METHODS, ASSAYS AND COMPOUNDS FOR TREATING BACTERIAL INFECTIONS BY INHIBITING METHYLMETHIONINE PHOSPHORYLASE

The present invention discloses methods for treating bacterial infections in a subject comprising administering to the subject a sub-growth inhibiting amount of a 5'-methylmethionine phosphorylase (MTPP) inhibitor, as well as assays for identifying such inhibitors, and compounds and pharmaceutical compositions comprising the inhibitors.

Abstract:

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CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/458,660, filed November 29, 2010.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. GM41916 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates to methods, assay, compounds and compositions for treating infections caused by bacteria that use 5'-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway, such as Pseudomonas aeruginosa.

BACKGROUND OF THE INVENTION

[0004] Throughout this application various publications are referred to by Arabic numerals in parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated by reference in their entireties into the subject application to more fully describe the art to which the subject application pertains.

[0005] Pseudomonas aeruginosa is a Gram-negative bacterium found in a wide range of environments including water, soil, and mammals (1). It is a major opportunistic human pathogen, infecting burns, and lungs in cystic fibrosis (2). Compromised immune systems and extended hospitalization are correlated with infections, making P. aeruginosa the causative agent of approximately 15% of all hospital infections (2, 3). P. aeruginosa infections are difficult to treat since the bacterium has multiple antimicrobial resistance mechanisms (4). Chronic infections can be severe in patients with cystic fibrosis, causing high rates of morbidity and mortality (5, 6). P. aeruginosa infections are related to quorum sensing (QS) pathways, which regulate virulence factors and biofilm formation.
Signal molecules of QS include N-acyl-homoserine lactones (AHLs). The concentration of AHLs increases during bacterial growth to allow AHL binding to specific receptors and the regulation of target genes. In *P. aeruginosa*, the *las* and *rhl* QS systems use AHLs of 3-oxo-C_{12}-homoserine lactone and C_4-homoserine lactone as signal molecules, respectively. Microarray studies on *P. aeruginosa* indicated that QS regulated 3-7% of the total open reading frames (7-9). Deletion of single or multiple QS genes reduced the virulence of *P. aeruginosa* in mouse studies, indicating a strong correlation between the QS system and *P. aeruginosa* pathogenesis (10-15). Quorum sensing blockade does not affect bacterial growth and is therefore expected to attenuate the virulence of infection without causing drug resistance (16, 17).

Potential therapeutic targets in the QS system include enzymes involved in the formation of AHLs that act as signaling molecules and as virulence factors in *P. aeruginosa* (16). AHLs are synthesized from 5-adenosylmethionine (SAM) and acylated-acyl carrier protein by AHL synthase with 5'-methylthioadenosine (MTA) as a by-product. MTA is recycled to ATP and methionine for SAM recycling (17). In bacteria, 5'-methylthioadenosine nucleosidase (MTAN) is the normal path to produce adenine and 5-methylthioribose-a-D-l-phosphate (MTR-l-P) from MTA for recycling. Transition state analogue inhibitors of *E. coli* and *V. cholerae* MTAN disrupted quorum sensing and reduced biofilm formation, supporting MTAN as a target for QS (17).

With the growing global threat of multi-drug resistance, nonconventional antibacterial discovery approaches are required that are nonlethal to bacteria where the potential to develop drug resistance is assumed to be less significant. The present invention addresses that need for infections caused by bacteria such as *Pseudomonas aeruginosa* that use 5'-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway.

**SUMMARY OF THE INVENTION**

The invention provides methods for treating infections caused by bacteria that use 5'-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway comprising administering to a subject having the infection a sub-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor. The invention also provides pharmaceutical compositions comprising a sub-bacterial-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor and a pharmaceutically acceptable carrier.

The invention also provides methods for determining whether or not a compound is a candidate for treating an infection caused by bacteria that use 5'-
methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway, the method comprising determining whether or not the compound inhibits MTIP, wherein a compound that inhibits MTIP is a candidate for treating an infection caused by bacteria that use MTIP in a quorum sensing pathway and wherein a compound that does not inhibit MTIP is not a candidate for treating an infection caused by bacteria that use MTIP in a quorum sensing pathway.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Early and late transition-state mimics as inhibitors for PaMTIP and PjPNP.

