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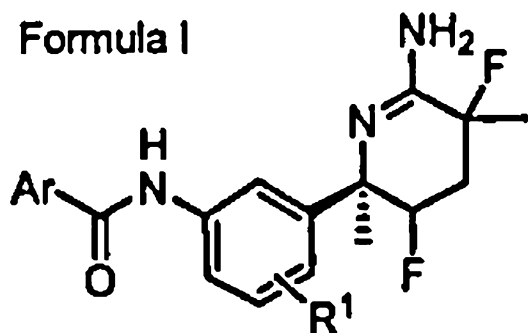
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54 Titre: 2-amino-3,5-difluoro-3,6-dimethyl-6-phenyl-3,4,5,6-tetrahydropyridines as bace1 inhibitors for treating Alzheimer's disease.

57 Abrégé :

The present invention is directed to compounds according to Formula (I)

Formula I



which compounds are inhibitors of the BACE1 enzyme. Separate aspects of the invention are directed to pharmaceutical compositions comprising said compounds and uses of the compounds to treat disorders for which the reduction of A β deposits is beneficial such as Alzheimer's disease.

2-Amino-3,5-Difluoro-3,6-Dimethyl-6-Phenyl-3,4,5,6-Tetrahydropyridines As Bace1 Inhibitors For Treating Alzheimer's Disease

FIELD OF THE INVENTION

- 5 The present invention provides compounds which act as BACE1 inhibitors. Separate aspects of the invention are directed to pharmaceutical compositions comprising said compounds and uses of the compounds to treat neurodegenerative and cognitive disorders.

BACKGROUND ART

Dementia is a clinical syndrome characterized by deficits in multiple areas of cognition that cannot be explained by normal aging, a noticeable decline in function, and an absence of delirium. In addition, neuropsychiatric symptoms and focal neurological findings are usually present. Dementia is further classified based on etiology. Alzheimer's disease (AD) is the most common cause of dementia, followed by mixed AD and vascular dementia, Lewy body dementia (DLB), and fronto-temporal dementia. β -Amyloid deposits and neurofibrillary tangles are considered to be major pathologic characterizations associated with AD which is characterized by the loss of memory, cognition, reasoning, judgment, and orientation. Also affected, as the disease progresses, are motor, sensory and linguistic abilities until global impairment of multiple cognitive functions occurs. β -Amyloid deposits are predominantly an aggregate of A β peptide, which in turn is a product of the proteolysis of amyloid precursor protein (APP) as part of the β -amyloidogenic pathway. A β peptide results from the cleavage of APP at the C-terminal by one or more γ -secretases and at the N-terminal by β -secretase 1 (BACE1) also known as aspartyl protease 2. BACE1 activity is correlated directly to the generation of A β peptide from APP.

Studies indicate that the inhibition of BACE1 impedes the production of A β peptide. Further, BACE1 co-localizes with its substrate APP in Golgi and endocytic compartments (Willem M, et al. Semin.Cell Dev. Biol, 2009, 20, 175-182). Knock-out studies in mice have demonstrated the absence of amyloid peptide formation while the animals are healthy and fertile (Ohno M, et al. *Neurobiol. Dis.*, 2007, 26, 134-145). Genetic ablation of BACE1 in APP-overexpressing mice has demonstrated absence of plaque formation, and the reverse of cognitive deficits (Ohno M, et al. *Neuron*; 2004, 41, 27-33). BACE1 levels are elevated in the brains of sporadic AD patients (Hampel and Shen, *Scand. J. Clin. Lab. Invest.* 2009, 69, 8-12).

These convergent findings indicate that the inhibition of BACE1 may be a therapeutic target for the treatment of AD as well as disorders for which the reduction of A β deposits is beneficial.

AstraZeneca announced the discovery of AZD3839, a potent BACE1 inhibitor clinical candidate for the treatment of AD (Jeppsson, F., et al. *J. Biol. Chem.*, 2012, 287, 41245-41257) In October 2012. The effort which led to the discovery of AZD3839 was further described in Ginman, T., et al. *J. Med. Chem.*, 2013, 56, 4181-4205. The Ginman publication describes the issues which were overcome in connection with the discovery and identification of AZD3839. These issues related to poor blood brain barrier penetration and P-glycoprotein mediated efflux of the compounds resulting in lack of brain exposure.

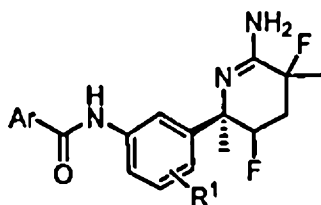
The Ginman manuscript hypothesized that the differences in brain exposure would largely be due to the core structures and Structure Activity Relationship data was provided wherein the in vitro properties on the reported compounds were given into four tables according to core sub-types. In table 4, a series of amidine containing compounds are described that were considered interesting from an activity perspective. However, the data suggests that the amidine containing core did not exhibit a favourable blood brain barrier permeability profile.

Researchers from Hoffmann-La Roche and Siena Biotech also reported the discovery of amidine containing compounds (Woltering, T. J., et al. *Bioorg. Med. Chem. Lett.* 2013, 23, 4239-4243). These compounds (compounds 17 and 18 in the paper) were found not to have any in vivo effect (lack of A β 40 reduction in brain in wild type mice).

Contrary to the teachings of Ginman, et al. and Woltering, T. J., et al., the inventors have discovered a series of amidine compounds which are brain penetrant. Accordingly, the present invention relates to novel compounds having BACE1 inhibitory activity, to their preparation, to their medical use and to medicaments comprising them.

SUMMARY OF THE INVENTION

It is an objective of the present invention to provide compounds that inhibit BACE1. Accordingly, the present invention relates to compounds of Formula I



Formula I

wherein Ar is selected from the group consisting of phenyl, pyridyl, pyrimidyl, pyrazinyl, imidazolyl, pyrazolyl, thiazolyl, oxazolyl, isoxazolyl, and where the Ar is optionally substituted with one or more substituent selected from halogen, CN, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ fluoroalkyl or C₁-C₆ alkoxy; and

R^1 is one or more hydrogen, halogen, C_1 - C_3 fluoroalkyl or C_1 - C_3 alkyl;
or a pharmaceutically acceptable salt thereof.

In one embodiment the present invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof for use in therapy.

5 The present invention further provides a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

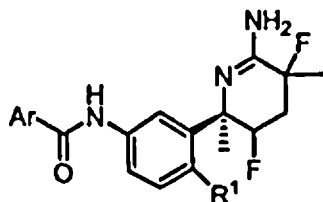
In one embodiment the invention provides the use of a compound of Formula I or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of a neurodegenerative or cognitive disorder.

10 In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof for use in a method for the treatment of a neurodegenerative or cognitive disorder.

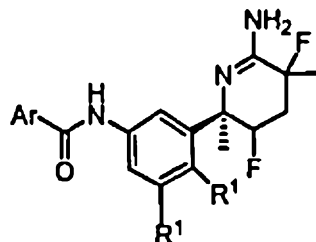
In one embodiment the present invention provides a method of treating a neurodegenerative or cognitive disorder comprising administering a therapeutically effective amount of a compound of
15 Formula I or a pharmaceutically acceptable salt thereof to a patient in need thereof.

Further embodiments of the invention are provided immediately below:

In one embodiment, the compound of the present invention is of formula Ia or Ib



Formula Ia



Formula Ib

or a pharmaceutically acceptable salt thereof.

In one embodiment, R^1 is F or H, particularly F.

20 In one embodiment, the compound of the present invention is of Formula Ia and R^1 is F.

In one embodiment, Ar is optionally substituted with one or more substituents selected from F, Cl, Br, CN, C_1 - C_3 alkyl, C_1 - C_3 fluoroalkyl or C_1 - C_3 alkoxy.

In one embodiment, Ar is optionally substituted phenyl.

In one embodiment, Ar is optionally substituted pyridyl.

25 In one embodiment, Ar is optionally substituted pyrimidyl.

In one embodiment, Ar is optionally substituted pyrazinyl.

In one embodiment, Ar is optionally substituted imidazolyl.

In one embodiment, Ar is optionally substituted pyrazolyl.

In one embodiment, Ar is optionally substituted thiazolyl.

In one embodiment, Ar is optionally substituted oxazolyl.

In one embodiment, Ar is optionally substituted isoxazolyl.

In one embodiment, the compound is selected from the group consisting of:

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide,

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypyrazine-2-carboxamide,

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypicolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypyrazine-2-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-2-methyloxazole-4-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypicolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-chloropicolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-1-methyl-1*H*-imidazole-2-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(trifluoromethyl)pyrazine-2-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-4-methylthiazole-2-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-2-(difluoromethyl)oxazole-4-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-1-(difluoromethyl)-1*H*-pyrazole-3-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(difluoromethyl)pyrazine-2-carboxamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-4-chlorobenzamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-cyanopicolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4,5-difluorophenyl)-5-(methoxy-*d*₃)picolinamide,

5 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide,

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)pyrazine-2-carboxamide,

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-

10 5-cyano-3-methylpicolinamide,

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4,5-difluorophenyl)-5-(methoxy-*d*₃)picolinamide,

N-(3-((2*R*,3*R*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide,

N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-bromopicolinamide

or a pharmaceutically acceptable salt thereof.

15 A separate embodiment is directed to a pharmaceutical composition comprising a compound from the above list and a pharmaceutically acceptable carrier.

Another embodiment is directed to a method of treating a neurodegenerative or cognitive disorder comprising administering a therapeutically effective amount of a compound from the above list to patient in need thereof.

20 Yet another embodiment is directed to a use of a compound from the above list for the manufacture of a medicament for treating a neurodegenerative or cognitive disorder.

In one embodiment the invention provides a compound from the above list for use in therapy.

Yet another embodiment of the invention is directed to a compound from the above list for use in the treatment of a neurodegenerative or cognitive disorder.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that the compounds of Formula I are inhibitors of BACE1, and as such, are useful for the treatment of related disorders. Certain aspects of the invention are explained in greater detail below but this description is not intended to be a detailed

30 catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. Hence, the following specification is intended to illustrate some

embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

As used herein, the term "C₁-C₆ alkyl" refers to a straight chained or branched saturated hydrocarbon having from one to six carbon atoms inclusive. Examples of C₁-C₆ alkyl include, but are not limited to, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, 2-methyl-2-propyl, 2-methyl-1-propyl, n-pentyl and n-hexyl. Similarly, the term "C₁-C₃ alkyl" refers to a straight chained or branched saturated hydrocarbon having from one to three carbon atoms inclusive. Examples of C₁-C₃ include, but are not limited to, methyl, ethyl and n-propyl.

Likewise, the term "C₁-C₆ alkoxy" refers to a straight chained or branched saturated alkoxy group having from one to six carbon atoms inclusive with the open valency on the oxygen. Examples of C₁-C₆ alkoxy include, but are not limited to, methoxy, ethoxy, n-butoxy, t-butoxy and n-hexyloxy. The "C₁-C₆ alkoxy" is optionally substituted with one or more fluorine atoms.

As used herein, the term "C₁-C₆ fluoroalkyl" refers to a straight chained or branched saturated hydrocarbon having from one to six carbon atoms inclusive substituted with one or more fluorine atoms. Examples of C₁-C₆ fluoroalkyl include, but are not limited to, trifluoromethyl, pentafluoroethyl, 1-fluoroethyl, monofluoromethyl, difluoromethyl, 1,2-difluoroethyl and 3,4 difluorohexyl. Similarly, the term "C₁-C₃ fluoroalkyl" refers to a straight chained or branched saturated hydrocarbon having from one to three carbon atoms inclusive substituted with one or more fluorine atoms per carbon atom.

The term "halogen" refers to fluorine, chlorine, bromine and iodine.

The term "C₂-C₆ alkenyl" refers to a branched or unbranched alkenyl group having from two to six carbon atoms and one double bond, including but not limited to ethenyl, propenyl, and butenyl.

The term "C₂-C₆ alkynyl" shall mean a branched or unbranched alkynyl group having from two to six carbon atoms and one triple bond, including but not limited to ethynyl, propynyl and butynyl.

As used herein, the phrase "effective amount" when applied to a compound of the invention, is intended to denote an amount sufficient to cause an intended biological effect. The phrase "therapeutically effective amount" when applied to a compound of the invention is intended to denote an amount of the compound that is sufficient to ameliorate, palliate, stabilize, reverse, slow or delay the progression of a disorder or disease state, or of a symptom of the disorder or disease. In an embodiment, the method of the present invention provides for administration of combinations of compounds. In such instances, the "effective amount" is the amount of the compound in said combination sufficient to cause the intended biological effect.

The term "treatment" or "treating" as used herein means ameliorating or reversing the progress or severity of a disease or disorder, or ameliorating or reversing one or more symptoms or side effects of such disease or disorder. "Treatment" or "treating", as used herein, also means to inhibit or block, as in retard, arrest, restrain, impede or obstruct, the progress of a system, condition

or state of a disease or disorder. For purposes of this invention, "treatment" or "treating" further means an approach for obtaining beneficial or desired clinical results, where "beneficial or desired clinical results" include, without limitation, alleviation of a symptom, diminishment of the extent of a disorder or disease, stabilized (i.e., not worsening) disease or disorder state, delay or slowing of a disease or disorder state, amelioration or palliation of a disease or disorder state, and remission of a disease or disorder, whether partial or total.

The present invention is based on the discovery that compounds of Formula I are inhibitors of BACE1, and as such, are useful for the treatment of disorders which pathological characteristics comprise β -amyloid deposits and neurofibrillary tangles, such as neurodegenerative or cognitive disorders.

The compounds of the present invention are, as discussed above, expected to be useful in the treatment of Alzheimer's disease due to their effects on β -amyloid deposits and neurofibrillary tangles. This includes familial Alzheimer's disease where patients carry mutations on specific genes intimately involved in the production of A β peptide. It is, however, important to note that aggregates of A β peptide is not limited to familial Alzheimer's disease but is similarly an important pathophysiological characteristics of the more common sporadic Alzheimer's disease [*Mol Cell Neurosci*, 66, 3-11, 2015].

The compounds of the present invention are also believed to be useful in the treatment of early-stage Alzheimer's disease, i.e. disease stages where the biological and structural changes have started but the clinical manifestations of the disease have not yet become evident or are not yet well developed. Early-stage Alzheimer's disease may, in fact, start years before any clinical sign of the disease becomes manifest. Early-stage Alzheimer's disease includes prodromal Alzheimer's disease, preclinical Alzheimer's disease and mild cognitive impairment. Although mild cognitive impairment may be unrelated to Alzheimer's disease it is often a transitional stage to Alzheimer's disease or due to Alzheimer's disease. Preclinical and prodromal Alzheimer's disease are asymptomatic stages, and they are typically diagnosed by the presence of Alzheimer's disease related biomarkers. In this context the compounds of the present invention are believed to be useful in slowing down the progression of early-stage Alzheimer's disease, such as mild cognitive impairment to Alzheimer's disease. The compounds of the present invention are also believed to be useful in the treatment of memory loss, attention deficits and dementia associated with Alzheimer's disease.

Other diseases, in addition to the continuum of Alzheimer's disease, are characterized by β -amyloid deposits and neurofibrillary tangles. This includes e.g. Trisomy 21 also known as Down's syndrome. Patients suffering from Down's syndrome have an extra chromosome 21 which chromosome contains the gene for the amyloid precursor protein (APP). The extra chromosome 21 leads to overexpression of APP, which leads to increased levels of A β peptide, which eventually

causes the markedly increased risk of developing Alzheimer's disease seen in Down's syndrome patients [*Alzheimer's & Dementia*, 11, 700-709, 201]. Cerebral amyloid angiopathy is also characterized by β -amyloid deposits and neurofibrillary tangles in blood vessels of the central nervous system [*Pharmacol Reports*, 67, 195-203, 2015] and is as such expected to be treatable with
 5 compounds of the present invention.

In one embodiment, the present invention provides a method of treating a disease selected from Alzheimer's disease (familial or sporadic), preclinical Alzheimer's disease, prodromal Alzheimer's disease, mild cognitive impairment, Down's syndrome and cerebral amyloid angiopathy, the method comprising the administration of a therapeutically effective amount of a compound of
 10 Formula I or a pharmaceutically acceptable salt thereof to a patient in need thereof.

The present invention further provides a method of inhibiting BACE1 in a patient comprising administering to a patient in need thereof a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof.

The present invention also provides a method of inhibiting β -secretase mediated cleavage of
 15 amyloid precursor protein comprising administering to a patient in need of such treatment a therapeutically effective amount a compound of Formula I or a pharmaceutically acceptable salt thereof.

In further embodiments, the present invention provides the use of a compound of Formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of
 20 disease selected from Alzheimer's disease (familial or sporadic), preclinical Alzheimer's disease, prodromal Alzheimer's disease, mild cognitive impairment, Down's syndrome or cerebral amyloid angiopathy.

The present invention also provides the use of a compound of Formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the inhibition of
 25 BACE1. The present invention further provides the use of a compound of Formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the inhibition of production or accumulation of A β peptide.

In one embodiment, the present invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof for use in a method for the treatment of a disease selected
 30 form Alzheimer's disease (familial or sporadic), preclinical Alzheimer's disease, prodromal Alzheimer's disease, mild cognitive impairment, Down's syndrome or cerebral amyloid angiopathy.

In one embodiment, the present invention relates to a compound of Formula I or a pharmaceutically acceptable salt thereof for use in a method for inhibiting of BACE1 or in a method for inhibiting of production or accumulation of A β peptide.

In a further embodiment, the invention provides a pharmaceutical formulation adapted for any of the above treatments and uses.

