

COMPOUNDS AND COMPOSITIONS AS HEDGEHOG PATHWAY MODULATORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Number 60/797,949, filed 5 May 2006. The full disclosure of this application is incorporated herein by reference in its entirety and for all purposes.

BACKGROUND

Field of the Invention

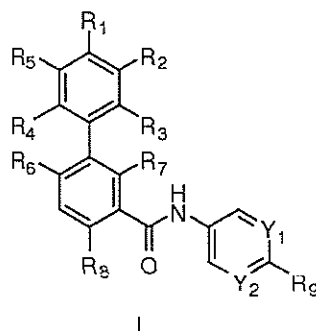
[0002] The invention provides a method for modulating the activity of the hedgehog signaling pathway. In particular, the invention provides a method for inhibiting aberrant growth states resulting from phenotypes such as Ptc loss-of-function, hedgehog gain-of-function, smoothened gain-of-function or Gli gain-of-function, comprising contacting a cell with a sufficient amount of a compound of Formula I.

Background of the Invention

[0002] During embryonic development, the hedgehog signaling pathway is essential for numerous processes such as the control of cell proliferation, differentiation and tissue patterning. The aberrant activity of the hedgehog signaling pathway, for example, as a result of enhanced activation, however may have pathological consequences. In this regard, activation of the hedgehog pathway in adult tissues can result in specific types of cancer that include, but are not limited to, cancers of the brain, muscle and skin, prostrate, medulloblastoma, pancreatic adenocarcinomas and small-cell lung carcinomas. Enhanced activation of the hedgehog signaling pathway contributes to the pathology and/or symptomology of a number of diseases. Accordingly, molecules that modulate the activity of the hedgehog signaling pathway are useful as therapeutic agents in the treatment of such diseases.

Summary of the Invention

[0003] In one aspect, the present invention provides compounds of Formula I:



[0004] in which

[0005] Y_1 and Y_2 are independently selected from N and CR_{10} ; wherein R_{10} is selected from hydrogen, halo, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy, halosubstituted- C_{1-6} alkoxy and $-OXNR_{10a}R_{10b}$; wherein R_{10a} and R_{10b} are independently selected from hydrogen and C_{1-6} alkyl;

[0006] R_1 is selected from cyano, halo, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy, halosubstituted- C_{1-6} alkoxy, C_{6-10} aryl, dimethyl-amino, C_{1-6} alkyl-sulfanyl and C_{3-8} heterocycloalkyl optionally substituted with up to 2 C_{1-6} alkyl radicals;

[0007] R_2 and R_5 are independently selected from hydrogen, cyano, halo, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy, halosubstituted- C_{1-6} alkoxy and dimethylamino;

[0008] R_3 and R_4 are independently selected from hydrogen, halo, cyano, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy and halosubstituted- C_{1-6} alkoxy; or either R_1 and R_2 or R_1 and R_5 together with the phenyl to which they are both attached form C_{5-10} heteroaryl;

[0009] R_6 and R_7 are independently selected from hydrogen, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy and halosubstituted- C_{1-6} alkoxy; with the proviso that R_6 and R_7 are not both hydrogen;

[0010] R_8 is selected from halo, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy and halosubstituted- C_{1-6} alkoxy;

[0011] R_9 is selected from $-S(O)_2R_{11}$, $-C(O)R_{11}$, $-OR_{11}$, $-NR_{12a}R_{12b}$ and $-R_{11}$; wherein R_{11} is selected from aryl, heteroaryl, cycloalkyl and heterocycloalkyl; R_{12a} and R_{12b} are independently selected from C_{1-6} alkyl and hydroxy-substituted- C_{1-6} alkyl;

[0012] wherein said aryl, heteroaryl, cycloalkyl and heterocycloalkyl of R_9 can be optionally substituted with 1 to 3 radicals independently selected from C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy, halosubstituted- C_{1-6} alkoxy, C_{6-10} aryl- C_{0-4} alkyl, C_{5-10} heteroaryl- C_{0-4} alkyl, C_{3-12} cycloalkyl and C_{3-8} heterocycloalkyl;

[0013] wherein said aryl-alkyl substituent of R_9 is optionally substituted with 1 to 3 radicals independently selected from halo, C_{1-6} alkyl, halosubstituted- C_{1-6} alkyl, C_{1-6} alkoxy, halosubstituted- C_{1-6} alkoxy and methyl-piperazinyl; and the N-oxide derivatives, prodrug derivatives, protected derivatives, individual isomers and mixture of isomers thereof; and the pharmaceutically acceptable salts and solvates (e.g. hydrates) of such compounds.

[0014] In a second aspect, the present invention provides a pharmaceutical composition which contains a compound of Formula I or a N-oxide derivative, individual isomers and mixture of isomers thereof; or a pharmaceutically acceptable salt thereof, in admixture with one or more suitable excipients.

[0015] In a third aspect, the present invention provides a method of treating a disease in an animal in which modulation of the hedgehog pathway activity, can prevent, inhibit or ameliorate the pathology and/or symptomology of the diseases, which method comprises administering to the animal a therapeutically effective amount of a compound of Formula I or a N-oxide derivative, individual isomers and mixture of isomers thereof, or a pharmaceutically acceptable salt thereof.

[0016] In a fourth aspect, the present invention provides the use of a compound of Formula I in the manufacture of a medicament for treating a disease in an animal in which hedgehog pathway activity, contributes to the pathology and/or symptomology of the disease.

[0017] In a fifth aspect, the present invention provides a process for preparing compounds of Formula I and the N-oxide derivatives, prodrug derivatives, protected derivatives, individual isomers and mixture of isomers thereof, and the pharmaceutically acceptable salts thereof.

Definitions

[0018] "Alkyl" as a group and as a structural element of other groups, for example halo-substituted-alkyl and alkoxy, can be either straight-chained or branched. C₁₋₄-alkoxy includes, methoxy, ethoxy, and the like. Halo-substituted alkyl includes trifluoromethyl, pentafluoroethyl, and the like.

[0019] "Aryl" means a monocyclic or fused bicyclic aromatic ring assembly containing six to ten ring carbon atoms. For example, aryl may be phenyl or naphthyl, preferably phenyl. "Arylene" means a divalent radical derived from an aryl group.

[0020] "Heteroaryl" is as defined for aryl above where one or more of the ring members is a heteroatom. For example C₅₋₁₀heteroaryl is a minimum of 5 members as indicated by the carbon atoms but that these carbon atoms can be replaced by a heteroatom. Consequently, C₅₋₁₀heteroaryl includes pyridyl, indolyl, indazolyl, quinoxaliny, quinoliny, benzofuranyl, benzopyranyl, benzothiopyranyl, benzo[1,3]dioxole, imidazolyl, benzo-imidazolyl, pyrimidinyl, furanyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, thienyl, etc.

[0011] "Cycloalkyl" means a saturated or partially unsaturated, monocyclic, fused bicyclic or bridged polycyclic ring assembly containing the number of ring atoms indicated. For example, C₃₋₁₀cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc.

[0012] "Heterocycloalkyl" means cycloalkyl, as defined in this application, provided that one or more of the ring carbons indicated, are replaced by a moiety selected from -O-, -N=, -NR-, -C(O)-, -S-, -S(O)- or -S(O)₂-, wherein R is hydrogen, C₁₋₄alkyl or a nitrogen protecting group. For example, C₃₋₈heterocycloalkyl as used in this application to describe compounds of the invention includes morpholino, pyrrolidinyl, pyrrolidinyl-2-one, piperazinyl, piperidinyl, piperidinylone, 1,4-dioxo-8-aza-spiro[4.5]dec-8-yl, thiomorpholino, sulfanomorpholino, sulfonomorpholino, etc.

[0013] "Halogen" (or halo) preferably represents chloro or fluoro, but may also be bromo or iodo.

[0014] "Hedgehog gain-of-function" refers to an aberrant modification or mutation of a Ptc gene, hedgehog gene, or smoothened gene, or a decrease (or loss) in the level of expression of such a gene, which results in a phenotype which resembles contacting a cell with a hedgehog protein, e.g., aberrant activation of a hedgehog pathway. The gain-of-function may include a loss of the ability of the Ptc gene product to regulate the level of expression of Gli genes, e.g., Gli1, Gli2, and Gli3. The term `hedgehog gain-of-function` is also used herein to refer to any similar cellular phenotype (e.g., exhibiting excess proliferation) which occurs due to an alteration anywhere in the hedgehog signal transduction pathway, including, but not limited to, a modification or mutation of hedgehog itself. For example, a tumor cell with an abnormally high proliferation rate due to activation of the hedgehog signaling pathway would have a `hedgehog gain-of-function` phenotype, even if hedgehog is not mutated in that cell.

[0015] "Patched loss-of-function" refers to an aberrant modification or mutation of a Ptc gene, or a decreased level of expression of the gene, which results in a phenotype which resembles contacting a cell with a hedgehog protein, e.g., aberrant activation of a hedgehog pathway. The loss-of-function may include a loss of the ability of the Ptc gene product to regulate the level of expression of Gli genes, e.g., Gli1, Gli2 and Gli3.

[0016] "Gli gain-of-function" refers to an aberrant modification or mutation of a Gli gene, or an increased level of expression of the gene, which results in a phenotype which resembles contacting a cell with a hedgehog protein, e.g., aberrant activation of a hedgehog pathway.

[0017] "Smoothened gain-of-function" refers to an aberrant modification or mutation of a Smo gene, or an increased level of expression of the gene, which results in a phenotype which resembles contacting a cell with a hedgehog protein, e.g., aberrant activation of a hedgehog pathway.

[0018] "Treat", "treating" and "treatment" refer to a method of alleviating or abating a disease and/or its attendant symptoms.

[0019] The present invention relates to the discovery that signal transduction pathways regulated by hedgehog, patched (Ptc), gli and/or smoothened can be modulated by compounds of Formula I.

Description of Preferred Embodiments

[0020] In one embodiment, with respect to compounds of Formula I, Y_1 and Y_2 are selected from N and CR_{10} ; wherein R_{10} is selected from hydrogen, methyl, fluoro, chloro, bromo, dimethylamino-ethoxy and trifluoromethyl; R_6 and R_7 are independently selected from hydrogen methyl, chloro, fluoro, bromo, trifluoromethyl and methoxy; with the proviso that R_6 and R_7 are not both hydrogen; and R_8 is selected from fluoro, chloro, methyl and trifluoromethyl.

[0020] In another embodiment, R_1 is selected from cyano, chloro, fluoro, methyl, ethyl, t-butyl, propyl, isobutyl, isopropyl, isopropoxy, butoxy, methoxy, dimethyl-amino, ethoxy, methyl-sulfanyl, phenyl, trifluoromethyl, trifluoromethoxy and piperazinyl optionally substituted with up to 2 methyl radicals; R_2 and R_5 are independently selected from hydrogen, chloro, fluoro, cyano, methyl, trifluoromethyl, isopropoxy, methoxy, ethoxy, trifluoromethoxy and dimethylamino; and R_3 and R_4 are independently selected from hydrogen, chloro, methyl, methoxy and cyano; or either R_1 and R_2 or R_1 and R_5 together with the phenyl to which they are both attached form quinoxaliny.

[0021] In another embodiment, R_9 is selected from $-S(O)_2R_{11}$, $-OR_{11}$, $-C(O)R_{11}$, $-NR_{12a}R_{12b}$ and $-R_{11}$; wherein R_{11} is selected from thiomorpholino, sulfonomorpholino, sulfanomorpholino, morpholino, cyclohexyl, phenyl, azepan-1-yl, 2-oxopiperazin-1-yl, 1,4-oxazepan-4-yl, piperidin-1-yl, tetrahydro-2H-pyran-4-yl, piperidin-3-yl, piperazinyl, pyrrolidinyl and 1,4-diazepan-1-yl; R_{12a} and R_{12b} are independently selected from isobutyl and hydroxy-ethyl; wherein said thiomorpholino, sulfonomorpholino, sulfanomorpholino, morpholino, cyclohexyl, phenyl, azepan-1-yl, 2-oxopiperazin-1-yl, 1,4-oxazepan-4-yl, piperidin-1-yl, tetrahydro-2H-pyran-4-yl, piperidin-3-yl, piperazinyl, pyrrolidinyl or 1,4-diazepan-1-yl of R_9 can be optionally substituted with 1 to 3 radicals independently selected from methyl, ethyl, methoxy, benzyl, thienyl-methyl, pyridinyl-methyl, benzo[d][1,3]dioxol-6-yl and 2,3-dihydrobenzo[b][1,4]dioxin-7-yl; wherein said phenyl or benzyl substituent of R_9 is

optionally substituted with 1 to 3 radicals independently selected from methoxy, ethoxy, methyl-piperazinyl, methyl, trifluoromethoxy, chloro, fluoro and trifluoromethyl.

[0022] Preferred compounds of Formula I are selected from 4'-cyano-6-methyl-biphenyl-3-carboxylic acid [4-(morpholine-4-sulfonyl)-phenyl]-amide, 4'-cyano-6-methyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Cyano-2-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Methoxy-2-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Methoxy-2-methyl-biphenyl-3-carboxylic acid (4-cyclohexyl-phenyl)-amide, 4'-Methoxy-2-methyl-biphenyl-3-carboxylic acid [6-(2-methyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Dimethylamino-2-methyl-biphenyl-3-carboxylic acid (4-cyclohexyl-phenyl)-amide, 4'-Dimethylamino-2-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 6-Chloro-4'-dimethylamino-biphenyl-3-carboxylic acid (6-[1,4]oxazepan-4-yl-pyridin-3-yl)-amide, 6-Chloro-4'-dimethylamino-biphenyl-3-carboxylic acid (6-morpholin-4-yl-pyridin-3-yl)-amide, 6-Chloro-4'-dimethylamino-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Chloro-4'-methoxy-biphenyl-3-carboxylic acid [6-(2-methyl-morpholin-4-yl)-pyridin-3-yl]-amide, 6-Chloro-4'-methoxy-biphenyl-3-carboxylic acid (6-[1,4]oxazepan-4-yl-pyridin-3-yl)-amide, 6-Chloro-4'-methoxy-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Chloro-4'-methoxy-biphenyl-3-carboxylic acid (6-morpholin-4-yl-pyridin-3-yl)-amide, 4'-Methoxy-6-methyl-biphenyl-3-carboxylic acid (6-morpholin-4-yl-pyridin-3-yl)-amide, 4'-Methoxy-6-methyl-biphenyl-3-carboxylic acid (6-[1,4]oxazepan-4-yl-pyridin-3-yl)-amide, 4'-Methoxy-6-methyl-biphenyl-3-carboxylic acid [6-(2-methyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Dimethylamino-6-methyl-biphenyl-3-carboxylic acid [6-(2-methyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Dimethylamino-6-methyl-biphenyl-3-carboxylic acid (6-[1,4]oxazepan-4-yl-pyridin-3-yl)-amide, 4'-Dimethylamino-6-methyl-biphenyl-3-carboxylic acid (6-morpholin-4-yl-pyridin-3-yl)-amide, 4'-Methoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Ethoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-4'-methylsulfanyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-

