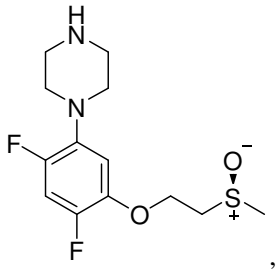
intermediates for A2AR inhibitors catalyzed by highly efficient enzymes.

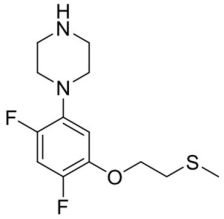
SUMMARY

[0006] In one aspect, provided herein is a process for preparing (+)-1-(2, 4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazine (Intermediate B), or a pharmaceutically acceptable salt or solvate thereof,



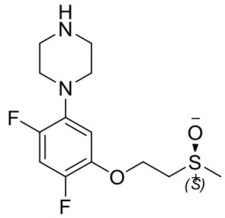
,

comprising the step of contacting 1-(2,4-difluoro-5-(2-(methylthio)ethoxy)phenyl)piperazine, or a pharmaceutically acceptable salt thereof:

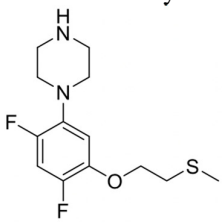


with an enzyme in a solvent.

[0006a] In one aspect, provided herein is a compound of formula Intermediate B:

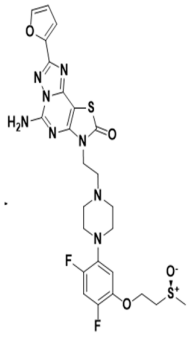


or a pharmaceutically acceptable salt thereof obtained by the step of contacting 1-(2,4-difluoro-5-(2-(methylthio)ethoxy)phenyl)piperazine, or a pharmaceutically acceptable salt thereof:

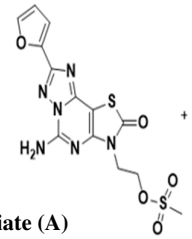


with an enzyme in a solvent.

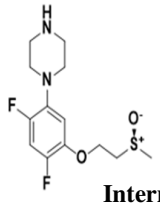
[0006b] In one aspect, provided herein is a process for preparing compund 3A



comprising the step of reacting Intermediate B with intermediate A:



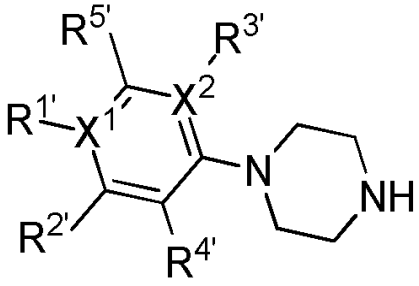
Intermediate (A)



Intermediate (B)

wherein Intermediate B was prepared using a process as disclosed herein.

[0007] Additionally, provided herein is an enzymatic biotransformation process for manufacturing compound of Formula (B) or a pharmaceutically acceptable salt or solvate thereof, wherein Formula (B) is at least 80%, at least 90%, at least 95%, at least 99%, at least 99.9% enantiomerically pure, wherein the enzymatic biotransformation comprises a solvent, and the temperature of the biotransformation is below the boiling temperature of the solvent



Formula (B)

heterocyclylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, alkylsulfoxide, alkylsulfoxidealkyl, alkylsulfonyl and alkylsulfonealkyl;

[0020] or U and Y form together with the atoms to which they are attached an aryl ring, a heteroaryl ring, a cycloalkyl ring or a heterocyclyl ring; optionally substituted by one or more substituent selected from oxo, halo, hydroxy, cyano, alkyl, alkenyl, aldehyde, heterocyclylalkyl, hydroxyalkyl, dihydroxyalkyl, hydroxyalkylaminoalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, (heterocyclyl)(alkyl)aminoalkyl, heterocyclyl, heteroaryl, alkylheteroaryl, alkyne, alkoxy, amino, dialkylamino, aminoalkylcarbonylamino, aminocarbonylalkylamino, (aminocarbonylalkyl)(alkyl)amino, alkenylcarbonylamino, hydroxycarbonyl, alkyloxycarbonyl, aminocarbonyl, aminoalkylaminocarbonyl, alkylaminoalkylaminocarbonyl, dialkylaminoalkylaminocarbonyl, heterocyclylalkylaminocarbonyl, (alkylaminoalkyl)(alkyl)aminocarbonyl, alkylaminoalkylcarbonyl, dialkylaminoalkylcarbonyl, heterocyclylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, alkylsulfoxide, alkylsulfoxidealkyl, alkylsulfonyl and alkylsulfonealkyl.

