Title: COMPOUNDS AND COMPOSITIONS FOR MODULATING ADENOSINE A3 RECEPTOR ACTIVITY

Abstract: The present invention provides for Adenosine A3 receptor agonists, and also methods and use of the compounds of the invention, by themselves or in combination with other therapies, for treating a disease, disorder, or condition.
DESCRIPTION

COMPOUNDS AND COMPOSITIONS FOR MODULATING
ADENOSINE A3 RECEPTOR ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to, and the benefit of, U.S. provisional application No. 61/700,924, filed September 14, 2012, the content of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to certain compounds which can modulate the activity of adenosine A3 receptor; and relates to processes for their preparation, as well as pharmaceutical compositions containing them as an active ingredient, and their use as medicaments.

BACKGROUND OF THE INVENTION

Extracellular adenosine can bind to a number of receptors on the cell membranes, including, among others, adenosine A1 receptor, adenosine A2A receptor, adenosine A2B receptor, and adenosine A3 receptor ("A3 receptor"). These receptors are G protein-coupled receptors that are involved in a variety of intracellular signaling pathways and physiological functions. The A3 receptor is widely expressed in human tissues with the most abundant expression in the lung and liver. It has been reported that A3 receptors are primarily expressed on inflammatory cells such as eosinophils in human lung, and also expressed on mast cells and neutrophils involved in inflammation, whereas low expression is found in other normal tissues. This pattern of expression on inflammatory cells is supportive of a role of A3 receptor in inflammation. A3 receptor was also found to be highly expressed in tumor cells and tissues, with low expression levels in normal cells or adjacent tissue. Further, A3 receptor expression in the tumor tissues was directly correlated to the severity of the cancer. This pattern of
expression on tumor cells and tissue, but not surrounding normal cells and tissue, is supportive of a role of A3 receptor in tumor pathology.

Further studies support the roles of A3 receptor in diseases involving inflammation. For example, it is known that inflammation plays a role in the pathogenesis of early brain injury following subarachnoid hemorrhage, and that a selective A3 receptor agonist (e.g., 2-chloro-N6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide (Cl-IB-MECA) significantly attenuated brain injury after administration of the selective A3 receptor agonist. Thus, activation of the A3 receptor by the selective A3 receptor agonist resulted in anti-inflammatory effects. In another example, selective A3 receptor agonist, methyl 1-[N6-(3-iodobenzyl)-adenin-9-yl]-b-D-ribofuronamide (IB-MECA) was shown in Phase I and Phase II trials in humans to have an anti-inflammatory effect when administered to individuals suffering from rheumatoid arthritis. In a further example, a selective A3 receptor agonist was tested in standard experimental models of liver inflammation. It was found that activating A3 receptor by an agonist resulted in protection of the liver against inflammation by a number of mechanisms, including preventing apoptosis of hepatocytes. A3 receptor agonists have also been reported to inhibit inflammation in a number of other experimental models of human diseases such as osteoarthritis, psoriasis, and inflammatory bowel disease. Thus, activation of A3 receptor by an A3 receptor agonist induces an anti-inflammatory effect, and such agonists are potential inhibitors of inflammatory diseases, including autoimmune diseases.

A3 receptors are also expressed on cardiac ventricular cells. Several studies have shown that activation of A3 receptor with one of a variety of A3 receptor agonists resulted in a cardioprotective effect against the deleterious effect of subsequent cardiac ischemia and reperfusion injury. Thus, activation of A3 receptor by an A3 receptor agonist induces a cardioprotective effect, and such agonists are potential inhibitors of cardiac ischemia and reperfusion injury.

High expression of A3 receptor in tumor cells and tumor tissues is thought to be, at least in part, attributable to overexpression of NF-kappaB, known to act as an A3 receptor transcription factor. Treatment of tumor cells, or individuals with tumor, with A3 receptor agonists has shown to induce inhibition of tumor growth. Selective A3 receptor agonist, Cl-IB-MECA (at an IC 50 of 5 µM, and 14µM, respectively), was shown to inhibit growth of melanoma cells and lung
carcinoma cells. A synergistic effect against tumor cell growth and metastasis has been observed when an A3 receptor agonist was combined with another chemotherapeutic agent (e.g., cyclophosphamide). Additionally, an A3 receptor agonist was shown to prevent the myelotoxic effects of cyclophosphamide during combinatorial therapy by increasing the number of white blood cells and the percentage of neutrophils. Thus, an A3 agonist can additionally function as a chemoprotective agent. Selective A3 receptor agonist, Cl-IB-MECA was shown (at nanomolar to micromolar levels) to inhibit growth of human hepatocellular carcinoma cells, human prostate carcinoma cells, human ovarian carcinoma cells, colon carcinoma cells, and lymphoma cells. Taken together, A3 receptors abundantly expressed in tumor cells may be targeted by A3 agonists, leading to inhibition of tumor growth.

SUMMARY OF THE INVENTION

The invention includes compounds comprising a C-nucleoside that selectively activates Adenosine A3 receptor. The compounds of the present invention, comprising A3 receptor agonists, surprisingly may be more chemically stable in body under physiological conditions, as compared to N-nucleoside compounds (e.g., C-C bond chemically more stable than C-N bond), as well as more stable to purine nucleoside phosphorylase enzyme activity.

It is an object of the present invention to provide a novel compound which is effective as an A3 receptor agonist.

It is another object of the present invention to provide a pharmaceutical composition for treating or preventing a disease, disorder, or condition modulated by activating adenosine A3 receptor on cells.

It is yet another object of the present invention to provide a method for treating or preventing a disease, disorder, or condition modulated by activating adenosine A3 receptor on cells in a mammal.

It is still another object of the present invention to provide a use of the inventive compound.
In accordance with one aspect of the present invention, there is provided a compound of Formula (I), or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof, wherein formula (I) is as defined herein.

In accordance with another aspect of the present invention, there is provided a pharmaceutical composition comprising the compound of formula (I) as an active ingredient, or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof, and a pharmaceutically acceptable carrier.

In accordance with yet another aspect of the present invention, there is provided a method for treating or preventing a disease, disorder, or condition modulated by activating adenosine A3 receptor on cells in a mammal, which comprises administering the compound of formula (I), or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof to the mammal.

In accordance with a further aspect of the present invention, there is provided a use of the compound of formula (I), or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof for the manufacture of a medicament for treating or preventing a disease, disorder, or condition modulated by activating adenosine A3 receptor on cells.

Other aspects, objects and features of the invention will be apparent from the following description.

DETAILED DESCRIPTION OF THE INVENTION

While the terms used in the description of the invention are believed to be well understood by one of ordinary skill in the pharmaceutical arts, definitions, where provided herein, are set forth to facilitate description of the invention, and to provide illustrative examples for use of the terms.

The terms "a," "an," and "the" may mean one or more, and may be used to reference both the singular and the plural.
The term "alkyl" is used herein to refer to a hydrocarbon containing normal, secondary, tertiary, or cyclic carbon atoms (e.g., linear saturated aliphatic hydrocarbon groups, branched saturated aliphatic hydrocarbon groups, or a saturated or unsaturated non-aromatic hydrocarbon mono or multi-ring system (e.g., cycloalkyl)). When the term "alkyl" is used without reference to a number of carbon atoms, it is to be understood to refer to a C₁₋₁₀ alkyl, preferably C₁₋₈ alkyl, more preferably C₁₋₄ alkyl; e.g., a C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉ or C₁₀ alkyl.

The term "aminoalkyl" is used herein to refer to at least one amino group that is appended to the parent molecular moiety through an alkyl group, as defined herein.

The term "alkylamino" is used herein to refer to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms, preferably from 1 to about 6 carbon atoms, connected to the parent molecular moiety through an NH group. Representative examples of alkylamino include, but are not limited to, methylamino, ethylamino, isopropylamino and tert-butylamino.

The term "alkylamido" is used herein to refer to an alkyl group as defined above, which is attached via —C(O)NH— or —NHC(O)—.

The term "aryl" is used herein to refer to cyclic, aromatic hydrocarbon groups which have 1 to 3 aromatic rings, for example phenyl or naphthyl. The aryl group may have fused thereto a second or third ring which is a heterocyclo, cycloalkyl, or heteroaryl ring, provided in that case the point of attachment will be to the aryl portion of the ring system. "Heteroaryl" refers to an aryl group in which at least one of the carbon atoms in the aromatic ring has been replaced by a heteroatom selected from oxygen, nitrogen and sulphur. The nitrogen and/or sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. The heteroaryl group may be a 5 to 6 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring system.

The term "alkenyl" is used herein to refer to a straight or branched chain hydrocarbon containing from 2 to 10 carbons, preferably from 2 to 8 carbon atoms, more preferably from 2 to 4 carbon atoms, and containing at least one carbon-carbon double bond formed by the removal of two hydrogens.
The term "alkynyl" is used herein to refer to a straight or branched chain hydrocarbon group containing from 2 to 10 carbon atoms, preferably from 2 to 8 carbon atoms, more preferably from 2 to 4 carbon atoms, and containing at least one carbon-carbon triple bond.

The term "BOC" is used herein to refer to tert-butyloxycarbonyl.

The term "carbocyclyl" (alone or in combination with another term(s)) is used herein to refer to a saturated cyclic (i.e., "cycloalkyl"), partially saturated cyclic (i.e., "cycloalkenyl"), or completely unsaturated (i.e., "aryl") hydrocarbyl substituent containing from 3 to 14 carbon ring atoms ("ring atoms" being the atoms bound together to form the ring or rings of a cyclic substituent). A carbocyclyl may be a single ring, which typically contains from 3 to 6 ring atoms. The term "carbocyclylalkyl" is used herein to refer to an alkyl group substituted with a carbocycle group.

The term "cycloalkyl" is used herein to refer to monocyclic or multicyclic (e.g., bicyclic, tricyclic, etc.) hydrocarbons containing from 3 to 12 carbon atoms, preferably from 3 to 10 carbon atoms, more preferably from 3 to 6 carbon atoms, that is completely saturated or has one or more unsaturated bonds but does not amount to an aromatic group.

The term "cyano" as used herein means a —CN group.

The term "halo" or "halogen" means —Cl, —Br, —I, or —F.

The term "haloalkyl" means that at least one hydrogen atom on an alkyl group is replaced with a halogen atom selected from fluorine, chlorine, bromine, iodine, and combinations thereof. The degree of halogenation may range from one hydrogen atom on the alkyl group being replaced by a halogen atom (e.g., a monofluoromethyl group) to full halogenation (e.g., perhalogenation) wherein all hydrogen atoms on the alkyl group have been replaced by a halogen atom (e.g., trifluoromethyl or perfluoromethyl). The haloalkyl groups useful in embodiments of the invention may be partially or fully halogenated and may be linear or branched.

The term "heterocyclyl" is used herein to include non-aromatic, ring systems, including, but not limited to, monocyclic, bicyclic and tricyclic rings, which can be completely saturated, and have 3 to 12 atoms including at least one heteroatom, such as nitrogen, oxygen, or sulfur.
The term "hydroxyalkyl" is used herein to refer to an alkyl that is substituted with at least one hydroxy substituent. Examples of hydroxyalkyl include hydroxymethyl, hydroxyethyl, hydroxypropyl and hydroxybutyl.