Dimethylamino-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-[1,1';4',1'']terphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Chloro-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 2',4'-Dichloro-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 2'-Chloro-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Chloro-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3',4'-Dichloro-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Chloro-6-methyl-4'-trifluoromethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6,4'-Dimethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Ethyl-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-tert-Butyl-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-4'-propyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Isobutyl-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Isopropyl-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6,2',6'-Trimethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6,2',3'-Trimethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-4'-trifluoromethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-3'-trifluoromethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-3', 5'-bistrifluoromethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Isopropoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Ethoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 2',6'-Dimethoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-4'-trifluoromethoxy-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-3'-trifluoromethoxy-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 6-Methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 4'-Methoxy-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 3'-Methoxy-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 4'-(2-Dimethylamino-ethoxy)-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 3'-Dimethylamino-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 4'-Fluoro-6-methyl-biphenyl-3-

carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 3'-Fluoro-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 2'-Fluoro-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 4-Methyl-N-(4-morpholin-4-yl-phenyl)-3-quinoxalin-6-yl-benzamide, 6-Methyl-4'-(4-methyl-piperazin-1-yl)-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 2'-Cyano-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 3'-Cyano-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (6-[1,4]oxazepan-4-yl-pyridin-3-yl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(2-methyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (3,4,5,6-tetrahydro-2H-[1,2']bipyridinyl-5'-yl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (6-morpholin-4-yl-pyridin-3-yl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-methyl-piperazin-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (3-fluoro-4-morpholin-4-yl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (3-chloro-4-morpholin-4-yl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (3-bromo-4-morpholin-4-yl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (3-methyl-4-morpholin-4-yl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (4-morpholin-4-yl-3-trifluoromethyl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (4-cyclohexyl-phenyl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid biphenyl-4-ylamide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (4'-methoxy-biphenyl-4-yl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [4-(4-benzyl-piperazin-1-yl)-phenyl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [4-(pyrrolidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-methoxy-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Cyano-2-methoxy-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Cyano-2-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Fluoro-4'-methoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Isopropoxy-6-methyl-

biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Butoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 3'-Chloro-4'-methoxy-6-methyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Methoxy-6,3'-dimethyl-biphenyl-3-carboxylic acid (6-azepan-1-yl-pyridin-3-yl)-amide, 4'-Cyano-2-methyl-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-fluoro-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 6-Bromo-4'-cyano-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-benzyl-[1,4]diazepan-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-thiophen-3-ylmethyl-[1,4]diazepan-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-2-methyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Methoxy-2-methyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 2-Methyl-4'-trifluoromethyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 2-Methyl-4'-trifluoromethoxy-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Cyano-2-methyl-biphenyl-3-carboxylic acid [6-(2-methyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Cyano-2-fluoro-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-trifluoromethyl-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-pyridin-4-ylmethyl-[1,4]diazepan-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-pyridin-3-ylmethyl-[1,4]diazepan-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2,6-dimethoxy-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2-ethoxy-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid (6-{4-[2-(4-methyl-piperazin-1-yl)-benzyl]-[1,4]diazepan-1-yl}-pyridin-3-yl)-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(4-methoxy-2,3-dimethyl-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2,3-dihydro-benzo[1,4]dioxin-6-ylmethyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-pyridin-2-ylmethyl-[1,4]diazepan-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic

acid [6-(4-benzo[1,3]dioxol-4-yl)methyl-[1,4]diazepan-1-yl]-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2-trifluoromethoxy-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2-dimethylamino-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2-chloro-5-trifluoromethyl-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2,3-difluoro-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2-chloro-4-fluoro-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2,6-difluoro-benzyl)-[1,4]diazepan-1-yl]-pyridin-3-yl}-amide, 2-Chloro-4'-cyano-biphenyl-3-carboxylic acid [4-(piperidine-1-sulfonyl)-phenyl]-amide, 4'-Cyano-6-trifluoromethyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 2-Chloro-4'-cyano-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-ethyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(3-fluoro-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(2-trifluoromethoxy-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(3-chloro-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(4-isobutyl-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(4-tert-butyl-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(7-methoxy-benzo[1,3]dioxol-5-yl)methyl]-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-benzyl-piperazin-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-pyridin-3-ylmethyl-piperazin-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(4-difluoromethoxy-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(4-cyano-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-quinolin-5-ylmethyl-piperazin-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-pyridin-4-ylmethyl-piperazin-1-yl)-

pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-pyridin-2-ylmethyl-piperazin-1-yl)-pyridin-3-yl]-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(4-imidazol-1-yl-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid {6-[4-(3-cyano-benzyl)-piperazin-1-yl]-pyridin-3-yl}-amide, 4'-Cyano-6-methyl-biphenyl-3-carboxylic acid [6-(4-isoquinolin-5-ylmethyl-piperazin-1-yl)-pyridin-3-yl]-amide, (R)-2-methyl-N-(6-(2-methylmorpholino)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 4'-cyano-2-methyl-N-(6-sulfonylmorpholinopyridin-3-yl)biphenyl-3-carboxamide, (S)-4'-cyano-2-methyl-N-(6-(2-methylmorpholino)pyridin-3-yl)biphenyl-3-carboxamide, (R)-6-chloro-N-(6-(2-methylmorpholino)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 4'-cyano-2-methyl-N-(6-sulfinylmorpholinopyridin-3-yl)biphenyl-3-carboxamide, 4'-cyano-N-(6-(diisobutylamino)pyridin-3-yl)-2-methylbiphenyl-3-carboxamide, 4'-cyano-N-(2-((2S,6R)-2,6-dimethylmorpholino)pyrimidin-5-yl)-2-methylbiphenyl-3-carboxamide, N-(2-((2S,6R)-2,6-dimethylmorpholino)pyrimidin-5-yl)-2-methyl-4'-(trifluoromethyl)biphenyl-3-carboxamide, N-(2-((2S,6R)-2,6-dimethylmorpholino)pyrimidin-5-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide, N-(2-(bis(2-hydroxyethyl)amino)pyrimidin-5-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 2-methyl-N-(6-(tetrahydro-2H-pyran-4-yloxy)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, N-(5-chloro-6-((2S,6R)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide, N-(6-((2R,6S)-2,6-dimethyltetrahydro-2H-pyran-4-yl)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide, N-(6-(4-ethylpiperazine-1-carbonyl)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 2-methyl-N-(6-(2-oxopiperazin-1-yl)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 2-methyl-N-(6-(1-(pyridin-4-ylmethyl)piperidin-4-yl)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 2-methyl-N-(6-(2-oxo-4-(pyridin-4-ylmethyl)piperazin-1-yl)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, 2-methyl-N-(6-(1-(pyridin-4-ylmethyl)piperidin-3-yl)pyridin-3-yl)-4'-(trifluoromethoxy)biphenyl-3-carboxamide, N-(6-(1-ethylpiperidin-3-yl)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-

carboxamide and N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide.

[0023] It is, therefore, specifically contemplated that compounds of Formula I which interfere with aspects of hedgehog, Ptc, or smoothened signal transduction activity will likewise be capable of inhibiting proliferation (or other biological consequences) in normal cells and/or cells having a patched loss-of-function phenotype, a hedgehog gain-of-function phenotype, a smoothened gain-of-function phenotype or a Gli gain-of-function phenotype. Thus, it is contemplated that in certain embodiments, these compounds may be useful for inhibiting hedgehog activity in normal cells, e.g., which do not have a genetic mutation that activates the hedgehog pathway. In preferred embodiments, the compounds are capable of inhibiting at least some of the biological activities of hedgehog proteins, preferably specifically in target cells.

[0024] Thus, the methods of the present invention include the use of compounds of Formula I which agonize Ptc inhibition of hedgehog signaling, such as by inhibiting activation of smoothened or downstream components of the signal pathway, in the regulation of repair and/or functional performance of a wide range of cells, tissues and organs, including normal cells, tissues, and organs, as well as those having the phenotype of Ptc loss-of-function, hedgehog gain-of-function, smoothened gain-of-function or Gli gain-of-function. For instance, the subject method has therapeutic and cosmetic applications ranging from regulation of neural tissues, bone and cartilage formation and repair, regulation of spermatogenesis, regulation of smooth muscle, regulation of lung, liver and other organs arising from the primitive gut, regulation of hematopoietic function, regulation of skin and hair growth, etc. Moreover, the subject methods can be performed on cells which are provided in culture (in vitro), or on cells in a whole animal (in vivo).

[0025] In another embodiment, the subject method can be to treat epithelial cells having a phenotype of Ptc loss-of-function, hedgehog gain-of-function, smoothened gain-of-function or Gli gain-of-function. For instance, the subject method can be used in treating or preventing basal cell carcinoma or other hedgehog pathway-related disorders.

[0026] In certain embodiments, a compound of Formula I can inhibit activation of a hedgehog pathway by binding to smoothened or its downstream proteins. In certain embodiments, a subject antagonist may inhibit activation of a hedgehog pathway by binding to patched.

[0027] In another preferred embodiment, the subject method can be used as part of a treatment regimen for malignant medulloblastomas and other primary CNS malignant neuroectodermal tumors.

[0028] In another aspect, the present invention provides pharmaceutical preparations comprising, as an active ingredient, a hedgehog signaling modulator such as a compound of Formula I, a Ptc agonist, a smoothened antagonist, or downstream hedgehog pathway protein antagonist such as described herein, formulated in an amount sufficient to inhibit, in vivo, proliferation or other biological consequences of Ptc loss-of-function, hedgehog gain-of-function, smoothened gain-of-function or Gli gain-of-function.

[0029] The subject treatments using a compound of Formula I, patched agonists, smoothened antagonists, or downstream hedgehog pathway protein antagonists can be effective for both human and animal subjects. Animal subjects to which the invention is applicable extend to both domestic animals and livestock, raised either as pets or for commercial purposes. Examples are dogs, cats, cattle, horses, sheep, hogs, and goats.

Pharmacology and Utility

[0030] The present invention makes available methods and compounds for inhibiting activation of the hedgehog signaling pathway, e.g., to inhibit aberrant growth states resulting from phenotypes such as Ptc loss-of-function, hedgehog gain-of-function, smoothened gain-of-function or Gli gain-of-function, comprising contacting the cell with a compound of Formula I, in a sufficient amount to agonize a normal Ptc activity, antagonize a normal hedgehog activity, antagonize smoothened activity, or antagonize Gli activity e.g., to reverse or control the aberrant growth state.

[0031] Members of the Hedgehog family of signaling molecules mediate many important short- and long-range patterning processes during vertebrate development. Pattern formation is the activity by which embryonic cells form

ordered spatial arrangements of differentiated tissues. The physical complexity of higher organisms arises during embryogenesis through the interplay of cell-intrinsic lineage and cell-extrinsic signaling. Inductive interactions are essential to embryonic patterning in vertebrate development from the earliest establishment of the body plan, to the patterning of the organ systems, to the generation of diverse cell types during tissue differentiation. The effects of developmental cell interactions are varied: responding cells are diverted from one route of cell differentiation to another by inducing cells that differ from both the uninduced and induced states of the responding cells (inductions). Sometimes cells induce their neighbors to differentiate like themselves (homeogenetic induction); in other cases a cell inhibits its neighbors from differentiating like itself. Cell interactions in early development may be sequential, such that an initial induction between two cell types leads to a progressive amplification of diversity. Moreover, inductive interactions occur not only in embryos, but in adult cells as well, and can act to establish and maintain morphogenetic patterns as well as induce differentiation.

[0032] The vertebrate family of hedgehog genes includes three members that exist in mammals, known as Desert (Dhh), Sonic (Shh) and Indian (Ihh) hedgehogs, all of which encode secreted proteins. These various Hedgehog proteins consist of a signal peptide, a highly conserved N-terminal region, and a more divergent C-terminal domain. Biochemical studies have shown that autoproteolytic cleavage of the Hh precursor protein proceeds through an internal thioester intermediate which subsequently is cleaved in a nucleophilic substitution. It is likely that the nucleophile is a small lipophilic molecule which becomes covalently bound to the C-terminal end of the N-peptide, tethering it to the cell surface. The biological implications are profound. As a result of the tethering, a high local concentration of N-terminal Hedgehog peptide is generated on the surface of the Hedgehog producing cells. It is this N-terminal peptide which is both necessary and sufficient for short- and long-range Hedgehog signaling activities.

[0033] An inactive Hedgehog signaling pathway is where the transmembrane protein receptor Patched (Ptc) inhibits the activity of Smoothened (Smo), a seven transmembrane protein. The transcription factor Gli, a downstream

component of Hh signaling, is prevented from entering the nucleus through interactions with cytoplasmic proteins, including Fused and Suppressor of fused (Sufu). As a consequence, transcriptional activation of Hedgehog target genes is repressed. Activation of the pathway is initiated through binding of any of the three mammalian ligands (Dhh, Shh or Ihh) to Ptc. Ligand binding results in a reversal of the repression of Smo, thereby activating a cascade that leads to the translocation of the active form of the transcription factor Gli to the nucleus. Nuclear Gli activates target gene expression, including Ptc and Gli itself.

[0034] Increased levels of Hedgehog signaling are sufficient to initiate cancer formation and are required for tumor survival. These cancers include, but are not limited to, prostate cancer ("Hedgehog signalling in prostate regeneration, neoplasia and metastasis", Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs JT, Berman DM, Beachy PA., Nature. 2004 Oct 7;431(7009):707-12; "Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling", Sanchez P, Hernandez AM, Stecca B, Kahler AJ, DeGueme AM, Barrett A, Beyna M, Datta MW, Datta S, Ruiz i Altaba A., Proc Natl Acad Sci U S A. 2004 Aug 24;101(34):12561-6), breast cancer ("Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer", Kubo M, Nakamura M, Tasaki A, Yamanaka N, Nakashima H, Nomura M, Kuroki S, Katano M., Cancer Res. 2004 Sep 1;64(17):6071-4), medulloblastoma ("Medulloblastoma growth inhibition by hedgehog pathway blockade", Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, Chen JK, Cooper MK, Taipale J, Olson JM, Beachy PA., Science. 2002 Aug 30;297(5586):1559-61), basal cell carcinoma ("Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions", Williams JA, Guicherit OM, Zaharian BI, Xu Y, Chai L, Wichterle H, Kon C, Gatchalian C, Porter JA, Rubin LL, Wang FY., Proc Natl Acad Sci U S A. 2003 Apr 15;100(8):4616-21; "Activating Smoothened mutations in sporadic basal-cell carcinoma", Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, Rosenthal A, Epstein EH Jr, de Sauvage FJ., Nature. 1998 Jan 1;391(6662):90-2), pancreatic cancer ("Hedgehog is an early and late mediator

of pancreatic cancer tumorigenesis”, Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernandez-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M., Nature. 2003 Oct 23;425(6960):851-6; “Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours”, Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, Parker AR, Shimada Y, Eshleman JR, Watkins DN, Beachy PA., Nature. 2003 Oct 23;425(6960):846-51), and small-cell lung cancer (“Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer”, Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB., Nature. 2003 Mar 20;422(6929):313-7).

[0035] In accordance with the foregoing, the present invention further provides a method for preventing or treating any of the diseases or disorders described above in a subject in need of such treatment, which method comprises administering to said subject a therapeutically effective amount (See, “Administration and Pharmaceutical Compositions”, infra) of a compound of Formula I or a pharmaceutically acceptable salt thereof. For any of the above uses, the required dosage will vary depending on the mode of administration, the particular condition to be treated and the effect desired.

Administration and Pharmaceutical Compositions:

[0036] In general, compounds of the invention will be administered in therapeutically effective amounts via any of the usual and acceptable modes known in the art, either singly or in combination with one or more therapeutic agents. A therapeutically effective amount may vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compound used and other factors. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 2.5mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5mg to about 100mg, conveniently administered, e.g. in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 1 to 50mg active ingredient.