In one embodiment, a mammal is a human.

In one embodiment, the patient is a human patient.

5 The present invention also comprises salts of the present compounds, typically, pharmaceutically acceptable salts. Such salts include pharmaceutically acceptable acid addition salts. Acid addition salts include salts of inorganic acids as well as organic acids.

Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, sulfamic, nitric acids and the like. Representative examples of
10 suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, itaconic, lactic, methanesulfonic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methane sulfonic, ethanesulfonic, tartaric, ascorbic, pantoic, bisphenylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, ethylenediaminetetraacetic acid (EDTA), glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-
15 toluenesulfonic acids, theophylline acetic acids, as well as the 8-halotheophyllines (for example, 8-bromotheophylline and the like). Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in S. M. Berge, et al., *J. Pharm. Sci.*, 1977, 66, 2.

Furthermore, the compounds of this invention may exist in unsolvated as well as in solvated
20 forms with pharmaceutically acceptable solvents such as water, ethanol and the like.

The compounds of the present invention may have one or more asymmetric centres and it is intended that any optical isomers (i.e. enantiomers or diastereomers), as separated, pure or partially purified optical isomers and any mixtures thereof including racemic mixtures, i.e. a mixture of stereoisomers, are included within the scope of the invention.

25 In this context is understood that when specifying the enantiomeric form, then the compound is in enantiomeric excess, e.g. essentially in a pure form. Accordingly, one embodiment of the invention relates to a compound of the invention having an enantiomeric excess of at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 96%, preferably at least 98%.

Racemic forms may be resolved into the optical antipodes by known methods, for example,
30 by separation of diastereomeric salts thereof with an optically active acid, and liberating the optically active amine compound by treatment with a base. Separation of such diastereomeric salts can be achieved, e.g. by fractional crystallization. The optically active acids suitable for this purpose may include, but are not limited to d- or l- tartaric, mandelic or camphorsulfonic acids. Another method for resolving racemates into the optical antipodes is based upon chromatography on an optically active
35 matrix. The compounds of the present invention may also be resolved by the formation and

chromatographic separation of diastereomeric derivatives from chiral derivatizing reagents, such as, chiral alkylating or acylating reagents, followed by cleavage of the chiral auxiliary. Any of the above methods may be applied either to resolve the optical antipodes of the compounds of the invention per se or to resolve the optical antipodes of synthetic intermediates, which can then be converted by methods described herein into the optically resolved final products which are the compounds of the invention.

Additional methods for the resolution of optical isomers, known to those skilled in the art, may be used. Such methods include those discussed by J. Jaques, A. Collet and S. Wilen in *Enantiomers, Racemates, and Resolutions*, John Wiley and Sons, New York, 1981. Optically active compounds can also be prepared from optically active starting materials.

The present invention further provides a pharmaceutical composition comprising a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of one of the specific compounds disclosed in the Experimental Section and a pharmaceutically acceptable carrier.

The compounds of the invention may be administered alone or in combination with pharmaceutically acceptable carriers or excipients, in either single or multiple doses. The pharmaceutical compositions according to the invention may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in *Remington: The Science and Practice of Pharmacy*, 22th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2013.

Pharmaceutical compositions for oral administration include solid dosage forms such as capsules, tablets, dragees, pills, lozenges, powders and granules. Where appropriate, the compositions may be prepared with coatings such as enteric coatings or they may be formulated so as to provide controlled release of the active ingredient such as sustained or prolonged release according to methods well known in the art. Liquid dosage forms for oral administration include solutions, emulsions, suspensions, syrups and elixirs. Pharmaceutical compositions for parenteral administration include sterile aqueous and nonaqueous injectable solutions, dispersions, suspensions or emulsions as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use. Other suitable administration forms include, but are not limited to, suppositories, sprays, ointments, creams, gels, inhalants, dermal patches and implants.

Typical oral dosages range from about 0.01 to about 100 mg/kg body weight per day.

The compounds of this invention are generally utilized as the free base or as a pharmaceutically acceptable salt thereof. A pharmaceutically acceptable salt of a compound of Formula I is prepared e.g. in a conventional manner by treating a solution or suspension of a free

base of Formula I with a molar equivalent of a pharmaceutically acceptable acid. Representative examples of suitable organic and inorganic acids are described above.

Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solutions and various organic solvents. Examples of solid carriers include lactose, terra alba, sucrose, cyclodextrin, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and lower alkyl ethers of cellulose. Examples of liquid carriers include, but are not limited to, syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The pharmaceutical compositions formed by combining the compounds of Formula I or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier are readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

If a solid carrier is used for oral administration, the preparation may be tableted, placed in a hard gelatin capsule in powder or pellet form or it may be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will range from about 25 mg to about 1 g per dosage unit. If a liquid carrier is used, the preparation may 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.

The compounds of the present invention are as demonstrated in the examples potent inhibitors of BACE1 and capable of lowering the level of A β peptide in rat brain and plasma, and said compounds are thus believed to be useful in the treatment of neurodegenerative and cognitive disorders which pathological characteristics comprise A β deposits and neurofibrillary tangles, such as e.g. Alzheimer's disease. It may be beneficial to combine a compound of the present invention with another treatment paradigm useful in the treatment of such disease, e.g. Alzheimer's disease.

Tau proteins are abundant in neurons. Tau proteins are soluble and highly phosphorylation labile and bind to tubulin providing regulation and modulation of tubulin assembly, i.e. eventually the microtubular structure and stability. Tau proteins can only associate with tubulin in the most de-phosphorylated state, and phosphorylation/de-phosphorylation acts as a switch controlling the tubulin association. Phosphorylated Tau constitutes an important part of the neurofibrillary tangles which are one of the hallmarks of Alzheimer's disease. The so-called Tau hypothesis suggests targeting these pathological tangles, a main constituent of which is phosphorylated Tau protein, as a treatment paradigm for Alzheimer's disease. In particular, immunotherapies, both active and passive, have been suggested as a way to target Tau neurofibrillary tangles. In active immunotherapy, a pathogenic antigen is injected into the patient and the innate immune system elicits an immune response. This triggers the maturation of B-cells generating high affinity

antibodies against the administered antigen. In a passive immunotherapy, the triggering of the innate immune system is circumvented by infusing a specific antibody against the antigen. It is suggested that the inherent clearance system then removes antibody bound ligand. Substantial evidence for the efficacy of both active and passive immunotherapy targeting phosphorylated Tau protein as a treatment for Alzheimer's disease exists [*Alzheimer's & Dementia*, 7(4, suppl) S480-481; *J Neurosci* 30, 16559-16556, 2010; *J Neurosci*, 27, 9115-9129, 2007].

In one embodiment the invention provides a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease, the method comprising the administration of a therapeutically effect amount of two components (1) a compound of Formula I or a pharmaceutically acceptable salt thereof and (2) a compound useful in active or passive Tau immunotherapy to a patient in need thereof. Said compound useful in passive Tau immunotherapy may be an antibody directed against phosphorylated Tau protein. Said compound useful in active Tau immunotherapy may be a fragment of the Tau protein amino acid sequence which upon injection in a patient elicits generation of an anti-phosphorylated Tau protein antibody in said patient. The administration according to this embodiment of the invention may be simultaneous, or there may be a time gap between the administration of the two components.

In one embodiment, the invention relates to the use of a compound of Formula I or a pharmaceutically acceptable salt thereof and a compound useful in active or passive Tau immunotherapy in the manufacture of a medicament for the treatment of neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof and a compound useful in active or passive Tau immunotherapy for use in a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof and a compound useful in active or passive Tau immunotherapy and a pharmaceutically acceptable carrier.

Another paradigm to treat neurodegenerative and cognitive disorder, e.g. Alzheimer's disease is to target the A β peptides. It has been suggested that this can be achieved by either passive or active immunotherapy targeting A β peptides [*J Neurosci*, 34, 11621-11630, 2014; *J Neurosci* 33, 4923-4934, 2013]. In combination with compounds of the present invention this would attempt to target the same pathological mechanism via two different routes. Anti-A β antibodies (either injected directly into the patient or generated in the patient as a result of active immunotherapy) clear A β deposits in the brain, while further accumulation of A β peptide is blocked or reduced by the compounds of the present invention.

In one embodiment the invention provides a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease, the method comprising the administration of a therapeutically effect amount of two components (1) a compound of Formula I or a pharmaceutically acceptable salt thereof and (2) a compound useful in active or passive A β peptide immunotherapy to a patient in need thereof. Said compound useful in passive A β peptide immunotherapy may be an anti-A β peptide antibody, such as gantenerumab, solanezumab, aducanumab or crenezumab. Said compound useful in active A β peptide immunotherapy may be a fragment of the A β peptide amino acid sequence which upon injection into a patient elicits anti-A β peptide antibodies in said patient. The administration according to this embodiment of the invention may be simultaneous, or there may be a time gap between the administration of the two components.

In one embodiment, the invention relates to the use of a compound of Formula I or a pharmaceutically acceptable salt thereof and a compound useful in active or passive A β peptide immunotherapy in the manufacture of a medicament for the treatment of neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof and a compound useful in active or passive A β peptide immunotherapy for use in a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof and a compound useful in active or passive A β peptide immunotherapy and a pharmaceutically acceptable carrier.

The NMDA (N-Methyl-D-Aspartate) receptor antagonist memantine and the acetylcholine esterase inhibitors donepezil, rivastigmine and galantamine are approved drugs for the treatment of Alzheimer's disease.

In one embodiment the invention provides a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease, the method comprising the administration of a therapeutically effect amount of two components (1) a compound of Formula I or a pharmaceutically acceptable salt thereof and (2) an NMDA receptor antagonist or an acetylcholine esterase inhibitor to a patient in need thereof. The administration according to this embodiment of the invention may be simultaneous, or there may be a time gap between the administration of the two components.

In one embodiment, the invention relates to the use of a compound of Formula I or a pharmaceutically acceptable salt thereof and an NMDA receptor antagonist or an acetylcholine

esterase inhibitor in the manufacture of a medicament for the treatment of neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof and an NMDA receptor antagonist or an acetylcholine esterase inhibitor for use in a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof and an NMDA receptor antagonist or an acetylcholine esterase inhibitor and a pharmaceutically acceptable carrier.

Seizures or epileptiform activity are also associated with Alzheimer's disease, including early stages of Alzheimer's disease, and treatment of said epileptic activity, which seeks to normalise hippocampal hyperactivity, may form part of an Alzheimer's disease treatment paradigm [*JAMA Neurol*, 70, 1158-1166, 2013; *J Neurosci Res*, 93, 454, 465, 2015; *Neuron*, 74, 647-474, 2012; *Neurepsychopharm*, 35, 1016-1025, 2010; *CNS Neurosci Ther*, 19, 871-881, 2013]. Useful antiepileptics include NMDA receptor antagonists and ion channel modulators, such as topiramate, levetiracetam and lamotrigine.

In one embodiment the invention provides a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease, the method comprising the administration of a therapeutically effect amount of two components (1) a compound of Formula I or a pharmaceutically acceptable salt thereof and (2) an antiepileptic to a patient in need thereof. The administration according to this embodiment of the invention may be simultaneous, or there may be a time gap between the administration of the two components.

In one embodiment, the invention relates to the use of a compound of Formula I or a pharmaceutically acceptable salt thereof and an antiepileptic in the manufacture of a medicament for the treatment of neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof and an antiepileptic for use in a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof and an antiepileptic and a pharmaceutically acceptable carrier.

Emerging evidence suggests that inflammation has a causal role in Alzheimer's disease pathogenesis and that neuroinflammation is not a passive system activated by emerging β -amyloid deposits and neurofibrillary tangles, but also contributes to pathogenesis itself [*Lancet Neurol*, 14, 388-405, 2015; *J Alz Dis*, 44, 385-396, 2015; *Neurol*, 84, 2161-2168, 2015]. It follows from this that

anti-inflammatory drugs, such as NSAID (non-steroid anti-inflammatory drugs), TNF α inhibitors, such as etanercept and p38 MAP kinase inhibitors, such as VX-745 (5-(2,6-Dichlorophenyl)-2-((2,4-difluorophenyl)thio)-6H-pyrimido[1,6-b]pyridazin-6-one) may be useful in the treatment of Alzheimer's disease.

5 In one embodiment the invention provides a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease, the method comprising the administration of a therapeutically effect amount of two components (1) a compound of Formula I or a pharmaceutically acceptable salt thereof and (2) an anti-inflammatory drug to a patient in need thereof. The administration according to this embodiment of the invention may be simultaneous, or
10 there may be a time gap between the administration of the two components.

In one embodiment, the invention relates to the use of a compound of Formula I or a pharmaceutically acceptable salt thereof and anti-inflammatory drug in the manufacture of a medicament for the treatment of neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

15 In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof and an anti-inflammatory drug for use in a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof and an anti-inflammatory drug and a pharmaceutically acceptable carrier.

20 In addition, efficacy in the treatment of Alzheimer's disease has been demonstrated for Tau protein aggregation inhibitors, such as TRX-0237, also known as Methylene Blue, and SSRIs (Selective Serotonin Reuptake Inhibitor), such as citalopram [*Behav Pharmacol*, 26, 353-368, 2015; *Sci Transl Med*, 6(236re4), 2014].

In one embodiment the invention provides a method for the treatment of a
25 neurodegenerative or cognitive disorder, e.g. Alzheimer's disease, the method comprising the administration of a therapeutically effect amount of two components (1) a compound of Formula I or a pharmaceutically acceptable salt thereof and (2) Tau protein aggregation inhibitor or an SSRI to a patient in need thereof. The administration according to this embodiment of the invention may be simultaneous, or there may be a time gap between the administration of the two components.

30 In one embodiment, the invention relates to the use of a compound of Formula I or a pharmaceutically acceptable salt thereof and a Tau protein aggregation inhibitor or an SSRI in the manufacture of a medicament for the treatment of neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a compound of Formula I or a pharmaceutically acceptable salt thereof and a Tau protein aggregation inhibitor or an SSRI drug for use in a method for the treatment of a neurodegenerative or cognitive disorder, e.g. Alzheimer's disease.

In one embodiment, the invention provides a pharmaceutical composition comprising a
 5 compound of Formula I or a pharmaceutically acceptable salt thereof and a Tau protein aggregation inhibitor or an SSRI drug and a pharmaceutically acceptable carrier.

EXPERIMENTAL SECTION

The compounds of the present invention of the general formula I, wherein R¹ and Ar are as defined
 10 above can be prepared by the methods outlined in the following reaction schemes 1-7 and in the examples. In the described methods, it is possible to make use of variants or modifications, which are themselves known to chemists skilled in the art or could be apparent to the person of ordinary skill in this art. Furthermore, other methods for preparing compounds of the invention will be readily apparent to the person skilled in the art in light of the following reaction schemes and examples.

For example, Scheme 2 describe the use of selective protecting groups during the synthesis
 15 of the compounds of the invention. One skilled in the art would be able to select the appropriate protecting group for a particular reaction. Moreover, it may be necessary to incorporate protection and deprotection strategies for substituents such as amino, amido, keto and hydroxyl groups in the synthetic methods described below to synthesize the compounds of Formula I. Methods for
 20 protection and deprotection of such groups are well known in the art, and may be found in T. Green, et al., Protective Groups in Organic Synthesis, 1991, 2nd Edition, John Wiley & Sons, New York.

For compounds, which can exist as a mixture or equilibrium between two or more
 25 tautomers, only one tautomer is represented in the schemes, although it may not be the most stable tautomer. For compounds, which can exist in enantiomeric, stereoisomeric or geometric isomeric forms their geometric configuration is specified; otherwise the structure represents a mixture of stereoisomers.

Analytical LC-MS data was obtained using the following methods.

30 Method A:

LC-MS was run on Waters Aquity UPLC-MS consisting of Waters Aquity including column manager, binary solvent manager, sample organizer, PDA detector (operating at 254 nm), ELS detector, and SQ-MS equipped with APPI-source operating in positive ion mode.

LC-conditions: The column was Acquity UPLC BEH C18 1.7 μ m ; 2.1x150mm operating at 60°C with 0.6 ml/min of a binary gradient consisting of water + 0.05 % trifluoroacetic acid (A) and acetonitrile + 5% water + 0.03 % trifluoroacetic acid (B). Gradient: 0.00 min: 10% B; 3.00 min: 99.9% B; 3.01 min: 10% B; 3.60 min: 10% B. Total run time: 3.60 min.

5 Method B:

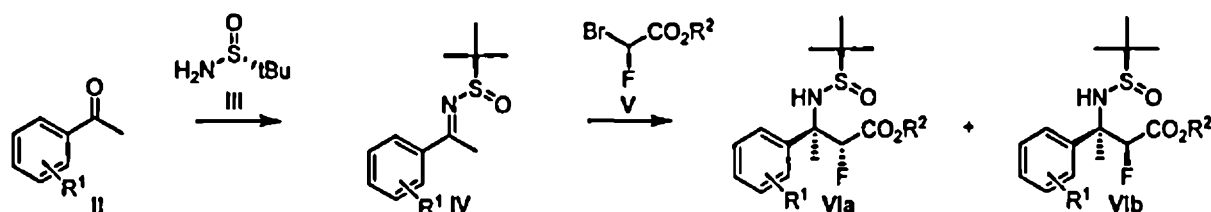
LC-MS was run on Waters Acquity UPLC-MS consisting of Waters Acquity including column manager, binary solvent manager, sample organizer, PDA detector (operating at 254 nm), ELS detector, and TQ-MS equipped with APPI-source operating in positive ion mode.