The terms "first" and "second" are used herein for purposes of distinguishing between two compounds, or between two compositions, as will be clearer from the description.

The phrase "medically effective amount" means an amount of a composition or compound that treats the particular disease, condition or disorder; ameliorates, relieves, or decreases one or more symptoms associated with the particular disease, condition or disorder; and/or delays or prevents the onset of symptoms of, or a pathological process associated, with the particular disease, condition or disorder described herein in more detail.

The terms "prevent", "prevents", or "preventing", as used herein, embrace one or more of preventative (prophylactically).

The terms "treat", "treats", or "treating", as used herein, embrace one or more of therapeutically (palliative).

The term "pharmaceutically acceptable carrier" is used herein to mean any compound or composition or carrier medium useful in any one or more of administration, delivery, storage, stability of a composition or compound described herein. These carriers are known in the art to include, but are not limited to, a diluent, water, saline, suitable vehicle (e.g., liposome, microparticle, nanoparticle, emulsion, capsule), buffer, medical parenteral vehicle, excipient, aqueous solution, suspension, solvent, emulsions, detergent, chelating agent, solubilizing agent, salt, colorant, polymer, hydrogel, surfactant, emulsifier, adjuvant, filler, preservative, stabilizer, oil, binder, disintegrant, absorbant, flavor agent, and the like as broadly known in the pharmaceutical art.

Hereinafter, the present invention is described in detail.

The present invention provides a compound selected from the group consisting of a compound of Formula 1 below, and pharmaceutically acceptable salts, a prodrug, a hydrate, a solvate, and an isomer thereof:
wherein,

Y is oxygen, sulfur, or carbon;

$X_i$ is H, alkyl, R$^a$R$^b$NC(=O)- or HOR$^e$-, wherein said R$^a$ and R$^b$ are each independently hydrogen, alkyl, amino, haloalkyl, aminoalkyl, BOC-aminoalkyl, or cycloalkyl, or optionally fuse together to form a heterocyclic ring containing two to five carbon atoms, and said R$^e$ is alkyl, amino, haloalkyl, aminoalkyl, BOC-aminoalkyl, or cycloalkyl;

$X_2$ is H, hydroxyl, alkylamino, alkylamido or hydroxyalkyl;

$X_3$ and $X_4$ are each independently hydrogen, hydroxyl, amino, amido, azido, halo, alkyl, carboxy, nitrile, nitro, trifluoro, aryl, alkaryl, thio, thioether, -OCOPh or -OC(=S)OPh, or both $X_3$ and $X_4$ are optionally oxygens connected to $\geq$C=S to form a 5-membered ring, or $X_2$ and $X_3$ optionally form the ring of formula (III),

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\begin{array}{c}
R'Si=O \\
Si=O \\
R'Si=O
\end{array}
\]

wherein R' and R" represent independently an alkyl group;

$X_5$ is H, cyano, C$_1$-8 alkyl, C$_2$-8 alkenyl, or C$_2$-8 alkynyl;

Z is nitrogen or carbon;

R$_1$ is halogen, NR$^{11}$R$^{12}$, N(R$^{11}$)OR$^{11}$, NR$^{11}$NR$^{11}$R$^{12}$, N$_3$, NO, NO$_2$, CHO, CN, -CH(=NR$^{11}$), -CH=NNHR$^{11}$, -CH=N(OH$^{11}$), -CH(OR$^{11}$)$_2$, -C(=O)NR$^{11}$R$^{12}$, -C(=S)NR$^{11}$R$^{12}$, -C(=O)OR$^{11}$, Cl-8 alkyl, C$_2$-8 alkenyl, C$_{2-8}$ alkynyl, C$_{4-8}$ carbocyclylalkyl, C$_{6-20}$ aryl, C$_{2-20}$ heterocyclyl, heteroaryl, -C(=O)-C$_{1-8}$ alkyl, -S(O)$_n$-C$_{1-8}$ alkyl, aryl-C$_{1-8}$ alkyl, OR$^{11}$ or SR$^{11}$, and said n is 0, 1, or 2; and
R² and R³ are each independently H, halogen, NR¹R¹², N(R¹¹)OR¹¹, 
NR¹¹NR¹¹R¹², N₃, NO, NO₂, CHO, CN, -CH(=NR¹¹), -CH=NNH, -CH=NO, 
-NH₂, -CH(OR¹²)₂, -C(=O)NR¹¹R¹², -C(=S)NR²R¹², -C(=O)OR¹¹, 
R¹¹, OR¹¹, or SR¹¹,

wherein said R¹¹ and R¹² are each independently H, C₁₋₈ alkyl, C₂₋₈ alkenyl, 
C₂₋₈ alkynyl, C₃₋₈ carbocyclyl, C₄₋₈ carbocyclylalkyl, aryl-C₁₋₈ alkyl, heterocyclyl-
C₁₋₈ alkyl, C₆₋₂₀ aryl, C₂₋₂₀ heterocyclyl, heteroaryl, -C(=O)-C₁₋₈ alkyl, or -S(O)₈-
C₁₋₈ alkyl, or R¹¹ and R¹² optionally fuse together with a nitrogen to which they 
are both attached to form 3 to 7 membered heterocyclic ring, wherein any carbon 
atom of said heterocyclic ring is optionally replaced with -O-, -S(O)₈- or -NRᵈ-, 
wherein, optionally, said C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₈ 
carbocyclyl, C₄₋₈ carbocyclylalkyl, aryl-C₁₋₈ alkyl, heterocyclyl-Cₙ₂₀ alkyl, C₆₋₂₀ 
aryl, C₂₋₂₀ heterocyclyl, and heteroaryl are each independently substituted with 
one or more halogen, hydroxyl, CN, N₃, N(Rᵈ)₂, NH(Rᵈ), NH₂, C(O)N(Rᵈ)₂, 
C(O)NH(Rᵈ), C(O)NH₂, OC(O)N(Rᵈ)₂, OC(O)NH(Rᵈ), OC(O)NH₂, C(O)ORᵈ, 
OC(O)Rᵈ, C(O)Rᵈ, OC(O)Rᵈ, S(O)₈Rᵈ, S(O)₂N(Rᵈ)₂, S(O)₂NH(Rᵈ), S(O)₂NH₂, 
ORᵈ or Rᵈ; wherein Rᵈ is C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₈ carbocyclyl, 
C₄₋₈ carbocyclylalkyl, aryl-C₈₂₀ alkyl, heterocyclyl-C₁₋₈ alkyl, C₆₋₂₀ aryl, C₂₋₂₀ 
heterocyclyl, or heteroaryl.

(Note: Ph is used to represent the phenyl group, C₆H₅).

In a preferred embodiment of the present invention, there is provided the 
compound of Formula (I), as defined below:

Y is oxygen;

X₁ is H or RᵃRᵇNC(=O)-, wherein said Rᵃ and Rᵇ are each independently 
hydrogen, C₁₋₈ alkyl, amino, haloalkyl, aminoalkyl, BOC-aminoalkyl, or 
cycloalkyl;

X₂ is H, hydroxyl, alkylamino, alkylamido or hydroxyalkyl;

X₃ and X₄ are each independently hydrogen, hydroxyl, amino, amido, 
azido, halo, alkyl, carboxy, nitrile, nitro, trifluoro, aryl, alkaryl, thio, thioester, 
thioether, -OCOPh or -OC(=S)OPh;

X₅ is H, cyano, C₁₋₈ alkyl, C₂₋₈ alkenyl, or C₂₋₈ alkynyl;

Z is nitrogen or carbon;
R, is halogen, NR^{11}R^{12}, N(R^{11})OR^{11}, NR^{11}NR^{11}R^{12}, -CH(=NR^{11}), -CH=NNHR^{11}, -CH=N(OR^{11}), -CH(OR^{11})_2, -C(=O)NR^{11}R^{12}, or -C(=S)NR^{11}R^{12}; and

R^2 and R^3 are each independently H, halogen, NR^{11}R^{12}, N(R^{11})OR^{11}, NR^{11}NR^{11}R^{12}, -CH(=NR^{11}), -CH=NNHR^{11}, -CH=N(OR^{11}), -CH(OR^{11})_2, -C(=O)NR^{11}R^{12}, or -C(=S)NR^{11}R^{12};

wherein said R^{11} and R^{12} are each independently H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, C_{3-8} carbocyclyl, C_{4-8} carbocyclylalkyl, aryl-C_{1-8} alkyl, heterocyclyl-C_{1-8} alkyl, C_{6-2}oaryl, C_{2-2}oheterocyclyl, heteroaryl, -C(=0)-C_{1-8} alkyl, or -S(O)_n-C_{1-8} alkyl,

wherein, optionally, said C_{1-8} alkyl, C_{2-8} alkenyl, and C_{2-8} alkynyl are each independently substituted with one or more halogen, hydroxyl, CN, or N_3.

In a more preferred embodiment of the present invention, there is provided the compound of Formula (I), as defined below:

Y is oxygen;
X_1 is R^aR^bNC(=O)-, wherein said R^a and R^b are each independently hydrogen or C_{1-8} alkyl;
X_2 is H;
X_3 and X_4 are hydroxyl;
X_5 is H or cyano;
Z is nitrogen or carbon;
R^1 is NR^{11}R^{12}; and

R^2 and R^3 are each independently H or halogen, wherein said R^{11} and R^{12} are each independently H or phenyl-d-alkyl.

Compounds especially useful in the present invention are selected from the group consisting of:

(1) (2S,3S,4R,5S)-3,4-dihydroxy-5-(4-((3-iodobenzyl)amino)imidazo[2, 1-f][1,2,4]triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide;

(2) (2S,3S,4R,5R)-5-cyano-3,4-dihydroxy-5-(4-((3-iodobenzyl)amino)imidazo[2, 1-j][1,2,4]triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide; and
The compounds of Formula (I) can form salts, and salts of the compounds are included within the scope of the invention. The terms "salt" or pharmaceutically acceptable salt", as used herein, refers to inorganic or organic salts of a compound. These salts can be prepared, for example, by reacting a compound of Formula (I) with an amount of acid or base, such as an equivalent amount, and in a medium such as one in which the salt formed then precipitates, or in an aqueous medium followed by lyophilization. Representative salts include bisulfate, sulfate, benzene sulfonate, camphorsulfonate, laurylsulphonate, methanesulfonate, toluenesulfonate, naphthalenesulfonmate, acetate, trifluoracetate, benzoate, borate, butyrate, citrate, formate, fumarate, hydorbromide, hydrochloride, hydroiodide, lactate, laurate, maleate, malonate, mesylate, nitrate, oxalate, phosphate, hexafluorophosphate, propionate, salicylate, stearate, succinate, tartrate, thiocyanate, and the like. The salts may include base salts based on the alkali and alkaline earth metals, such as calcium, sodium, lithium, magnesium, and potassium; or with organic bases such as with organic amines (e.g., dicyclohexylamine, t-butyl amine, methylamine, dimethylamine, triethylamine, ethylamine, procaine, morpholine, N-methylpiperidined, dibenzyamine, and the like); or as an ammonium salt.