[0037] Compounds of the invention can be administered as pharmaceutical compositions by any conventional route, in particular enterally, e.g., orally, e.g., in the form of tablets or capsules, or parenterally, e.g., in the form of injectable solutions or suspensions, topically, e.g., in the form of lotions, gels, ointments or creams, or in a nasal or suppository form. Pharmaceutical compositions comprising a compound of the present invention in free form or in a pharmaceutically acceptable salt form in association with at least one pharmaceutically acceptable carrier or diluent can be manufactured in a conventional manner by mixing, granulating or coating methods. For example, oral compositions can be tablets or gelatin capsules comprising the active ingredient together with a) diluents, e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; b) lubricants, e.g., silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol; for tablets also c) binders, e.g., magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and or polyvinylpyrrolidone; if desired d) disintegrants, e.g., starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or e) absorbents, colorants, flavors and sweeteners. Injectable compositions can be aqueous isotonic solutions or suspensions, and suppositories can be prepared from fatty emulsions or suspensions. The compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. Suitable formulations for transdermal applications include an effective amount of a compound of the present invention with a carrier. A carrier can include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. For example, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin. Matrix transdermal formulations may also be used. Suitable formulations for topical application, e.g., to the skin and eyes, are preferably aqueous

solutions, ointments, creams or gels well-known in the art. Such may contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.

[0038] Compounds of the invention can be administered in therapeutically effective amounts in combination with one or more therapeutic agents (pharmaceutical combinations). For example, synergistic effects can occur with immunomodulatory or anti-inflammatory substances or other anti-tumor therapeutic agents. Where the compounds of the invention are administered in conjunction with other therapies, dosages of the co-administered compounds will of course vary depending on the type of co-drug employed, on the specific drug employed, on the condition being treated and so forth.

[0039] The invention also provides for a pharmaceutical combinations, e.g. a kit, comprising a) a first agent which is a compound of the invention as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent. The kit can comprise instructions for its administration.

[0040] The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

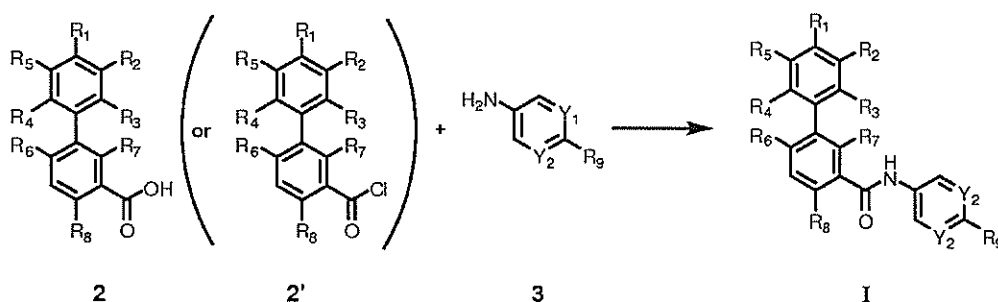
[0041] The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of Formula I and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of Formula I and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the 2 compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

Processes for Making Compounds of the Invention

[0042] The present invention also includes processes for the preparation of compounds of the invention. In the reactions described, it can be necessary to protect reactive functional groups, for example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups can be used in accordance with standard practice, for example, see T.W. Greene and P. G. M. Wuts in "Protective Groups in Organic Chemistry", John Wiley and Sons, 1991.

[0043] Compounds of Formula I can be prepared by proceeding as in the following Reaction Scheme I:

Reaction Scheme I:



in which Y_1 , Y_2 , R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 and R_9 are as defined for Formula I in the Summary of the Invention. A compound of Formula I can be prepared by reacting a compound of formula 2 (or 2') with a compound of formula 3 in the presence of a suitable solvent (e.g., dichloromethane, *N,N*-dimethylformide or the like), in a temperature range of about -20 to about 100°C. The reaction can take up to about 20 hours to complete.

[0044] Detailed examples of the synthesis of compounds of Formula I can be found in the Examples, *infra*.

Additional Processes for Making Compounds of the Invention

[0045] A compound of the invention can be prepared as a pharmaceutically acceptable acid addition salt by reacting the free base form of the compound with a pharmaceutically acceptable inorganic or organic acid.

Alternatively, a pharmaceutically acceptable base addition salt of a compound of the invention can be prepared by reacting the free acid form of the compound with a pharmaceutically acceptable inorganic or organic base.

[0046] Alternatively, the salt forms of the compounds of the invention can be prepared using salts of the starting materials or intermediates.

[0047] The free acid or free base forms of the compounds of the invention can be prepared from the corresponding base addition salt or acid addition salt form, respectively. For example a compound of the invention in an acid addition salt form can be converted to the corresponding free base by treating with a suitable base (e.g., ammonium hydroxide solution, sodium hydroxide, and the like). A compound of the invention in a base addition salt form can be converted to the corresponding free acid by treating with a suitable acid (e.g., hydrochloric acid, etc.).

[0048] Compounds of the invention in unoxidized form can be prepared from N-oxides of compounds of the invention by treating with a reducing agent (e.g., sulfur, sulfur dioxide, triphenyl phosphine, lithium borohydride, sodium borohydride, phosphorus trichloride, tribromide, or the like) in a suitable inert organic solvent (e.g. acetonitrile, ethanol, aqueous dioxane, or the like) at 0 to 80°C.

[0049] Prodrug derivatives of the compounds of the invention can be prepared by methods known to those of ordinary skill in the art (e.g., for further details see Saulnier et al., (1994), Bioorganic and Medicinal Chemistry Letters, Vol. 4, p. 1985). For example, appropriate prodrugs can be prepared by reacting a non-derivatized compound of the invention with a suitable carbamylating agent (e.g., 1,1-acyloxyalkylcarbanochloridate, para-nitrophenyl carbonate, or the like).

[0050] Protected derivatives of the compounds of the invention can be made by means known to those of ordinary skill in the art. A detailed description of techniques applicable to the creation of protecting groups and their removal can be found in T. W. Greene, "Protecting Groups in Organic Chemistry", 3rd edition, John Wiley and Sons, Inc., 1999.

[0051] Compounds of the present invention can be conveniently prepared, or formed during the process of the invention, as solvates (e.g., hydrates). Hydrates of compounds of the present invention can be conveniently prepared by

recrystallization from an aqueous/organic solvent mixture, using organic solvents such as dioxin, tetrahydrofuran or methanol.

[0052] Compounds of the invention can be prepared as their individual stereoisomers by reacting a racemic mixture of the compound with an optically active resolving agent to form a pair of diastereoisomeric compounds, separating the diastereomers and recovering the optically pure enantiomers. While resolution of enantiomers can be carried out using covalent diastereomeric derivatives of the compounds of the invention, dissociable complexes are preferred (e.g., crystalline diastereomeric salts). Diastereomers have distinct physical properties (e.g., melting points, boiling points, solubilities, reactivity, etc.) and can be readily separated by taking advantage of these dissimilarities. The diastereomers can be separated by chromatography, or preferably, by separation/resolution techniques based upon differences in solubility. The optically pure enantiomer is then recovered, along with the resolving agent, by any practical means that would not result in racemization. A more detailed description of the techniques applicable to the resolution of stereoisomers of compounds from their racemic mixture can be found in Jean Jacques, Andre Collet, Samuel H. Wilen, "Enantiomers, Racemates and Resolutions", John Wiley And Sons, Inc., 1981.

[0053] In summary, the compounds of Formula I can be made by a process, which involves:

- (a) those of reaction scheme I; and
- (b) optionally converting a compound of the invention into a pharmaceutically acceptable salt;
- (c) optionally converting a salt form of a compound of the invention to a non-salt form;
- (d) optionally converting an unoxidized form of a compound of the invention into a pharmaceutically acceptable N-oxide;
- (e) optionally converting an N-oxide form of a compound of the invention to its unoxidized form;
- (f) optionally resolving an individual isomer of a compound of the invention from a mixture of isomers;

(g) optionally converting a non-derivatized compound of the invention into a pharmaceutically acceptable prodrug derivative; and

(h) optionally converting a prodrug derivative of a compound of the invention to its non-derivatized form.

[0055] Insofar as the production of the starting materials is not particularly described, the compounds are known or can be prepared analogously to methods known in the art or as disclosed in the Examples hereinafter.

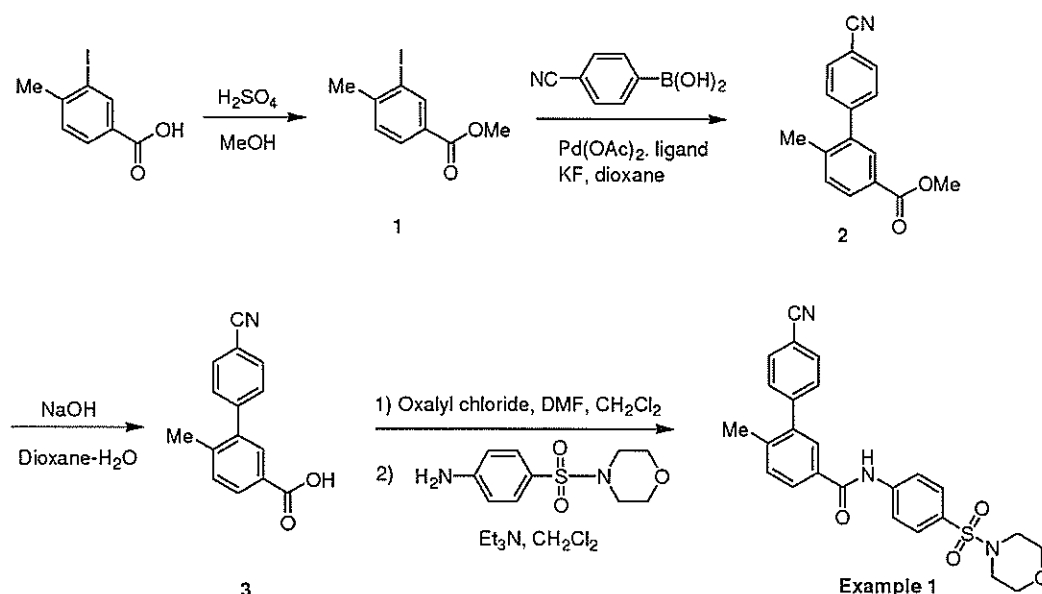
[0056] One of skill in the art will appreciate that the above transformations are only representative of methods for preparation of the compounds of the present invention, and that other well known methods can similarly be used.

Examples

[0057] The present invention is further exemplified, but not limited, by the following example that illustrates the preparation of compounds of Formula I according to the invention.

Example 1

4'-cyano-6-methyl-biphenyl-3-carboxylic acid [4-(morpholine-4-sulfonyl)-phenyl]-amide



[0058] Step 1: To a solution of 3-iodo-4-methyl-benzoic acid (10.0 g, 38.2 mmol) in methanol (70 ml) is added concentrated sulfuric acid (0.5 ml). The reaction mixture is heated at 70°C for 48 hours, cooled to room ambient temperature and then concentrated. After that, ethyl acetate (100 ml) and aqueous NaHCO₃ (saturated, 100 ml) solution are added to the residue. The organic layer is separated and washed again with aqueous NaHCO₃ (saturated, 100 ml) solution. The organic layer is separated, dried over anhydrous Na₂SO₄ and concentrated to yield 3-iodo-4-methyl-benzoic acid methyl ester 1. It is used without further purification in the next step. ¹H NMR (400 MHz, DMSO-d₆) δ 8.31 (s, 1 H), 7.87 (d, 1 H, J = 8.4 Hz), 7.48 (d, 1 H, J = 8.4 Hz), 3.85 (s, 3 H), 3.35 (s, 3H); LC-MS m/z: 277.0 (M+1).

[0059] Step 2: To a round-bottom flask containing 3-iodo-4-methyl-benzoic acid methyl ester (1.38 g, 5.00 mmol), 4-cyanophenylboronic acid (1.10 g, 7.48 mmol), palladium acetate (168 mg, 0.748 mmol), 2-(dicyclohexylphosphino)biphenyl (0.526 g, 1.50 mmol) and potassium fluoride (0.870 g, 15.0 mmol) is added anhydrous 1,4-dioxane (15 ml). The flask is purged with argon and sealed. The mixture is stirred at 130°C for 18 hours, cooled to ambient temperature and then water (20 ml) and ethyl acetate (20 ml) are added. Solid is removed under vacuum filtration. The filtrate is extracted with EtOAc (20 ml x 2). The organic layers are combined, washed with aqueous HCl (5%, 20 ml) and saturated NaHCO₃ (20 ml). It is dried over MgSO₄, and concentrated. The residue is purified by silica gel column chromatography (EtOAc/Hexane, gradient) to give 4'-cyano-6-methyl-biphenyl-3-carboxylic acid methyl ester 2; LC-MS m/z: 252.1 (M+1).

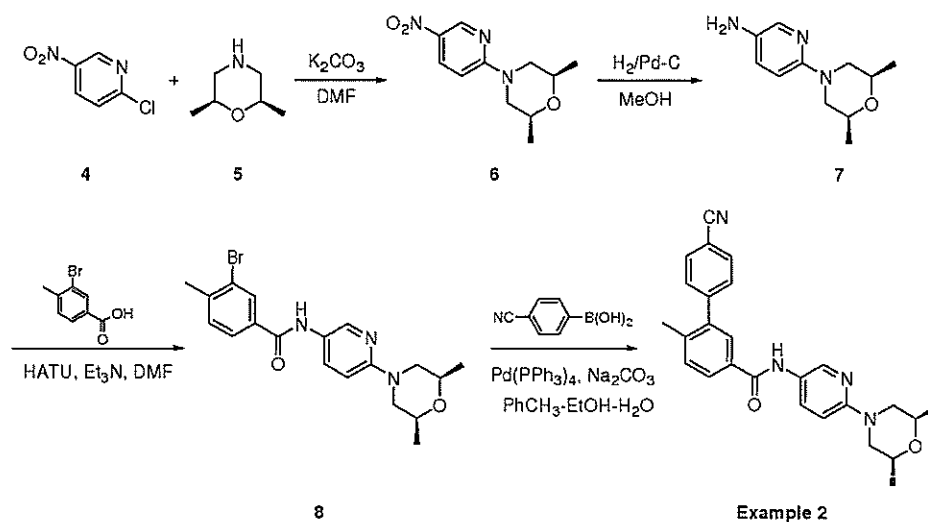
[0060] Step 3: To a solution of 4'-cyano-6-methyl-biphenyl-3-carboxylic acid methyl ester 2 (2.56 g, 10.3 mmol) in 1,4-dioxane-H₂O (1:1 mixture, 20 ml) is added NaOH (1.22 g, 30.2 mmol). The reaction is stirred at ambient temperature for 24 hours. To this mixture is added aqueous HCl (1 N, 36 ml) and it is then extracted with ethyl acetate (40 ml x 3). The organic layers are combined, dried over anhydrous Na₂SO₄. The solvent is removed. The solid obtained is washed with small amount of

acetonitrile and air dried to give 4'-cyano-6-methyl-biphenyl-3-carboxylic acid 3: ^1H NMR (DMSO-d_6) δ 7.94 (d, 2 H, $J = 8.0$ Hz), 7.84 (dd, 1 H, $J_1 = 8.4$ Hz, $J_2 = 1.2$ Hz), 7.75 (d, 1 H, $J = 1.2$ Hz), 7.61 (d, 2 H, $J = 8.0$ Hz), 7.48 (d, 1 H, $J = 8.4$ Hz), 2.29 (s, 3 H); LC-MS m/z 238.1 (M+1).