LC-conditions: The column was Acquity UPLC BEH C18 1.7 μ m ; 2.1x50mm operating at 60°C with
10 1.2 ml/min of a binary gradient consisting of water + 0.05 % trifluoroacetic acid (A) and acetonitrile + 5% water + 0.05 % trifluoroacetic acid (B). Gradient: 0.00 min: 10% B; 1.00 min: 100% B; 1.01 min: 10% B; 1.15 min: 10% B. Total run time: 1.15 min.

¹H NMR spectra were recorded at 600 MHz on a Bruker Avance AV-III-600 Instrument or at 400 MHz on a Bruker Avance AV-III-400 instrument or a Varian 400 instrument. Chemical shift
15 values are expressed in ppm-values relative. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, ddd = double double doublet, dt = double triplet, br = broad, and m = multiplet.

Compounds of the general formulae VIa and VIb may be prepared as shown in Scheme 1.

20 Scheme 1



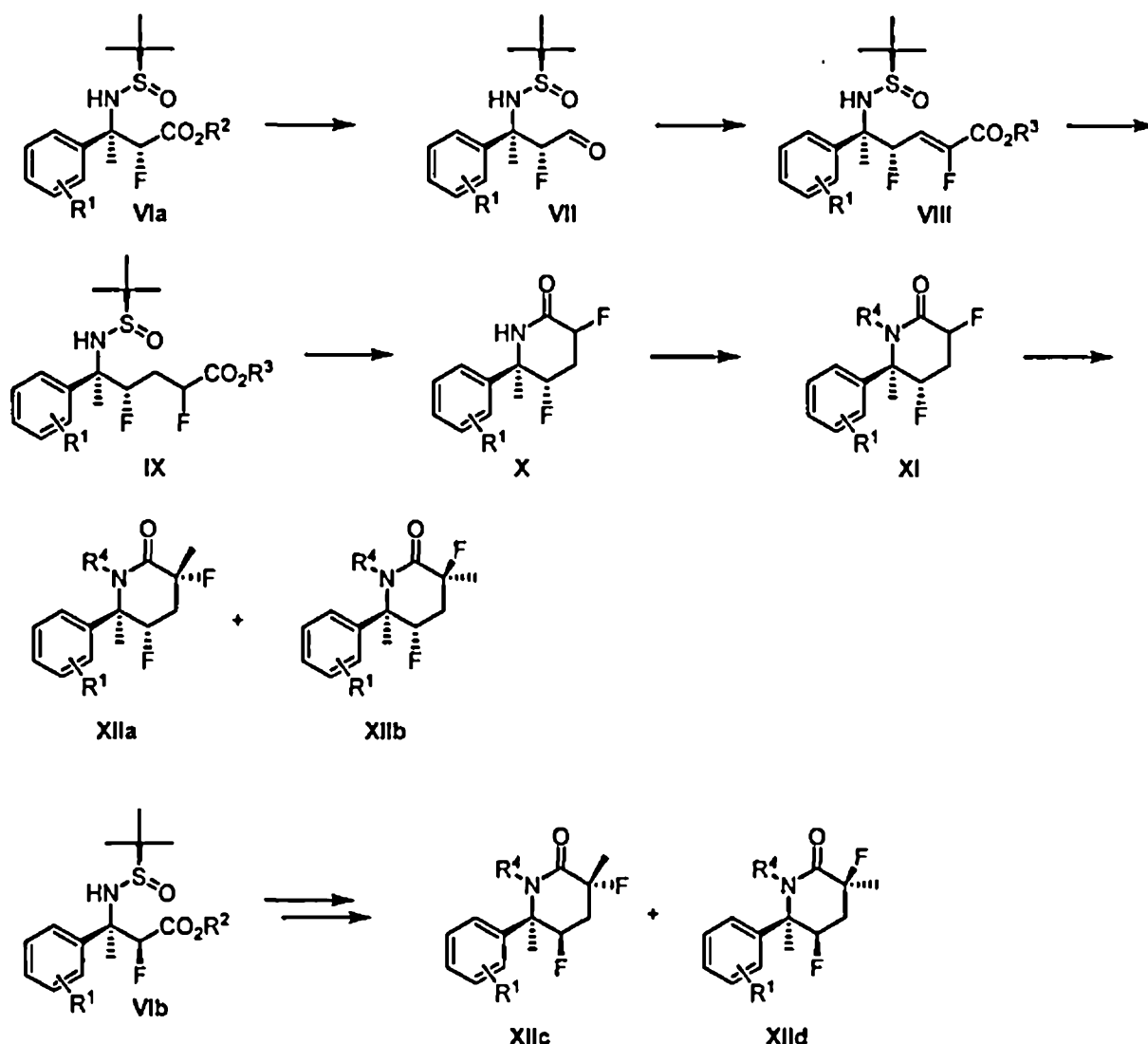
where R¹ is as defined under formula I and R² is as an alkyl group such as methyl or ethyl.

Compounds of the general formula IV (Scheme 1) may be prepared by reacting compounds of formula II with a sulfinamide such as III in the presence of a Lewis acid/drying agent such as
25 titanium tetraethoxide. Treatment of compounds of the formula IV with compounds of the general formula V such as ethyl bromofluoroacetate in the presence of Zn powder or in the presence of diethyl zinc and tris(triphenylphosphine)rhodium(I) chloride gives compounds of the general formulae VIa and VIb which can be separated by chromatography.

Compounds of the general formulae XIIa and XIIb may be prepared as shown in Scheme 2.

30

Scheme 2



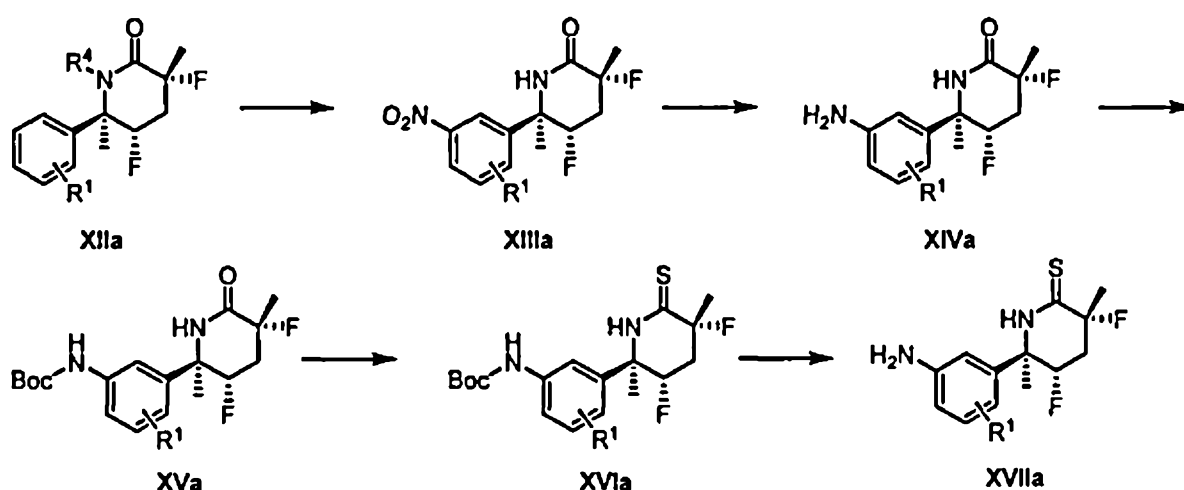
where R^2 and R^3 are alkyl groups such as methyl or ethyl and R^4 is protecting group such as *tert*-butoxy carbonyl.

Compounds of formula VII (Scheme 2) may be prepared by treatment of compounds of formula VIa with a reducing agent such as diisobutylaluminium hydride. In some cases, compound VII might be in equilibrium with the hydrate form. Treatment of compounds of the general formula VII with conditions such as ethyl 2-(diethoxyphosphoryl)-2-fluoroacetate in the presence of lithium chloride and a base such as *N,N*-diisopropylethylamine gives compounds of the general formula VIII as either the *E* or *Z* form or a mixture thereof. Compounds of the general formula IX are obtained by hydrogenation of compounds of the general formula VIII in the presence of a catalyst such as palladium on carbon. Compounds of the general formula X are obtained by treatment of compounds of the general formula IX with an acid such as hydrochloric acid in methanol followed by heating the intermediate in a higher boiling solvent such as toluene or xylenes in the presence of a base such as triethylamine. Compounds of the general formula XI are obtained by treatment of

compounds of the general formula X with di-*tert*-butyl dicarbonate in the presence of a catalytic amount of *N,N*-dimethyl-4-amino-pyridine. Compounds of the general formulae XIIa and XIIb are obtained by treatment of compounds of the general formula XI with a base such as lithium hexamethyldisilazide followed by alkylation with an alkylhalide. The same procedures described above can also be used for the synthesis of compounds of the general formulae XIIc and XIId starting from VIb.

As an example and wherein R¹ is fluorine in the 2-position of the phenyl ring or wherein the phenyl ring is substituted with two fluorines in the 2- and 3-positions, compound of the general formula XVIIa may be prepared as shown in Scheme 3.

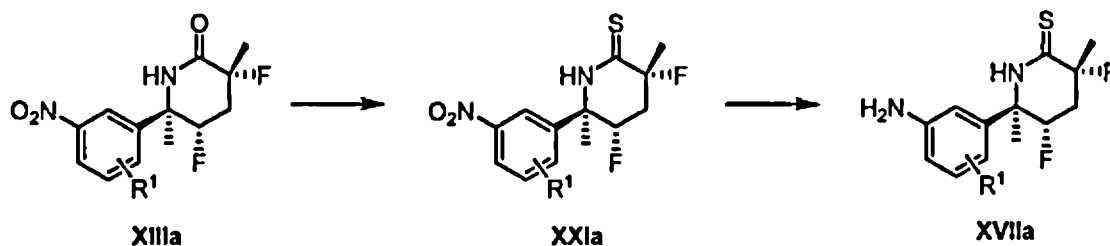
Scheme 3



where R⁴ is a protecting group such as *tert*-butoxy carbonyl.

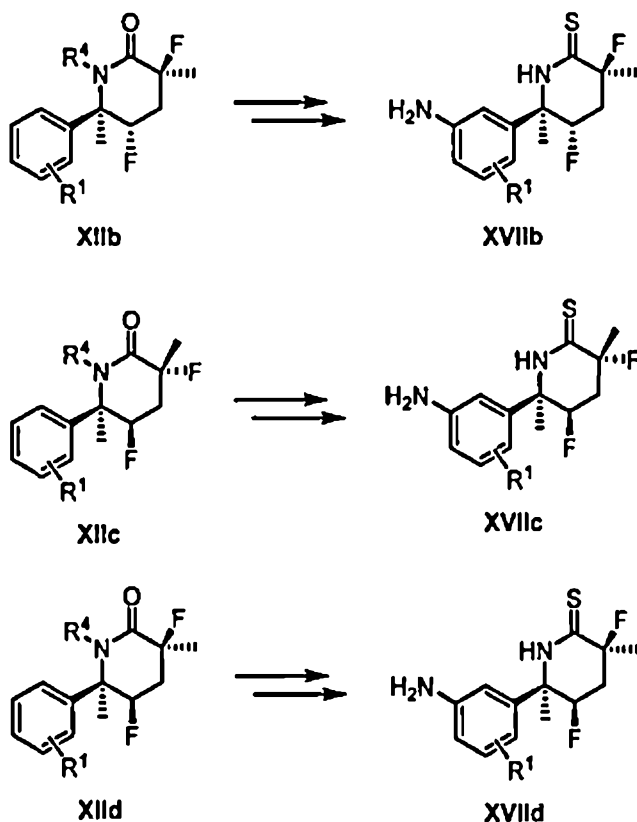
Compounds of the general formula XIIIa are obtained by treating compounds of the general formula XIIa with nitric acid. This will both deprotect the amide moiety and nitrate the benzene ring in the indicated position. Reduction of the nitro group of compounds of the general formula XIIIa followed by protection of the formed aniline moiety gives compounds of the general formula XVa. Treatment of compounds of the general formula XVa with a reagent such as Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide) gives compounds of the general formula XVIa. Deprotection of XVIa under conditions such as trifluoroacetic acid in dichloromethane gives compound XVIIa. Alternatively, the order of reactions can be changed and compounds of the general formula XIIIa can be reacted with a reagent such as Lawesson's reagent to give compounds of the general formula XXia, which after reduction of the nitro group also gives compounds of the general formula XVIIa (Scheme 4).

Scheme 4



As an example and wherein R¹ is fluorine in the ortho position of the phenyl ring or wherein the phenyl ring is substituted with two fluorines in the 2- and 3-positions, compound of the general formula XVIIb (Scheme 5) may be prepared by the same procedures as shown in Scheme 3 and 4 but starting from compound of general formula XIIb. Likewise, the same procedures can be used for the synthesis of compounds of the general formulae XVIIc and XVIId starting from XIIc and XIId, respectively.

10 **Scheme 5**

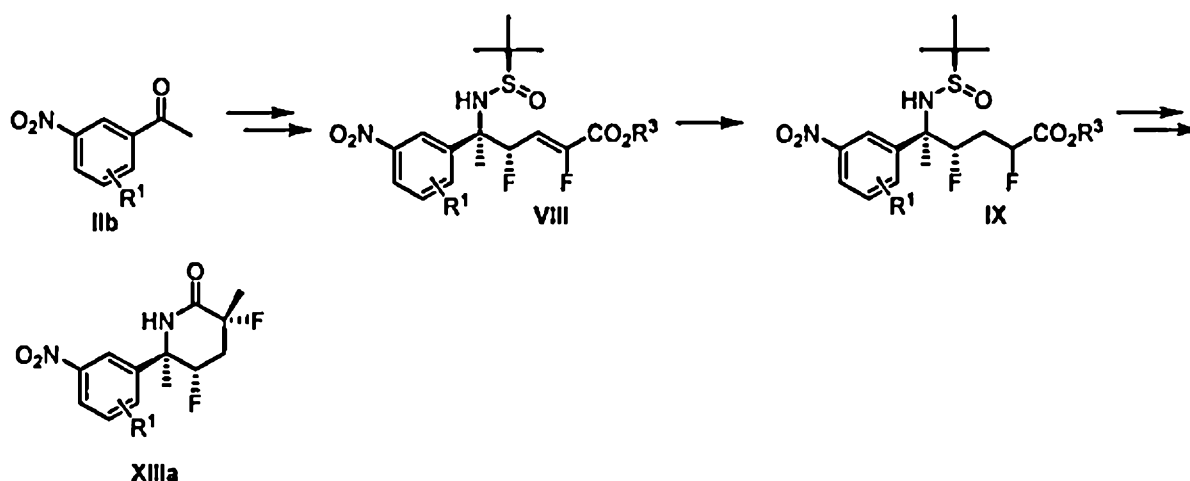


where R⁴ is a protecting group such as *tert*-butoxy carbonyl.

Compounds of the general formula XIIIa may also be prepared as shown in Scheme 6. Starting from nitro substituted acetophenones of general formula IIb, compounds of the general formula VIII may

be prepared as described in Schemes 1 and 2. Compounds of the general formula IX may be obtained by selective reduction of the olefin moiety of compounds of the general formula VIII. Compounds of the general formula XIIIa may be prepared as described for compounds of general formula XIIa in Scheme 2. Likewise, compounds of general formulae XIIIb, XIIIc and XIId may also be prepared as shown in Scheme 6 starting from nitro substituted acetophenones of general formula IIb.

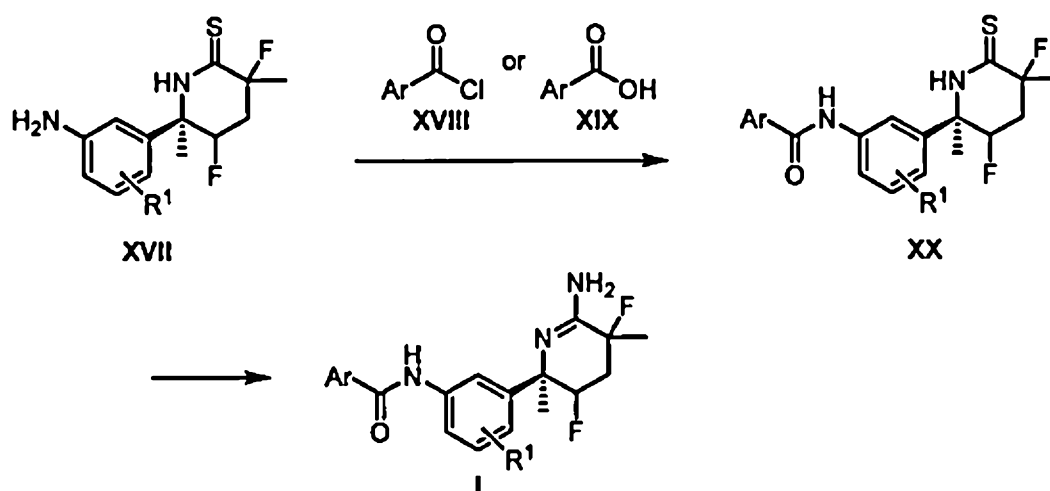
Scheme 6



10 where R^1 is as defined under formula I and R^3 is an alkyl group such as methyl or ethyl

Compounds of the general formula I may be prepared as shown in Scheme 7.

Scheme 7



15 where R^1 and Ar are as defined under formula I.