Prodrugs of the compounds of Formula (I), or salts thereof, are included within the scope of the invention. The term "prodrug", as used herein, refers to a compound that is transformed in vivo (e.g., by a metabolic, physiological, or chemical process) to yield a compounds of Formula (I), or a pharmaceutically acceptable salt, hydrate or solvate of the compound. Prodrugs, made by synthesizing one or more prodrug moieties as part of an active compound, can serve to enhance one or more of solubility, absorption, lipophilicity, pharmacodynamics, pharmacokinetics, and efficacy, as compared to the active compound without the one or more prodrug moieties. Various forms of prodrugs are known in the art. Examples of prodrugs of the compounds of the invention include an in vivo cleavable ester of a carboxy group (e.g., lower alkyl esters, cycloalkyl esters, lower alkenyl esters, benzyl esters, mono- or di-substituted
lower alkyl esters, and the like); or S-acyl and O-acyl derivatives of thiols, alcohols, or phenols. "Prodrug moiety" refers to a labile functional group, including but not limited to a protective group, which can be removed or reduced from the active compound during a process elected from one or more of metabolism, systemic circulation, intracellular, hydrolysis, or enzymatic cleavage. Enzymes which are capable of an enzymatically activating a phosphonate prodrug include, but are not limited to, amidases, esterases, phospholipases, cholinesterases, and phosphases. Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A prodrug moiety may include an active metabolite or drug itself.

Exemplary prodrug moieties include the hydrolytically sensitive or labile acyloxymethyl esters -CH₂OC(=O)R³ and acyloxymethyl carbonates esters -CH₂OC(=O)OR³ where R³ is C₁-C₆ alkyl, C₁-C₆ substituted alkyl, C₆-C₁₀ aryl or C₆-C₁₀ substituted aryl. Phosphonate prodrug moieties may include, but are not limited, to groups such as phosphate esters, phosphonate esters, phosphoramidate esters, wherein the ester may be further substituted with groups that confer a pharmaceutical advantage such as one or more of improved solubility in aqueous solutions, or prolonged in vivo exposure, or enhanced absorption through a mucosa of a compound according to Formula (I). Thio-containing prodrug moieties, reported to be useful for the intracellular delivery of drugs, contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Following deesterification or reduction of the disulfide, generated is a free thio intermediate which, in turn, breaks down.

The compounds of Formula (I) may exist in a solvated form or unsolvated form. Solvates of a compound of the invention may be formed in the synthetic process in which the compound becomes physically associated with one or more solvent molecules (e.g., such as by ionic and/or covalent bonding) or, optionally, may be converted to a solvate such as by dissolving the compound in desired amounts of a solvent of choice (e.g., organic solvent, water, or mixtures thereof) in forming a solution, heating the solution to a temperature higher that ambient temperature, and cooling the solution at a rate sufficient to form crystals of the solvate, which may then be further isolated using methods known the art.
Examples of suitable solvents include methanolates, ethanolates, hydrates (where the solvent molecule is water), and the like.

The terms "purified" or "isolated" for a compound according to Formula (I) refers to the physical state of the compound following isolation from a synthetic process or purification step described herein or well known to those in the art, and in sufficient purity to be characterizable by standard analytical methods described herein or well known in the art.

The compounds of Formula (I) may contain asymmetric or chiral centers, and thus exist in different stereoisomeric forms. All stereoisomers (e.g., geometric isomers, optical isomers, and the like), enantiomeric forms, diastereomeric forms, tautomeric forms, positional isomers, of the compounds of the invention are embraced within the scope of the invention. A first conformational form of the compound can be separated from a second and different conformational form of the compound using methods well known in the chemical arts such as by chromatography, crystallization, and methods of synthesis which selectively result in a particular desired conformational form.

Further, the present invention provides a pharmaceutical composition for preventing or treating cancer, inflammation, cardiac ischemia or reperfusion injury, comprising a compound of Formula (I), or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof, and a pharmaceutically acceptable carrier.

The pharmaceutical composition according to the present invention may comprise a medically effective amount (e.g., therapeutically effective amount, or prophylactically effective amount, or a combination thereof, as the medical condition, disease or disorder warrants and as known to a skilled medical practitioner) of a compound according to the present invention, as an A3 receptor agonist, for activating A3 receptor activity in modulating (for inhibiting, ameliorating, treating, preventing) a disease, disorder, or condition in which A3 receptor activation inhibits the disease, disorder, or condition. The pharmaceutical composition, in addition to comprising a compound according to the present invention, may also contain at least one additional pharmaceutical agent (i.e., other than an A3 receptor agonist) in a medically effective amount.
For example, one or more compounds having A3 receptor agonist activity, when used to treat cancer, may be used in combination with one or more additional chemotherapeutic agents, with the potential for synergistically enhancing one or more of apoptosis of cancer cells, growth inhibition of cancer cells, or reducing the myelotoxic effect of the one or more additional chemotherapeutic agents in the combination. Examples of chemotherapeutic agents which can be used in combination with an A3 receptor agonist of the invention include, but are not limited to, LSD1 blockers PPAR (peroxisome proliferating-activator receptor) ligands (e.g., rosiglitazone); alkylating agents (e.g., nitrogen mustards, such as mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, and melphalan; nitrosoureas, such as streptozocin, carmustine, and lomustine; alkyl sulfonates, such as busulfan; triazines, such as dacarbazine and temozolomide; ethylenimines, such as thiota and altretamine; and platinum-based drugs, such as cisplatin, carboplatin, and oxalaplatin); antimetabolites (e.g., 5-fluorouracil, 6-mercaptopurine, capecitabine, cladribine, clofarabine, cytarabine, flouxuridine, fludarabine, gemcitabine, hydroxyurea, methotrexate, pemetrexed, pentostatin, and thioguanine); anti-tumor antibiotics (e.g., anthracyclines, such as daunorubicin, doxorubicin, epirubicin, and idarubicin; and actinomycin-D, bleomycin, mitomycin-C, and mitoxantrone); topoisomerase inhibitors (e.g., topoisomerase I inhibitors such as topotecan and irinotecan; and topoisomerase II inhibitors, such as etoposide, teniposide, and mitoxantrone); mitotic inhibitors (e.g., taxanes, such as paclitaxel and docetaxel; epothilones such as ixabepilone; Vinca alkaloids, such as vinblasteine, vincristine, and vinorelbine; and estramustine); corticosteroids (e.g., prednisone, methylprednisolone, and dexamethasone); proteosome inhibitors (e.g., bortezomib); targeted therapies (e.g., imatinib, gefitinib, sunitinib, rituximab, alemtuzumab, trastuzumab, and bortezomib); differentiating agents (e.g., retinoids, tretinoin, and bexarotene); and hormonal agents (e.g., anti-estrogens, such as fulvestrant, tamoxifen, and toremifene; aromatase inhibitors, such as anastrozole, exemestane, and letrozole; progestins, such as megestrol acetate; estrogens; anti-androgens, such as bicalutamide, flutamide, and nilutamide; gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH) agonists or analogs, such as leuprolide and goserelin).
In another example, one or more compounds of the present invention having A3 receptor agonist activity, when used to treat an inflammatory disease or condition (e.g., comprising osteoarthritis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, liver inflammation, lung inflammation, brain inflammation) may be used in combination with one or more agents that include, but are not limited to, non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, naproxen, ketoprofen, flurbiprofen, misoprostol, diclofenac potassium, diclofenac sodium, sulindac, oxaprozin, diflunisal, piroxicam, indomethacin, etodolac, meclofenamate, meloxicam, fenoprofen, mefanamic acid, nabumetone, and tolmetin); and COX-2 inhibitors (e.g., celecoxib).

In another example, one or more compounds of the present invention having A3 receptor agonist activity, when used to treat an ischemic disease or condition (e.g., comprising cardiac ischemia and reperfusion injury) may be used in combination with one or more agents that include, but are not limited to, aspirin, nitroglycerin, beta-blockers (e.g., acebutolol, atenolol, bisoprolol, metoprolol, nadolol, nebivolol, and propranolol), cholesterol lowering medications (statins (e.g., lovastatin, amlodipine and atorvastatin, rosuvastatin, sitagliptin/simvastatin, fluvastatin, atorvastatin, pitavastatin, and pravastatin); niacin; fibrates; and bile acid sequestrants), calcium channel blockers (e.g., amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, nisoldipine, and verapamil); angiotensin converting enzyme inhibitors (e.g., benazepril, captopril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, and trandolapril); and ranolazine.

The pharmaceutical composition according to the present invention may be administered once, or multiple times, as needed, to deliver a medically effective amount of the composition, e.g., an amount effective to mediate modulation of a disease, disorder, or condition by activating A3 receptor on cells in the individual receiving the composition. For example, a medically effective amount of the composition comprising the compound of the present invention may be an amount that enters into cells which are contacted with the compound, and which results in activating A3 receptor on the cells. Such a medically effective amount of the composition will depend on such factors as the mode of administration, the formulation for administration, disease to be modulated, the size and health of the individual to receive such a composition, and other factors which can be taken...
into consideration by a medical practitioner whom is skilled in the art of determining appropriate dosages for treatment. An amount of compound of the present invention in the composition to be administered may vary from 0.01 milligrams to about 500 milligrams, and more typically from about 1 milligram per day to about 200 milligram per day. One skilled in the art can apply known principles and models of drug delivery and pharmacokinetics to ascertain a likely range of dosages to be tested in preclinical and clinical studies for determining a medically effective amount of a compound of the invention. The pharmaceutically acceptable carrier, used in the composition of the present invention may facilitate one or more of storage, stability, administration, and delivery, of the composition. The carrier may be particulate, so that the composition may be in, for example, powder or solid form. The carrier may be in a semi-solid, gel, or liquid formula, so that the composition may be ingested, injected, applied, or otherwise administered. The carrier may be gaseous, so that the composition may be inhaled.

For oral administration of the composition containing the compound of the present invention, suitable formulations may be presented in the form of tablets, caplets, capsules, and the like, in which typically the compound of the invention may be present in a predetermined amount as a powder, granules, solution, or suspension as the sole active agent, or in combination with an additional one or more pharmaceutical agents. As known in the art, such oral formulations typically involve one or more of a binder (e.g., syrup, sorbitol, gum, corn starch, gelatin, and acacia), a filler (e.g., lactose, sugar, starch, calcium phosphate), an excipient (e.g., dicalcium phosphate), a disintegrating agent (e.g., vegetable starch, alginic acid), a lubricant (e.g., magnesium stearate), a flavoring agent (sweetening agent, natural or artificial flavors). Such oral formulations may be coated or uncoated to modify their disintegration and/or absorption. Coating may be performed using conventional coating agents and methods known in the art.