[0061] Step 4: To a suspension of 4'-cyano-6-methyl-biphenyl-3-carboxylic acid 3 (40 mg, 0.17 mmol) in anhydrous methylene chloride (5 ml) is added 2 drops of DMF. Then oxalyl chloride (32 mg, 22 μl , 0.25 mmol) is added. The mixture is stirred at ambient temperature until it turns clear. After that, it is concentrated, re-dissolved in anhydrous methylene chloride (3 ml), and added to a solution of 4-(morpholine-4-sulfonyl)-phenylamine (61 mg, 0.25 mmol) and triethylamine (34 mg, 47 μl , 0.33 mmol) in methylene chloride (2 ml). The mixture is stirred for 2 hours, concentrated and the residue is purified by preparative mass triggered HPLC (C_{18} column, eluted with $\text{CH}_3\text{CN-H}_2\text{O}$ containing 0.05% TFA) to give 4'-cyano-6-methyl-biphenyl-3-carboxylic acid [4-(morpholine-4-sulfonyl)-phenyl]-amide: ^1H NMR (DMSO-d_6) δ 10.64 (s, 1 H), 8.07 (d, 2 H, $J = 8.8$ Hz), 7.97 (d, 2 H, $J = 8.4$ Hz), 7.95 (d, 1 H, $J = 8.8$ Hz), 7.89 (s, 1 H), 7.43 (d, 2 H, $J = 8.4$ Hz), 7.67 (d, 2 H, $J = 8.8$ Hz), 7.53 (d, 2 H, $J = 8.8$ Hz), 3.63 (m, 4 H), 2.84 (m, 4 H) 2.32 (s, 3 H); LC-MS m/z : 462.1 (M+1).

Example 2

4'-cyano-6-methyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide



[0062] Step 1: To a solution of 2-chloro-5-nitro-pyridine **4** (2.38 g, 15 mmol) and *cis*-2,6-dimethylmorpholine (1.73 g, 15 mmol) is added K_2CO_3 (4.14 g, 30 mmol). The mixture was heated at 50°C overnight. After concentration, the residue is partitioned between EtOAc and water. The EtOAc layer is dried over anhydrous Na_2SO_4 and concentrated to give crude product **6** as a yellow solid. The crude product is used directly in next step without further purification. LC-MS m/z : 238.1 ($\text{M}+1$).

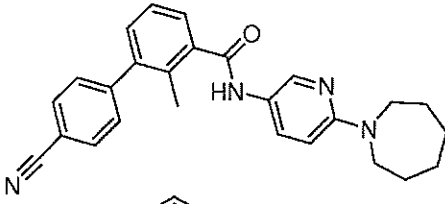
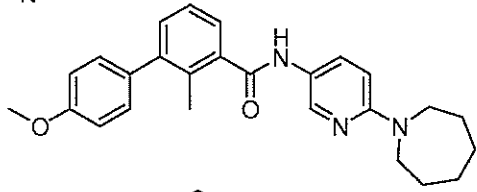
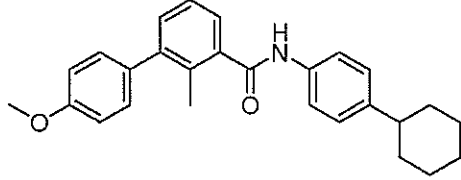
[0063] Step 2: The above crude material **6** is hydrogenated in the presence of Pd-C (0.2 g) in MeOH (100 mL) under hydrogen over 10 h. The suspension is filtered through celite and the filtrate is concentrated to give the crude product **7** as a dark brown oil which is used directly in the next step without further purification. LC-MS m/z : 208.1 ($\text{M}+1$).

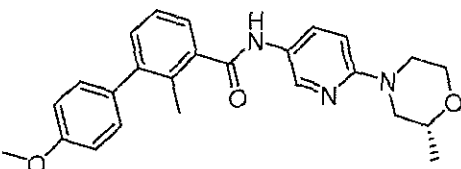
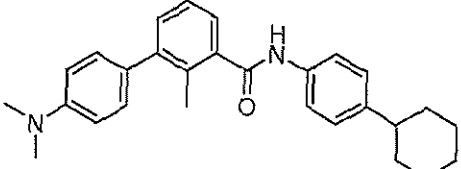
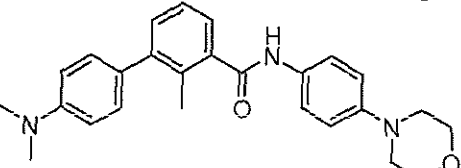
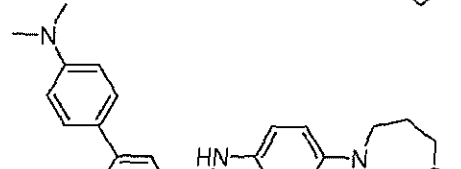
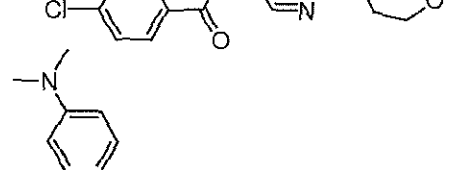
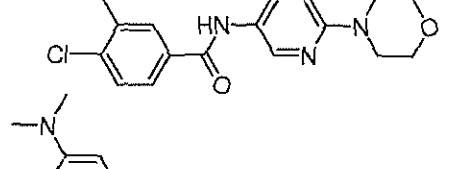
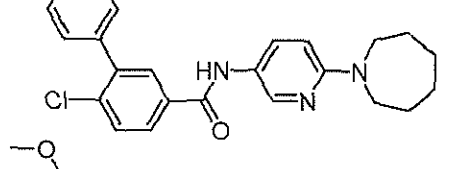
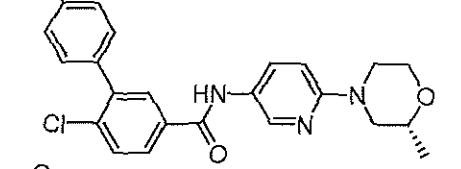
[0064] Step 3: To a solution of 3-bromo-4-methyl benzoic acid (108 mg, 0.5 mmol), 6-(2,6-Dimethyl-morpholin-4-yl)-pyridin-3-ylamine **7** (104 mg, 0.5 mmol), and HATU (190 mg, 0.5 mmol) in dry DMF (5 mL) is added triethylamine (139 μL , 1.0 mmol) dropwise. The resulting mixture is stirred at room temperature for 2 h. After concentration, the residue is partitioned between EtOAc and water. The organic layer is dried and concentrated to give the crude product. The final compound is purified by flash column chromatography using 50% EtOAc in hexane as eluent to give **8** as a white solid. LC-MS m/z : 404.1 ($\text{M}+1$).

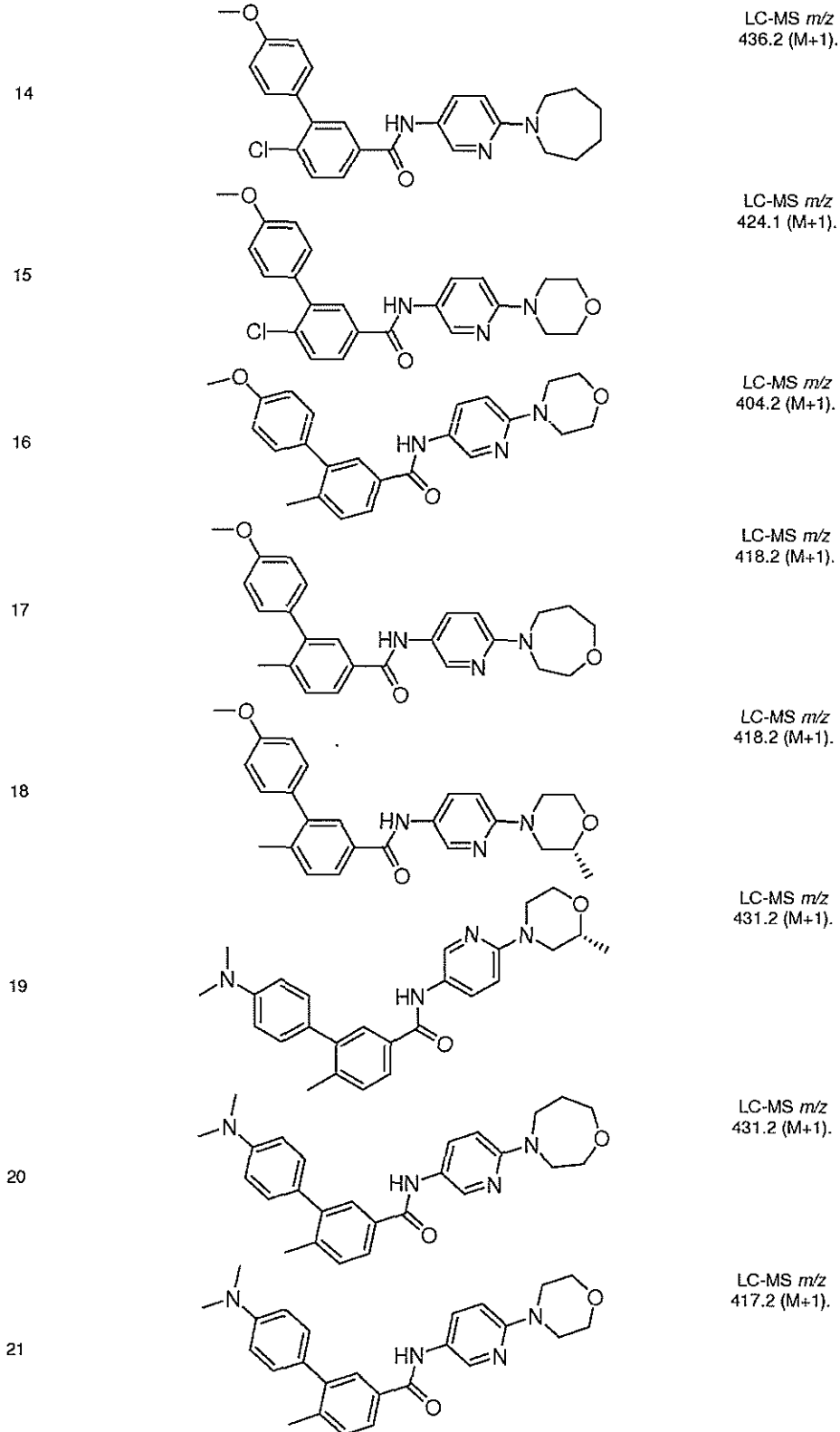
[0065] Step 4: A mixture of 4-cyanophenyl boronic acid (18 mg, 0.12 mmol), 3-bromo-N-[6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-4-methyl-benzamide **8** (40 mg, 0.1 mmol), Pd(PPh₃)₄ (11 mg, 0.01 mmol), and Na₂CO₃ (42 mg, 0.4 mmol) in a combined solvent system of toluene (0.2 mL) and water (0.2 mL) and ethanol (0.05 mL) is heated at 140°C under microwave irradiation for 30 min. The reaction mixture is diluted with EtOAc and water. The aqueous layer is extracted with EtOAc. The combined organic layer is washed with brine and concentrated to give the crude product which is purified by preparative mass triggered HPLC (C₁₈ column, eluted with CH₃CN-H₂O containing 0.05% TFA) to give 4'-cyano-6-methyl-biphenyl-3-carboxylic acid [6-(2,6-dimethyl-morpholin-4-yl)-pyridin-3-yl]-amide. LC-MS *m/z*: 427.2 (M+1).

[0066] By repeating the procedures described in the above examples, using appropriate starting materials, the following compounds of Formula I, as identified in Table 1, are obtained.

Table 1

Compound Number	Structure	Physical Data MS (<i>m/z</i>) LC-MS <i>m/z</i> 411.2 (M+1).
3		LC-MS <i>m/z</i> 416.2 (M+1).
4		LC-MS <i>m/z</i> 400.2 (M+1).
5		

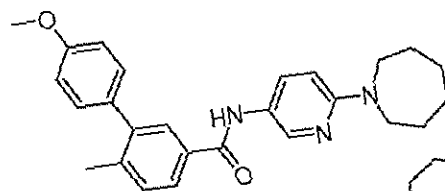
6		LC-MS m/z 418.2 (M+1).
7		LC-MS m/z 413.2 (M+1).
8		LC-MS m/z 416.2 (M+1).
9		LC-MS m/z 451.2 (M+1).
10		LC-MS m/z 437.2 (M+1).
11		LC-MS m/z 449.2 (M+1).
12		LC-MS m/z 438.2 (M+1).
13		LC-MS m/z 438.2 (M+1).



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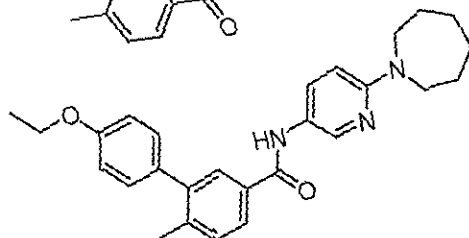
LC-MS m/z
416.2 (M+1).

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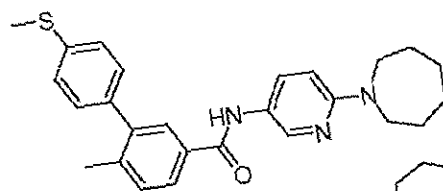
LC-MS m/z
430.2 (M+1).

23



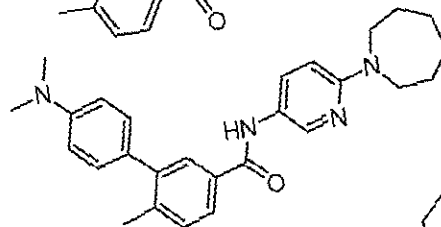
LC-MS m/z
432.2 (M+1).

24



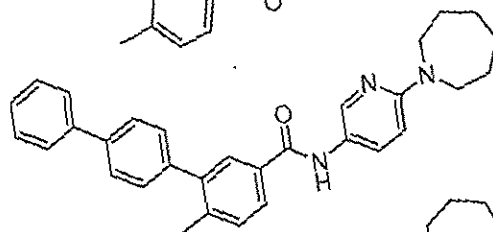
LC-MS m/z
429.2 (M+1).

25



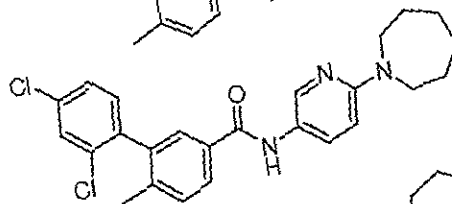
LC-MS m/z
462.2 (M+1).

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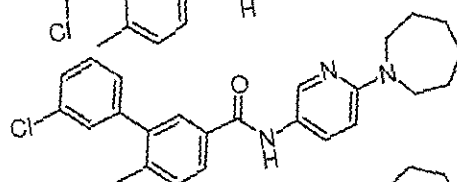
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454.1 (M+1).

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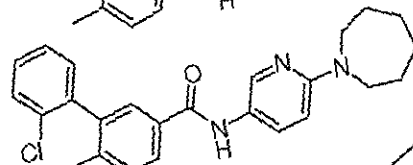
LC-MS m/z
420.2 (M+1).