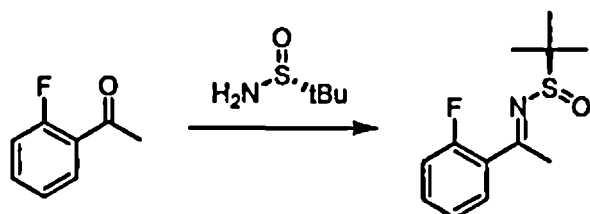
Compounds of the general formula XX (Scheme 7) may be prepared by reacting compounds of the general formula XVII with a carboxylic acid chloride of general formula XVIII or by reaction with a

carboxylic acid of general formula XIX using procedures known to chemists skilled in the art. Treatment of compounds of the general formula XX with ammonia gives compounds of the general formula I. In some cases, the addition of an oxidizing reagent such as *tert*-butyl hydroperoxide might be necessary to facilitate the reaction.

5

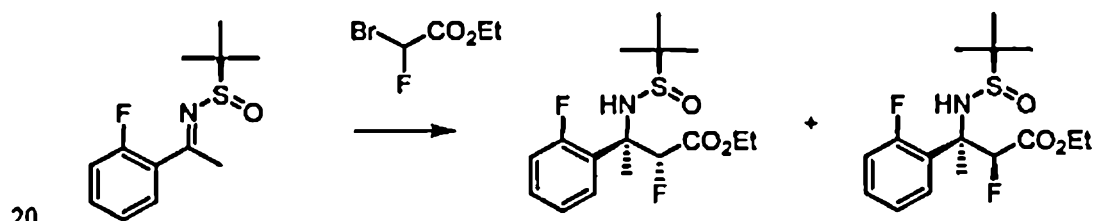
PREPARATION OF INTERMEDIATES

INTERMEDIATE: (*R*)-*N*-[1-(2-fluorophenyl)ethylidene]-2-methyl-propane-2-sulfinamide



To a solution of 1-(2-fluorophenyl)ethanone (100 g, 723.9 mmol) and (*R*)-2-methylpropane-2-sulfinamide (105.28 g, 868.68 mmol) in THF (2.00 L) was added tetraethoxytitanium (330.26 g, 1.45 mol). The solution was warmed to 80°C and stirred for 18 hours. The reaction was quenched by water (500 mL), then extracted with ethyl acetate (500 mL, 4 times). The combined organic phases were washed with brine (500 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, petroleum ether/ethyl acetate=10/1, 5/1) to give (*R*)-*N*-[1-(2-fluorophenyl)ethylidene]-2-methyl-propane-2-sulfinamide (150 g, 86% yield).

INTERMEDIATES: Ethyl (2*R*,3*R*)-3-[[(*R*)-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)-butanoate and ethyl (2*S*,3*R*)-3-[[(*R*)-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)butanoate

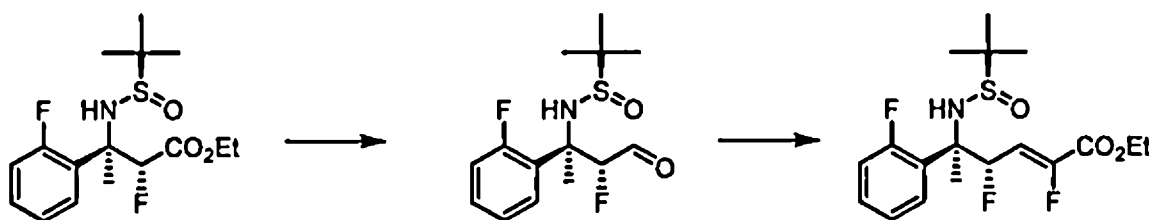


To a suspension of (*R*)-*N*-[1-(2-fluorophenyl)ethylidene]-2-methyl-propane-2-sulfinamide (20. g, 82.9 mmol), ethyl 2-bromo-2-fluoroacetate (30.66 g, 165.8 mmol) and Rh(PPh₃)₃Cl (2.30 g, 2.49 mmol) in anhydrous THF (400 mL) was added Et₂Zn (1M in THF, 249 mL) dropwise at 0°C over a period of 10 minutes under argon during which the temperature was maintained below 5°C. The reaction mixture was warmed to 30°C and stirred at 30°C for 18 hours. The reaction was quenched with water (150 mL), filtered and then extracted with ethyl acetate (3 × 500 mL). The combined organic phases were washed with saturated brine (40 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel

(petroleum ether: ethyl acetate = 100:1 to 3:1) to give ethyl (2*R*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)butanoate (11.2 g, 39% yield) and an impure fraction containing (2*S*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)butanoate. After preparative HPLC purification of the latter fraction, (2*S*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)butanoate was isolated (0.8 g, 3% yield)..

Ethyl (2*R*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-3-(2,3-difluorophenyl)-2-fluorobutanoate was prepared in a similar way starting from (*R*)-*N*-[1-(2,3-difluorophenyl)ethylidene]-2-methylpropane-2-sulfonamide

INTERMEDIATE: Ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hex-2-enoate

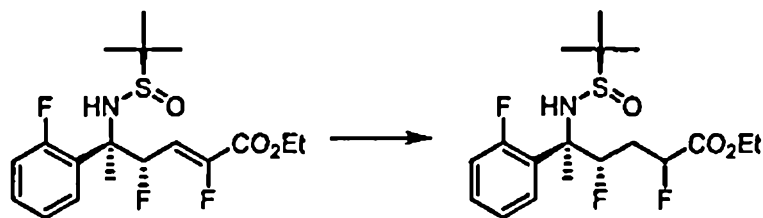


To a solution of ethyl (2*R*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)butanoate (10.0 g, 28.8 mmol) in THF (200 mL) was added DIBAL-H (diisobutylaluminum hydride) (1M in THF, 143.9 mL) dropwise at -78°C over a period of 30 minutes under N₂. The yellow reaction solution was stirred at -78°C for 3.5 hours. The reaction was quenched with water (200 mL), filtered and extracted with ethyl acetate (200 mL, 4 times). The combined organic phases were washed with saturated brine (200 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give the crude product (*R*)-*N*-((2*R*,3*R*)-3-fluoro-2-(2-fluorophenyl)-4-oxobutan-2-yl)-2-methylpropane-2-sulfonamide (8.0 g, crude). To a suspension of ethyl 2-diethoxyphosphoryl-2-fluoroacetate (5.13 g, 21.2 mmol), DIPEA (*N,N*-diisopropylethylamine) (1.18 g, 9.1 mmol) and LiCl (386 mg, 9.1 mmol) in acetonitrile (150 mL) was added a solution of (*R*)-*N*-((2*R*,3*R*)-3-fluoro-2-(2-fluorophenyl)-4-oxobutan-2-yl)-2-methylpropane-2-sulfonamide (2.30 g, 7.58 mmol) in acetonitrile (20 mL) dropwise at 0°C over a period of 10 minutes under N₂. The suspension was warmed to 25°C stirred for 18 hours. The reaction was concentrated *in vacuo*, the residue was extracted with ethyl acetate (100 mL, 3 times). The combined organic phases were washed with saturated brine (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 5:1, 1:1) to give ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hex-2-enoate (3.44 g, 8.79 mmol, 49.8% yield).

Ethyl (4*R*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hex-2-enoate was prepared in a similar way starting from (2*S*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-2-fluoro-3-(2-fluorophenyl)butanoate

Ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2,3-difluorophenyl)hex-2-enoate was prepared in a similar way starting from ethyl (2*R*,3*R*)-3-[[*(R)*-*tert*-butylsulfinyl]amino]-3-(2,3-difluorophenyl)-2-fluorobutanoate

INTERMEDIATE: Ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)-hexanoate

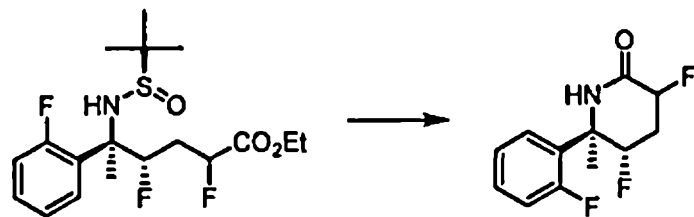


To a solution of ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hex-2-enoate (13.77 g, 35.18 mmol) in ethyl acetate (500 mL) was added Pd/C (10%, 2.00 g, wet) . The black suspension was degassed under vacuum and purged with H₂ several times. The mixture was stirred under H₂ (50 psi) at 30°C for 18 hours. The suspension was filtered and the filter was concentrated to give product of ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hexanoate (10.5 g, 75.9% yield).

Ethyl (4*R*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hexanoate was prepared in a similar way starting from ethyl (4*R*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hex-2-enoate

Ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2,3-difluorophenyl)hexanoate was prepared in a similar way starting from ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2,3-difluorophenyl)hex-2-enoate

INTERMEDIATE: (5*S*,6*R*)-3,5-difluoro-6-(2-fluorophenyl)-6-methylpiperidin-2-one



To a solution of ethyl (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hexanoate (10.50 g, 26.69 mmol) in MeOH (100 mL) was added HCl/MeOH (4 M, 50 mL) in one portion at 30°C under N₂. The colorless solution was stirred at 30°C for 2 hours. The solution was

concentrated in vacuum to give crude ethyl (4*S*,5*S*)-5-amino-2,4-difluoro-5-(2-fluorophenyl)hexanoate (6.50 g, 21.35 mmol, 80% yield) which was dissolved in toluene (100 mL) and triethylamine (2.19 g, 21.64 mmol) was added. The colorless solution was stirred at 110°C for 18 hours. The reaction was concentrated *in vacuo*, the residue was purified by flash

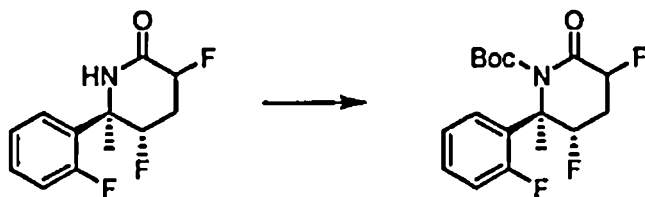
5 chromatography on silica gel (petroleum ether: ethyl acetate =3:1~1:1) to give (5*S*,6*R*)-3,5-difluoro-6-(2-fluorophenyl)-6-methyl-piperidin-2-one (4.50 g, 17.6 mmol, 78.2% yield).

(5*R*,6*R*)-3,5-difluoro-6-(2-fluorophenyl)-6-methylpiperidin-2-one was prepared in a similar way starting from (4*R*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2-fluorophenyl)hexanoate.

(5*S*,6*R*)-3,5-difluoro-6-(2,3-difluorophenyl)-6-methylpiperidin-2-one was prepared in a similar way

10 starting from (4*S*,5*R*)-5-[[*(R)*-*tert*-butylsulfinyl]amino]-2,4-difluoro-5-(2,3-difluorophenyl)hexanoate

INTERMEDIATE: *tert*-Butyl (2*R*,3*S*)-3,5-difluoro-2-(2-fluorophenyl)-2-methyl-6-oxo-piperidine-1-carboxylate



15 To a solution of (5*S*,6*R*)-3,5-difluoro-6-(2-fluorophenyl)-6-methyl-piperidin-2-one (4.50 g, 18.50 mmol) and di-*tert*-butyl dicarbonate (8.08 g, 37.0 mmol) in THF (100 mL) was added *N,N*-dimethylpyridin-4-amine (113 mg, 925 μ mol) in one portion at 25°C under N_2 . The colorless solution was stirred at 25°C for 48 hours. The solution was quenched with water (100 mL) and extracted with ethyl acetate (100 mL, 3 times). The combined organic phases were washed with saturated

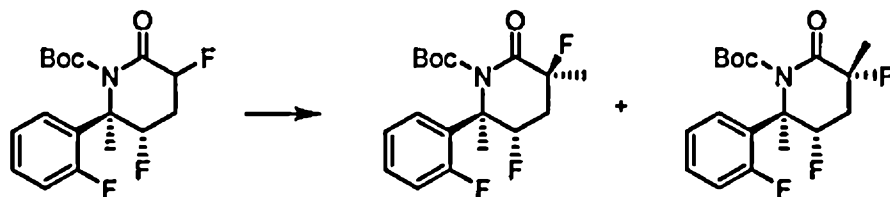
20 brine (50 mL), dried with anhydrous Na_2SO_4 , filtered and concentrated in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate=20/1, 5/1) to give *tert*-butyl (2*R*,3*S*)-3,5-difluoro-2-(2-fluorophenyl)-2-methyl-6-oxo-piperidine-1-carboxylate (4.70 g, 13.0 mmol, 70.3% yield).

tert-Butyl (2*R*,3*R*)-3,5-difluoro-2-(2-fluorophenyl)-2-methyl-6-oxo-piperidine-1-carboxylate was

25 prepared in a similar way starting from (5*R*,6*R*)-3,5-difluoro-6-(2-fluorophenyl)-6-methylpiperidin-2-one.

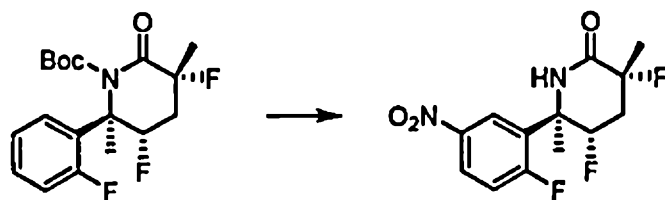
tert-Butyl (2*R*,3*S*)-3,5-difluoro-2-(2,3-difluorophenyl)-2-methyl-6-oxo-piperidine-1-carboxylate was prepared in a similar way starting from (5*S*,6*R*)-3,5-difluoro-6-(2,3-difluorophenyl)-6-methylpiperidin-2-one

INTERMEDIATES: *tert*-butyl (2*R*,3*S*,5*R*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate and *tert*-butyl (2*R*,3*S*,5*S*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate



- 5 To a solution of *tert*-butyl (2*R*,3*S*)-3,5-difluoro-2-(2-fluorophenyl)-2-methyl-6-oxo-piperidine-1-carboxylate (1.50 g, 4.37 mmol) in THF (30 mL) was added LiHMDS (Lithium bis(trimethylsilyl)amide) (1 M, 8.74 mL) dropwise at -78°C over a period of 10 minutes under N₂. The solution was stirred at -78°C for 1 hour, then to the solution was added iodomethane (3.10 g, 21.9 mmol). The reaction solution was stirred at -78°C for another 45 minutes. Then the solution
- 10 was allowed to be warmed to 25°C and stirred for 18 hours. The reaction was slowly quenched by ice and then extracted with ethyl acetate (30 mL, 3 times). The combined organic phases were washed with saturated brine (30 mL, twice), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate = 15:1, 10:1) to give the products *tert*-butyl (2*R*,3*S*,5*R*)-3,5-difluoro-2-(2-
- 15 fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate (550 mg, 1.54 mmol, 35.2% yield) and *tert*-butyl (2*R*,3*S*,5*S*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate (600 mg, 1.68 mmol, 38.4% yield).
- tert*-butyl (2*R*,3*R*,5*S*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate was prepared in a similar way starting from *tert*-butyl (2*R*,3*R*)-3,5-difluoro-2-(2-fluorophenyl)-2-
- 20 methyl-6-oxo-piperidine-1-carboxylate
- tert*-butyl (2*R*,3*S*,5*R*)-3,5-difluoro-2-(2,3-difluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate and *tert*-butyl (2*R*,3*S*,5*S*)-3,5-difluoro-2-(2,3-difluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate were prepared in a similar way starting from *tert*-butyl (2*R*,3*S*)-3,5-difluoro-2-(2,3-
- 25 difluorophenyl)-2-methyl-6-oxo-piperidine-1-carboxylate

INTERMEDIATE: (3*R*,5*S*,6*R*)-3,5-Difluoro-6-(2-fluoro-5-nitro-phenyl)-3,6-dimethyl-piperidin-2-one



tert-Butyl-(2*R*,3*S*,5*R*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate (1.20 g, 3.36 mmol) in trifluoroacetic acid (5.69 mL, 73.9 mmol) was added H₂SO₄ (1.27 mL, 25.9 mmol), then to the solution was added HNO₃ (1.16 mL, 16.8 mmol), the solution was stirred at 0°C for 30 min, then the solution was stirred at 50°C for 18 hours, LCMS showed no starting material, the solution was poured into ice-water (w/w = 1/1) (50 mL) and basified to pH > 11 using 5N aqueous NaOH. The aqueous phase was extracted with ethyl acetate (50 mL, 3 times). The combined organic phases were washed with saturated brine (100 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate=10/1, 2/1) to give (3*R*,5*S*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitro-phenyl)-3,6-dimethyl-piperidin-2-one (1.0 g, 99% yield).

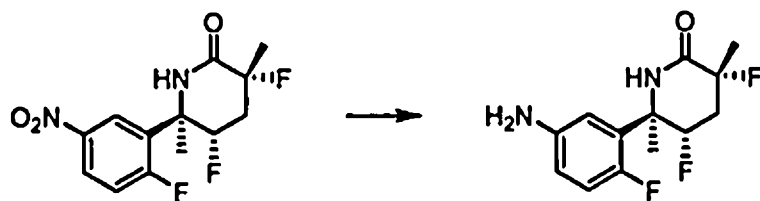
(3*S*,5*S*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one was prepared in a similar way starting from *tert*-butyl (2*R*,3*S*,5*S*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate.

(3*S*,5*R*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one was prepared in a similar way starting from *tert*-butyl (2*R*,3*R*,5*S*)-3,5-difluoro-2-(2-fluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate.

(3*R*,5*S*,6*R*)-3,5-difluoro-6-(2,3-difluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one was prepared in a similar way starting from *tert*-butyl (2*R*,3*S*,5*R*)-3,5-difluoro-2-(2,3-difluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate.