The mode of administration of the compound or composition of the present invention to an individual (such as a human) in need of such composition or compound may be any mode known in the art to be suitable for delivering a pharmaceutical composition, and particularly suitable for treating a disease, disorder or condition by activating A3 receptors, and may include but is not limited to, intravenously, intraperitoneally, orally, subcutaneously,
intramuscularly, intranasally, transdermally, by perfusion, and by peristaltic
techniques. The compositions of the present invention may also be combined
with other therapies, such as one or more additional pharmaceutical agents, to
treat a disease, disorder or condition. Such combination therapy may be
administered in concurrently, sequentially, or in regimen alternating between the
composition of the invention and the other therapy. Such combination therapies
may include the following treatments: a compound of Formula (I) with one or
more additional chemotherapeutic agents, for treating cancer; a compound of
Formula (I) with one or more anti-inflammatory agents (e.g., non-steroidal anti-
inflammatory drugs, COX-2 inhibitors, and a combination thereof) for treating
inflammation; a compound of Formula (I) with one or more agents for treating
cardiac ischemia and reperfusion injury that include, but are not limited to, aspirin,
nitroglycerin, beta-blockers, cholesterol lowering medications, calcium channel
blockers, angiotensin converting enzyme inhibitors, ranolazine, and a
combination thereof. The structure of the agents, for combination with the
compound of Formula (I), identified herein, and their generic or trademark names,
are readily available to those skilled in the art, such as from the standard
compendium of drugs (e.g., The Merck Index) or from the applicable
pharmaceutical company's web site, as well as dosages applicable for treatment
(see also The Physician's Desk Reference). Alternatively, the doses and dosage
regimen of an additional pharmaceutical agent, used in conjunction with the
compound of the present invention in combination therapy, can be determined by
a physician, taking into account the medical literature, the health, age and sex of
the patient, the disease or condition or disorder to be treated, the mode of
administration and dosing schedule of the pharmaceutical agent, and other
relevant considerations. Generally, dosages of such agents can range from about
0.1 mg to 1000 mg per day, with more specific dosages dependent on the
aforementioned factors.

Accordingly, provided herein is a pharmaceutical composition or
medicament comprising a medically effective amount of the compound of one or
more of Formula (I), in combination with a medically effective amount of one or
more chemotherapeutic agents, anti-inflammatory drugs, agents for treating
cardiac ischemia and reperfusion injury; and optionally further comprising a
pharmaceutically acceptable carrier. Also provided herein is a pharmaceutical
composition or medicament comprising a medically effective amount of the compound of one or more of Formula (I), and a pharmaceutically acceptable carrier.

Further, the present invention provides a method for preventing or treating cancer, inflammation, cardiac ischemia or reperfusion injury in a mammal, which comprises administering the compound of formula (I), or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof to the mammal.

Furthermore, the present invention provides a method for preventing or treating cancer, inflammation, cardiac ischemia or reperfusion injury in a mammal, comprising the steps of: (i) administering to the mammal in need thereof a first composition comprising a compound of Formula (I), and a pharmaceutically acceptable carrier; and (ii) administering to the mammal in need thereof a second composition comprising at least one additional pharmaceutical agent comprising one or more selected from the group consisting of a chemotherapeutic agent, anti-inflammatory agent, and an agent for treating cardiac ischemia and reperfusion injury.

In these methods, one or more compounds of the invention may be administered in a medically effective amount as the sole pharmaceutical agent, or may be administered in combination therapy wherein a medically effective amount of a compound of the invention is administered with a medically effective amount of at least one additional pharmaceutical agent. Such combination therapy may comprise (a) a single pharmaceutical composition comprised of a compound of formula (I), at least one additional pharmaceutical agent, and a pharmaceutically acceptable carrier; or (b) two separate pharmaceutical compositions, which can be administered simultaneously or sequentially, comprising a first composition comprising a compound of the invention and a pharmaceutically acceptable carrier; and a second composition comprising at least one additional pharmaceutical agent and a pharmaceutically acceptable carrier.

In addition, the present invention provides a use of a compound of Formula (I), or a pharmaceutically acceptable salt, a prodrug, a hydrate, a solvate, or an isomer thereof for the manufacture of a medicament for preventing or treating a cancer, inflammation, cardiac ischemia or reperfusion injury.
General Methods of Synthesis and Testing

The general methods for preparing the compounds of this invention are illustrated in the following schemes (in case $R_1$ is $NR^{11}R^{12}$).

**<Scheme I>**

![Scheme I]

**EXAMPLES**

Herein after, the present invention is described in more detail. The following Examples are given for the purpose of illustration only, and are not intended to limit the scope of the invention.

**Example 1: Preparation of $(2^\alpha,3^\alpha,4\alpha,5\alpha)$-3,4-dihydroxy-5-((3-iodobenzyl)amino)imidazo[2,1-$\alpha$][1,2,4]triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide**

$<$1-1$>$ Preparation of $(3R,4R,5R)\alpha$-(4-aminoimidazol-$2,1-$/)[[1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (2)
A flask equipped with a thermocouple was added with 7-bromoimidazo[2,1-][1,2,4]triazin-4-amine (500 mg, 2.34 mmol, PharmaBlock) and anhydrous THF (5 mL). The reaction mixture was cooled down to 0 °C and treated dropwise with a solution of MeMgCl in THF (3 M, 0.78 mL). The rate of addition was controlled to maintain internal temperature below 10 °C. After completion of addition, the reaction mixture was cooling to 0 °C, treated with 1,2-bis(chlorodimethylsilyl)ethane (503 mg, 2.34 mmol) in a single portion and dropwise with the second portion of MeMgCl solution (3 M, 0.78 mL) while internal temperature was kept below 10 °C. The reaction mixture was cooled down to 0 °C again and treated with a solution of i-PrMgCl-LiCl in THF (1.3 M, 2.0 mL). The reaction mixture was stirred at room temperature, and the disappearance of 7-bromoimidazo[2,1-][1,2,4]triazin-4-amine was checked with TLC. When about 95% of starting material was disappeared, the reaction mixture was cooled down to 0 °C and treated with a solution of (3R,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)dihydrofuran-2(3H)-one (1.96 g, 4.67 mmol) in THF (2.5 mL) via cannula. The resulting orange solution was stirred at room temperature for 12 h. The reaction was quenched with aq. 13% NH₄Cl (5 mL), and resulting mixture was diluted with EtOAc (20 mL). Phases were separated, and the organic layer was dried (Na₂SO₄) and concentrated under reduced pressure to provide crude product. Flash chromatography (SiO₂, EtOAc) afforded 2 as a colorless oil (560 mg, 45%).

MS (EI) for C₃₁H₃₁N₅O₅ (M+H)⁺, (calcd: 554.2); found 554.3.

<1-2> Preparation of 7-((2S,3S,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)imidazor2. 2,41triazin-4-amine (3)
To a stirred solution of the compound 2 (1.0 g, 1.81 mmol) in CH₂Cl₂ (25 mL) at 0 °C was added boron trifluoride etherate (1.3 mL, 10.84 mmol) and triethylsilane (2.3 mL, 14.45 mmol). The reaction mixture was stirred at room temperature for 16 h and diluted with CH₂Cl₂ (30 mL) and saturated aq. NaHCO₃ (30 mL). Phases were separated, and the organic layer was washed with water, saturated aq. NH₄Cl and brine, dried over MgSO₄ and concentrated under reduced pressure to provide crude product. Flash chromatography (SiO₂, 3:1 EtOAc:hexane) to give the compound 3 as a yellow solid (675 mg, 70%).

**1H NMR** (400 MHz, methanol-d₆): 7.98 (s, 1H), 7.46 (s, 1H), 7.36-7.26 (m, 11H), 7.21-7.19 (m, 4H), 5.45 (d, J = 5.2 Hz, 1H), 4.68-4.44 (m, 7H), 4.44 (t, J = 5.0 Hz, 1H), 4.29-4.27 (m, 1H), 4.20 (t, J = 5.2 Hz, 1H), 3.71 (dd, J = 10.8, 4.0 Hz, 1H), 3.62 (dd, J = 10.8, 4.4 Hz, 1H).

**Preparation of 7-((2S,3S,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)-4-chloroimidazo[1,2,4]triazine (4)**

To a solution of the compound 3 (400 mg, 0.74 mmol) in CH₂Cl₂ (10 mL) at 0 °C was added dropwise TMSCI (0.19 mL, 1.49 mmol) and t-butyl nitrite (0.28 mL, 3.72 mmol). The reaction mixture allowed to warm at room temperature 12 h, and the reaction was quenched saturated aq. NaHCO₃ (30 mL). Resulting mixture was extracted with CH₂Cl₂ (50 mL). Organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The
residue was purified with flash chromatography (SiO$_2$, 2:1 EtOAc:hexane) to provide the compound 4 as a yellow solid (100 mg, 24%).

**NMR** (400 MHz, DMSO-d$_6$) : $\delta$ 8.80 (s, 1H), 8.00 (s, 1H), 7.38-7.27 (m, 11H), 7.26-7.21 (m, 4H), 5.46 (d, $J = 5.2$ Hz, 1H), 4.64-4.50 (m, 7H), 4.26-4.24 (m, 1H), 3.69 (dd, $J = 10.8, 3.6$ Hz, 1H).

Preparation of 7-((2S,3S,4R,5S)-2-(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)-N-(3-iodobenzyl)imidazo[2,1-f][1,2,4]triazin-4-amine (5)

![Chemical structure of 4](image)

To a solution of the compound 4 (25 mg, 0.04 mmol) in ethanol (5 mL) was added 3-iodobenzylamine (63 mg, 0.27 mmol). The reaction mixture was stirred at 81 °C for 3 h. The reaction mixture concentrated under reduced pressure, and the residue was purified with flash chromatography (SiO$_2$, 1:5 EtOAc:hexane) to afford the compound 5 as a colorless oil (22 mg, 65%).

**NMR** (400 MHz, DMSO-d$_6$) : $\delta$ 9.39 (t, $J = 5.2$ Hz, 1H), 8.12 (s, 1H), 7.75 (brs, 1H), 7.61 (d, $J = 8.0$ Hz, 1H), 7.50 (s, 1H), 7.38-7.19 (m, 16H), 7.12 (t, $J = 8.0$ Hz, 1H), 5.35 (d, $J = 5.2$ Hz, 1H), 4.68-4.48 (m, 8H), 4.45 (t, $J = 5.0$ Hz, 1H), 4.21-4.15 (m, 2H), 3.65 (dd, $J = 10.8, 3.6$ Hz, 1H).

Preparation of (2R,3S,4R,5S)-2-(hydroxymethyl)-5-(4-((3-iodobenzyl)amino)imidazo[2,1-f][1,2,4]triazin-7-yl)tetrahydrofuran-3,4-diol (6)
A solution of the compound 5 (148 mg, 0.20 mmol) in anhydrous CH₂Cl₂ (5 mL) at -78 °C under N₂ was treated with a solution of boron trichloride in CH₂Cl₂ (IN, 0.8 mL). The reaction mixture stirred at -78 °C for 1 h, and the reaction was quenched with methanol (5 mL). Resulting mixture was warm to room temperature and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:10 MeOH:CH₂Cl₂) to give the compound 6 as a white powder (53 mg, 56%).

¹H NMR (400 MHz, methanol^δ) : S 8.27. (s, 1H), 7.83-7.80 (m, 2H), 7.65 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.13 (t, J = 8.0 Hz, 1H), 5.25 (d, J = 6.0 Hz, 1H), 4.83 (s, 2H), 4.50 (t, J = 5.8 Hz, 1H), 4.20 (t, J = 4.8 Hz, 1H), 4.05-4.04 (m, 1H), 3.81 (dd, J = 12.0, 3.2 Hz, 1H), 3.70 (dd, J = 12.0, 4.0 Hz, 1H).