[0021] In one example, the compound of Formula (B) is selected from the group consisting of:

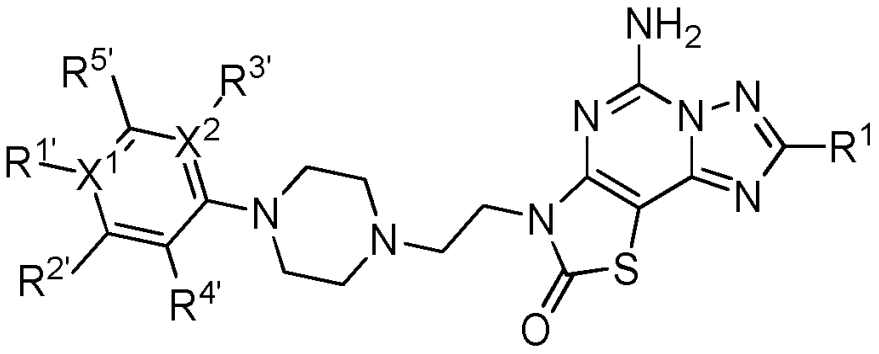
[0022] (S)- 1-(2-fluoro-4-(2-(methylsulfinyl)ethoxy)phenyl)piperazine;

[0023] (R)- 1-(2-fluoro-4-(2-(methylsulfinyl)ethoxy)phenyl)piperazine;

[0024] (S)- 1-(2,4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazine; and

[0025] (R)- 1-(2,4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazine.

[0026] Provided herein, a process for manufacturing a compound of Formula (Ia), or a pharmaceutically acceptable salt or solvate thereof,



(Ia)

[0027] wherein

[0028] R^1 represents 5- or 6-membered heteroaryl or 5- or 6-membered aryl, wherein heteroaryl or aryl groups are optionally substituted by one or more substituent selected from C1-C6 alkyl (preferably methyl) and halo (preferably fluoro or chloro); preferably R1 represents 5-membered heteroaryl; more preferably R1represents furyl;

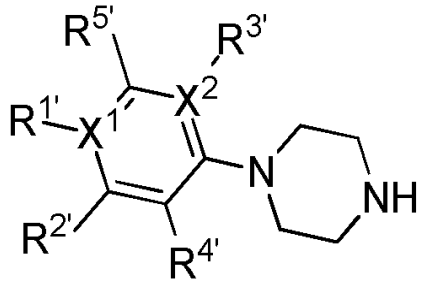
[0029] X^1 and X^2 represent each independently C or N; R1 is absent when X1 is N; or when X1 is C, R1′ represents H, halo, alkyl, heterocyclyl, alkoxy, cycloalkyloxy, heterocyclyloxy, carbonyl, alkylcarbonyl,

[0032] R3′ is absent when X2 is N; or when X2 is C, R3 represents H or halo, preferably H or F;

[0033] R4′ represents H or halo, preferably H or F; and R5′ represents H or halo, preferably H or F,

[0034] In one example, the process for manufacturing a compound of Formula (Ia), or a pharmaceutically acceptable salt or solvate thereof comprises the steps of:

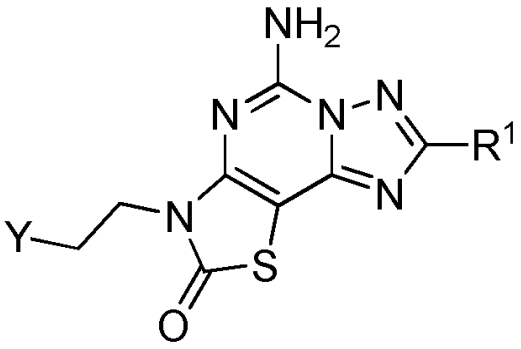
[0035] synthesis of diamine intermediate of Formula (B) by an enzymatic biotransformation, wherein the synthesis produces the intermediate of Formula (B) at least 80%, at least 90%, at least 95%, at least 99%, or at least 99.9% enantiomerically purity:



(B)

[0036] wherein, $R^{1'}$, $R^{2'}$, $R^{3'}$, $R^{4'}$, and $R^{5'}$ are as defined above in this claim, the enzymatic biotransformation is performed in a solvent, and the temperature of the biotransformation is below the boiling temperature of the solvent;

[0037] introduction of an intermediate of Formula (A) and the intermediate of Formula (B) into a suitable solvent:



(A)

wherein, R^1 is as defined above in this claim and Y represents halo, alkylsulfonyloxy having 1 to 6 carbon atoms or arylsulfonyloxy having 6 to 10 carbon atoms; and

[0038] coupling between intermediate of Formula (B) and intermediate of Formula (A) in the suitable solvent at a temperature ranging from about 20 °C to about 180 °C, thereby forming the compound of Formula (Ia).

[0039] In one example, the intermediate of Formula (B) is selected from the group consisting of:

[0040] (S)- 1-(2-fluoro-4-(2-(methylsulfinyl)ethoxy)phenyl)piperazine;

[0041] (R)- 1-(2-fluoro-4-(2-(methylsulfinyl)ethoxy)phenyl)piperazine;

[0042] (S)- 1-(2,4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazine; and

[0043] (R)- 1-(2,4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazine.

[0044] In one example, the compound of Formula (Ia) is selected from the group consisting of: (S)-5-amino-3-(2-(4-(2-fluoro-4-(2-(methylsulfinyl)ethoxy)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0045] (R)-5-amino-3-(2-(4-(2-fluoro-4-(2-(methylsulfinyl)ethoxy)phenyl)-piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0046] (+)-(S)-5-amino-3-(2-(4-(2,4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0047] (-)-(R)-5-amino-3-(2-(4-(2,4-difluoro-5-(2-(methylsulfinyl)ethoxy)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0048] (S)-5-amino-8-(furan-2-yl)-3-(2-(4-(4-(methylsulfinyl) phenyl)piperazin-1-yl)ethyl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0049] (R)-5-amino-8-(furan-2-yl)-3-(2-(4-(4-(methylsulfinyl) phenyl)piperazin-1-yl)ethyl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0050] (S)-5-amino-3-(2-(4-(2,4-difluoro-5-(methylsulfinyl)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0051] (R)-5-amino-3-(2-(4-(2,4-difluoro-5-(methylsulfinyl)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0052] (S)-5-(4-(2-(5-amino-8-(furan-2-yl)-2-oxothiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-3(2H)-yl)ethyl)piperazin-1-yl)-2,4-difluoro-N-(2-(methylsulfinyl)ethyl)benzamide;

[0053] (R)-5-(4-(2-(5-amino-8-(furan-2-yl)-2-oxothiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-3(2H)-yl)ethyl)piperazin-1-yl)-2,4-difluoro-N-(2-(methylsulfinyl)ethyl)benzamide;

[0054] (S)-5-(4-(2-(5-amino-8-(furan-2-yl)-2-oxothiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-3(2H)-yl)ethyl)piperazin-1-yl)-2,4-difluoro-N-methyl-N-(2-(methylsulfinyl)ethyl)benzamide;

[0055] (R)-5-(4-(2-(5-amino-8-(furan-2-yl)-2-oxothiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-3(2H)-yl)ethyl)piperazin-1-yl)-2,4-difluoro-N-methyl-N-(2-(methylsulfinyl)ethyl)benzamide;

[0056] (R)-5-amino-3-(2-(4-(2-fluoro-4-(methylsulfinyl)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0057] (S)-5-amino-3-(2-(4-(2-fluoro-4-(methylsulfinyl)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one;