Figure 2. Structure-based sequence alignment. Protein are listed by PDB ID. The top 7 sequences are PNPs, the bottom 4 sequences are MTAPs, and PaMTIP is 30ZB. The conserved residues in the phosphate binding site of PNPs (bold font) and MTAPs (bold font) are indicated. The conserved residues in the purine binding site of PNPs and MTAPs are underlined. The conserved Phe ("F") and His ("H") in ribose-binding site of PNPs are in italics. The conserved His and small hydrophobic amino acid in the (methylthio)ribose-binding site of MTAPs are in lower case letters. The residues in columns marked by * are conserved (but not identical) in all species. The Leu ("L") of PaMTIP is not conserved in either MTAPs or PNPs and is in underlined italic. The PDB IDs are as follows: 1YR3, E. coli PNP II (SEQ ID NO:1); 1N3I, Mycobacterium tuberculosis PNP (SEQ ID NO:2); 3KHS, Grouper Iridoviurs PNP (SEQ ID NO:3); 2P4S, Anopheles Gambia PNP (SEQ ID NO:4); 1VMK, Thermotoga maritima PNP (SEQ ID NO:5); 1A9T, bovine PNP (SEQ ID NO:6); 1RR6, human PNP (SEQ ID NO:7); 30ZB, PaMTIP (SEQ ID NO:8); 1V4N, Sulfolobus tokodaii MTAP (SEQ ID NO:9); 1K27, human MTAP (SEQ ID NO:10); 1WTA, Aeropyrum pernix K1 MTAP (SEQ ID NO:11); 2A8Y, Sulfolobus solfataricus MTAP (SEQ ID NO:12).

Figure 3. Metabolism of [8-14C]MTA in P. aeruginosa. P. aeruginosa lysate was incubated with [8-14C]MTA for 0, 10, and 25 min, respectively. The 14C-metabolites MTA, MTI, adenine and hypoxanthine were purified using RP-HPLC and quantitated by scintillation counting. Bar graphs from left to right of figure for hypoxanthine, adenine, MTI and MTA.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a method for treating an infection caused by bacteria that use 5’-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway comprising
administering to a subject having the infection a sub-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor.

[0015] The bacteria can be, for example, Pseudomonas aeruginosa, Pseudomonas syringae or Xanthomonas campestris.

[0016] The subject can be, for example, an animal, such as a mammal, such as a primate, such as a human, or a plant.

[0017] For example, in one embodiment, the bacteria is Pseudomonas aeruginosa and the subject is a human. In another embodiment, for example, the bacteria is Pseudomonas syringae or Xanthomonas campestris and the subject is a plant.

[0018] As used herein, to treat a bacterial infection in a subject means to reduce the virulence of the bacteria in the subject. The term "bacterial infection" shall mean any deleterious presence of bacteria in the subject.

[0019] The term "sub-growth inhibiting amount" of a MTIP inhibitor as used herein means an amount of the inhibitor, which when contacted with a population of bacteria, does not reduce the growth of the bacterial population. Preferably, the sub-growth inhibiting amount of the MTIP inhibitor inhibits quorum sensing in the bacteria. Preferably, the sub-growth inhibiting amount of the MTIP inhibitor is effective to reduce virulence of the bacteria without promoting the development of resistance by the bacteria to the MTIP inhibitor.

[0020] The term "quorum sensing" as used herein refers to the process by which bacteria produce and detect signaling molecules with which to coordinate gene expression and regulate processes beneficial to the microbial community. The term "inhibit quorum sensing" as used herein means altering this process such that coordination of gene expression and process regulation in microbial communities are impaired or prevented.

[0021] The invention also provides a pharmaceutical composition or an agrochemical composition comprising a sub-bacterial-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor and a pharmaceutically or agrochemically acceptable carrier. Preferably, the pharmaceutical composition is formulated in dosage form. Preferably, the sub-bacterial-growth inhibiting amount of the MTAN inhibitor inhibits quorum sensing in bacteria. Preferred bacteria include, for example, Pseudomonas aeruginosa, Pseudomonas syringae and Xanthomonas campestris.

[0022] As used herein, "pharmaceutically acceptable carriers" are materials that (i) are compatible with the other ingredients of the composition without rendering the
composition unsuitable for its intended purpose, and (ii) are suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, and emulsions such as oil/water emulsions and microemulsions.