<1-6> Preparation of ((3aR,4R,6S,6aSV6-i4-(Y3-iodobenzyl)amino)imidazof2, 1-f\1,2,4]triazin-7-yl)-2,2-dimethyltetrahydrofurol3,4-dl[1,3]dioxol-4-y]methanol (7)
To a solution of the compound 6 (53 mg, 0.11 mmol) in acetone (5 mL) at room temperature were added 2,2-dimethoxy propane (206 mg, 1.97 mmol) and camphor-10-sulfonic acid (25 mg, 0.11 mmol). The reaction mixture was stirred for 2 h and quenched with NaHCO$_3$ (28 mg, 0.33 mmol). Resulting mixture was stirred for 1 h and diluted with H$_2$O (30 mL) and chloroform (20 mL). Phases were separated, and organic phase was washed with saturated aq. NaHCO$_3$ (30 mL) and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO$_2$, 1:1 EtOAc:hexane) to afford the compound 7 as a white powder (53 mg, 93%).

H NMR (400 MHz, methanol-d$_4$): $\delta$ 8.13 (s, 1H), 7.78 (brs, 1H), 7.64-7.61 (m, 2H), 7.40 (d, $J = 7.6$ Hz, 1H), 7.11 (t, $J = 7.8$ Hz, 1H), 5.31 (d, $J = 4.8$ Hz, 1H), 5.16-5.13 (m, 1H), 4.86-4.84 (m, 1H), 4.77 (s, 2H), 4.17 (q, $J = 4.1$ Hz, 1H), 3.74-3.64 (m, 2H), 1.59 (s, 3H), 1.37 (s, 3H).

Preparation of (3αS,4S,6αS,6aαS)-6-(4-ri3-iodobenzylamino)imidazo[2, 1-f\\1,2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-f][1,3]dioxole-4-carboxylic acid (8)

A mixture of the compound 7 (53 mg, 0.10 mmol), bis(acetoxy)iodobenzene (BAIB, 72 mg, 0.22 mmol) and 2,2,6,6-tetramethylpiperidine-l-oxyl (TEMPO, 3 mg, 0.02 mmol) in 1:1 CH$_3$CN:H$_2$O (4 mL) was stirred at room temperature for 5 h. The reaction mixture was diluted with CH$_2$Cl$_2$ (15 mL) and water (15 mL). Phases were separated, and the aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 15 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated under reduced pressure to provide the compound 8 as yellow oil (54 mg, 100%).
**H** NMR (400 MHz, methanol-\(d_4\)): \(\delta\) 8.09 (s, 1H), 7.78 (s, 1H), 7.72 (dd, \(J = 8\) Hz, 1.2 Hz, 1H), 7.63 (dd, 1H), 7.41 (d, \(J = 7.6\) Hz, 1H), 7.13 (m, 1H), 5.54 (m, 1H), 5.37 (m, 1H), 5.37 (dd, \(J = 6.0\) Hz, 2.0 Hz, 1H), 5.26 (dd, \(J = 6.4\) Hz, 1.6 Hz, 1H), 4.77 (s, 2H), 4.53 (m, 1H), 1.56 (s, 3H), 1.38 (s, 3H).

**<l-8>** Preparation of (2\(S\),3\(S\),4\(R\),5\(S\))-ethyl 3,4-dihydroxy-5-(4-(Y3-iodobenzyl)amino)imidazo[2,1-f]1,2,4-triazin-7-yl)tetrahydrofuran-2-carboxylate (9)

To a solution of the compound 8 (40 mg, 0.07 mmol) in anhydrous ethanol (5 mL) at 0 °C was added SOCl\(_2\) (43 mg, 0.37 mmol). The reaction mixture was stirred at room temperature for 15 h and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO\(_2\), 1:1 EtOAc:hexane) to give the compound 9 as a colorless oil (25 mg, 64%).

**H** NMR (400 MHz, methanol-\(d_4\)): \(\delta\) 8.12 (s, 1H), 7.77 (brs, 1H), 7.74 (s, 1H), 7.61 (d, \(J = 8.0\) Hz, 1H), 7.40 (d, \(J = 7.2\) Hz, 1H), 7.10 (t, \(J = 7.8\) Hz, 1H), 5.42 (d, \(J = 5.6\) Hz, 1H), 4.77 (s, 2H), 4.50-4.44 (m, 2H), 4.39 (t, \(J = 4.6\) Hz, 1H), 4.24 (dd, \(J = 7.2\) Hz, 1.6 Hz, 2H), 1.29 (t, \(J = 7.2\) Hz, 3H).

**<l-9>** Preparation of (2\(S\),3\(S\),4\(R\),5\(S\))-3,4-dihydroxy-5-(4-(T3-iodoebenzyl)amino)imidazo[2,1-f]1,2,4-triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide (10)
A flask containing the compound 9 (25 mg, 0.05 mmol) was treated with a solution of methylamine in methanol (2M, 2 mL). The reaction mixture was stirred at -20 °C for 3 h. Volatiles were removed under reduced pressure, and the residue was purified with flash chromatography (SiO₂, 1:20 MeOH:CH₂Cl₂) to give the compound 10 as a white powder (15 mg, 63%).

**H NMR (400 MHz, methanol-d₄):** δ 8.21 (s, 1H), 7.78 (brs, 1H), 7.66 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 5.23 (d, J = 8.8 Hz, 1H), 4.79 (d, J = 2.8 Hz, 2H), 4.63-4.59 (m, 1H), 4.39 (d, J = 3.6 Hz, 1H), 4.29 (dd, J = 5.0, 1.8 Hz, 1H), 2.84 (s, 3H). MS (EI) for C₉H₁₄N₂O₅(M+H)⁺ (calcd: 511.0); found 511.0.

**Example 2:** Preparation of (2S,3S,4R,5R)-5-cyano-3,4-dihydroxy-5-(4-((3-iodobenzyI)amino)imidazo[2,l-f][1,2,4]triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide

To a stirred solution of the compound 2 (2.2 g, 3.97 mmol) in CH₂Cl₂ (50 mL) at 0 °C was added trimethylsilyl trifluoromethanesulfonate (4.3 mL, 23.82 mmol) and trimethylsilyl cyanide (2.5 mL, 19.85 mmol). The reaction
mixture was stirred at room temperature for 16 h and diluted with CH$_2$Cl$_2$ (30 mL) and saturated aq. NaHCO$_3$ (50 mL). Phases were separated, and the organic layer is washed sequentially with water, saturated aq. NH$_4$Cl and brine, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO$_2$, 3:1:1 EtOAc:hexane:CH$_2$Cl$_2$) to result in an isomeric mixture of the compound 11 (1.4 g, 62%).

MS (EI) for C$_{32}$H$_{30}$N$_6$O$_4$ (M+H)$^+$, (calcd: 563.2); found 563.0.

<2-2> Preparation of (3R,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-2-(4-chloroimidazo[2,1-f][1,2,4]triazin-7-yl)tetrahydrofuran-2-carbonitrile (12)

To a solution of the compound 11 (144 mg, 0.25 mmol) in anhydrous CH$_2$Cl$_2$ (4.5 mL) at 0 °C was added dropwise TMSCI (0.12 mL, 1.02 mmol) and t-butyl nitrite (0.18 mL, 2.56 mmol). The reaction mixture was stirred at room temperature for 12 h, and the reaction was quenched with saturated aq. NaHCO$_3$ (15 mL). The resulting mixture was extracted with CH$_2$Cl$_2$ (10 mL). The organic extract was washed with brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO$_2$, 1:1 EtOAc:CH$_2$Cl$_2$) to give the compound 12 as an isomeric mixture (78 mg, 52%).

MS (EI) for C$_{32}$H$_{28}$ClN$_5$O$_4$ (M+H)$^+$, (calcd: 582.1); found 582.4.

<2-3> Preparation of (3R,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-2-(4-(f3-iodobenzyl)amino)imidazo[2,1-f][1,2,4]triazin-7-yl)tetrahydrofuran-2-carbonitrile (13)
To a solution of the compound 12 (634 mg, 1.1 mmol) in ethanol (150 mL) was added 3-iodobenzylamine (0.87 mL, 6.5 mmol). The reaction mixture was stirred at 80 °C for 1 h and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:2 EtOAc:hexane) to give the compound 13 as an isomeric mixture (950 mg, 88%).

MS (EI) for C₃₉H₃₅N₆O₄ ((M+H)⁺, (calcd: 779.2) found 779.0.

<2-4> Preparation of (2R3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)-2-(4-((3-iodobenzyl)amino)imidazo[2, 1-f|1,2,4]triazin-7-yl)tetrahydrofuran-2-carbonitrile (14)

A solution of the compound 13 (950 mg, 1.23 mmol) in anhydrous CH₂Cl₂ (50 mL) at -78 °C under N₂ was treated with a solution of boron trichloride in CH₂Cl₂ (1 M, 7 mL). The reaction mixture was stirred at same temperature for 1 h, and the reaction was quenched with methanol (10 mL). The resulting mixture was allowed to warm to room temperature and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:19 methanol :CH₂Cl₂) to afford the compound 14 as a white powder (53 mg, 56%).
NMR (400 MHz, methanol-\(\text{d}L\)): \(\delta\) 8.14 (s, 1H), 7.77 (s, 1H), 7.75 (s, 1H), 7.60 (d, 1H), 7.39 (d, 1H), 7.08 (t, \(J = 8.0\) Hz, 1H), 4.76 (s, 2H), 4.25-4.22 (m, 2H), 3.82 (dd, 1H), 3.73 (dd, 1H).

Preparation of (3aR,4R,6R,6aR)-\((\text{hydroxymethyl})V4-(4-((3-iodobenzyl)amino)imidazo[2,1-f][1,2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuror3,4-dirL31dioxole-4-carbonitrile (15)

To a solution of the compound 14 (370 mg, 0.73 mmol) in acetone (50 mL) at room temperature were added 2,2-dimethoxy propane (1.6 mL, 13 mmol) and camphor-10-sulfonic acid (170 mg, 0.73 mmol). The reaction mixture was stirred at room temperature for 8 h, and the reaction mixture was quenched with saturated aq. NaHCO\(_3\). The resulting mixture was diluted with chloroform (20 mL), and phases were separated. Organic layer was washed with brine, dried over Na\(_2\)SO\(_4\) and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO\(_2\), 3% methanol-\(\text{CH}_2\text{Cl}_2\)) to provide the compound 15 as a white powder (280 mg, 70%).

NMR (400 MHz, CDC13): \(\delta\) 8.15 (s, 1H), 7.81 (s, 1H), 7.69 (s, 1H), 7.63 (d, \(J = 4.4\) Hz, 1H), 7.29 (d, \(J = 8.4\) Hz, 1H), 7.07 (t, \(J = 7.6\) Hz, 1H), 5.30 (m, 1H), 5.21 (dd, \(J = 6.8\) Hz, 2.4 Hz, 1H), 4.78 (d, 2H), 4.66 (d, 1H), 4.22 (d, 1H), 3.97 (d, 1H), 3.84 (d, 1H), 1.78 (s, 3H), 1.37 (s, 3H).

Preparation of (3aS,4S,6R,6aR)-6-cvano-6-((3-iodobenzyl)amino)imidazor2J-/irL2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuror3,4-dirL31dioxole-4-carboxylic acid (16)
A mixture of the compound 15 (280 mg, 0.51 mmol), BAIB (360 mg, 1.12 mmol) and TEMPO (16 mg, 0.10 mmol) in 1:1 CH₃CN:H₂O (5 mL) was stirred at room temperature for 1 h. The reaction mixture was diluted with CH₂Cl₂ (15 mL) and treated with water (15 mL). Phases were separated, and the aqueous layer was extracted with CH₂Cl₂ (2 x 15 mL). Combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to provide the crude the compound 16 as a yellow oil (470 mg, 100%), which used in the next step without purification.