(3*S*,5*S*,6*R*)-3,5-difluoro-6-(2,3-difluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one was prepared in a similar way starting from *tert*-butyl (2*R*,3*S*,5*S*)-3,5-difluoro-2-(2,3-difluorophenyl)-2,5-dimethyl-6-oxo-piperidine-1-carboxylate.

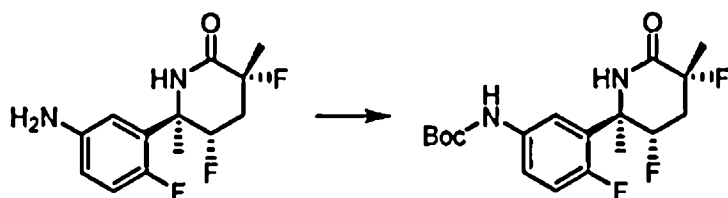
INTERMEDIATE: (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidin-2-one



To a solution of (3*R*,5*S*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitro-phenyl)-3,6-dimethyl-piperidin-2-one (1.10 g, 3.64 mmol) in ethyl acetate (100 mL) was added Pd/C (10%, wet, 0.5 g) under N₂. The suspension was degassed under vacuum and purged with H₂ several times. The mixture was stirred under H₂ (15 psi) at 25°C for 18 hours. The reaction mixture was filtered and the filtrate was concentrated to give (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidin-2-one (950 mg, 3.49 mmol, 96% yield).

(3*S*,5*S*,6*R*)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethylpiperidin-2-one was prepared in a similar way starting from (3*S*,5*S*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one.

- 5 INTERMEDIATE: *tert*-Butyl *N*-[3-[(2*R*,3*S*,5*R*)-3,5-difluoro-2,5-dimethyl-6-oxo-2-piperidyl]-4-fluorophenyl]carbamate

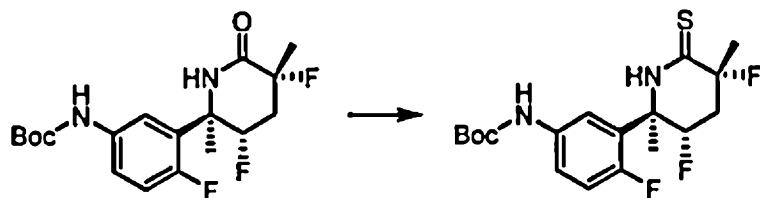


- To a mixture of (3*R*,5*S*,6*R*)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethyl-piperidin-2-one (750 mg, 2.75 mmol) in aqueous NaHCO₃ (5 mL) and dichloromethane (20 mL) was added di-*tert*-butyl dicarbonate (1.80 g, 8.26 mmol) in one portion at 25°C under N₂. The colorless mixture was stirred at 25°C for 18 hours. The mixture was quenched with water (20 mL) and extracted with ethyl acetate (50 mL, 3 times). The combined organic phases were washed with saturated brine (50 mL, twice), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate=10/1, 1/1) to give *tert*-butyl *N*-[3-[(2*R*,3*S*,5*R*)-3,5-difluoro-2,5-dimethyl-6-oxo-2-piperidyl]-4-fluorophenyl]carbamate (850mg, 83% yield).

tert-Butyl *N*-[3-[(2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-oxo-2-piperidyl]-4-fluorophenyl]carbamate was prepared in a similar way starting from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethyl-piperidin-2-one.

20

- INTERMEDIATE: *tert*-Butyl *N*-[3-[(2*R*,3*S*,5*R*)-3,5-difluoro-2,5-dimethyl-6-thio-2-piperidyl]-4-fluorophenyl]carbamate

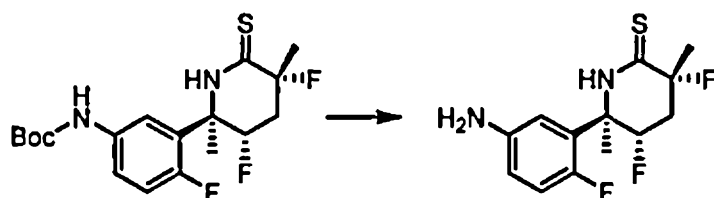


- To a suspension of *tert*-butyl *N*-[3-[(2*R*,3*S*,5*R*)-3,5-difluoro-2,5-dimethyl-6-oxo-2-piperidyl]-4-fluorophenyl]carbamate (250 mg, 671 μmol) in toluene (10 mL) was added Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide) (149 mg, 369 μmol) in one portion at 90°C under N₂. The mixture was stirred at 90°C for 1 hour. The mixture was concentrated in reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum

ether/ethyl acetate=10/1, 1/1) to give *tert*-butyl *N*-[3-[(2*R*,3*S*,5*R*)-3,5-difluoro-2,5-dimethyl-6-thioxo-2-piperidyl]-4-fluoro-phenyl]carbamate (230 mg, 88% yield).

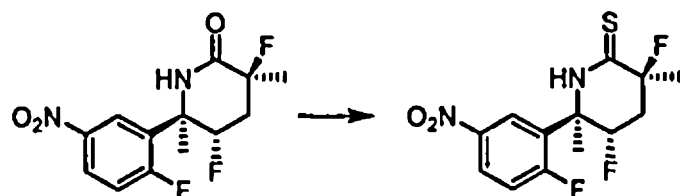
tert-butyl *N*-[3-[(2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxo-2-piperidyl]-4-fluoro-phenyl]carbamate was prepared in a similar way starting from *tert*-butyl *N*-[3-[(2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-oxo-2-piperidyl]-4-fluoro-phenyl]carbamate.

INTERMEDIATE: (3*R*,5*S*,6*R*)-6-(5-Amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione



- 10 To a solution of *tert*-butyl *N*-[3-[(2*R*,3*S*,5*R*)-3,5-difluoro-2,5-dimethyl-6-thioxo-2-piperidyl]-4-fluoro-phenyl]carbamate (500 mg, 1.29 mmol) in dichloromethane (20 mL) was added HCl/MeOH (10 mL) in one portion at 25°C under N₂. The mixture was stirred at 25°C for 18 hours. The mixture was concentrated in reduced pressure. The residue was dissolved in water (10 mL), the pH of the solution was basified to pH=7-8 using saturated aqueous NaHCO₃ solution, the aqueous phase
- 15 was extracted with ethyl acetate (20 mL, 3 times). The combined organic phases were washed with saturated brine (20 mL, twice), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate=10/1, 1/1) to give (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione (318 mg, 85.5% yield).
- 20 (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione was prepared in a similar way starting from *tert*-butyl *N*-[3-[(2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxo-2-piperidyl]-4-fluoro-phenyl]carbamate.

INTERMEDIATE: (3*S*,5*S*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione



- 25 To a suspension of (3*S*,5*S*,6*R*)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one (4.50 g, 14.89 mmol) in toluene (100 mL) was added Lawesson's reagent (3.31 g, 8.19 mol) in one portion at 90°C under N₂. The mixture was stirred at 90°C for 2 hours. The mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum

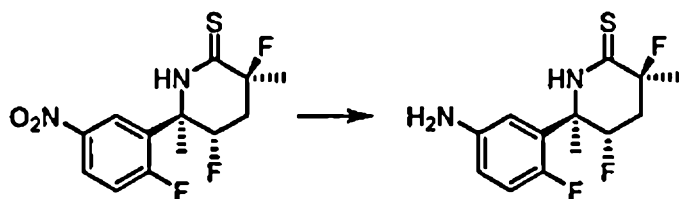
ether/ethyl acetate) to give (3S,5S,6R)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione (4.0 g, 84% yield).

(3S,5R,6R)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione was prepared in a similar way starting from (3S,5R,6R)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidin-2-one.

(3S,5S,6R)-6-(2,3-difluoro-5-nitrophenyl)-3,5-difluoro-3,6-dimethylpiperidine-2-thione was prepared in a similar way starting from (3S,5S,6R)-6-(2,3-difluoro-5-nitrophenyl)-3,5-difluoro-3,6-dimethylpiperidin-2-one.

(3R,5S,6R)-6-(2,3-difluoro-5-nitrophenyl)-3,5-difluoro-3,6-dimethylpiperidine-2-thione was prepared in a similar way starting from (3R,5S,6R)-6-(2,3-difluoro-5-nitrophenyl)-3,5-difluoro-3,6-dimethylpiperidin-2-one.

INTERMEDIATE: (3S,5S,6R)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethylpiperidine-2-thione



To a solution of (3S,5S,6R)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione (4.50 g, 14.14 mmol) and NH_4Cl (3.78 g, 70.70 mmol) in ethanol (80 mL) and water (20 mL) was added Iron powder (3.95 g, 70.70 mmol) in one portion at 25°C under N_2 . The black suspension was stirred at 25 °C for 18 hours. The mixture was filtered and the filtrate was concentrated in reduced pressure. The residue was extracted with ethyl acetate (100 mL $\times$ 3). The combined organic phases were washed with saturated brine (100 mL), dried with anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate) to give (3S,5S,6R)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethylpiperidine-2-thione (2.59 g, 61% yield). ^1H NMR (CDCl_3 , 400 MHz TMS): δ 8.33 (s, 1H), 6.93-6.87 (m, 1H), 6.62-6.60 (m, 1H), 6.45-6.44 (m, 1H), 5.38-5.26 (m, 1H), 3.73 (s, 2H), 2.65-2.58 (m, 1H), 2.00-1.86 (m, 1H), 1.80 (s, 3H), 1.68 (d, $J = 20.8$ Hz, 3H). $[\alpha]_{\text{D}}^{20} = -58^\circ$ (c = 0.1 g/100mL, MeOH).

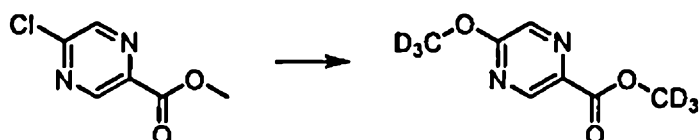
(3S,5R,6R)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione was prepared in a similar way starting from (3S,5R,6R)-3,5-difluoro-6-(2-fluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione.

(3*S*,5*S*,6*R*)-6-(5-amino-2,3-difluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione was prepared in a similar way starting from (3*S*,5*S*,6*R*)-3,5-difluoro-6-(2,3-difluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione.

(3*R*,5*S*,6*R*)-6-(5-amino-2,3-difluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione was

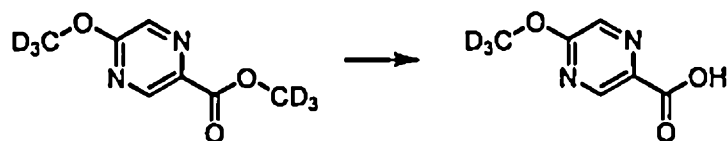
- 5 prepared in a similar way starting from (3*R*,5*S*,6*R*)-3,5-difluoro-6-(2,3-difluoro-5-nitrophenyl)-3,6-dimethylpiperidine-2-thione.

INTERMEDIATE: methyl-*d*₃-5-(methoxy-*d*₃)pyrazine-2-carboxylate



- 10 Sodium (0.094 g, 4.10 mmol) was added in small portions methanol-*d*₄ (2.94 ml) and the reaction mixture was stirred until all sodium has reacted. The solution was then added to another solution of methyl-5-chloropyrazine-2-carboxylate (0.6 g, 3.48 mmol) in methanol-*d*₄ (0.98 ml). The reaction mixture was stirred for 1.5 hours at room temperature. The reaction mixture was concentrated *in vacuo*. 2 ml of water was added. The mixture was extracted with ethyl acetate. The organic phase
- 15 was washed with brine, dried over MgSO₄ and concentrated *in vacuo* to give methyl-*d*₃-5-(methoxy-*d*₃)pyrazine-2-carboxylate.

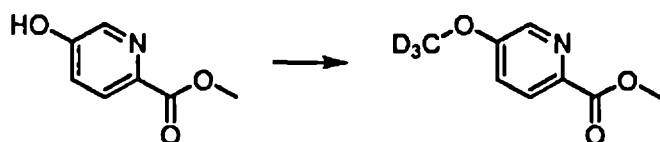
INTERMEDIATE: 5-(methoxy- *d*₃)pyrazine-2-carboxylic acid



- 20 Methyl-*d*₃-5-(methoxy-*d*₃)pyrazine-2-carboxylate (424 mg, 2.43 mmol) was dissolved in water (3 ml) and 1,4-dioxane (3 ml). Lithium hydroxide (146 mg, 6.09 mmol) was added and the reaction mixture was stirred for 1 hour. The reaction mixture was evaporated to about 2 ml and extracted with diethylether. The organic phase was extracted with 1M NaOH and the combined aqueous phases were acidified to pH 2 with 6N HCl (aq). The mixture was cooled on an icebath, and the solid compound collected to give 5-(methoxy-*d*₃)pyrazine-2-carboxylic acid.

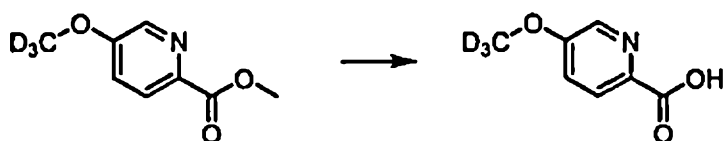
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INTERMEDIATE: methyl 5-(methoxy-*d*₃)picolinate



Methyl 5-hydroxypicolinate (2.88 g, 18.8 mmol) was dissolved in dimethylformamide (108 ml) under argon. Potassium carbonate (7.20 g, 52.1 mmol) was added and the orange suspension was stirred for 45 minutes at room temperature. Iodomethane- d_3 (1.41 ml, 22.6 mmol) was added. The reaction mixture was stirred for 2 hours. Water was added. The mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* and purified by column chromatography on silica gel (heptane: ethyl acetate) to give methyl 5-(methoxy- d_3)picolinate.

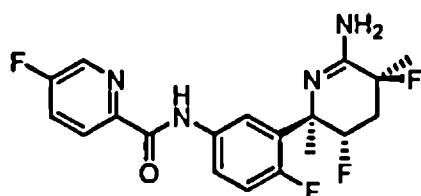
INTERMEDIATE: 5-(methoxy- d_3)picolinic acid



Methyl 5-(methoxy- d_3)picolinate (200mg, 1.175 mmol) was dissolved in water (1.5 ml) and 1,4-dioxane (3 ml). Lithium hydroxide (70.4 mg, 2.94 mmol) was added and the reaction mixture was stirred for 1 hour. The reaction mixture was evaporated to about 2 ml and extracted with diethylether. The organic phase was extracted with 1M NaOH and the combined aqueous phases were acidified to pH 2 with 6N HCl (aq). The mixture was cooled on an icebath and a precipitate was formed. The precipitate was collected to give 5-(methoxy- d_3)picolinic acid.

PREPARATION OF THE COMPOUNDS OF THE INVENTION

Example 1 *N*-(3-((2*R*,3*S*,5*S*)-6-Amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide (compound 1)



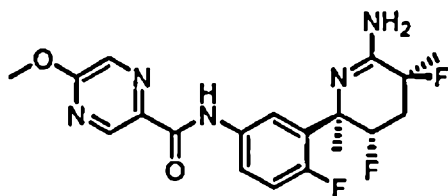
To a solution 5-fluoropicolinic acid (0.019 g, 0.14 mmol) in DMF (1 mL), HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate) (0.046 g, 0.12 mmol) was added, the solution was stirred 5 minutes. Then (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione (0.025 g, 0.092 mmol) and DIPEA

(*N,N*-diisopropylethylamine) (0.08 mL, 0.46 mmol) were added. The mixture was stirred at room temperature for 1 hour. Saturated aqueous NH_4Cl was added and the mixture was extracted with ethyl acetate. The organic phase was washed with brine, dried over MgSO_4 , filtered and concentrated under reduced pressure. The residue was dissolved in 7M ammonia in methanol (2 mL). The mixture was stirred at 65°C overnight, concentrated under reduce pressure and purified by column chromatography on silica gel (heptane: ethyl acetate). The obtained oil was dissolved in ethyl acetate and washed with saturated aqueous NaHCO_3 /water (1/1) five times. The organic phase was concentrated under reduced pressure to afford *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide (0.019 mmol, 21% yield). ^1H NMR (600 MHz, DMSO) δ 10.64 (s, 1H), 8.74 (d, J = 2.8 Hz, 1H), 8.23 (dd, J = 8.7, 4.6 Hz, 1H), 7.98 (td, J = 8.7, 2.9 Hz, 1H), 7.89 – 7.75 (m, 2H), 7.16 (dd, J = 11.8, 8.6 Hz, 1H), 6.11 (s, 2H), 5.23 – 5.03 (m, 1H), 2.37 – 2.26 (m, 1H), 2.01 – 1.84 (m, 1H), 1.62 (d, J = 23.9 Hz, 3H), 1.52 (d, J = 1.1 Hz, 3H).

LC-MS (m/z) 395.4 (MH^+); t_R = 0.47 minutes (Method B)

The following compounds were prepared in a way similar to example 1:

Example 2 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypyrazine-2-carboxamide (compound 2)

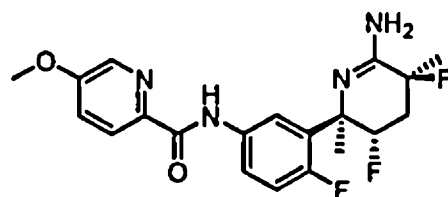


Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-methoxypyrazine-2-carboxylic acid.

