushed of fecal matter and examined for gross changes including strictures, adhesions and ulcers. The colon length, weight of the distal 2 cm, and wall thickness was recorded. Oral delivery of 1 mg/kg of Compound 85 reduced TNBS induced colon shortening from 31% in the diseased rats to 15%.

Description of Influenza A H1N1 Model in Mice

[0187] Male C57B1/6 (6-8 weeks of age) were acclimatized for seven days and then assigned to 5-8 mice per group so that each group has approximately the same mean weight. Mice were infected with 10^4 PFUs mouse-adapted influenza A virus (A/WSN/33) via the intratracheal route. Mice were then treated with 0.2-1.5 mg/kg compound p.o. 1 hr post-infection. Forty eight hours after infection mice were euthanized by cervical dislocation and bronchoalveolar lavage fluid was collected. Quantitative cytokine analysis was performed via ELISA. In some experiments whole body perfusion was performed and lungs were collected for cellular enumeration of inflammatory cells. Longevity studies were performed by infection with 3-$10x10^4$ PFUs mouse-adapted influenza A virus over 14 days. Intratracheal delivery of 0.5 mg/kg of Compound 85, 1 hr after virus infection suppressed cellular infiltrate into the lungs by 40%.

Comparative Data

[0188] Comparative potency data for $S1P_1$-$S1P_5$ is shown in *Table* 4. The agonist values (EC_{50}) are reported in nM.

TABLE 4

COMPOUND NUMBER	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
49	0.17	1080	8945	9034	20.11
50	0.19	7717	8914	7866	44.55
85	0.16	5690	4501	1610	15.06
86	0.16	9559	9938	4192	55.20
90	0.13	6662	8816	>10000	12.90
91	0.09	>10000	>10000	>10000	15.23

[0189] Comparative PK and lymphopenia data is shown in *Table 5.*

TABLE 5

Compound Number	Rat -Oral bioavailability Solution	Mouse Lymphopenia (AUEC)
49	93%	1762
50	91%	1632
85	69%	1425
86	N/A	1342
90	N/A	1486
91	N/A	1408

[0190] *Table 6* shows the therapeutic index (TI) obtained after 5 or 14 day toxicology studies in rats for selected compounds. The dose producing a 10% increase in lung to body weight ratio was interpolated from a plot of dose versus lung to body weight. The lymphopenia response was measured 24 hours following the final dose of a 3-5 day multiple dose regimen.

TABLE 6

Compound Number	Dose Resulting in 10% increase in lung weight (mg/kg)	Dose Producing 50% lymphopenia (mg/kg)	TI 5 days	TI 14 days
49	0.2	0.10	N/A	2
50	2.0	0.10	N/A	20
85	2.8	0.15	N/A	14
86	2.7	0.15	N/A	18
90	5.5	0.40	N/A	14
91	0.3	0.30	N/A	1

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

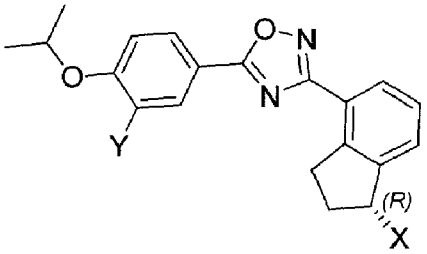
- WO2009151529A1 [0005]
- WO2004058149A2 [0005]
- US5180741A [0005]

Non-patent literature cited in the description

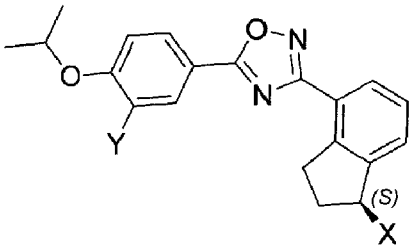
- **ROSEN et al.**Immunol. Rev., 2003, vol. 195, 160-177 [0003]
- **MIRON et al.**Ann. Neurol., 2008, vol. 63, 61-71 [0004]
- **GREENEWUTS**Protective Groups in Organic SynthesisJohn Wiley & Sons19910000 [0051]
- **LIT et al.**Salt Selection for Basic DrugsInt J. Pharm., 1986, vol. 33, 201-217 [0073]
- Remington: The Science and Practice of Pharmacy19950000 [0084]
- Design of ProdrugsElsevier19850000 [0098]
- J Biol Chem, 1990, vol. 265, 9308-9313 [0165]

PATENTKRAV

1. Forbindelse med Formel I-R eller Formel I-S eller et farmaceutisk acceptabelt salt deraf til anvendelse ved en fremgangsmåde til behandling af en inflammatorisk tarmsygdom (IBD) med en hyppighed og i en tidsvarighed, der er tilstrækkelig til at tilvejebringe en fordelagtig effekt for patienten,



I-R



I-S

hvor X er -$NHCH_2CH_2OH$, og Y er -CN.

2. Forbindelse til anvendelse ifølge krav 1, hvor forbindelsen er en racemisk blanding af Formel I-R og Formel I-S.

3. Forbindelse til anvendelse ifølge krav 1, hvor forbindelsen er beriget i Formel I-R sammenlignet med Formel I-S.

4. Forbindelse til anvendelse ifølge krav 3, hvor forbindelsen er en i det væsentlige enantiomerisk ren form af Formel I-R.

5. Forbindelse til anvendelse ifølge krav 4, hvor berigelsesniveauet af Formel I-R i forhold til Formel I-S er mindst 95 %.

6. Forbindelse til anvendelse ifølge krav 4, hvor berigelsesniveauet af Formel I-R i forhold til Formel I-S er mindst 99 %.

7. Forbindelse til anvendelse ifølge krav 1, hvor forbindelsen er beriget i Formel I-S sammenlignet med Formel I-R.

8. Forbindelse til anvendelse ifølge krav 7, hvor forbindelsen er en i det væsentlige enantiomerisk ren form af Formel I-S.

9. Forbindelse til anvendelse ifølge krav 8, hvor berigelsesniveauet af Formel I-S i forhold til Formel I-R er mindst 95 %.

10. Forbindelse til anvendelse ifølge krav 8, hvor berigelsesniveauet af Formel I-S i forhold til Formel I-R er mindst 99 %.

11. Forbindelse til anvendelse ifølge et af kravene 1-10, hvor IBD'en er colitis ulcerosa.

12. Forbindelse til anvendelse ifølge et af kravene 1-10, hvor IBD'en er Crohns sygdom.