[0058] (S)-4-(4-(2-(5-amino-8-(furan-2-yl)-2-oxothiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-3(2H)-yl)ethyl)piperazin-1-yl)-3-fluoro-N-(2-(methylsulfinyl)ethyl)benzamide;

[0059] (R)-4-(4-(2-(5-amino-8-(furan-2-yl)-2-oxothiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-3(2H)-yl)ethyl)piperazin-1-yl)-3-fluoro-N-(2-(methylsulfinyl)ethyl)benzamide;

[0060] (S)-5-amino-3-(2-(4-(2,4-difluoro-5-(3-(methylsulfinyl)propoxy)phenyl)piperazin-1-yl)ethyl)-8-(furan-2-yl)thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidin-2(3H)-one; and pharmaceutically acceptable salts or solvates thereof.

[0061] In some examples, the enzymatic biotransformation is in a cell free system.

[0062] In some examples, the enzymatic biotransformation comprises at least one enzyme selected from oxidoreductases.

[0063] In some examples, the oxidoreductases are selected from the group of monooxygenases and/or alcohol dehydrogenases.

[0064] In a preferred example, the monooxygenase is derived from Rhodococcus jostii.

[0065] In a preferred example, the monooxygenase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:1.

[0066] In a preferred example, the alcohol dehydrogenase comprises a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 2.

[0067] In a preferred example, the alcohol dehydrogenase is derived from Thermoanaerobium brockii.

[0068] In some examples, the enzymes are in crude or purified or in immobilized form.

[0069] In some examples, the temperature in step (i) is between about 0° C and about 45° C.

[0070] In some examples, the solvent in step (i) comprises water and/or isopropanol.

[0071] In some examples, the step (i) further comprises nicotinamide adenine dinucleotide phosphate (NADP+).

[0072] In some examples, the steps (i), (ii) and (iii) are performed in a reactor. In some examples, the reactor is different for each step (i), (ii) and (iii).

[0073] In some examples, enantiomerically pure compound of Formula (B) is 'R' isomer.

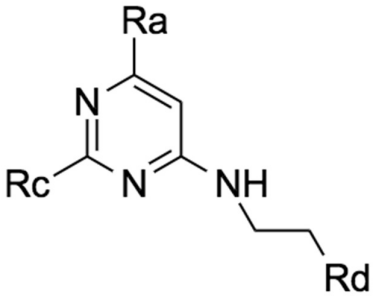
[0074] In some examples, enantiomerically pure compound of Formula (B) is 'S' isomer.

[0075] In some examples, the synthesis of intermediate of Formula (B) comprises a reaction yield. In one example, the reaction yield is at least about 50%.

[0076] In one example, the synthesis of intermediate of Formula (B) comprises overoxidation of an undesired enantiomer, whereby undesired enantiomer is converted to a sulfone.

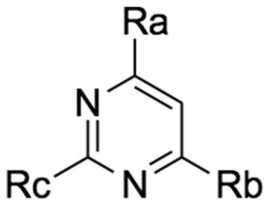
[0077] In some examples, the sulfone is removed by recrystallization.

[0078] In one example, provided herein is a process for the synthesis of a compound of formula (IV)



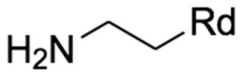
(IV)

[0079] wherein, Ra is absent or is halo; Rb is halo; Rc is absent or is amino; Rd is absent or is methoxy; comprising the steps of: reacting the compound of formula (II)



(II)

[0080] with a compound of formula (III)



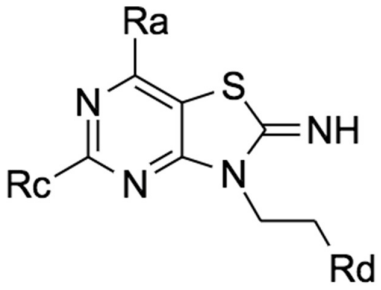
(III)

[0081] in the presence of a suitable base.