^1H NMR (600 MHz, DMSO) δ 10.53 (s, 1H), 8.88 (d, J = 1.3 Hz, 1H), 8.41 (d, J = 1.3 Hz, 1H), 7.82 (m, 1H), 7.16 (dd, J = 11.9, 8.7 Hz, 1H), 6.10 (broad s, 2H), 5.18-5.07 (m, 1H), 4.03 (s, 3H), 2.37 – 2.26 (m, 1H), 1.99-1.83 (m, 1H), 1.53 (d, J = 23.9 Hz, 3H), 1.52 (s, 3H). LC-MS (m/z) 408.4 (MH^+);

t_R = 0.47 minutes (Method B)

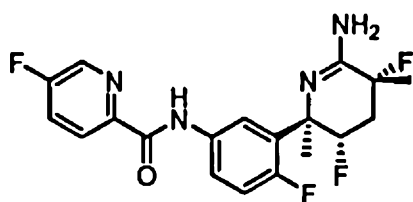
Example 3 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypicolinamide (compound 3)



Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-methoxypicolinic acid.

¹H NMR (600 MHz, DMSO) δ 10.46 (s, 1H), 8.43 – 8.34 (m, 1H), 8.16 – 8.06 (m, 1H), 7.85 (ddd, *J* = 8.7, 4.1, 2.8 Hz, 1H), 7.79 (dd, *J* = 7.3, 2.8 Hz, 1H), 7.61 (dd, *J* = 8.7, 2.8 Hz, 1H), 7.15 (dd, *J* = 11.9, 8.8 Hz, 1H), 6.46 – 5.73 (m, 2H), 5.20 – 5.06 (m, 1H), 3.93 (s, 3H), 2.37 – 2.22 (m, 1H), 2.02-1.84 (m, 1H), 1.62 (d, *J* = 23.9 Hz, 3H), 1.52 (m, 3H). LC-MS (*m/z*) 407.4 (MH⁺); *t_R* = 0.48 minutes (Method B)

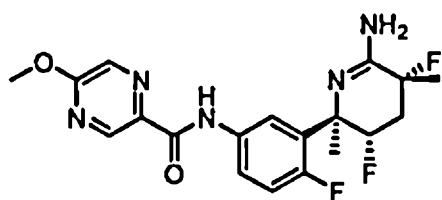
Example 4 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide (compound 4)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-fluoropicolinic acid.

¹H NMR (600 MHz, DMSO) δ 10.77 (s, 1H), 8.73 (d, *J* = 2.8 Hz, 1H), 8.22 (dd, *J* = 8.7, 4.6 Hz, 1H), 7.97 (td, *J* = 8.7, 2.8 Hz, 1H), 7.82 – 7.73 (m, 1H), 7.71 (dd, *J* = 7.2, 2.6 Hz, 1H), 7.17 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.25 (s, 2H), 5.12 (dd, *J* = 48.0, 2.5 Hz, 1H), 2.45 – 2.25 (m, 1H), 1.76 – 1.58 (m, 1H), 1.56 (s, 3H), 1.53 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 395.1 (MH⁺); *t_R* = 0.46 minutes (Method A)

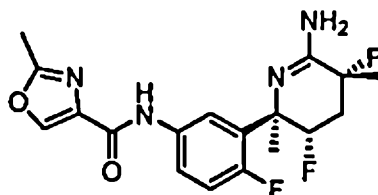
Example 5 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypyrazine-2-carboxamide (compound 5)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-methoxypyrazine-2-carboxylic acid.

¹H NMR (600 MHz, DMSO) δ 10.67 (s, 1H), 8.67 (s, 1H), 8.41 (d, *J* = 1.3 Hz, 1H), 7.76 (m, 1H), 7.71 (dd, *J* = 7.2, 2.6 Hz, 1H), 7.17 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.30 (broad s, 2H), 5.12 (dd, *J* = 47.9, 2.5 Hz, 1H), 4.02 (s, 3H), 2.38 – 2.28 (m, 1H), 1.73-1.58 (m, 1H), 1.56 (s, 3H), 1.53 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 408.2 (MH⁺); *t_R* = 0.48 minutes (Method A)

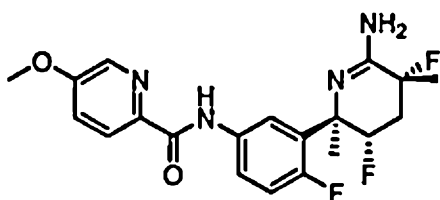
Example 6 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-2-methyloxazole-4-carboxamide (compound 6)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 2-methyloxazole-4-carboxylic acid.

¹H NMR (600 MHz, DMSO) δ 10.37 (s, 1H), 8.65 (s, 1H), 7.78 – 7.72 (m, 1H), 7.71 – 7.66 (m, 1H), 7.18 (dd, *J* = 11.6, 8.9 Hz, 1H), 5.22 – 5.09 (m, 1H), 2.50 (s, 3H), 2.43 – 2.33 (m, 1H), 1.75 – 1.62 (m, 1H), 1.60 (s, 3H), 1.56 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 381.1 (MH⁺); *t_R* = 0.43 minutes (Method A)

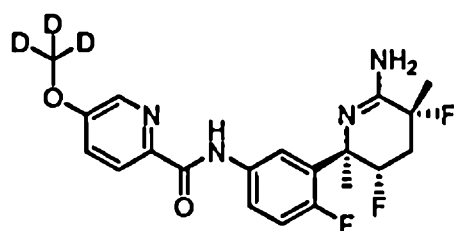
Example 7 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypicolinamide (compound 7)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-methoxypicolinic acid.

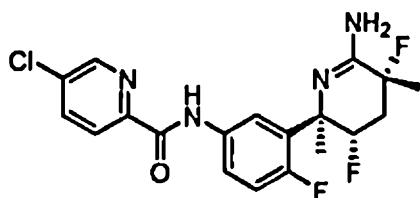
¹H NMR (600 MHz, DMSO) δ 10.61 (s, 1H), 8.41 – 8.30 (m, 1H), 8.14 – 8.07 (m, 1H), 7.80 – 7.74 (m, 1H), 7.71 (dd, *J* = 7.3, 2.8 Hz, 1H), 7.60 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.15 (dd, *J* = 11.8, 8.8 Hz, 1H), 6.27 (s, 2H), 5.19 – 5.05 (m, 1H), 3.93 (s, 3H), 2.45 – 2.27 (m, 1H), 1.76 – 1.59 (m, 1H), 1.56 (s, 3H), 1.53 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 407.1 (MH⁺); *t_R* = 0.5 minutes (Method A)

Example 8 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide (Compound 8)



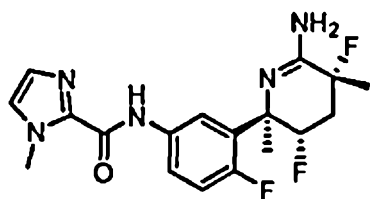
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(methoxy-*d*₃)picolinic acid. ¹H NMR (600 MHz, DMSO) δ 10.72 (s, 1H), 8.39 (d, *J* = 2.8 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 1H), 7.90 (s, 1H), 7.80 – 7.75 (m, 1H), 7.60 (dd, *J* = 8.7, 2.9 Hz, 1H), 7.21 (dd, *J* = 11.6, 9.0 Hz, 1H), 5.21 (d, *J* = 47.5 Hz, 1H), 2.46 – 2.38 (m, 1H), 1.82 – 1.66 (m, 1H), 1.64 (s, 3H), 1.61 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 410 (MH⁺); *t_R* = 0.49 (Method A)

Example 9 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-chloropicolinamide (Compound 9)



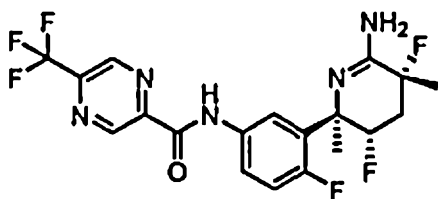
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 4-chlorobenzoic acid. ¹H NMR (600 MHz, DMSO) δ 10.83 (s, 1H), 8.78 (dd, *J* = 2.4, 0.7 Hz, 1H), 8.18 (dd, *J* = 8.4, 2.4 Hz, 1H), 8.15 (dd, *J* = 8.4, 0.7 Hz, 1H), 7.78 (ddd, *J* = 8.7, 4.1, 2.8 Hz, 1H), 7.71 (dd, *J* = 7.3, 2.7 Hz, 1H), 7.17 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.21 (s, 2H), 5.11 (ddd, *J* = 48.2, 4.7, 2.1 Hz, 1H), 2.34 (dddd, *J* = 20.7, 15.6, 8.9, 4.8 Hz, 1H), 1.72 – 1.57 (m, 1H), 1.56 (s, 3H), 1.53 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 411.1 (MH⁺); *t_R* = 0.53 (Method A)

Example 10 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-1-methyl-1*H*-imidazole-2-carboxamide (Compound 10)



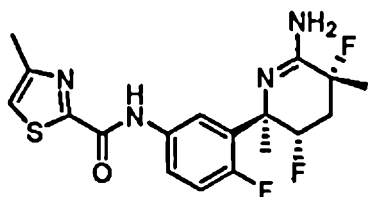
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 1-methyl-1*H*-imidazole-2-carboxylic acid. ¹H NMR (600 MHz, DMSO) δ 10.53 (s, 1H), 7.72 (ddd, *J* = 8.7, 4.2, 2.8 Hz, 1H), 7.61 (dd, *J* = 7.3, 2.7 Hz, 1H), 7.43 (d, *J* = 0.7 Hz, 1H), 7.12 (dd, *J* = 11.8, 8.8 Hz, 1H), 7.07 (d, *J* = 1.0 Hz, 1H), 6.16 (s, 2H), 5.15 – 5.04 (m, 1H), 3.97 (s, 3H), 2.32 (dddd, *J* = 20.8, 15.5, 8.9, 4.9 Hz, 1H), 1.71 – 1.56 (m, 1H), 1.54 (s, 3H), 1.51 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 380.1 (MH⁺); *t_R* = 0.42 (Method A)

Example 11 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(trifluoromethyl)pyrazine-2-carboxamide (Compound 11)



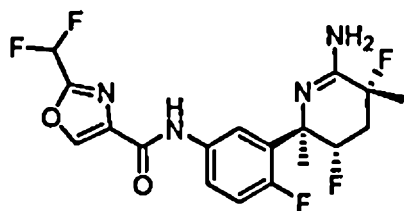
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(trifluoromethyl)pyrazine-2-carboxylic acid. ¹H NMR (600 MHz, DMSO) δ 11.13 (s, 1H), 9.44 (d, *J* = 1.0 Hz, 1H), 9.30 (d, *J* = 1.0 Hz, 1H), 7.81 (ddd, *J* = 8.7, 4.1, 2.8 Hz, 1H), 7.74 (dd, *J* = 7.2, 2.7 Hz, 1H), 7.20 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.23 (s, 2H), 5.18 – 5.06 (m, 1H), 2.34 (dddd, *J* = 20.7, 15.6, 9.0, 4.8 Hz, 1H), 1.71 – 1.58 (m, 1H), 1.56 (s, 3H), 1.53 (d, *J* = 22.0 Hz, 3H). LC-MS (*m/z*) 446.1 (MH⁺); *t_R* = 0.53 (Method A)

Example 12 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-4-methylthiazole-2-carboxamide (Compound 12)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 4-methylthiazole-2-carboxylic acid. ¹H NMR (600 MHz, DMSO) δ 10.91 (s, 1H), 7.78 – 7.72 (m, 1H), 7.71 – 7.67 (m, 2H), 7.16 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.21 (s, 2H), 5.16 – 5.05 (m, 1H), 2.50 (t, *J* = 2.0 Hz, 3H), 2.38 – 2.27 (m, 1H), 1.70 – 1.56 (m, 1H), 1.55 (s, 3H), 1.52 (d, *J* = 22.0 Hz, 3H). LC-MS (*m/z*) 397.1 (MH⁺); *t_R* = 0.5 (Method A)

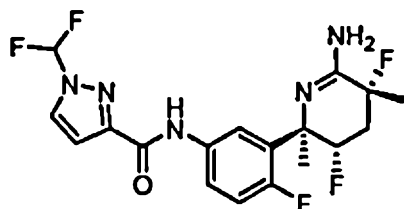
Example 13 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-2-(difluoromethyl)oxazole-4-carboxamide (Compound 13)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 1-(difluoromethyl)-1*H*-pyrazole-3-carboxylic acid. ¹H NMR (600 MHz, DMSO) δ 10.54 (s, 1H), 9.04 (s, 1H), 7.67 (ddd, *J* = 8.7, 4.2, 2.8 Hz, 1H), 7.62 (dd, *J* = 7.2, 2.7 Hz, 1H), 7.33 (t, *J* = 51.9 Hz, 1H), 7.15 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.17 (s, 2H), 5.10 (ddd, *J* = 48.2, 4.5, 2.1 Hz, 1H), 2.38 – 2.27

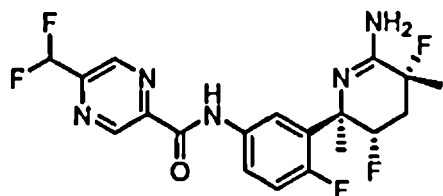
(m, 1H), 1.69 – 1.56 (m, 1H), 1.55 (s, 3H), 1.51 (d, $J = 22.0$ Hz, 3H). LC-MS (m/z) 417.1 (MH^+); $t_R = 0.47$ (Method A)

Example 14 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-1-(difluoromethyl)-1*H*-pyrazole-3-carboxamide (Compound 14)



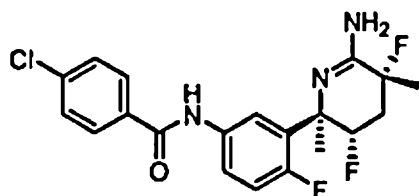
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(methoxy- d_3)pyrazine-2-carboxylic acid. ^1H NMR (600 MHz, DMSO) δ 10.52 (s, 1H), 8.41 (d, $J = 2.7$ Hz, 1H), 7.92 (t, $J = 58.7$ Hz, 1H), 7.69 (ddd, $J = 8.7, 4.1, 2.8$ Hz, 1H), 7.63 (dd, $J = 7.3, 2.7$ Hz, 1H), 7.15 (dd, $J = 11.8, 8.8$ Hz, 1H), 7.01 (d, $J = 2.7$ Hz, 1H), 6.19 (s, 2H), 5.11 (ddd, $J = 48.2, 4.5, 2.1$ Hz, 1H), 2.39 – 2.26 (m, 1H), 1.73 – 1.56 (m, 1H), 1.55 (s, 3H), 1.51 (d, $J = 22.0$ Hz, 3H). LC-MS (m/z) 416.1 (MH^+); $t_R = 0.46$ (Method A)

Example 15 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(difluoromethyl)pyrazine-2-carboxamide (Compound 15)



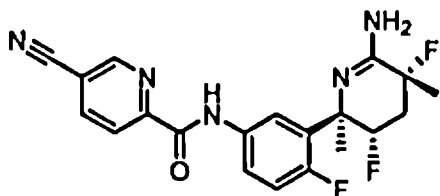
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-cyano-3-methylpicolinic acid. ^1H NMR (600 MHz, DMSO) δ 11.06 (s, 1H), 9.38 (d, $J = 1.4$ Hz, 1H), 9.09 (d, $J = 0.9$ Hz, 1H), 7.81 (ddd, $J = 8.7, 4.1, 2.8$ Hz, 1H), 7.74 (dd, $J = 7.2, 2.7$ Hz, 1H), 7.26 (t, $J = 54.0$ Hz, 1H), 7.20 (dd, $J = 11.7, 8.8$ Hz, 1H), 6.22 (s, 2H), 5.12 (ddd, $J = 48.2, 4.6, 2.1$ Hz, 1H), 2.41 – 2.28 (m, 1H), 1.72 – 1.58 (m, 1H), 1.56 (s, 3H), 1.53 (d, $J = 22.0$ Hz, 3H). LC-MS (m/z) 428.1 (MH^+); $t_R = 0.48$ (Method A)

Example 16 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-4-chlorobenzamide (Compound 16)



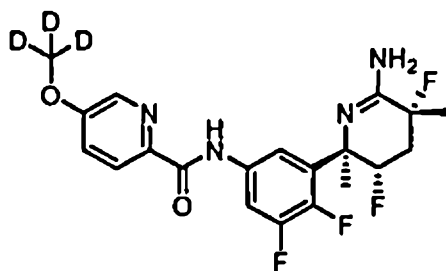
Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(methoxy-*d*₃)picolinic acid. ¹H NMR (600 MHz, DMSO) δ 10.45 (s, 1H), 7.96 – 7.92 (m, 2H), 7.70 (ddd, *J* = 8.7, 4.1, 2.8 Hz, 1H), 7.62 – 7.58 (m, 2H), 7.54 (dt, *J* = 7.2, 3.6 Hz, 1H), 7.16 (dd, *J* = 11.8, 8.8 Hz, 1H), 6.17 (s, 2H), 5.11 (ddd, *J* = 48.2, 4.6, 2.1 Hz, 1H), 2.38 – 2.28 (m, 1H), 1.71 – 1.57 (m, 1H), 1.56 (s, 3H), 1.50 (d, *J* = 22.0 Hz, 3H). LC-MS (*m/z*) 410.1 (MH⁺); *t_R* = 0.54 (Method A)