28



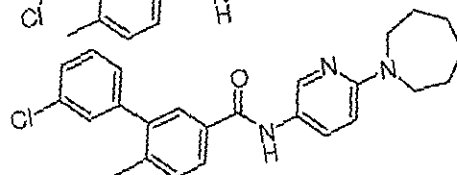
LC-MS m/z
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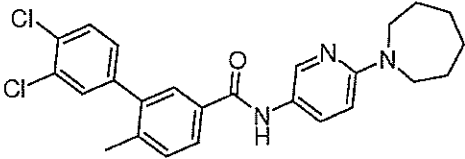
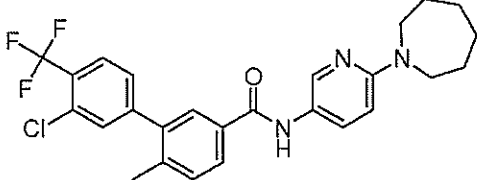
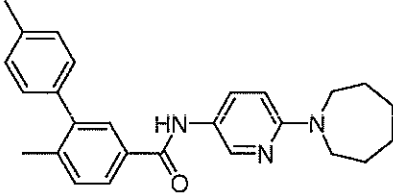
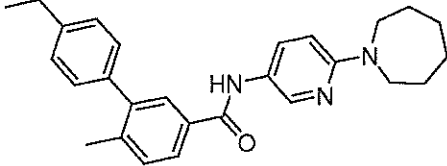
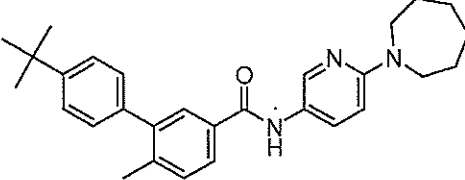
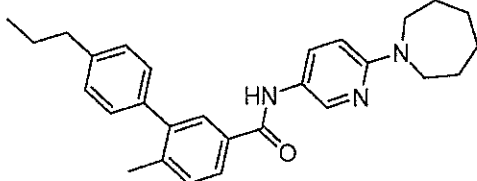
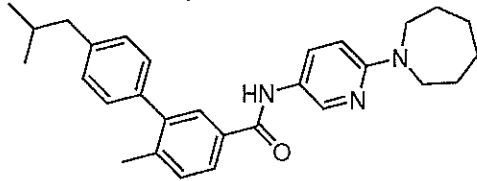
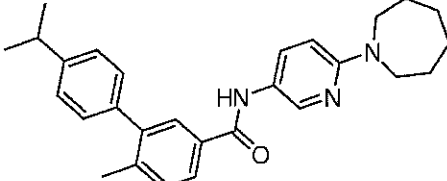
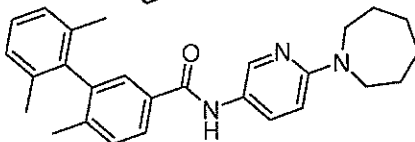
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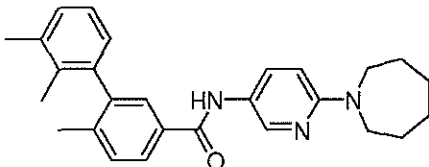
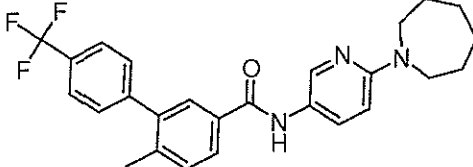
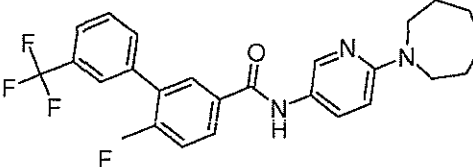
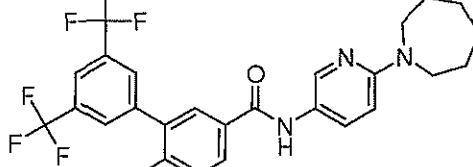
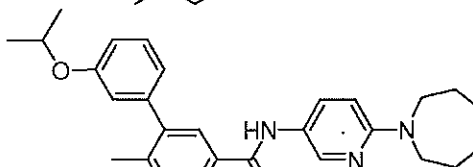
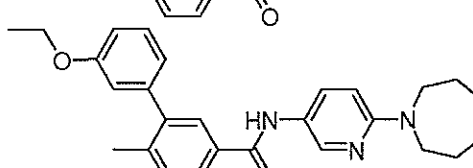
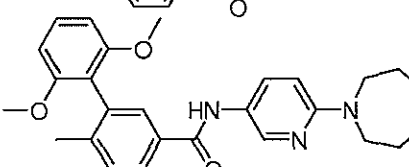
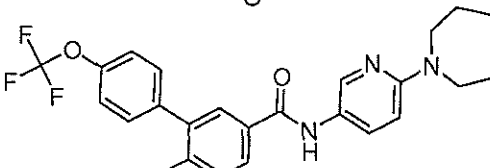
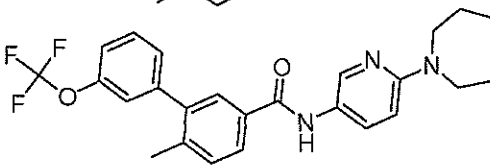


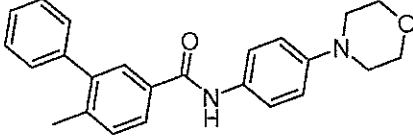
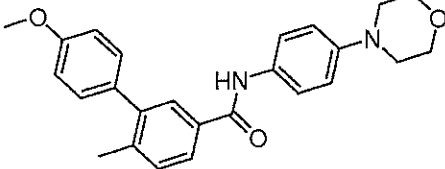
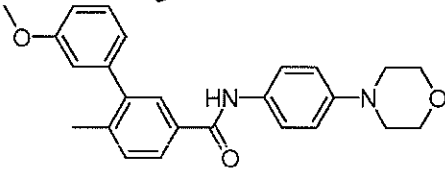
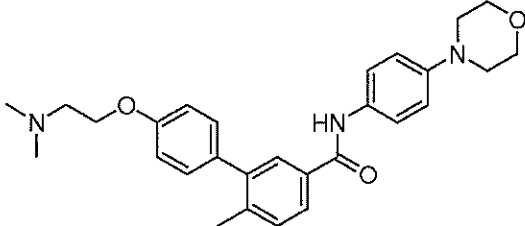
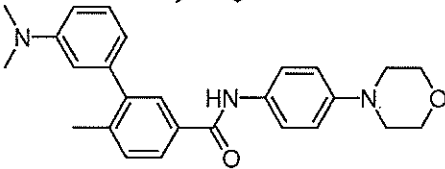
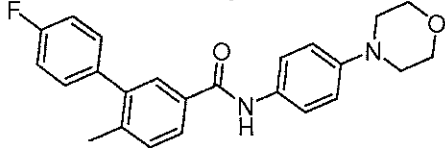
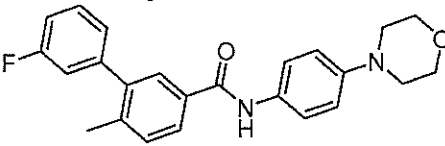
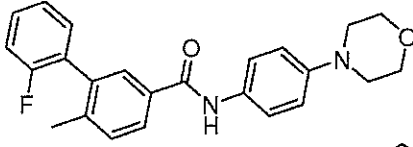
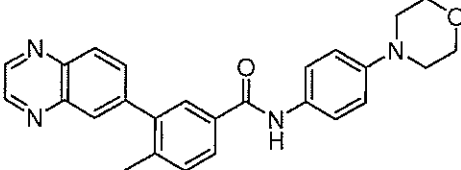
LC-MS m/z
420.2 (M+1).

30

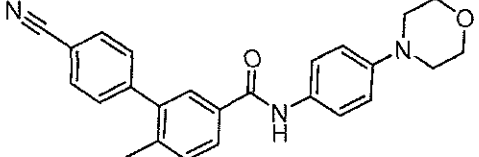
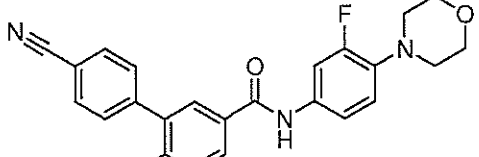
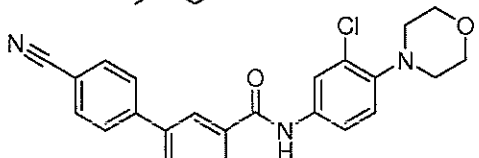
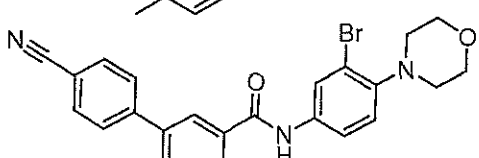
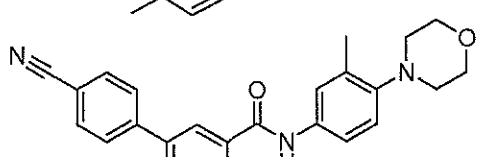
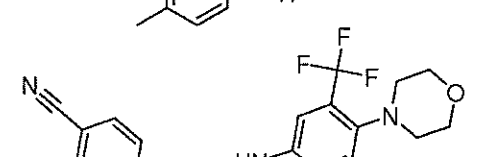
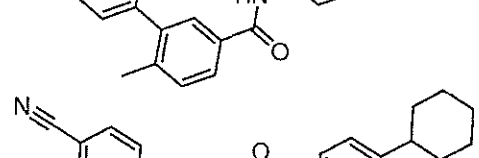
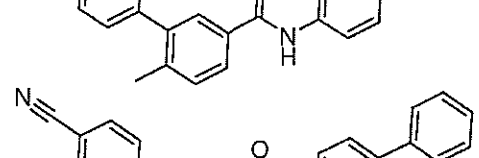
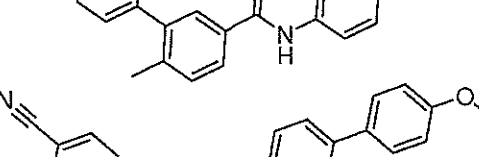


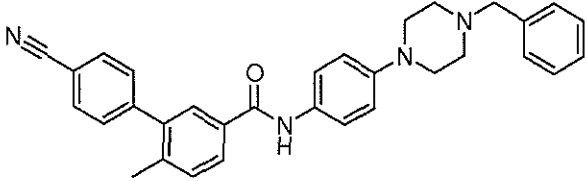
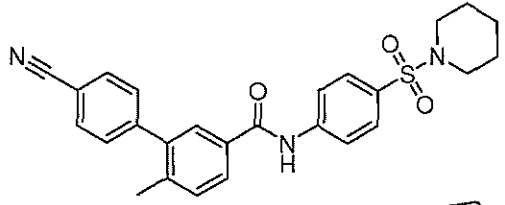
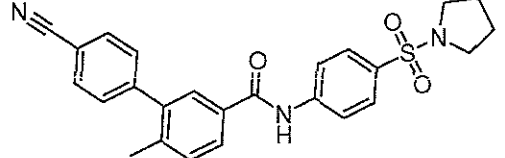
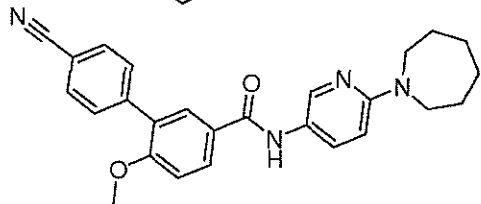
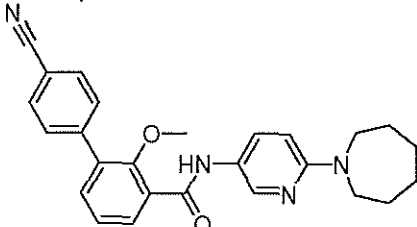
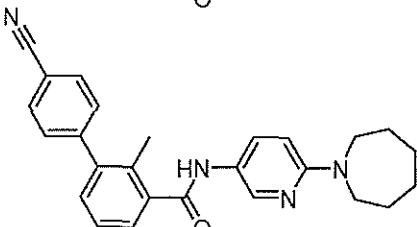
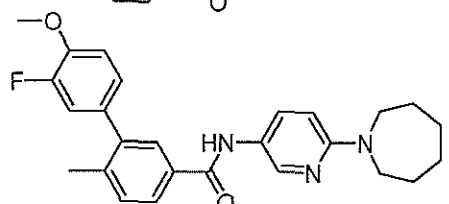
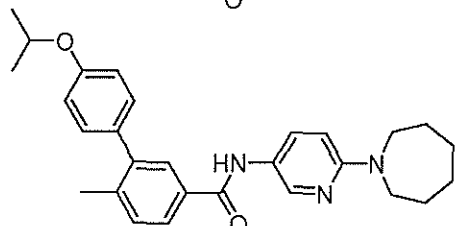
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32		LC-MS <i>m/z</i> 488.1 (M+1).
33		LC-MS <i>m/z</i> 400.2 (M+1).
34		LC-MS <i>m/z</i> 414.2 (M+1).
35		LC-MS <i>m/z</i> 442.2 (M+1).
36		LC-MS <i>m/z</i> 428.2 (M+1).
37		LC-MS <i>m/z</i> 442.2 (M+1).
38		LC-MS <i>m/z</i> 428.2 (M+1).
39		LC-MS <i>m/z</i> 414.2 (M+1).

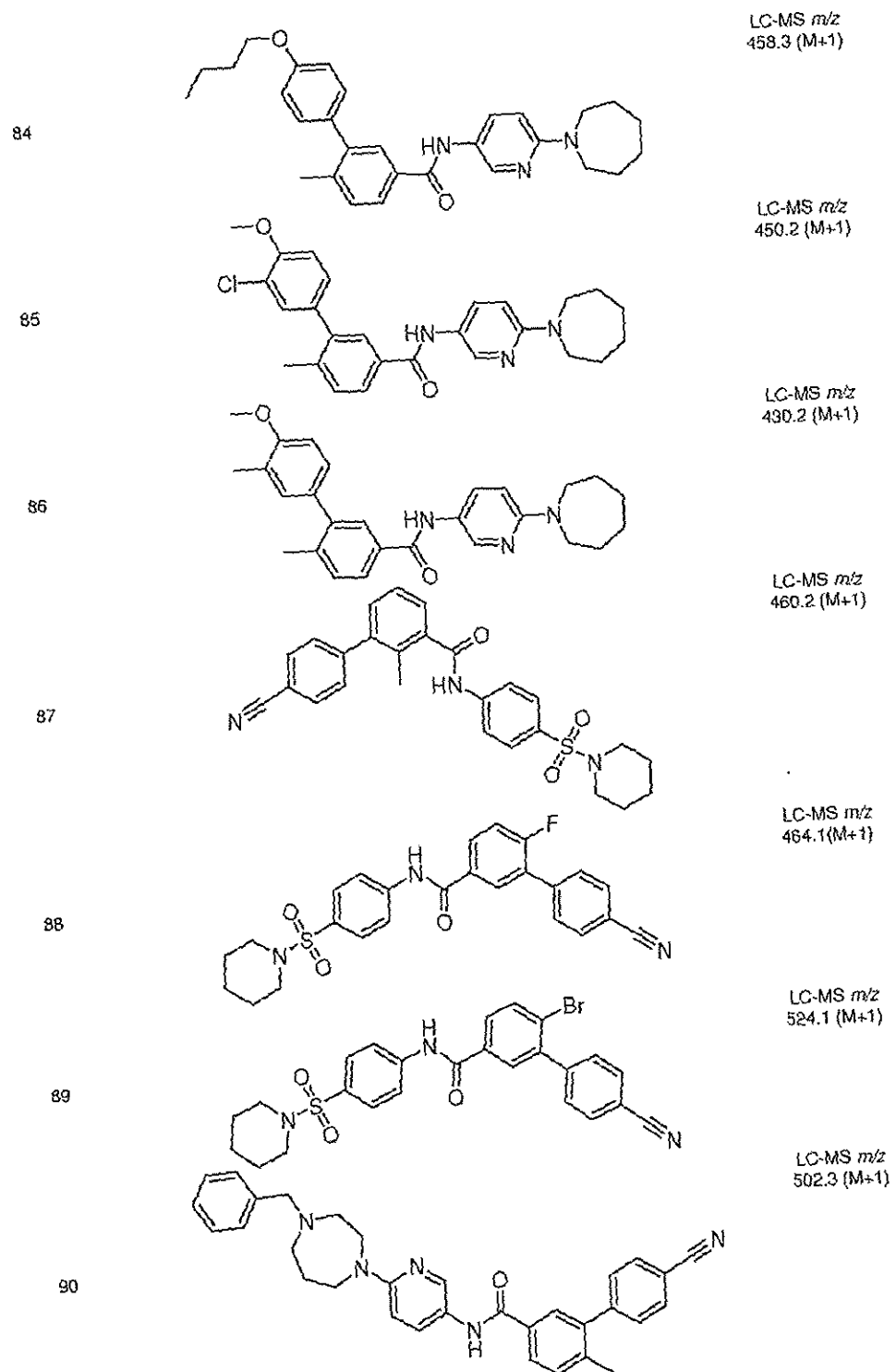
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41		LC-MS <i>m/z</i> 454.2 (M+1).
42		LC-MS <i>m/z</i> 454.2 (M+1).
43		LC-MS <i>m/z</i> 522.2 (M+1).
44		LC-MS <i>m/z</i> 444.2 (M+1).
45		LC-MS <i>m/z</i> 430.2 (M+1).
46		LC-MS <i>m/z</i> 446.2 (M+1).
47		LC-MS <i>m/z</i> 470.2 (M+1).
48		LC-MS <i>m/z</i> 470.2 (M+1).