H NMR (400 MHz, methanol-d₄) : δ 8.12 (s, 1H), 7.74 (s, 1H), 7.59 (m, 1H), 7.39 (m, 1H), 7.32 (t, 1H), 7.09 (t, 1H), 5.51 (m, 1H), 5.48 (m, 1H), 4.79 (m, 1H), 4.73 (m, 2H), 1.65 (s, 3H), 1.41 (s, 3H).

Preparation of (2S,3S,4R,5S)-ethyl 3,4-dihydroxy-5-(4-(Y3-iodobenzyl)amino)imidazo[2,1-f][1,2,4]triazin-7-yl)tetrahydrofuran-2-carboxylate (17)
To a solution of crude the compound 16 (470 mg, 0.84 mmol) in anhydrous ethanol (80 mL) at 0 °C was added SOCl₂ (0.3 mL, 4.2 mmol). The reaction mixture was stirred at room temperature for 15 h. Volatiles were removed under reduced pressure, and the residue was purified with flash chromatography (SiO₂, 1:2 EtOAc:hexane) to afford the compound 17 as a colorless oil (292 mg, 63%).

¹H NMR (400 MHz, CDC13): δ 8.30 (s, 1H), 8.09 (s, 1H), 7.95 (brs, 1H), 7.75 (s, 1H), 7.65 (d, 1H), 7.38 (d, 1H), 7.10 (t, 1H), 4.96 (s, 1H), 4.87 (m, 2H), 4.70 (m, 1H), 4.60 (m, 1H), 4.20 (q, J = 7.2 Hz, 2H), 2.88 (s, 3H), 1.25 (t, J = 7.2 Hz, 3H).

<2-8> Preparation of (2S,SS,4R,SR)-5-cyano-3,4-dihydroxy-S-(4-((3-iodobenzyl)aminylimidazo[2, 1-f]1,2,4-triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide (18)

The compound 17 (292 mg, 0.53 mmol) in a flask was treated with a solution of methylamine in methanol (2 M, 40 mL) and stirred at -20 °C for 3 h. Upon completion of reaction, volatiles were removed under reduced pressure, and the residue was purified with flash chromatography (SiO₂, 1:19 methanol:CH₂Cl₂) to give the compound 18 as a white powder (123 mg, 43%).

¹H NMR (400 MHz, methanol-d₄): δ 8.19 (s, 1H), 7.83 (s, 1H), 7.75 (s, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 7.6 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H), 4.98 (d, J = 5.2 Hz, 1H), 4.77 (d, J = 2.8 Hz, 2H), 4.60 (d, J = 2.8 Hz, 1H), 4.38 (dd, J = 5.2 Hz, 2.4 Hz, 1H), 2.78 (s, 3H). MS (EI) for C₁₉H₁₈IN₇O₄ (M+H)⁺, (calcd; 536.05); found 536.05.
Example 3: Preparation of (2S,3S,4R,5S)-3,4-dihydroxy-5-(4-((3-iodobenzyl)amino)pyrrolo[2,1-\text{\textgreek{f}}][1,2,4]triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide

Preparation of (3R,4R,5R)-2-(4-aminopyrrolor2-f][1,2,4]triazin-7-yl)-3^-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (20)

A flask equipped with a thermocouple was added with 7-bromopyrrole[2,1-\text{\textgreek{f}}][1,2,4]triazin-4-amine (1 g, 4.69 mmol) and anhydrous THF (10 mL). The reaction mixture was cooled down to 0 °C and treated dropwise with a solution of MeMgCl in THF (3 M, 1.57 mL). The rate of addition was controlled to maintain internal temperature below 10 °C. After completion of the addition, the reaction mixture was cooled to 0 °C, treated with 1,2-bis(chlorodimethylsilyl)ethane (1.0 g, 4.69 mmol) in a single portion and dropwise with the second portion of MeMgCl solution (3 M, 1.57 mL) while internal temperature was kept below 10 °C. The reaction mixture was cooled down to 0 °C again and treated with a solution of /-PrMgCl-LiCl in THF (1.3 M, 4.71 mL). The resulting dark solution was allowed to warm to room temperature, and the conversion was checked by TLC. Once the conversion of 7-bromomimidazo[2,1-\text{\textgreek{f}}][1,2,4]triazin-4-amine was > 95% complete (6 h), the solution was cooled down to 0 °C, and treated with a solution of (3R,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)dihydrofuran-2(3 \text{H})-one (2.56 g, 6.1 mmol) in THF (5 mL) via cannula. The resulting orange solution was stirred at room temperature for 12 h, and the reaction was found to be complete by TLC. At this point,aq. 13% NH\textsubscript{4}Cl (10 mL) was added, and phased were separated. Organic layer was added with EtOAc (40 mL), dried over NaSO\textsubscript{4} and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO\textsubscript{2}, 100% EtOAc) to afford the compound 20 (650 mg, 26%) as a yellow oil.

MS (EI) for C\textsubscript{32}H\textsubscript{32}N\textsubscript{4}O\textsubscript{5} (M+H\textsuperscript{+}), (calcd; 553.2); found 553.3.
Preparation of 7-((2S,3S,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)pyrrolo[2,1-f][1,2,4]triazin-4-amine (21)

To a stirred solution of the compound 20 (0.65 g, 1.21 mmol) in \( \text{CH}_2\text{Cl}_2 \) (17 mL) at -78 °C was added boron trifluoride etherate (0.89 mL, 7.26 mmol) and triethylsilane (1.55 mL, 9.68 mmol), and the reaction mixture was allowed to warm to room temperature for 16 h. The reaction mixture was diluted with \( \text{CH}_2\text{Cl}_2 \) (20 mL) and saturated aq. NaHCO$_3$ (20 mL). Phases were separate, and the organic layer is washed with water, saturated aq. NH$_4$Cl and brine, dried over MgSO$_4$, and concentrated. The residue was purified with flash chromatography (SiO$_2$, 2:1 EtOAc:hexane) to give the compound 21 as a yellow oil (324 mg, 50%).

$^1$H NMR (400 MHz, methanol-d$_4$): $\delta$ 7.74 (s, 1H), 7.3-7.23 (m, 15H), 6.77 (d, $J$ = 4.4 Hz, 1H), 6.61 (d, $J$ = 4.4 Hz, 1H), 5.55 (d, $J$ = 4.8 Hz, 1H), 4.61 (s, 2H), 4.54 (m, 2H), 4.47 (m, 2H), 4.30 (t, $J$ = 4.8 Hz, 1H), 4.12 (t, $J$ = 4.4 Hz, 1H), 3.67 (dd, $J$ = 10.8 Hz, 3.6 Hz, 1H), 3.59 (dd, $J$ = 10.8 Hz, 3.2 Hz, 1H).

Preparation of 7-((2S3S,4R,5R)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)-4-chloropyrrolo[2,1-f][1,2,4]triazine (22)

To a solution of the compound 21 (1 g, 1.86 mmol) in anhydrous \( \text{CH}_2\text{Cl}_2 \) (40 mL) at 0 °C was added dropwise TMSCl (0.5 mL, 3.72 mmol) and t-butyl nitrite (0.70 mL, 9.3 mmol). The reaction mixture was allowed to warm at room
temperature for 12 h. Upon completion of reaction, the reaction mixture was quenched with saturated aq. NaHCO₃, and the resulting mixture was extracted with CH₂Cl₂. The organic layer was washed brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:2 EtOAc:hexane) to afford the compound 22 as an oil (273 mg, 30%).

<3-3> Preparation of 7-([2S,3S,4R,5R]-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)-N-(3-iodobenzyl)pyrrolo[2,1-\(\text{a}\)pyrazin-4-amine (23)

![Chemical structure of compounds 22 and 23]

To a solution of the compound 22 (734 mg, 1.32 mmol) in ethanol (250 mL) was added 3-iodobenzylamine (1.05 mL, 7.92 mmol). The reaction mixture was stirred at 81 °C for 1 h. Upon completion of reaction, the reaction mixture concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:4 EtOAc:hexane) to give the compound 23 as yellow oil (486 mg, 50%).

\(^1\)H NMR (400 MHz, CDCl₃) : δ 7.96 (s, 1H), 7.70 (s, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.34-7.23 (m, 11H), 7.07 (t, J = 7.6 Hz, 1H), 6.63 (d, J = 4.4 Hz, 1H), 6.42 (d, J = 4.4 Hz, 1H), 5.67 (d, 1H), 5.49 (brs, 1H), 4.77-4.72 (m, 3H), 4.54-4.49 (m, 2H), 4.40-4.36 (m, 2H), 4.27 (t, J = 4.8 Hz, 1H), 4.12-4.09 (m, 1H), 3.77 (dd, J = 10.8 Hz, 3.2 Hz, 1H), 3.65 (dd, J = 4.0 Hz, 10.8 Hz, 1H).
A solution of the compound 23 (311 mg, 0.41 mmol) in anhydrous CH₂Cl₂ (20 mL) under N₂ at -78 °C was treated with a solution of boron trichloride in CH₂Cl₂ (IV, 1.23 mL). The reaction mixture stirred at -78 °C for 10 min. When the reaction was complete by TLC, methanol (5 mL) was added to quench the reaction. The resulting mixture was allowed to room temperature and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:1 EtOAc:hexane) to give the compound 24 as a white solid (148 mg, 75%).

H NMR (400 MHz, methanol-d₄): δ 7.96 (s, 1H), 7.79 (s, 1H), 7.72 (m, 1H), 7.42 (m, 2H), 7.19 (m, 1H), 5.59 (d, 1H), (s, 2H), 4.25-4.22 (m, 2H), 3.82 (dd, 1H), 3.73 (dd, 1H).
To a solution of the compound 24 (148 mg, 0.30 mmol) in acetone (30 mL) at room temperature was added 2,2-dimethoxy propane (0.2 mL, 1.53 mmol) and camphor-10-sulfonic acid (70 mg, 0.30 mmol). The reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, the reaction mixture was quenched with saturated aq. NaHCO₃. The resulting mixture was diluted with chloroform (20 mL), and phases were separated. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 1:1 EtOAc:hexane) to give the compound 25 as yellow oil (63.2 mg, 40%).

**1H NMR** (400 MHz, CDC13): δ 7.96 (s, 1H), 7.69 (s, 1H), 7.61 (d, 1H), 7.29 (d, \( J = 7.6 \) Hz, 1H), 7.08 (t, \( J = 7.6 \) Hz, 1H), 6.87 (d, \( J = 4.8 \) Hz, 1H), 6.57 (d, \( J = 4.4 \) Hz, 1H), 5.68 (d, 1H), 5.53 (brs, 1H), 5.02 (m, 1H), 4.78-4.69 (m, 3H), 4.33 (m, 1H), 3.78-3.70 (m, 2H), 1.49 (s, 3H), 1.30 (s, 3H).