DEFINITIONS as applied to MTIP Inhibitors:

[0023] The term "alkyl" is intended to include straight- and branched-chain alkyl groups, as well as cycloalkyl groups and having up to 30 carbon atoms, and includes any C1-C25, C1-C20, C1-C15, C1-C10, or C1-C6 alkyl group. Cycloalkyl groups may have one or two carbon atoms substituted by a nitrogen, oxygen or sulfur atom. The same terminology applies to the non-aromatic moiety of an aralkyl radical. Examples of alkyl groups include, but are not limited to: methyl group, ethyl group, w-propyl group, iso-propyl group, w-butyl group, iso-butyl group, sec-butyl group, t-butyl group, w-pentyl group, 1,1-dimethylpropyl group, 1,2-dimethylpropyl group, 2,2-dimethylpropyl group, 1-ethylpropyl group, 2-ethylpropyl group, n-hexyl group and 1-methyl-2-ethylpropyl group. Examples of cycloalkyl group include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, 2-tetrahydrofuranyl, 2-thietaneyl, 3-piperidinyl, 2-pyrrolidinyl and 4-thiacyclohexanyl.

[0024] The term "alkenyl" means any hydrocarbon radical having at least one double bond, and having up to 30 carbon atoms, and includes any C2-C25, C2-C20, C2-C15, C2-C10, or C2-C6 alkenyl group, as well as cycloalkenyl groups, and is intended to include both straight- and branched-chain alkenyl groups. The same terminology applies to the non-aromatic moiety of an aralkenyl radical. Examples of alkenyl groups include but are not limited to: ethenyl group, prop-1-en-1-yl group, prop-2-en-1-yl group, prop-1-en-2-yl group, cis-but-2-en-1-yl group, trans-but-2-en-1-yl group, but-3-en-1-yl group, but-1-en-1-yl group, but-3-en-2-yl group, pent-4-en-1-yl group, 2-methyl-but-3-en-2-yl group, hex-5-en-1-yl group, cyclohex-1-enyl group, cyclohex-2-enyl group and cyclohex-3-enyl group.

[0025] The term "alkynyl" means any hydrocarbon radical having at least one triple bond, and having up to 30 carbon atoms, and includes any C2-C25, C2-C20, C2-C15, C2-C10, or C2-C6 alkynyl group, and is intended to include both straight- and branched-chain alkynyl groups. The same terminology applies to the non-aromatic moiety of an aralkynyl radical. Examples of alkynyl groups include but are not limited to: ethynyl group, prop-1-yn-1-yl group, prop-2-yn-1-yl group, but-1-yn-1-yl group, but-2-yn-1-nyl group, but-3-yn-1-yl group,
pent-l-yn-l-yl group, 3,3-dimethyl-but-l-yn-l-yl group, 2-methyl-but-3-yn-2-yl group and hex-l-yn-l-yl group.

The term "aryl" means an aromatic radical having 1 to 18 carbon atoms and includes heteroaromatic radicals. Examples include monocyclic groups, as well as fused groups such as bicyclic groups and tricyclic groups. Examples include but are not limited to: phenyl group, indenyl group, 1-naphthyl group, 2-naphthyl group, azulenyl group, heptalenyl group, biphenyl group, indacenyl group, acenaphthyl group, fluorenyl group, phenalenyl group, phenanthrenyl group, anthracenyl group, cyclopentacyclooctenyl group, and benzocyclooctenyl group, pyridyl group, pyrrolyl group, pyrazinyl group, pyrimidinyl group, pyrazolyl group, triazolyl group, tetrazolyl group, benzotriazolyl group, pyrazolyl group, imidazolyl group, benzimidazolyl group, indolyl group, isoindolyl group, indolizinyl group, purinyl group, indazolyl group, furyl group, pyranyl group, benzofuryl group, isobenzofuryl group, thienyl group, thiazolyl group, isothiazolyl group, benzothiazolyl group, oxazolyl group, and isoxazolyl group.

The term "aralkyl" means an alkyl radical having an aryl substituent.

The term "aralkenyl" means an alkenyl radical having an aryl substituent.

The term "alkoxy" means an hydroxy group with the hydrogen replaced by an alkyl group.

The term "halogen" includes fluorine, chlorine, bromine and iodine.

The term "prodrug" as used herein means a pharmacologically acceptable derivative of the MTIP inhibitor, such that an in vivo biotransformation of the derivative gives the MTIP inhibitor. Prodrugs of MTIP inhibitors may be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved in vivo to give the parent compound.

As used herein, the structural formulae showing the "wedge" notation, e.g.:

\[\text{\ldots} \]

are intended to represent pure enantiomeric forms of a trans isomer.