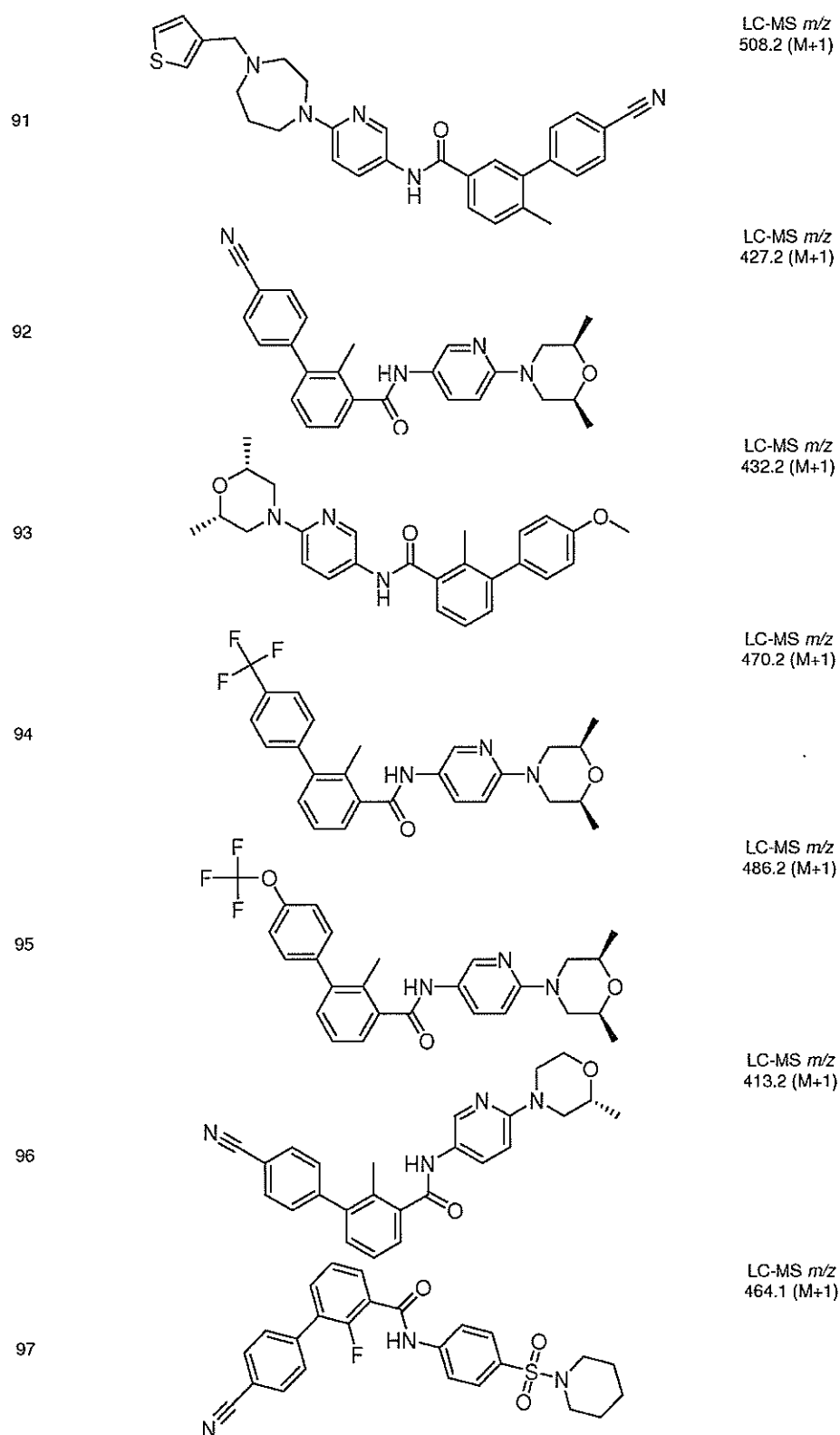
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50		LC-MS m/z 403.2 (M+1).
51		LC-MS m/z 403.2 (M+1).
52		LC-MS m/z 460.2 (M+1).
53		LC-MS m/z 416.2 (M+1).
54		LC-MS m/z 391.2 (M+1).
55		LC-MS m/z 391.2 (M+1).
56		LC-MS m/z 391.2 (M+1).
57		LC-MS m/z 425.2 (M+1).

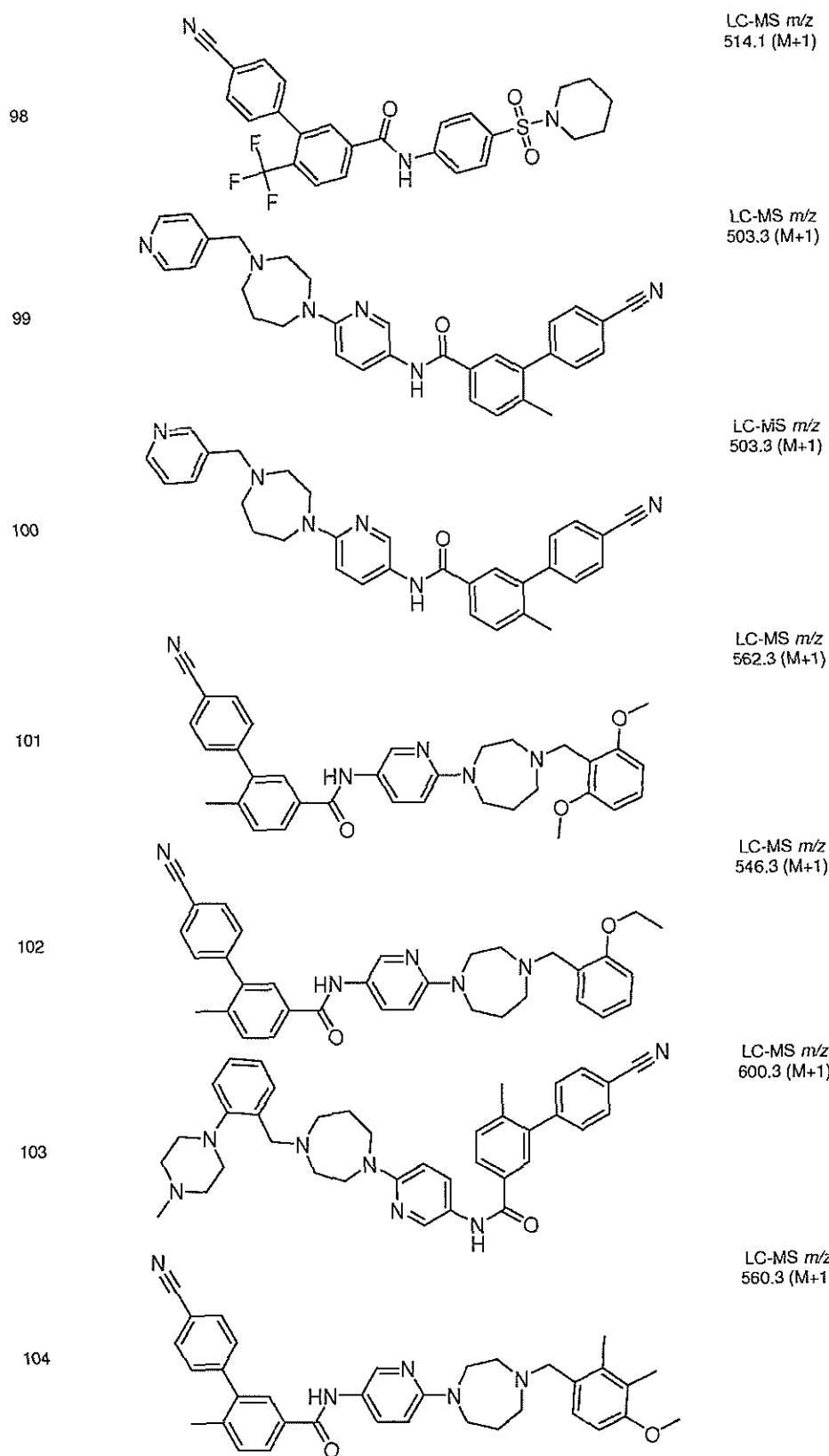
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59		LC-MS <i>m/z</i> 398.2 (M+1).
60		LC-MS <i>m/z</i> 398.2 (M+1).
61		LC-MS <i>m/z</i> 413.2 (M+1).
62		LC-MS <i>m/z</i> 411.2 (M+1).
63		LC-MS <i>m/z</i> 413.2 (M+1).
64		LC-MS <i>m/z</i> 397.2 (M+1).
65		LC-MS <i>m/z</i> 399.2 (M+1).
66		LC-MS <i>m/z</i> 412.2 (M+1).

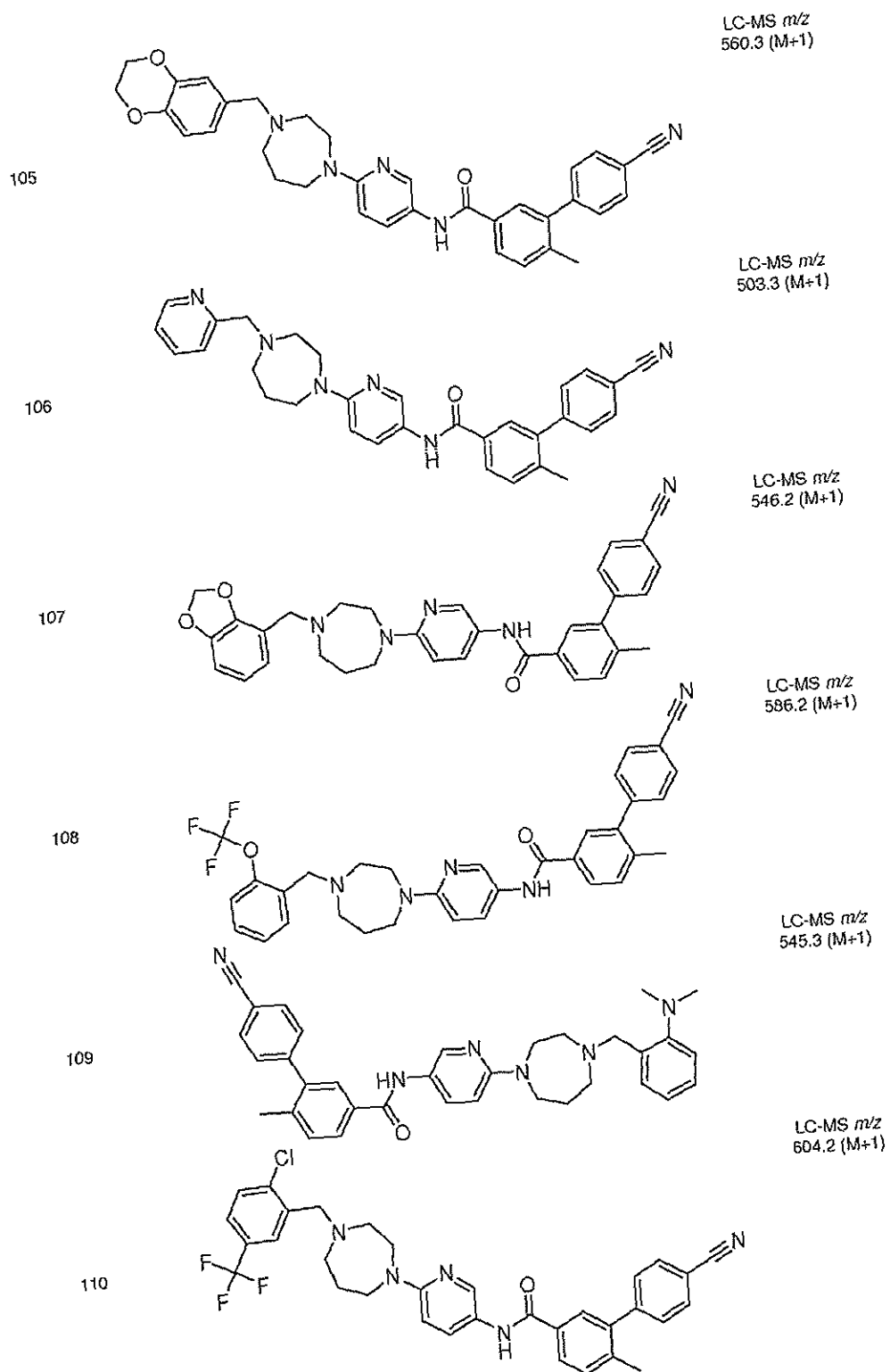
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68		LC-MS <i>m/z</i> 416.2 (M+1).
69		LC-MS <i>m/z</i> 432.1 (M+1).
70		LC-MS <i>m/z</i> 476.1 (M+1).
71		LC-MS <i>m/z</i> 412.2 (M+1).
72		LC-MS <i>m/z</i> 466.2 (M+1).
73		LC-MS <i>m/z</i> 385.2 (M+1).
74		LC-MS <i>m/z</i> 389.1 (M+1).
75		LC-MS <i>m/z</i> 419.2 (M+1).