**Preparation of (3aS,4S,6S,6aS)-6-(4-rf3-iodobenzylamino)pyrrolo[2J-\( L \)2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuro|3,4-d|1,3dioxole-4-carboxylic acid (26)**
A mixture of the compound 25 (63 mg, 0.12 mmol), BAIB (147 mg, 0.24 mmol) and TEMPO (6.4 mg, 0.0024 mmol) in 1.5 mL of mixture solvent (CH$_3$CN:H$_2$O, 1:1) was stirred at room temperature for 1 hour. Upon completion of reaction, the reaction mixture was diluted with CH$_2$Cl$_2$ (2 mL) and then added with water (2 mL). Phases were separated, and the aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 5 mL). The combined organic layers were dried with Na$_2$SO$_4$ and concentrated under reduced pressure to provide crude the compound 26 as yellow oil (32 mg), which was used in the next step without purification.

MS (EI) for C$_{21}$H$_{21}$IN$_4$O$_5$ (M+H)$^+$, calcd; 537.1; found 537.1.

Preparation of (3aS,4S,6S,6aS)-methyl 6-(4-((3-iodobenzyl)amino)pyrrolo[2,1-$f$/1,2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carboxylate (27)

To a solution of the compound 26 (32 mg, 0.06 mmol) in 1:1 THF:MeOH (1 mL) was added a solution of TMSCHN$_2$ in hexane (1M, 0.26 mL), and the reaction mixture was stirred at room temperature for 3 h. The
solvent was removed under reduced pressure, and the residue was purified with flash chromatography (SiO$_2$, 1:1 EtOAc:hexane) to give the compound 27 as a yellow oil (7 mg, 21%).

$^1$H NMR (400 MHz, CDC13): $\delta$ 7.93 (s, 1H), 7.64 (s, 1H), 7.58 (d, $J = 8.0$ Hz, 1H), 7.26 (d, $J = 7.6$ Hz, 1H), 7.03 (t, $J = 7.6$ Hz, 1H), 6.81 (d, $J = 4.4$ Hz, 1H), 6.52 (d, $J = 4.8$ Hz, 1H), 5.68 (d, $J = 3.6$ Hz, 1H), 5.50 (brs, 1H), 5.08 (d, $J = 6.0$ Hz, 1H), 4.99-4.96 (m, 1H), 4.74-4.65 (m, 3H), 3.73 (s, 3H), 1.40 (s, 3H), 1.26 (s, 3H).

The compound 27 (7 mg, 0.012 mmol) was treated with a solution of methylamine in methanol (2M, 0.5 mL) and stirred at -20 °C for 3 h. Upon completion of the reaction, volatiles were removed under reduced pressure, and the residue was purified with flash chromatography (SiO$_2$, 1:1 EtOAc:hexane) to give the compound 28 as a yellow powder (7 mg, 100%).

$^1$H NMR (400 MHz, CDC13): $\delta$ 7.93 (s, 1H), 7.64 (s, 1H), 7.59 (d, $J = 8.4$ Hz, 1H), 7.26 (d, $J = 7.6$ Hz, 1H), 7.09 (brs, 1H), 7.04 (t, $J = 8.0$ Hz, 1H), 6.93 (d, $J = 4.4$ Hz, 1H), 5.55 (brs, 1H), 5.28-5.23 (m, 3H), 4.91 (m, 1H), 4.73 (m, 2H), 4.51 (s, 1H), 2.83 (d, $J = 5.2$ Hz, 1H), 1.45 (s, 3H), 1.29 (s, 3H).

The compound 27 (7 mg, 0.012 mmol) was treated with a solution of methylamine in methanol (2M, 0.5 mL) and stirred at -20 °C for 3 h. Upon completion of the reaction, volatiles were removed under reduced pressure, and the residue was purified with flash chromatography (SiO$_2$, 1:1 EtOAc:hexane) to give the compound 28 as a yellow powder (7 mg, 100%).

$^1$H NMR (400 MHz, CDC13): $\delta$ 7.93 (s, 1H), 7.64 (s, 1H), 7.59 (d, $J = 8.4$ Hz, 1H), 7.26 (d, $J = 7.6$ Hz, 1H), 7.09 (brs, 1H), 7.04 (t, $J = 8.0$ Hz, 1H), 6.93 (d, $J = 4.4$ Hz, 1H), 5.55 (brs, 1H), 5.28-5.23 (m, 3H), 4.91 (m, 1H), 4.73 (m, 2H), 4.51 (s, 1H), 2.83 (d, $J = 5.2$ Hz, 1H), 1.45 (s, 3H), 1.29 (s, 3H).
The compound 28 (7mg, 0.012 mmol) was dissolved in 1 : 1 TFA:H₂O (2 mL) and stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was diluted with CH₂Cl₂ (2 mL), and the reaction was quenched with saturated aq. NaHCO₃ (2 mL). Phases were separated, and the organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified with flash chromatography (SiO₂, 3% MeOH-CH₂Cl₂) to give the compound 29 as a white solid (3 mg, 50%).

**H NMR (400 MHz, CDCl₃) :** δ 7.92 (s, 1H), 7.64 (s, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.27 (d, J = 7.2 Hz, 1H), 7.04 (t, J = 7.6 Hz, 1H), 6.82 (d, J = 4.4 Hz, 1H), 6.54 (d, J = 4.4 Hz, 1H), 5.76 (m, 2H), 4.74 (m, 2H), 4.49-4.44 (m, 3H), 3.97 (brs, 1H), 3.26 (brs, 1H) 2.79 (m, 3H). MS (EI) for C₁₉H₂₀IN₅O₄ (M+H)⁺, (calcd: 510.1); found 510.06.

**Experimental Examples**

Adenosine receptor assays were done in DiscoverX in California, USA. The activities of the compounds as agonist on adenosine receptors were assay as followings.

For A1 and A3 receptors, the inhibition of folksonlin-induced cAMP production was measured. Cyclopentyladenosine (Discover X) and chloro-IB-MECA (Discover X) were used as standards, respectively.
(1) cAMP Hunter cell lines (DiscoverX) expressing the A1 or A3 receptor were seeded in a total volume of 20 µL into white walled, 384-well microplates and incubated at 37°C.

(2) Media (DMEM w/10% serum, DiscoverX) was aspirated from cells and replaced with 15 µL 2:1 HBSS/10mM Hepes : cAMP XS+ Ab reagent.

(3) Prepare a series of dilution of sample stocks was made to 4X containing EC80 forskolin in media, and added 5 µL and incubated at 37°C for 60 minutes. Final assay vehicle concentration was 1%.

(4) Cells were lysed through incubation with 20 µL cAMP XS+ ED/CL lysis cocktail for one hour followed by incubation with 20 µL cAMP XS+ EA reagent for three hours at room temperature to generate signals. Microplates were read for chemiluminescence using the PerkinElmer EnvisionTM instrument.

For A2A receptor, calcium mobilization was measured using NECA (DiscoverX) as a standard.

Cells were seeded in a total volume of 20 µL into blackwalled, clear bottom, Poly-D-lysine coated 384-well microplates and incubated at 37°C.

Cells were loaded with dye prior to testing by incubation with 20 µL Dye Loading Buffer consisting of 1x Dye, 1x Additive A and 2.5 mM Probenecid in HBSS/20 mM Hepes (Probenecid was prepared fresh) for 30-60 minutes at 37°C.

Prepare a series of dilution of sample stocks were made to 3X, and added 10 ul to cells. Calcium mobilization was monitored on a FLIPR Tetra (MDS).

For A2B receptor, stimulation of cAMP production was measured using NECA as a standard.

(1) cAMP Hunter cell line expressing the A2B receptor were seeded in a total volume of 20 µL into white walled, 384-well microplates and incubated at 37°C.

(2) Media (DMEM w/10% serum, DiscoverX) was aspirated from cells and replaced with 15 µL 2:1 HBSS/10mM Hepes : cAMP XS+ Ab reagent.

(3) Prepare a series of dilution of sample stocks was made to 4X in media, and added 5 µL and incubated at 37°C for 60 minutes. Final assay vehicle concentration was 1%.
Cells were lysed through incubation with 20 µL cAMP XS+ ED/CL lysis cocktail for one hour followed by incubation with 20 µL cAMP XS+ EA reagent for three hours at room temperature to generate signals. Microplates were read for chemiluminescence using the PerkinElmer Envision™ instrument.

Data were analyzed using CBIS data analysis suite (Chemlnnovation, CA).

For A2A and A2B receptor agonist mode assays, percentage activity was calculated using the following formula:

\[
\% \text{Activity} = \frac{100 \% \times (\text{mean RLU of test sample} - \text{mean RLU of vehicle control})}{(\text{mean RLU of MAX control} - \text{mean RLU of vehicle control})}.
\]

For A1 and A3 Gi agonist mode assays, percentage activity was calculated using the following formula:

\[
\% \text{Activity} = 100 \% \times \left(1 - \frac{\text{mean RLU of test sample} - \text{mean RLU of vehicle control}}{(\text{mean RLU of MAX control} - \text{mean RLU of vehicle control})}\right).
\]

Hep-3B Cell proliferation assays were done in Crown Biosciences in Beijing, China.

Hep-3B cells (Crown Biosciences) were cultured in the media supplemented with 10% FBS at 37°C, 5% CO2 and 95% humidity.

(1) Harvest cells and adjust cell concentrations to 5.56 *10^4 cells/ml, and add 90µl cell suspensions to two 96-well plates (A and B) with the final cell density of 5×10^3 cells/well. Incubate seeded plates at 37°C.

(2) Remove cell culture medium next day and replace with 90µl culture medium containing 1% serum. Starve the cells overnight.

(3) Dissolve the test article or reference controls with DMSO. Prepare 200x solution of test article (2 mM) using DMSO. Then, dilute DMSO solution 20-fold with culture medium containing 1% serum to 10x working solution. Prepare 10x reference control solution with culture medium containing 1% serum.
(4) Dispense 10µl drug solution (10x) to each well (triplicate for each concentration) of the plate B. Incubate the test plates for 48h in the humidified incubator at 37°C with 5% CO2.

(5) Equilibrate the plate and its contents at room temperature for approximately 30 minutes. Add a volume of CellTiter-Glo® Reagent equal to the volume of cell culture medium present in each well, mix contents for 2 minutes on an orbital shaker to induce cell lysis, and incubate at room temperature for 10 minutes to stabilize luminescent signal.

(6) Record luminescence using EnVision Multi Label Reader.

(7) In order to calculate IC50, a dose-responsive curve will be fitted using nonlinear regression model with a sigmoidal dose response.