Example 17 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-cyanopicolinamide (Compound 17)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-cyanopicolinic acid. ¹H NMR (600 MHz, DMSO) δ 10.99 (s, 1H), 9.19 (dd, *J* = 2.1, 0.9 Hz, 1H), 8.57 (dd, *J* = 8.2, 2.1 Hz, 1H), 8.28 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.79 (ddd, *J* = 8.7, 4.1, 2.9 Hz, 1H), 7.72 (dd, *J* = 7.2, 2.7 Hz, 1H), 7.18 (dd, *J* = 11.7, 8.8 Hz, 1H), 6.20 (s, 2H), 5.17 – 5.05 (m, 1H), 2.41 – 2.26 (m, 1H), 1.69 – 1.56 (m, 1H), 1.55 (s, 3H), 1.52 (d, *J* = 22.1 Hz, 3H). LC-MS (*m/z*) 402.1 (MH⁺); *t_R* = 0.47 (Method A)

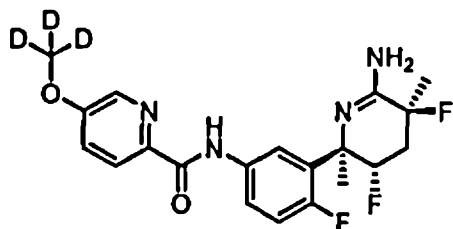
Example 18 *N*-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4,5-difluorophenyl)-5-(methoxy-*d*₃)picolinamide (Compound 18)



Prepared from (3*R*,5*S*,6*R*)-6-(5-amino-2,3-difluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 2-(difluoromethyl)oxazole-4-carboxylic acid. ¹H NMR (600 MHz, DMSO) δ 10.89 (s, 1H), 8.38 (d, *J* = 2.8 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 1H), 8.08 – 8.01 (m, 1H), 7.63 – 7.58 (m, 2H), 5.22 –

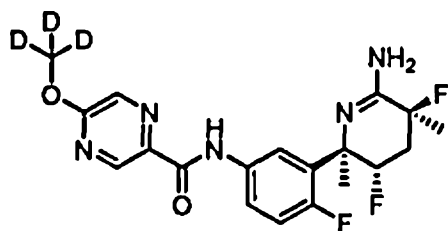
5.09 (m, 1H), 2.47 – 2.35 (m, 1H), 1.85 – 1.69 (m, 1H), 1.62 (s, 3H), 1.58 (d, $J = 22.1$ Hz, 3H). LC-MS (m/z) 428.1 (MH^+); $t_R = 0.54$ (Method A)

Example 19 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy- d_3)picolinamide (Compound 19)



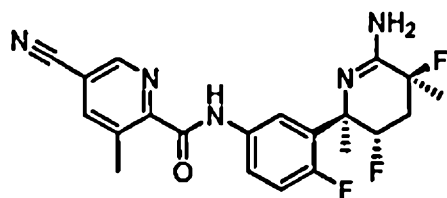
Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(methoxy- d_3)picolinic acid. ^1H NMR (600 MHz, DMSO) δ 10.46 (s, 1H), 8.39 (dd, $J = 2.9, 0.5$ Hz, 1H), 8.12 (dd, $J = 8.7, 0.5$ Hz, 1H), 7.86 – 7.80 (m, 1H), 7.78 (dd, $J = 7.3, 2.7$ Hz, 1H), 7.61 (dd, $J = 8.7, 2.9$ Hz, 1H), 7.15 (dd, $J = 11.8, 8.8$ Hz, 1H), 6.07 (s, 2H), 5.12 (dd, $J = 48.5, 6.1$ Hz, 1H), 2.35 – 2.27 (m, 1H), 1.98 – 1.84 (m, 1H), 1.61 (d, $J = 23.9$ Hz, 3H), 1.51 (s, 3H). LC-MS (m/z) 410 (MH^+); $t_R = 0.49$ (Method A)

Example 20 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy- d_3)pyrazine-2-carboxamide (Compound 20)



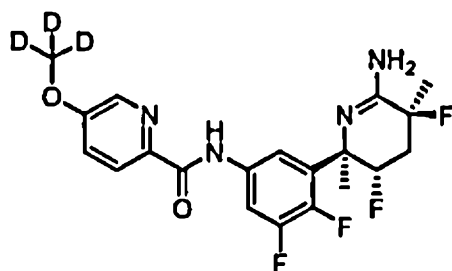
Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(methoxy- d_3)picolinic acid. ^1H NMR (600 MHz, DMSO) δ 10.52 (s, 1H), 8.88 (s, 1H), 8.40 (s, 1H), 7.86 – 7.78 (m, 2H), 7.16 (dd, $J = 11.6, 8.9$ Hz, 1H), 6.13 (s, 2H), 5.12 (dd, $J = 48.6, 5.3$ Hz, 1H), 2.35 – 2.28 (m, 1H), 2.01 – 1.84 (m, 1H), 1.62 (d, $J = 23.9$ Hz, 3H), 1.52 (s, 3H). LC-MS (m/z) 411.2 (MH^+); $t_R = 0.48$ (Method A)

Example 21 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-cyano-3-methylpicolinamide (Compound 21)



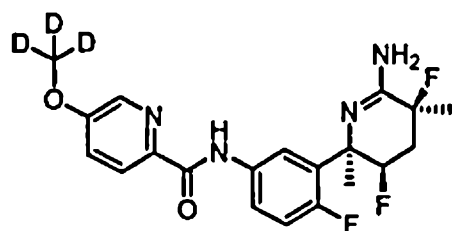
Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-chloropicolinic acid. ¹H NMR (600 MHz, DMSO) δ 10.77 (s, 1H), 8.98 (s, 1H), 8.39 (s, 1H), 7.89 – 7.82 (m, 1H), 7.64 (d, *J* = 5.1 Hz, 1H), 7.17 (dd, *J* = 11.5, 9.1 Hz, 1H), 6.20 (s, 2H), 5.18 – 5.06 (m, 1H), 2.50 (s, 3H), 2.37 – 2.28 (m, 1H), 1.97 – 1.83 (m, 1H), 1.62 (d, *J* = 23.9 Hz, 3H), 1.52 (s, 3H). LC-MS (*m/z*) 416 (MH⁺); *t_R* = 0.51 (Method B)

Example 22 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4,5-difluorophenyl)-5-(methoxy-*d*₃)picolinamide (Compound 22)



Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2,3-difluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(difluoromethyl)pyrazine-2-carboxylic acid. ¹H NMR (600 MHz, DMSO) δ 10.68 (s, 1H), 8.39 (d, *J* = 2.8 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 1H), 8.04 – 7.98 (m, 1H), 7.68 (d, *J* = 5.1 Hz, 1H), 7.62 (dd, *J* = 8.7, 2.9 Hz, 1H), 6.36 (s, 2H), 5.10 (dd, *J* = 48.6, 6.2 Hz, 1H), 2.38 – 2.29 (m, 1H), 2.08 – 1.91 (m, 1H), 1.63 (d, *J* = 23.7 Hz, 3H), 1.53 (s, 3H). LC-MS (*m/z*) 428.2 (MH⁺); *t_R* = 0.54 (Method A)

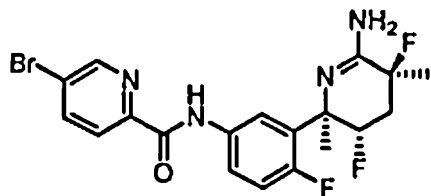
Example 23 *N*-(3-((2*R*,3*R*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide (Compound 23)



Prepared from (3*S*,5*R*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-(methoxy-*d*₃)picolinic acid. ¹H NMR (600 MHz, DMSO) δ 10.27 (s, 1H), 8.39 (dd, *J* = 2.9, 0.4 Hz, 1H), 8.14 – 8.11 (m, 1H), 8.08 (dd, *J* = 7.3, 2.8 Hz, 1H), 7.87 – 7.82 (m, 1H), 7.62 (dd, *J* = 8.7,

2.9 Hz, 1H), 7.13 (dd, $J = 12.0, 8.8$ Hz, 1H), 6.00 (s, 2H), 5.23 – 5.13 (m, 1H), 2.46 – 2.33 (m, 2H), 1.62 (d, $J = 22.0$ Hz, 3H), 1.36 (s, 3H). LC-MS (m/z) 410.1 (MH^+); $t_R = 0.52$ (Method B)

Example 24 *N*-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-bromopicolinamide (Compound 24)



Prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluoro-phenyl)-3,5-difluoro-3,6-dimethyl-piperidine-2-thione and 5-bromopicolinic acid.

^1H NMR (600 MHz, DMSO) δ 10.70 (s, 1H), 8.86 (dd, $J = 2.3, 0.7$ Hz, 1H), 8.32 (dd, $J = 8.4, 2.3$ Hz, 1H), 8.08 (dd, $J = 8.4, 0.6$ Hz, 1H), 7.92 – 7.76 (m, 2H), 7.17 (dd, $J = 11.8, 8.7$ Hz, 1H), 6.17 (s, 2H), 5.19 – 5.07 (m, 1H), 2.39 – 2.25 (m, 1H), 2.03 – 1.83 (m, 1H), 1.62 (d, $J = 23.9$ Hz, 3H), 1.53 (s, 3H). LC-MS (m/z) 455.1 (MH^+); $t_R = 0.54$ (Method B)

Stereochemistry

Crystals were obtained by recrystallization of 5-bromo-*N*-(3-((2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxopiperidin-2-yl)-4-fluorophenyl)picolinamide from a mixture of heptane and ethyl acetate. The structure of 5-bromo-*N*-(3-((2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxopiperidin-2-yl)-4-fluorophenyl)picolinamide was elucidated by X-ray crystallography of said crystals. The structure shows the absolute and relative configuration of 5-bromo-*N*-(3-((2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxopiperidin-2-yl)-4-fluorophenyl)picolinamide. 5-Bromo-*N*-(3-((2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxopiperidin-2-yl)-4-fluorophenyl)picolinamide was prepared as described in example 1 starting from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethylpiperidine-2-thione and 5-bromopicolinic acid.

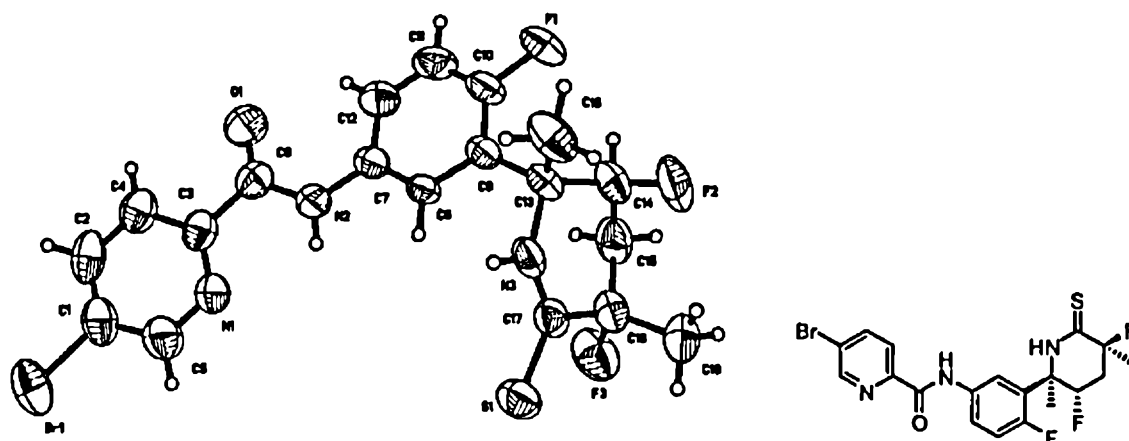
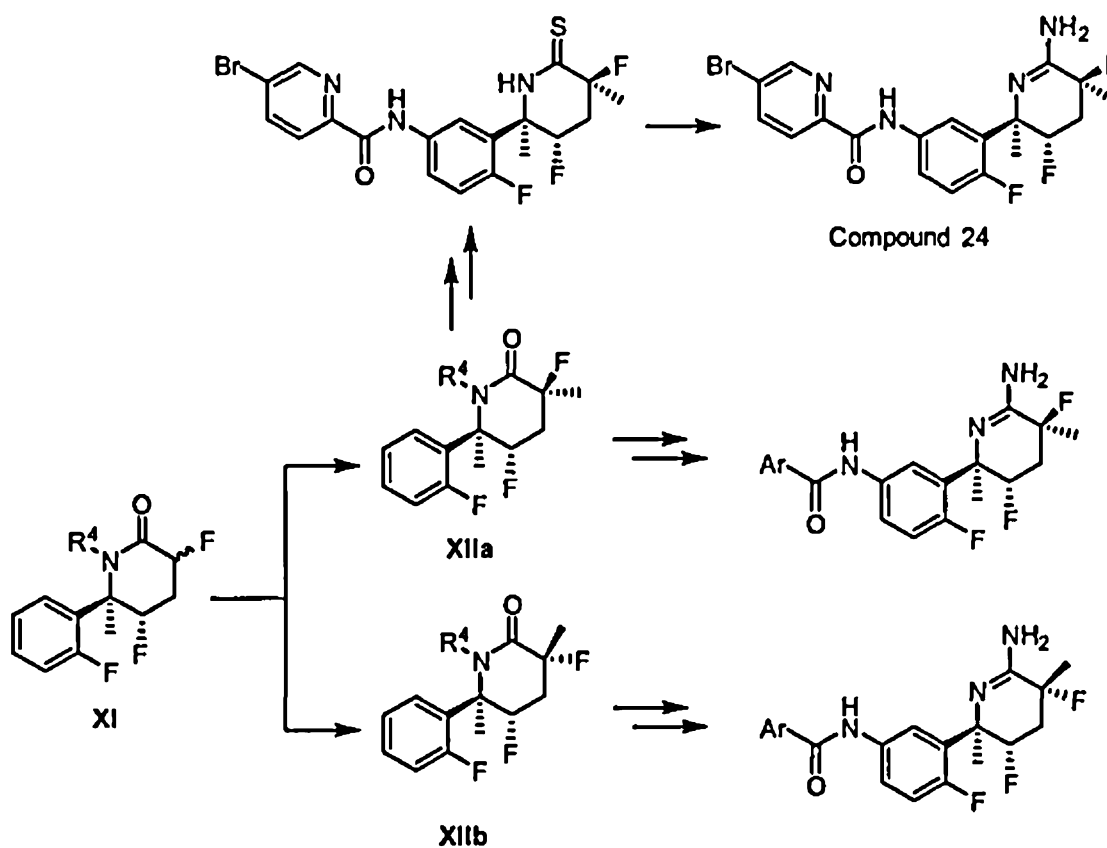


Figure 1: X-ray structure of 5-bromo-*N*-(3-((2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxopiperidin-2-yl)-4-fluorophenyl)picolinamide

The absolute configurations of the exemplified compounds of the present invention can thus be rationalized. 5-Bromo-*N*-(3-((2*R*,3*S*,5*S*)-3,5-difluoro-2,5-dimethyl-6-thioxopiperidin-2-yl)-4-fluorophenyl)picolinamide was prepared from (3*S*,5*S*,6*R*)-6-(5-amino-2-fluorophenyl)-3,5-difluoro-3,6-dimethylpiperidine-2-thione which was prepared from XIa ($R^4 = \textit{tert}$ -butoxy carbonyl) as shown in Scheme 7, thus confirming the absolute configuration of XIa. As a consequence thereof, the absolute configuration of XIb ($R^4 = \textit{tert}$ -butoxy carbonyl) is also confirmed.



Scheme 8. Rationale for assignment of absolute and relative stereochemistry of the exemplified compounds. R^4 = *tert*-butoxy carbonyl

Pharmacological Testing

5

BACE1 binding assay

The binding assay was performed as SPA-based assay using a biotinylated form of human BACE1 recombinantly expressed and subsequently purified from Freestyle HEK293 cells. The binding assay was run in a 50 mM sodium acetate buffer, pH 4.5 containing 50 mM NaCl and 0.03%

10 Tween-20 in white clear bottom 384 plates (Corning #3653). 10 nM (final concentration) radioligand ($[^3\text{H}]\text{-N-}((1\text{S},2\text{R})\text{-1-benzyl-3-cyclopropylamino-2-hydroxy-propyl})\text{-5-(methanesulfonyl-methyl-amino)-N-}((\text{R})\text{-1-phenyl-ethyl})\text{-isophthalamide}$) (TRQ11569 purchased from GE Healthcare) was mixed with test compound at a given concentration, 6 nM (final concentration) human BACE1 and 25 μg Streptavidin coated PVT core SPA beads (RPNQ0007, GE Healthcare Life Sciences) in a
15 total volume of 40 μl . Several concentrations of each test compound were tested in the assay for IC_{50} determination. The plates were incubated for one hour at room temperature and counted in a Wallac Trilux counter. Total and non-specific binding were determined using buffer and 1 μM (final concentration) of the high affinity BACE1 reference inhibitor (S)-6-[3-chloro-5-(5-prop-1-ynyl-pyridin-3-yl)-thiophen-2-yl]-2-imino-3,6-dimethyl-tetrahydro-pyrimidin-4-one, respectively. For each
20 test compound, a IC_{50} value (the concentration mediating 50% inhibition of the specific binding of the radioligand) was determined from concentration-response curve and used to calculate the K_i from the equation $K_i = \text{IC}_{50}/(1 + L/K_d)$, where L and K_d are the final concentration of the radioligand used in the assay and the dissociation constant of the radioligand, respectively. The K_d of the radioligand was determined from saturation binding experiments.