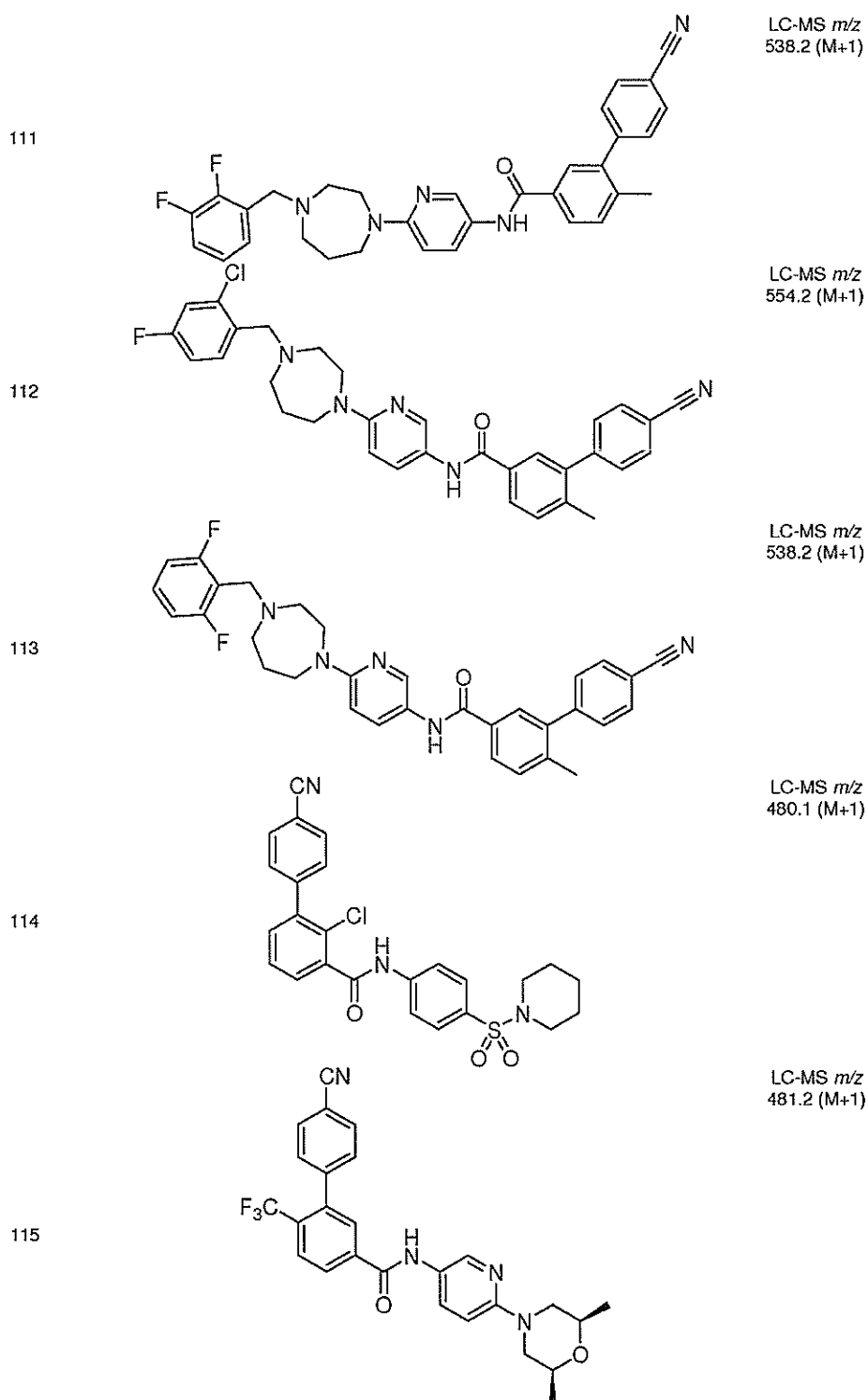
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78		LC-MS m/z 446.1 (M+1).
79		LC-MS m/z 427.2 (M+1).
80		LC-MS m/z 427.2 (M+1).
81		LC-MS m/z 411.2 (M+1).
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83		LC-MS m/z 444.3 (M+1).

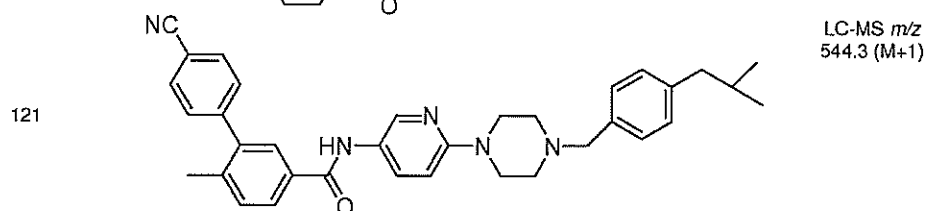
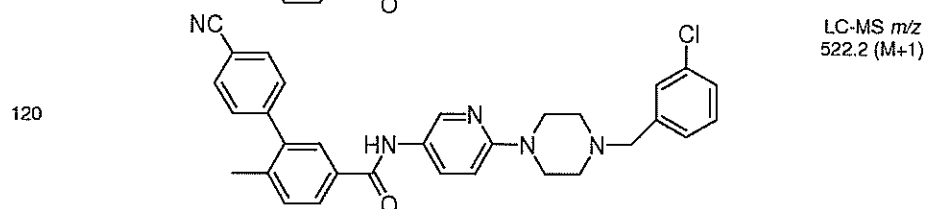
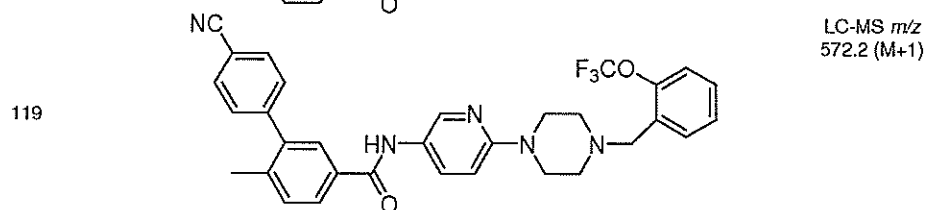
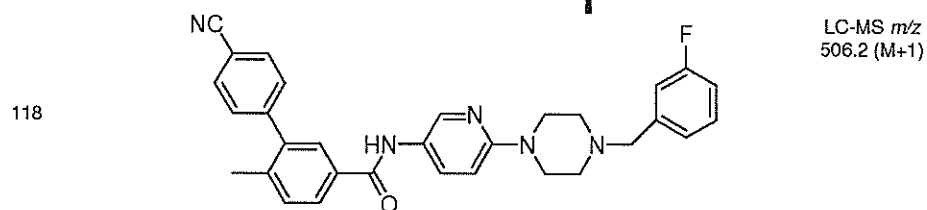
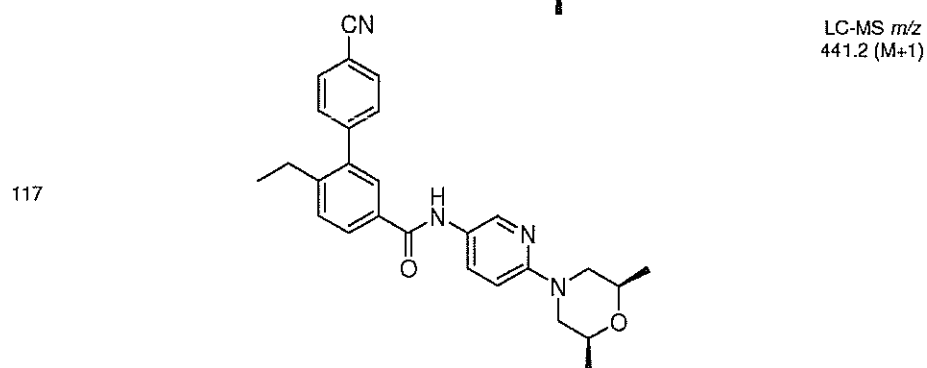
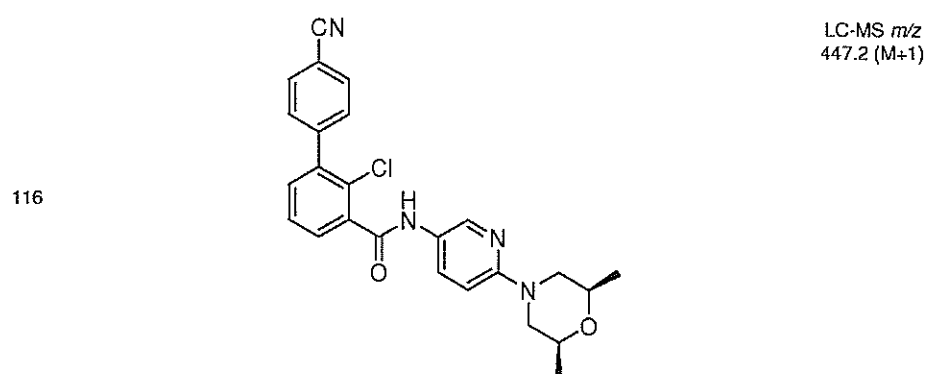


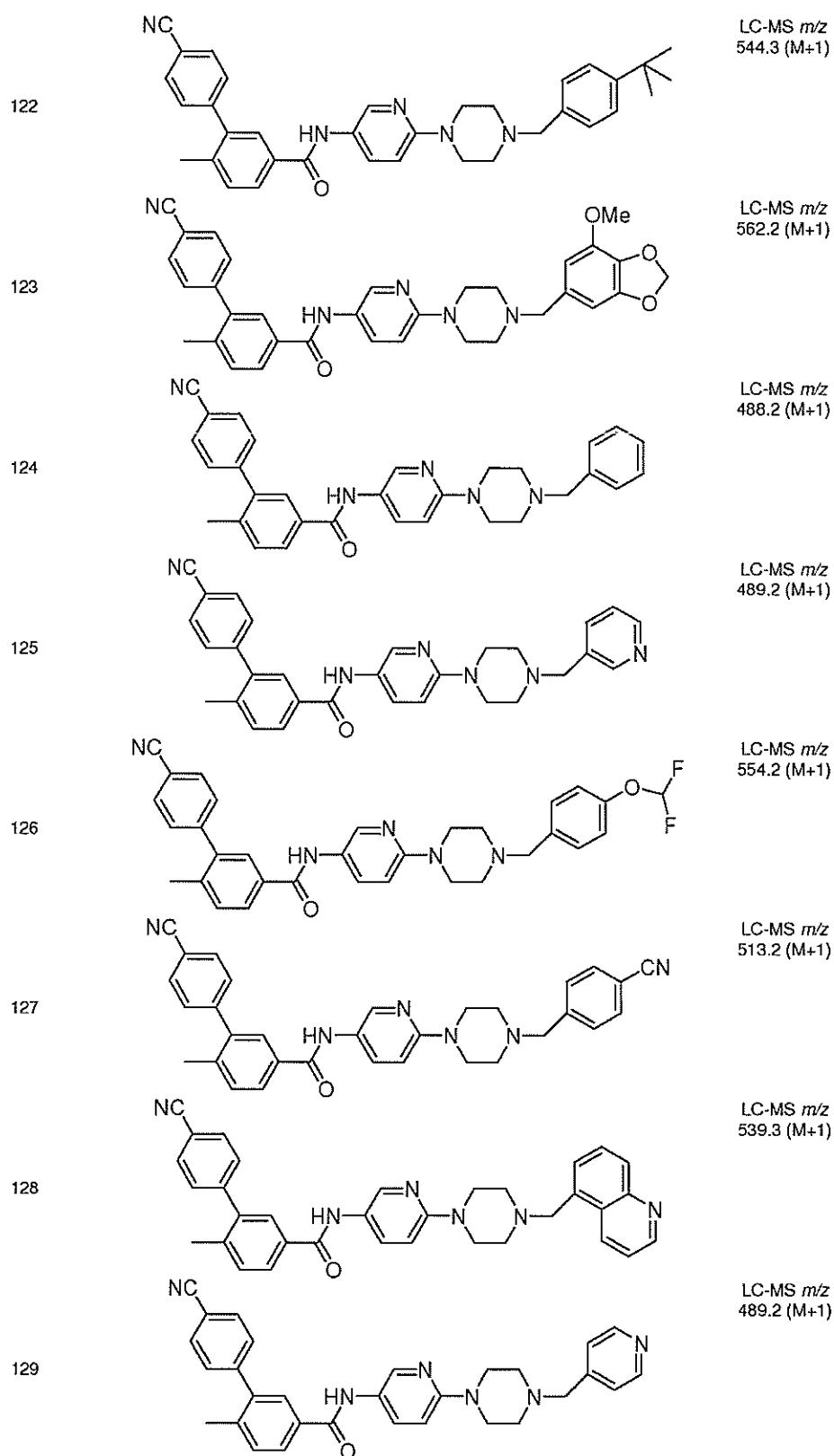


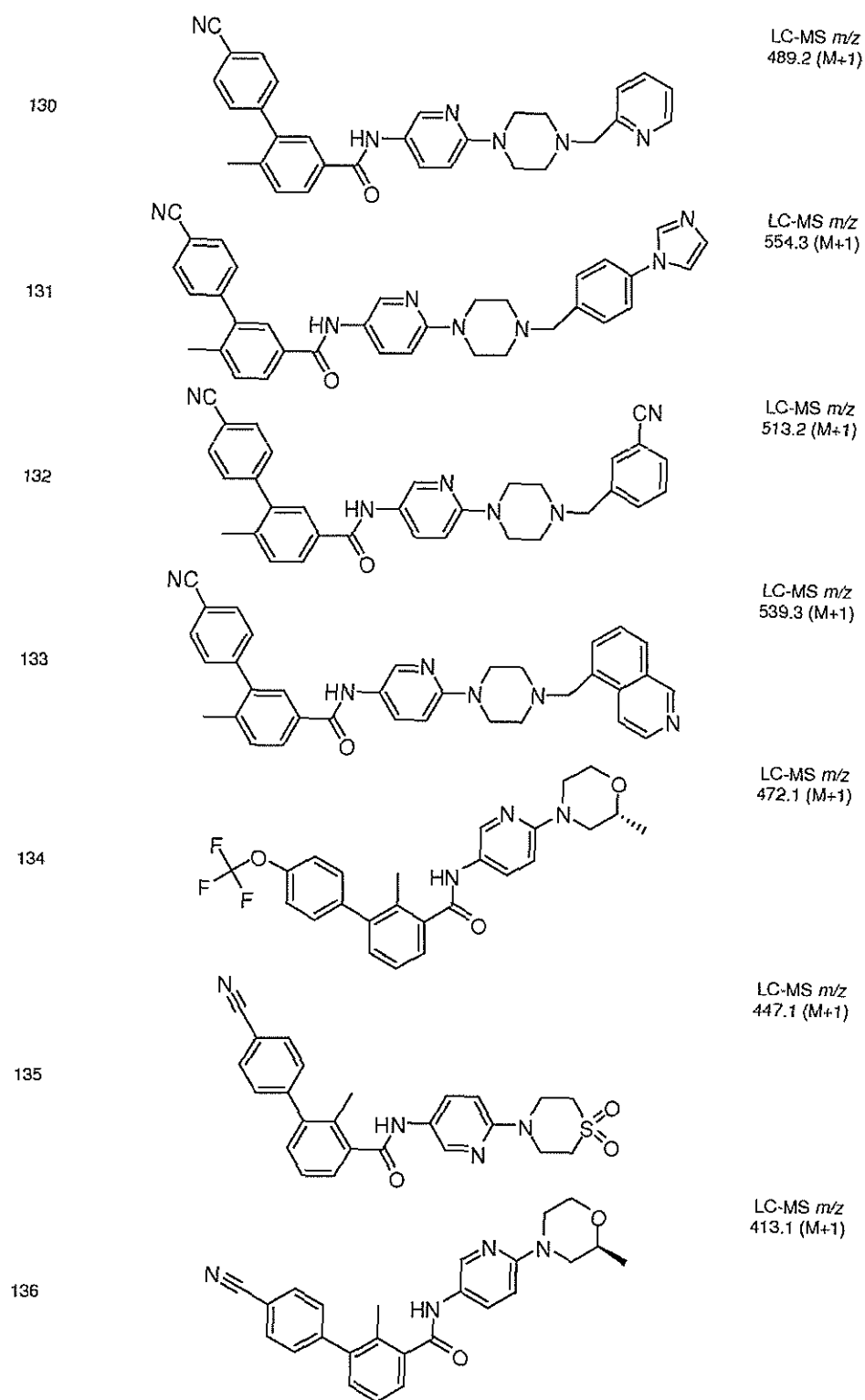


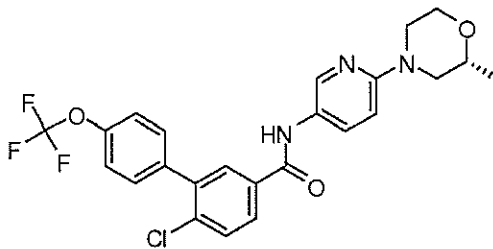
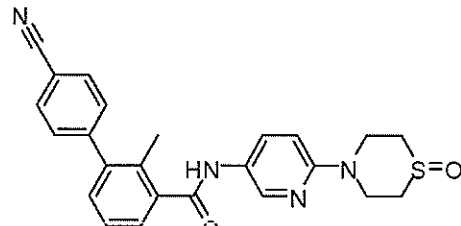
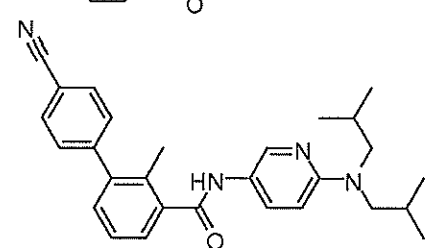
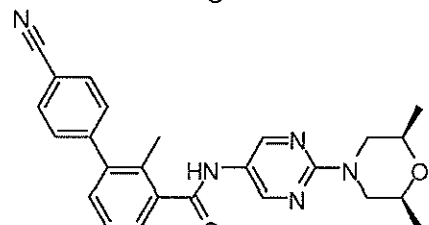
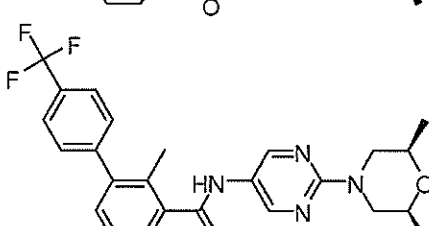
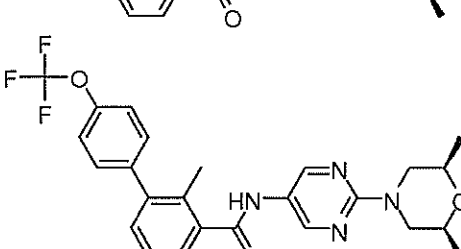