The formula of surviving rate is shown below, and the IC50 was automatically produced by GraphPad Prism 5.0.

\[
\text{The surviving rate} \% = \frac{(\text{Lum}_{\text{Test}} - \text{Lum}_{\text{Medium control}})}{(\text{Lum}_{\text{None treated}} - \text{Lum}_{\text{Medium control}})} \times 100\%.
\]

[Table 1]

<table>
<thead>
<tr>
<th>compound</th>
<th>Adenosine receptor EC50 (uM)</th>
<th>Hepo-3B proliferation IC50 (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound N°</td>
<td>A1              A2A          A2B          A3</td>
<td></td>
</tr>
<tr>
<td>Compound 10</td>
<td>413.2          74.5         801.3        5.23</td>
<td>125</td>
</tr>
<tr>
<td>Compound 18</td>
<td>1263.7         87.4         864.1        29.08</td>
<td>nd</td>
</tr>
</tbody>
</table>

Mouse pharmacokinetics experiments were done in Crown Biosciences in Taican, China, using ICR male mice (DiscoverX). The compounds were formulated in appropriate solutions, and IV and oral (PO.) administration were performed. Blood samples were collected by cardiac puncture over 24 hr time course, and the compounds were quantified by analyzing on LC/MS/MS. The pharmacokinetic parameters were calculated using the software WinNonlin 5.3.
As shown in Table 1, the compounds of the present invention selectively activated Adenosine A3 receptor. Pharmacokinetic data showed that the compounds of the present invention demonstrated improved oral bioavailability but moderate Cm and T1/2 (Table 2). The results indicate that the compounds of the present invention selectively activated Adenosine A3 receptor.
What is claimed is:

1. A compound selected from the group consisting of a compound of Formula 1 below, and pharmaceutically acceptable salts, a prodrug, a hydrate, a solvate, and an isomer thereof:

\[
\text{(I)}
\]

wherein,

Y is oxygen, sulfur, or carbon;

\[X_1\] is H, alkyl, \(R^aR^b\text{NC}(=O)\)- or \(\text{HOR}^c\); wherein said \(R^a\) and \(R^b\) are each independently hydrogen, alkyl, amino, haloalkyl, aminoalkyl, BOC-aminoalkyl, or cycloalkyl, or optionally fuse together to form a heterocyclic ring containing two to five carbon atoms, and said \(R^c\) is alkyl, amino, haloalkyl, aminoalkyl, BOC-aminoalkyl, or cycloalkyl;

\[X_2\] is H, hydroxyl, alkylamino, alkylamido or hydroxyalkyl;

\[X_3\] and \[X_4\] are each independently hydrogen, hydroxyl, amino, amido, azido, halo, alkyl, carboxy, nitrile, nitro, trifluoro, aryl, alkaryl, thio, thioester, thioether, \(-\text{OCOPh}\) or \(-\text{OC}(=\text{S})\text{OPh}\), or both \[X_3\] and \[X_4\] are optionally oxygens connected to \(>\text{C}=\text{S}\) to form a 5-membered ring, or \[X_2\] and \[X_3\] optionally form the ring of formula (III),

\[
\text{(III)}
\]

wherein \(R'\) and \(R''\) represent independently an alkyl group;

\[X_5\] is H, cyano, \(\text{C}_1\text{--}_8\) alkyl, \(\text{C}_2\text{--}_8\) alkenyl, or \(\text{C}_2\text{--}_8\) alkynyl;

\(Z\) is nitrogen or carbon;
R is halogen, NR \textsuperscript{11}R \textsuperscript{12}, N(R \textsuperscript{11})OR \textsuperscript{11}, NR \textsuperscript{11}NR \textsuperscript{11}R \textsuperscript{12}, N\textsubscript{3}, NO, NO\textsubscript{2}, CHO, CN, -CH(=NR \textsuperscript{11}), -CH=NNHR \textsuperscript{11}, -CH=N(OR \textsuperscript{11}), -CH(OR \textsuperscript{11})\textsubscript{2}, -C(=O)NR \textsuperscript{11}R \textsuperscript{12}, -C(=S)NR \textsuperscript{11}R \textsuperscript{12}, -C(=O)OR \textsuperscript{11}, -C(=O)OR \textsuperscript{11}, -C(=S)NR \textsuperscript{11}R \textsuperscript{12}, CN, -CH(=NR \textsuperscript{11}), -CH=NNHR \textsuperscript{11}, -CH=N(OR \textsuperscript{11}), -CH(OR \textsuperscript{11})\textsubscript{2}, -C(=O)NR \textsuperscript{11}R \textsuperscript{12}, -C(=S)NR \textsuperscript{11}R \textsuperscript{12}, -C(=O)OR \textsuperscript{11}, -C(=O)OR \textsuperscript{11}, or SR \textsuperscript{11},

wherein said R \textsuperscript{11} and R \textsuperscript{12} are each independently H, C\textsubscript{1-8} alkyl, C\textsubscript{2-8} alkenyl, C\textsubscript{2-8} alkynyl, C\textsubscript{3-8} carboxycycl, C\textsubscript{4-8} carboxycyclalkyl, aryl-C\textsubscript{1-8} alkyl, heterocyclyl-C\textsubscript{1-8} alkyl, C\textsubscript{6-20} aryl, C\textsubscript{2-20} heterocycl, heteroaryl, -C(=O)-C\textsubscript{1-8} alkyl, or -S(O)\textsubscript{N} C\textsubscript{1-8} alkyl, or R \textsuperscript{11} and R \textsuperscript{12} optionally fuse together with a nitrogen to which they are both attached to form 3 to 7 membered heterocyclic ring, wherein any carbon atom of said heterocyclic ring is optionally replaced with -O-,-S(O)\textsuperscript{a} or -NR \textsuperscript{d}.

wherein, optionally, said C\textsubscript{1-8} alkyl, C\textsubscript{2-8} alkenyl, C\textsubscript{2-8} alkynyl, C\textsubscript{3-8} carboxycycl, C\textsubscript{4-8} carboxycyclalkylalkyl, aryl-C\textsubscript{1-8} alkyl, heterocyclyl-C\textsubscript{1-8} alkyl, C\textsubscript{6-20} aryl, C\textsubscript{2-20} heterocycl, and heteroaryl are each independently substituted with one or more halogen, hydroxyl, CN, N\textsubscript{3}, N(R \textsuperscript{D} \textsubscript{2}), NH(R \textsuperscript{D}), NH\textsubscript{2}, C(O)N(R \textsuperscript{D} \textsubscript{2}), C(O)NH(R \textsuperscript{D}), C(O)NH\textsubscript{2}, OC(O)N(R \textsuperscript{D} \textsubscript{2}), OC(O)NH(R \textsuperscript{D}), OC(O)NH\textsubscript{2}, C(O)OR \textsuperscript{D}, OC(O)R \textsuperscript{D}, C(O)R \textsuperscript{D}, OC(O)R \textsuperscript{D}, S(O)\textsubscript{R}R\textsubscript{D}, S(O)\textsubscript{2}N(R \textsuperscript{D} \textsubscript{2}), S(O)\textsubscript{2}NH(R \textsuperscript{D}), S(O)\textsubscript{2}NH\textsubscript{2}, OR\textsuperscript{P} or R\textsuperscript{P}, wherein R\textsuperscript{D} is C\textsubscript{1-8} alkyl, C\textsubscript{2-8} alkenyl, C\textsubscript{2-8} alkynyl, C\textsubscript{3-8} carboxycycl, C\textsubscript{4-8} carboxycyclalkylalkyl, aryl-C\textsubscript{1-8} alkyl, heterocyclyl-C\textsubscript{1-8} alkyl, C\textsubscript{6-20} aryl, C\textsubscript{2-20} heterocycl, or heteroaryl.

The compound of claim 1,

wherein,

Y is oxygen;

X\textsubscript{1} is H, or R\textsuperscript{A}R\textsuperscript{B}NC(=O)-, wherein said R\textsuperscript{A} and R\textsuperscript{B} are each independently hydrogen, C\textsubscript{1-8} alkyl, amino, haloalkyl, aminoalkyl, BOC-aminoalkyl, or cycloalkyl;

\textbullet{} X\textsubscript{2} is H, hydroxyl, alkylamino, alkylamido or hydroxyalkyl;
X₃ and X₄ are each independently hydrogen, hydroxyl, amino, amido, azido, halo, alkyl, carboxy, nitrile, nitro, trifluoro, aryl, alkaryl, thio, thioester, thioether, -OCOPh or -OC(=S)OPh;

X₅ is H, cyano, C₁₋₈ alkyl, C₂₋₈ alkenyl, or C₂₋₈ alkynyl;

Z is nitrogen or carbon;

Rᵢ is halogen, NR¹¹R₁², N(R¹¹)OR¹¹, NR¹¹NR¹¹R¹², -CH(=NR¹¹), -CH=NNHR¹¹, -CH=N(OR¹¹), -CH(OR¹¹)₂, -C(=O)NR¹¹R¹², or -C(=S)NR¹¹R¹²; and

R² and R³ are each independently H, halogen, NR¹¹R¹², N(R¹¹)OR";

NR¹¹NR¹¹R¹², -CH(=NR¹¹), -CH=NNHR¹¹, -CH=N(OR¹¹), -CH(OR¹¹)₂, -C(=O)NR¹¹R¹², or -C(=S)NR¹¹R¹²,

wherein said R¹¹ and R¹² are each independently H, C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, C₃₋₈ carbocyclyl, C₄₋₈ carbocyclylalkyl, aryl-C₁₋₈ alkyl, heterocyclyl-C₁₋₈ alkyl, C₆₋₂₀ aryl, C₂₋₂₀ heterocyclyl, heteroaryl, -C(=O)-C₁₋₈ alkyl, or -S(O)ₙ-C₁₋₈ alkyl,

wherein, optionally, said C₁₋₈ alkyl, C₂₋₈ alkenyl, and C₂₋₈ alkynyl are each independently substituted with one or more halogen, hydroxyl, CN, or N₃.

3. The compound of claim 1,

wherein,
Y is oxygen;

X₁ is R²R⁵NC(=O)⁻, wherein said R² and R⁵ are each independently hydrogen or C₁₋₈ alkyl;

X₂ is H;

X₃ and X₄ are hydroxyl;

X₅ is H or cyano;

Z is nitrogen or carbon;

Rᵢ is NR¹¹R¹²; and

R² and R³ are each independently H or halogen, wherein said R¹¹ and R¹² are each independently H or phenyl-C₁₋₈ alkyl.

4. The compound of claim 1, which is selected from the group consisting of:
(1) (2S,3S,4R,5S)-3,4-dihydroxy-5-(4-((3-iodobenzyl)amino)imidazo[2,1-f][1,2,4]triazin-7-yl)-N-methyltetrahydrofuran-2-carboxamide;
5. A pharmaceutical composition for preventing or treating cancer, inflammation, cardiac ischemia or reperfusion injury, comprising the compound of claim 1, and a pharmaceutically acceptable carrier.

6. The pharmaceutical composition of claim 5, which further comprises at least one additional pharmaceutical agent.

7. A method for preventing or treating cancer, inflammation, cardiac ischemia or reperfusion injury in a mammal, which comprises administering the compound of claim 1 to the mammal.

8. A method for preventing or treating cancer, inflammation, cardiac ischemia or reperfusion injury in a mammal, comprising the steps of:
   (i) administering to the mammal in need thereof a first composition comprising the compound of claim 1, and a pharmaceutically acceptable carrier; and
   (ii) administering to the mammal in need thereof a second composition comprising at least one additional pharmaceutical agent comprising one or more selected from the group consisting of a chemotherapeutic agent, anti-inflammatory agent, and an agent for treating cardiac ischemia and reperfusion injury.

9. The method of claim 8, wherein the first composition and second composition are administered simultaneously.

10. The method of claim 8, wherein the first composition and second composition are administered sequentially and in any order.
11. The use of the compound of claim 1 for the manufacture of a medicament for preventing or treating a cancer, inflammation, cardiac ischemia or reperfusion injury.