[0082] In some examples, the base is an organic base. In some examples, the organic base is selected from an acyclic amine (e.g., triethylamine, diisopropylethylamine (DIPEA), etc.) or a cyclic amine (e.g., pyrrolidine, piperidine, etc.).

[0083] In some examples, Ra is chlorine; Rb is chlorine; Rc is amino and Rd is methoxy.

[0084] Provided herein is a process for the synthesis of a compound of formula (V)



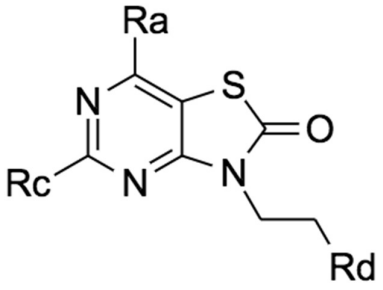
(V)

[0085] wherein, Ra is absent or is halo; Rc is absent or is amino; Rd is absent or is methoxy; comprising the steps of: (i) preparing the compound of formula (IV) according to process described above in this disclosure; (ii) reacting compound of formula (IV) with KSCN and Br_2 in the presence of an acid at a temperature below 0 ^{O}C; and (iii) addition of a base selected from ammonia, barium hydroxide, calcium hydroxide, cesium hydroxide, magnesium hydroxide, potassium hydroxide, or sodium hydroxide to yield a compound of formula (V).

[0086] The base can be made available in the form of a resin (such as Amberlite® and the like). In some further examples, the base can be provided in the form of a solution in water such as about 2N solution (e.g., about 0.5N solution, about IN solution, about 1.5N solution, about 2.5N solution, from about 3N to about 5N solution, from about 5N to about 10N solution).

[0087] In some examples, the step (ii) can be performed at a temperature from about from about -10 °C to about 0 °C, from about -5 °C to about -15 °C, from about -10 °C to about -25 °C, or from about -15 °C to about -35 °C.

[0088] Provided herein is a process for the synthesis of a compound of formula (VI)



(VI)

[0089] wherein, Ra is absent or is halo; Rc is absent or is amino; Rd is absent or is methoxy; comprising the steps of: (i) preparing the compound of formula (IV) according to process described above in this disclosure; (ii) dissolving the compound of formula (IV) in a water miscible organic solvent.
(iii) addition of an acid to yield a compound of formula (VI).

[0090] In some examples, water miscible organic solvent can be selected from dioxane, THF, water,

acetic acid, DME, DMF, DMSO, acetonitrile, diglyme, alcohol (e.g. methanol, ethanol or tert-butanol) methyl-ethyl ketone, NMP, or mixtures thereof.

[0091] In some examples, the acid can be selected from TFA, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, acetic acid, propionic acid, hydroxyacetic acid, lactic acid, pyruvic acid, oxalic acid (ie ethanedioic acid), malonic acid, succinic acid (ie butanedioic acid), maleic acid, fumaric acid, malic acid (ie hydroxyl-butanedioic acid), tartaric acid, citric acid, methanesulfonic acid, ethanesulfonic acid , benzenesulfonic acid, p-toluenesulfonic acid, cyclamic acid, salicylic acid, p-aminosalicylic acid, pamoic acid, or combination thereof.

DETAILED DESCRIPTION

Definitions

[0092] Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this disclosure pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodologies by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 4th ed. (2012) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer-defined protocols and conditions unless otherwise noted.

[0093] As used herein, the singular forms “a,” “an,” and “the” include the plural referents unless the context clearly indicates otherwise. The terms “include,” “such as,” and the like are intended to convey inclusion without limitation, unless otherwise specifically indicated.

[0094] As used herein, the term “comprising” also specifically includes embodiments “consisting of” and “consisting essentially of” the recited elements, unless specifically indicated otherwise.

[0095] The term “about” indicates and encompasses an indicated value and a range above and below that value. In certain embodiments, the term “about” indicates the designated value ± 10%, ± 5%, or ± 1%. In certain embodiments, where applicable, the term “about” indicates the designated value(s) ± one standard deviation of that value(s).

[0096] The term “intermediate” or “intermediate compound” refers to a compound which is