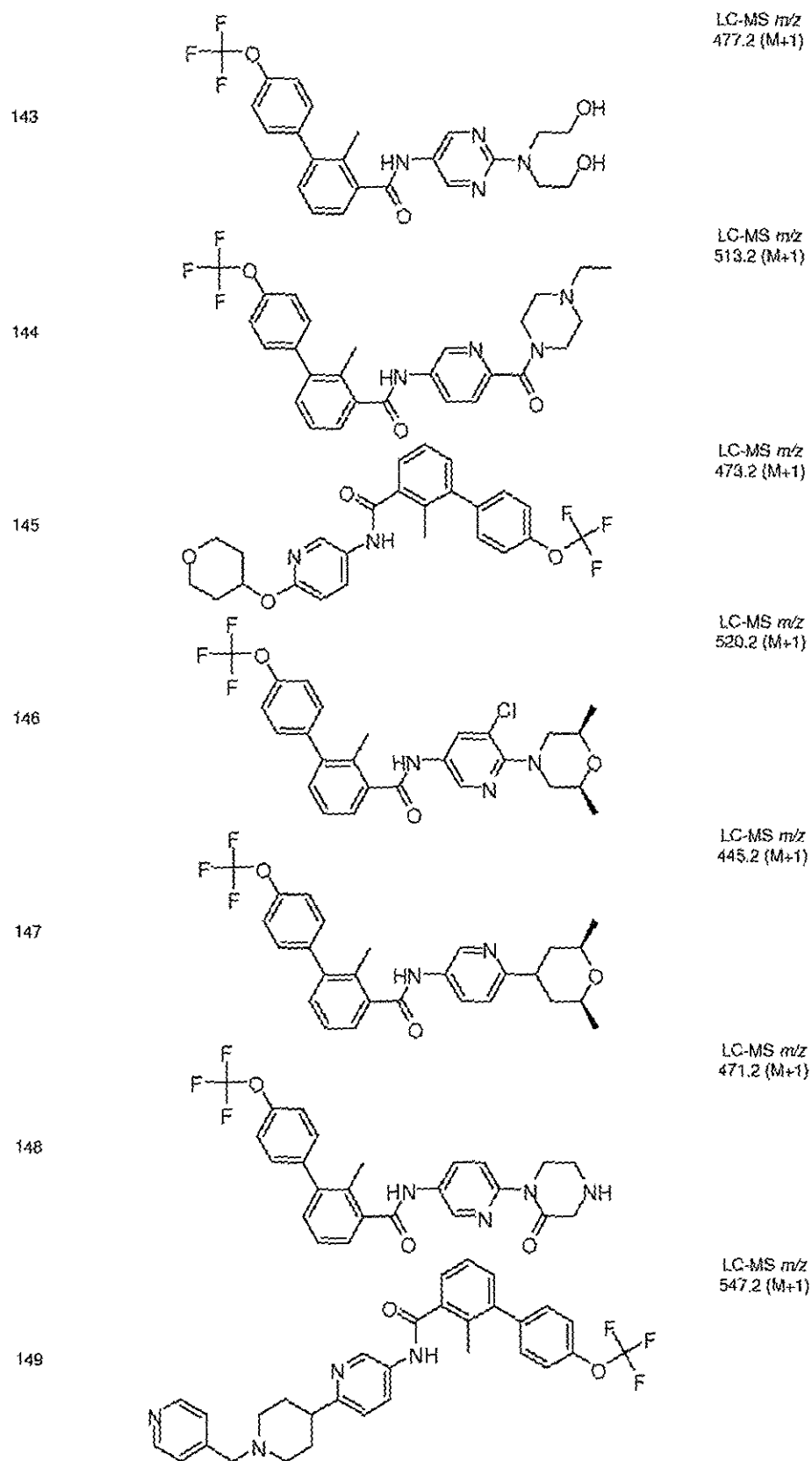


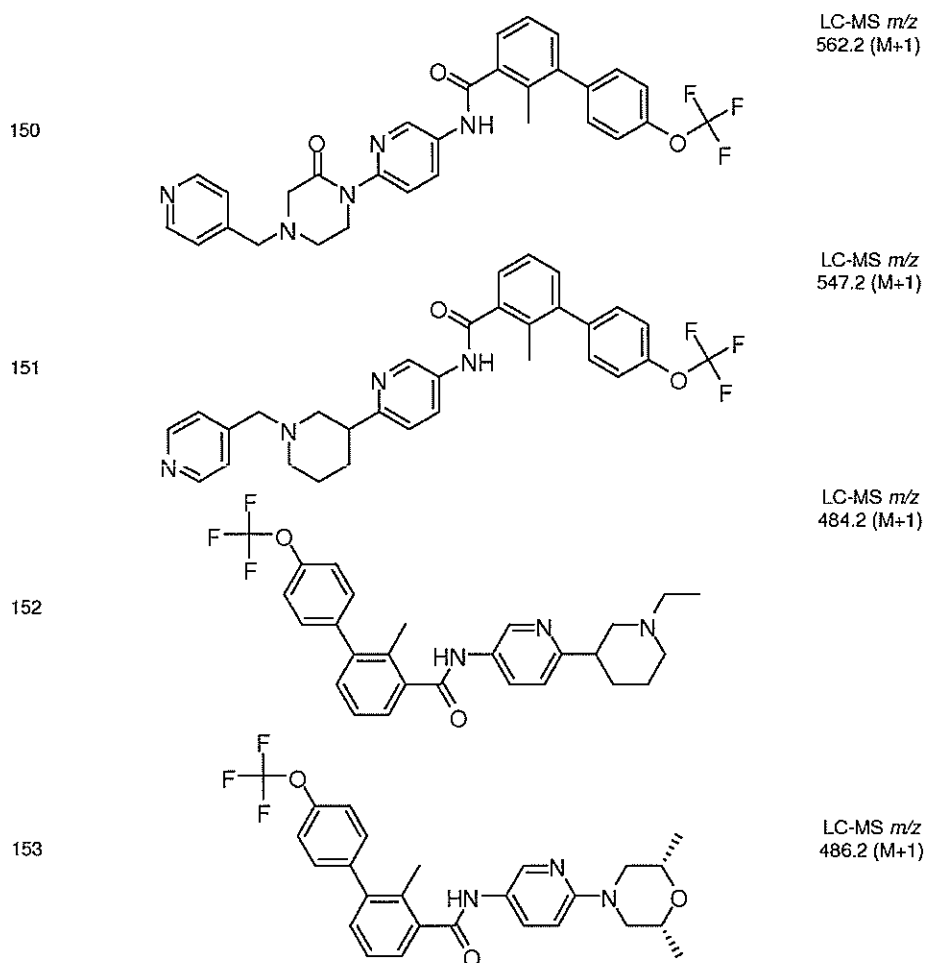






137		LC-MS <i>m/z</i> 492.1 (M+1)
138		LC-MS <i>m/z</i> 431.1 (M+1)
139		LC-MS <i>m/z</i> 441.1 (M+1)
140		LC-MS <i>m/z</i> 428.2 (M+1)
141		LC-MS <i>m/z</i> 471.2 (M+1)
142		LC-MS <i>m/z</i> 487.2 (M+1)





[0067] Compounds of the present invention are assayed to evaluate their capacity to inhibit the hedgehog signaling pathway.

Gli-Luc Reporter Assay for Hh Pathway Inhibition

[0068] Mouse TM3 cells (obtained from American Type Culture Collection, ATCC, Manassas, VA) are cultured in DMEM/F12 medium (Gibco/Invitrogen, Carlsbad, CA) supplemented with 5% heat inactivated horse serum and 2.5% FBS (Gibco/Invitrogen, Carlsbad, CA), 50 unit/mL penicillin and 50 μ g/mL of streptomycin (Gibco/Invitrogen, Carlsbad, CA) at 37°C with 5% CO₂ in air atmosphere. TM3 cells were transfected with pTA-8xGli-Luc reporter plasmid. A stably transfected clone termed TMHh-12 was selected. TMHh-12 clone showed good response to Shh-N stimulation. To evaluate the IC₅₀s of the antagonists, 8000 TMHh-12 cells were plated

into each wells in 384-well plates with 50% DMEM/F12 medium supplemented with 2% FBS. After 12 hours, Hh pathway is activated by adding recombinant mouse Shh protein (expressed in E.coli, 8 µg/mL) or by adding Smo agonists. The testing compounds are added into plates with different concentrations. After 48 hours, the firefly luciferase activities are assayed with the Bright-Glo™ Luciferase Assay System (Promega, Madison, WI). The IC₅₀ is measured when the effect of the compound reduces the luminescence signal by 50%. Toxicity of these compounds are evaluated in TM3 cells using CellTiter Glo assays or by TM3-Luc cell line (a TM3 cell stably transfected with a constitutive luciferase expression vector).

[0069] Compounds of Formula I preferably have an EC₅₀ of less than 500nM, more preferable less than 200nM.

Cyto-toxicity Assay

[0070] A cytotoxicity assay is performed to compare the effects of a compound of the invention on medulloblastoma cells (Daoy cells), basal cell carcinoma cells (TE354.T cells) and control cells (human normal fibroblast) according to the following procedure:

[0071] Daoy cells (medulloblastoma cell line) are purchased from ATCC, and cultured in Minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate and 10% FBS at 37°C with 5% CO₂ in an air atmosphere.

[0072] TE354.T cells (from ATCC) are cultured in Dulbecco's modified Eagle's medium with 4 mM L-glutamine fetal bovine serum and 10% of FBS.

[0073] Normal human dermal fibroblast cells (Clonetics) are cultured in Fibroblast Growth Medium (Clonetics).

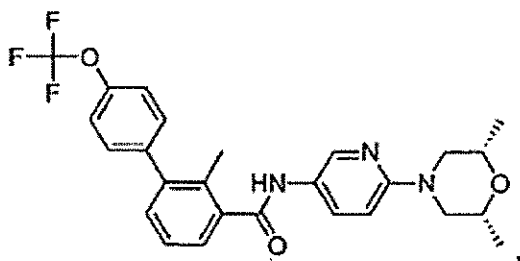
[0074] Each of the above cell lines are independently seeded into 96-well plates and cultured to a density of 5,000-10,000 cells/well. A compound of the invention, at different concentrations, is added into the cell cultures. After 2 days, the cell viability is evaluated with Cell Titer-Glo Luminescent Cell Viability Assay Kit (Promega) following the manufacturer's protocol. The cell viability is directly measured by luminescent signaling and EC₅₀s are measured when the signal is inhibited 50%.

[0075] Compounds of Formula I preferably have an EC_{50} of less than 500nM, more preferable less than 200nM.

[0076] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

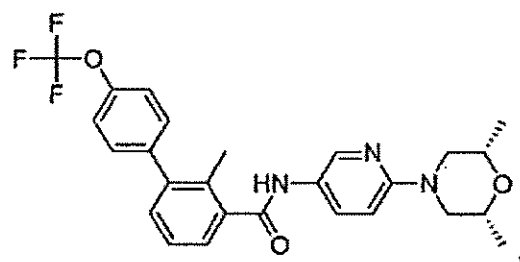
Claims

1. The compound, N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide of formula:



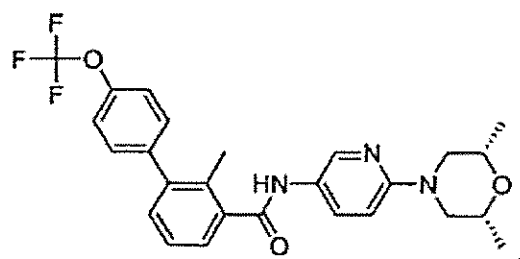
or a pharmaceutically acceptable salt, hydrate, or solvate thereof.

2. The compound, N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide of formula:

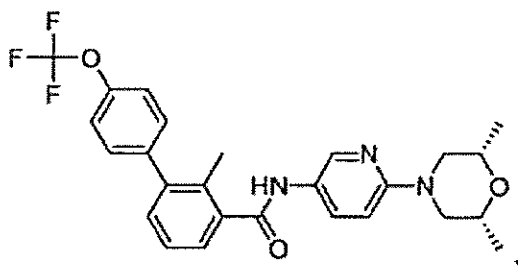


or a pharmaceutically acceptable salt thereof.

3. The compound, N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide of formula:

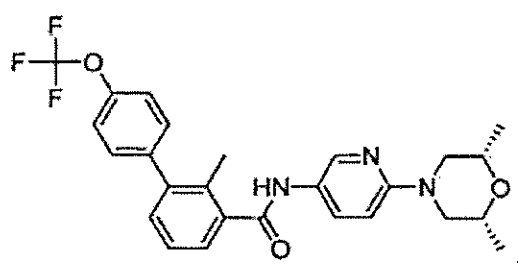


4. A pharmaceutical composition comprising the compound, N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4'-(trifluoromethoxy)biphenyl-3-carboxamide of formula:



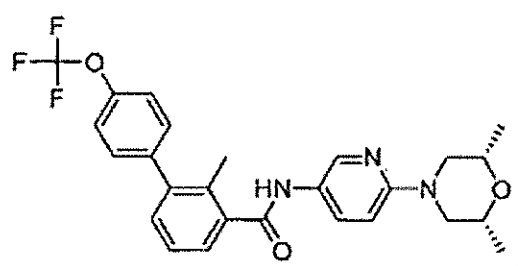
in free form or in a pharmaceutically acceptable salt form in association with at least one pharmaceutically acceptable carrier or diluent.

5. A pharmaceutical composition comprising a therapeutically effective amount of the compound, N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4-(trifluoromethoxy)biphenyl-3-carboxamide of formula:



or a pharmaceutically acceptable salt thereof, in combination with one or more therapeutic agents.

6. A pharmaceutical combination according to claim 5 wherein the additional therapeutic agent is selected from immunomodulatory, anti-inflammatory substances or other anti-tumor therapeutic agents.
7. The compound, N-(6-((2R,6S)-2,6-dimethylmorpholino)pyridin-3-yl)-2-methyl-4-(trifluoromethoxy)biphenyl-3-carboxamide of formula:



or a pharmaceutically acceptable salt, thereof, for use in the treatment of pancreatic cancer, prostate cancer, medulloblastoma, basal cell carcinoma and small-cell lung cancer.