25

Table 1: binding affinity of selected compounds

Compound No	BACE1 K_i (nM)
1	30
2	26
3	23
4	98
5	51
6	58
7	39

8	58
9	36
10	820
11	190
12	99
13	62
14	45
15	100
16	220
17	39
18	6.8
19	20
20	21
21	4.1
22	3.6
23	81
24	4.3

BACE1 efficacy assay

The efficacy assay was performed as a FRET-based assay using a commercially available BACE1 kit (Life Technologies, P2985). 2 µl test compound at 10 µM (final concentration) and 15 µl BACE1 enzyme from the kit (final concentration 3 nM) were preincubated for 15 minutes at room temperature before addition of 15 µl of substrate from the kit (250 nM final concentration) and incubated for additional 90 minutes at room temperature. The assay plate was subsequently read in a Pherastar (Ex540/Em590). The enzyme activity observed in presence of test compound were normalized to the enzyme activity observed in presence of buffer and 10 µM (final concentration) of the high affinity BACE1 reference inhibitor (S)-6-[3-Chloro-5-(5-prop-1-ynyl-pyridin-3-yl)-thiophen-2-yl]-2-imino-3,6-dimethyl-tetrahydropyrimidin-4-one, respectively. The efficacy of the test compounds was evaluated at 10 µM (final concentration) and defined as the percent inhibition of the enzyme activity using the equation %inhibition = 100% - normalized enzyme activity in percent.

Table 2: BACE1 activity of selected compounds

Compound No	BACE1 inhibition at 10µM (%)
-------------	------------------------------

1	103
2	99
3	102
4	101
6	101
7	102
8	100
9	109
10	115
11	104
12	118
13	108
14	112
15	108
16	103
18	101
19	106
20	106
21	117
22	101
23	107
24	103

Assessment of A β levels in rat brain and plasma following BACE1 inhibition.

Animals.

All rat care and experimental procedures were approved by Lundbeck Veterinary Staff, according to Danish legislature. The rats were maintained in a barrier facility with a 12/12-h light/dark cycle and ad libitum food and water access.

Treatment of naïve Rats.

Young adult Male Sprague Dawley rats of approximately 250g weight were purchased from Charles River and received 0-30 mg/kg of vehicle (10% HP betaCD + 1M MeSO₄, pH 2.5) or test compounds (dissolved in vehicle) only by oral gavage (p.o). The compounds are dosed at a volume of 5ml/kg. Cohorts of 5-10 animals were established for each treatment condition.

The animals undergoing treatment were closely monitored by veterinary staff for any signs of toxicity. Monitoring parameters included body weight, physical appearance, changes in coat appearance, occurrence of unprovoked behavior, and blunted or exaggerated responses to external stimuli.

5

Tissue collection.

At T =180 minutes after initial dosing the animals were stunned and decapitated with a guillotine. Trunk-blood was sampled in EDTA coated tubes after decapitation of the animal. The blood was centrifuged at 2200G at 4°C for 15 minutes and the plasma was collected and frozen at -80°C. The blood was aliquoted for A β ELISA and DMPK analysis. Immediately following sacrifice, the brain was extracted and split into 2 halves. The right hemibrains were snap frozen on dry ice and stored at -80°C. The left half was dissected; with the front forebrain taken for A β ELISA and the remainder used for DMPK analysis. These samples were also snap frozen on dry ice and stored at -80°C until use for analysis.

15

Tissue processing.

The cortex samples were thawed slightly on wet ice before they were homogenized with a small volume dispersing instrument (T10 basic ULTRA-TURRAX®) which was set at speed 5 for approximately 5-7 sec. The tissue was processed in a 10 times volume of the weight, for example 100mg of tissue was homogenized in 1000 μ L of Homogenization buffer. Homogenization buffer: 50ml Milli Q water + 50nM NaCl + 0.2% Diethylamin (DEA) + 1 tablet of Complete Protease Inhibitor cocktail + 1nM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride Irreversible serine protease inhibitor (AEBSF).

20

After homogenization 450 μ L aliquots of the samples are collected into a 1.5ml Eppendorf tube and placed on wet ice, 0.5% NP-40 (50ul) was added to all samples and then they were incubated on ice for 30 min. After which all samples were sonicated using an Ultrasonic homogenizer with 20 kHz homogeneous sound (SONOPLUS HD2070, Bandelin Electronic) 10 pulse set at 12-13 % power to extract all the A β species. The samples were then centrifuged (Ole Dich 157 MPRF Micro centrifuge) at 20000G for 20 minutes at 4°C. After centrifugation 285 μ L of the supernatant was pipetted into 600 μ L microtubes tubes and neutralized with 15 μ L of 1M Tris-HCL buffer.

30

ELISA protocol.

WAKO 294-62501 Human/Rat Abeta amyloid (40) kit was used for all ELISA analyses. 30 μ L plasma samples or 30 μ L of the cortex supernatants generated as described above were placed in

35

600 μ L microtubes tubes on wet ice. To this 30 μ L of 8M Urea (AppliChem A1049, 9025) are added to generate a 2-fold dilution. Both plasma and cortex supernatants are incubated on ice for 30 min. Standard rows were prepared from the standard peptide stock provided in the kit and standard diluent containing 1.6M Urea (200 μ L 8M Urea + 800 μ L of standard diluent) and 0.8M Urea (400 μ L
 5 8M Urea + 3600 μ L Standard diluent). A serial 2-fold dilution of A β 40 from 100 pmol/ml to 0 pmol/L was prepared for the assay.

After incubation with urea, all samples were further diluted by addition of 5 times standard diluent from the Kit. This was done by adding 240 μ L Standard Diluent to 60 μ L sample/urea mixture,
 10 which was then mixed well. 100 μ L of each diluted sample was pipetted into designated wells of the ELISA plate in duplicates. The plate was then covered and incubated overnight at 4°C. The following day, the ELISA kit was brought to room temperature before use. The incubated plate was washed 5 times with the 20x washing solution diluted in Milli Q water. 100 μ L HRP-conjugate was applied to each well, and the plate was covered and incubates at 4°C for 1 hr. The wash was
 15 repeated again for 5 times. 100 μ L 3,3',5,5'-Tetramethylbenzidine (TMB) solution was applied to each well and the plate was covered and incubated in the dark at room temperature for 30 minutes. 100 μ L STOP-solution was next applied to each well, and the plate was read at 450 nm wavelength in a spectrophotometer (Labsystems Multiscan Ascent) within 30 min of adding the STOP-solution to the wells.

20 Concentration of A β in the samples was determined based on a standard curve generated from standards containing known concentrations of synthetic A β 40. Those skilled in the art will appreciate that diethylamine (DEA) and urea extractions will release soluble A β , and insoluble A β respectively. Since the ELISA kit is validated and widely used, it is accepted that as long as the
 25 treatment conditions and assay conditions are the same for each compound tested, then the assay should yield consistent robust data for the compounds tested and produce minimal discrepancies.

Data analysis

To determine the concentration of A β 40 in the samples, the interpolated values of the samples
 30 loaded on plates are multiplied by 20 to account for the dilutions made when the volumes of DEA, urea and neutralization solution were added up. Values are calculated as percentage change in A β 40 compared to vehicle treated animals.

Compound 4 was administered at doses of 10 mg/kg p.o. and brain and plasma samples were collected at 3 hours post dose and the following exposures were measured as described above.

Bioanalysis of brain and plasma samples

TC was determined in plasma and brain homogenate using UltraPerformance LC® (UPLC®) chromatography followed by tandem-MS (MS/MS) detection.

Apparatus:

- 5 Tecan Genesis RSP 200; Biomek NXP, Beckman Coulter; Sigma 4K15 centrifuge; Acquity UPLC, Waters; Sciex API4000 TQ, Applied Biosystems; MS software: Analyst version 1.4.1

Chemicals

Acetonitrile, HPLC-grade, Fluka, No. 34967N; Methanol, HPLC-grade, Sigma-Aldrich, Lot 9003S; Formic acid, HPLC-grade, Riedel-de Haën, Lot 51660; Purified water, Millipore Synergy UV

10 **Sample preparation**

- Brain homogenate was prepared by homogenizing the brain 1:4 (v/v) with water:2-propanol:DMSO (50:30:20 v/v/v) followed by centrifugation and collection of the supernatant. Calibration standards and QC samples were prepared using a Hamilton robot. 150 µL of ISTD in acetonitrile (1 ng/mL ISTD) was added to 25 µL of calibration standards, QC samples and test samples (plasma and brain
- 15 homogenate) using a Biomek robot. After centrifugation (6200 g, 4 °C, 20 min) 100 µL supernatant from each sample was transferred to a new plate and mixed with 100 µL water with 0.1 % formic acid using a Biomek robot (method file InVivo transfer). After a quick centrifugation (6200 g, 4 °C, 5 min) the samples were placed in the auto-sampler.

UPLC-MS/MS analysis

- 20 MS/MS detection was done with an Applied Biosystems Sciex API 4000 instrument in positive-ion electrospray ionisation mode. TC and ISTD were detected at a parent > daughter mass to charge ratio (m/z). Nitrogen was used for the nebulizer and collision gases. The peak area correlated linearly with the plasma and brain concentration of the analytes in the range of 1.00 – 1000 ng/mL plasma and 5.00 – 5000 ng/g brain (corrected for dilution). If the plasma/brain sample drug concentration was
- 25 above 1000 ng/mL or 5000 ng/g, the sample was diluted appropriately in blank plasma/blank brain homogenate before analysis.

Chromatographic system

Analytical columns:

Waters Acquity UPLC HSS C18 SB (pH 2-8) 1.8 µm, 2.1x30mm.

- 30 Mobile phase A: 0.1 % aq. formic acid or 0.1 % aq. ammonium hydroxide

Mobile phase B: Acetonitrile with 0.1 % aq. formic acid or 0.1 % aq. ammonium hydroxide.

Weak wash: Methanol

Strong wash: Acetonitrile/Isopropanol/formic acid (50/50/2 v/v/v)

Flow: 0.6 mL/min

- 35 Run time: 3 min.

To waste: 0-0.5 min

Temperature: 40°C

Gradient:

	Time (min)	% A	% B
5	0	98	2
	0.01	98	2
	1.5	5	95
	2	5	95
	2.2	98	2
10	3	98	2

Table 3: Results for compound 3

	Dose (mg/kg)	Exp (ng/g)	Brain/Plasma ratio	A β 40 reduction (%)
Brain Rat	10	671	1.4	35
Plasma Rat		489		24
Brain Rat	30	1510	0.83	61
Plasma Rat		1826		53

Table 4: Results for compound 19

	Dose (mg/kg)	Exp (ng/g)	Brain/Plasma ratio	A β 40 reduction (%)
Brain Rat	10	1025	2.2	56
Plasma Rat		473		49
Brain Rat	30	2772	2.3	66
Plasma Rat		1198		53

15

Table 5: Results for compound 20

	Dose (mg/kg)	Exp (ng/g)	Brain/Plasma ratio	A β 40 reduction (%)
Brain Rat	3	610	2.2	52
Plasma Rat		272		64

As shown in tables 3, 4 and 5 compounds of the present invention are able to penetrate the blood brain barrier and show efficacy in the CNS.

5

MDCK-MDR1 assay

The permeability of the test compounds was assessed in MDCK-MDR1 cells that were cultured to confluency (4-6 days) in a 96 transwell plate. Test compounds were diluted with the transport buffer (HBSS + 1% BSA) to a concentration of 0.5 μ M and applied to the apical or basolateral side of the cell monolayer. Permeation of the test compounds from A to B direction or B to A direction was determined in triplicate over a 60-minute incubation time at 37°C and 5% CO₂ with a relative humidity of 95%. Test compounds were quantified by LC-MS/MS analysis based on the peaks area ratios of analyte/IS in both the receiver and donor wells of the transwell plate.

10

The apparent permeability coefficient Papp (cm/s) was calculated using the equation:

$$15 \quad P_{app} = (dC_r/dt) \times V_r / (A \times C_0)$$

Where dCr/dt is the cumulative concentration of compound in the receiver chamber as a function of time (μ M/s); V_r is the solution volume in the receiver chamber (0.05 mL on the apical side; 0.25 mL on the basolateral side); A is the surface area for the transport, i.e. 0.0804 cm² for the area of the monolayer; C₀ is the initial concentration in the donor chamber (μ M).

20 Compounds are classified Pgp substrates when efflux ratio (Papp BA / Papp AB) is ≥ 2 .

Table 5: BACE1 activity of selected compounds

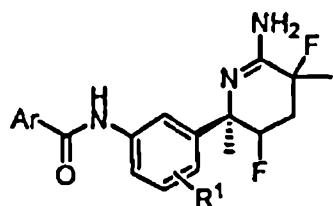
Compound	MDCK- MDR1 efflux ratio
1	1.09

2	0.79
3	0.96
4	1.17
5	1.53
6	1.1
7	5.67
8	0.95
9	1.31
13	11.7
14	15.76
15	2.07
16	1.6
17	0.45
18	1.24
19	0.79
20	0.85
21	1.15
22	0.52
23	0.95

As shown in tables 5, the majority of the exemplified compounds of the present invention have MDCK- MDR1 efflux ratios below 2 and are thus likely to be able to cross the blood brain barrier (E Kerns, L Di, Drug-like Properties: Concepts, Structure Design and Methods (2008) Elsevier).

CLAIMS

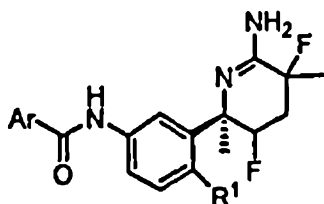
1. A compound of formula I



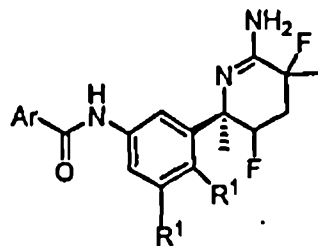
Formula I

wherein Ar is selected from the group consisting of phenyl, pyridyl, pyrimidyl, pyrazinyl, imidazolyl, pyrazolyl, thiazolyl, oxazolyl, isoxazolyl, and where the Ar is optionally substituted with one or more substituent selected from halogen, CN, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ fluoroalkyl or C₁-C₆ alkoxy; and R¹ is one or more hydrogen, halogen, C₁-C₃ fluoroalkyl or C₁-C₃ alkyl; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, wherein the compound is of formula Ia or Ib



Formula Ia



Formula Ib

or a pharmaceutically acceptable salt thereof.

3. The compound according to claim 1 or 2, wherein R¹ is F or H.
4. The compound according to anyone of claims 1-3, wherein Ar is optionally substituted with one or more F, Cl, Br, CN, C₁-C₃ alkyl, C₁-C₃ fluoroalkyl or C₁-C₃ alkoxy.
5. The compound according to anyone of claims 1-4, wherein Ar is optionally substituted phenyl.
6. The compound of anyone of claims 1-4, wherein Ar is optionally substituted pyridyl.

7. The compound according to anyone of claims 1-4, wherein Ar is optionally substituted pyrazinyl.
8. The compound according to anyone of claims 1-4, wherein Ar is optionally substituted imidazolyl.
9. The compound according to anyone of claims 1-4, wherein Ar is optionally substituted pyrazolyl.
10. The compound according to anyone of claims 1-4, wherein Ar is optionally substituted oxazolyl.

5

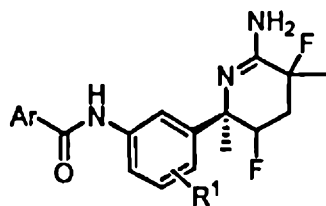
11. The compound according to anyone of claims 1-4, wherein Ar is optionally substituted thiazolyl.
12. The compound according to claim 1, wherein the compound is selected from the group consisting of:
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypyrazine-2-carboxamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypicolinamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-fluoropicolinamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypyrazine-2-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-2-methyloxazole-4-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-methoxypicolinamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide,

N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-chloropicolinamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-1-methyl-1*H*-imidazole-2-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(trifluoromethyl)pyrazine-2-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-4-methylthiazole-2-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-2-(difluoromethyl)oxazole-4-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-1-(difluoromethyl)-1*H*-pyrazole-3-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(difluoromethyl)pyrazine-2-carboxamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-4-chlorobenzamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-cyanopicolinamide,
N-(3-((2*R*,3*S*,5*R*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4,5-difluorophenyl)-5-(methoxy-*d*₃)picolinamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)pyrazine-2-carboxamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-cyano-3-methylpicolinamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4,5-difluorophenyl)-5-(methoxy-*d*₃)picolinamide,
N-(3-((2*R*,3*R*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-(methoxy-*d*₃)picolinamide,
N-(3-((2*R*,3*S*,5*S*)-6-amino-3,5-difluoro-2,5-dimethyl-2,3,4,5-tetrahydropyridin-2-yl)-4-fluorophenyl)-5-bromopicolinamide
 or a pharmaceutically acceptable salt thereof.

13. A pharmaceutical composition comprising the compound according to anyone of claims 1-12 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.
14. The use of a compound according to anyone of claims 1-12 for the manufacture of a medicament for treating a disease selected from Alzheimer's disease (familial or sporadic), preclinical Alzheimer's disease, prodromal Alzheimer's disease, mild cognitive impairment, Down's syndrome and cerebral amyloid angiopathy.
15. A compound according to anyone of claims 1-12 for use in therapy.
16. A compound according to anyone of claims 1-12 for use in a method for the treatment of a disease selected from Alzheimer's disease (familial or sporadic), preclinical Alzheimer's disease, prodromal Alzheimer's disease, mild cognitive impairment, Down's syndrome and cerebral amyloid angiopathy.

ABSTRACT

The present invention is directed to compounds according to Formula I



which compounds are inhibitors of the BACE1 enzyme. Separate aspects of the invention are

- 5 directed to pharmaceutical compositions comprising said compounds and uses of the compounds to treat disorders for which the reduction of A β deposits is beneficial such as Alzheimer's disease.