Similarly, the structural formulae showing the "rectangular" notation, e.g.:

\[\text{\ldots} \]

are intended to represent racemic mixtures of trans isomers.

It will be appreciated that the representation of the compound of the invention where B is a hydroxy group, is of the enol-type tautomeric form of a corresponding amide, and this will largely exist in the amide form. The enol-type tautomeric representation is
reproduced the referenced patents and patent applications simply to allow direct comparison the compounds of the invention with those in the references.

[0034] In one embodiment, as described in part in U.S. Patent No. 5,985,848 and in PCT International Patent Application Publication No. WO 99/19338, the contents of which are herein incorporated by reference, the MTIP inhibitor comprises a compound having formula (I):

![Chemical Structure](image)

wherein A is CH or N; B is OH; D is chosen from H, OH, NH₂, or SCH₃; and X and Y are independently selected from H, OH or halogen, except that when one of X and Y is hydroxy or halogen, the other is hydrogen; Z is selected from CH₂SQ, CH₂OQ or Q, where Q is an optionally substituted alkyl, alkenyl, aralkyl, aralkenyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C₁-Cₑ alkyl; provided that Q is not CH₂OH; or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0035] Preferably A is CH. Preferably D is H. Preferably X is OH and Y is H. Preferably Z is CH₂SQ. Preferably, when Z is CH₂SQ, Q is alkyl, preferably an optionally substituted C₁-Cₑ alkyl group such as methyl, ethyl, propyl or butyl, or Q is an aryl group such as a phenyl group or an aralkyl group such as a benzyl group. It is also preferred that Q is an alkyl group substituted with one or more amino groups. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups.

[0036] Alternatively preferably, Z is Q. Preferably, when Z is Q, Q is an optionally substituted alkyl group, e.g. a C₁-Cₑ alkyl group such as a methyl, ethyl, propyl or butyl group. It is also preferred that Q is an alkyl group substituted with one or more amino groups. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups.
Alternatively, preferably, Z is Q and Q is an optionally substituted aralkyl group, e.g. a phenylethyl group. In still other preferred embodiments, Q is an optionally substituted aralkenyl group, e.g. a phenylethenyl group, or an optionally substituted phenyl.

It is preferred that Z is selected from the group consisting of: phenylthiomethyl, p-chlorophenylthiomethyl, methylthiomethyl, ethylthiomethyl, propylthiomethyl, e.g. n-propylthiomethyl or isopropylthiomethyl, butylthiomethyl, -CH2,S(CH2)2CH(NH2)COOH, methyl, ethyl, propyl, butyl, benzyl, cis-phenylethenyl, trans-phenylethenyl and -(CH2)nCH3C(NH2)COOH.

Preferably when one or more halogens are present they are chosen from chlorine and fluorine.

In one embodiment, A is CH or N; B is OH; D is chosen from H, OH, NH2, or SCH2; and X is OH; Y is H; Z is Q, where Q is a methyl group optionally substituted with a halogen, a methoxy, an amino or a carboxy group, or Q is an optionally substituted aralkyl, aryl or C1-C6 alkyl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C1-C6 alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

Preferred compounds include those having the formula (II):

where Q is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C1-C6 alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt thereof, or an ester thereof. Preferably, Q is alkyl, preferably a C1-C6 alkyl group such as methyl, ethyl, propyl or butyl, or Q is an aryl group such as a phenyl group or an aralkyl group such as a benzyl group. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups. Preferred compounds include those where Q is methyl or optionally substituted phenyl.

Additional preferred compounds include those having the formula (Ha):
where Q is is a methyl group optionally substituted with a halogen, a methoxy, an amino or a carboxy group, or Q is an optionally substituted aralkyl, aryl or C2-C1₀ alkyl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain Ci-Ce alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt thereof, or an ester thereof. Preferably, Q is alkyl, preferably a Ci-Ce alkyl group such as methyl, ethyl, propyl or butyl, or Q is an aryl group such as a phenyl group or an aralkyl group such as a phenylethenyl group e.g. a cis-phenylethenyl group or a trans-phenylethenyl group. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups.

Examples of compounds of formula (I) include:
7-((2S,3S,4R,5S)-3,4-dihydroxy-5-(phenylthiomethyl)pyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5S)-5-(4-chlorophenylthio)methyl)-3,4-dihydroxypyrrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5S)-3,4-dihydroxy-5-(methylthiomethyl)pyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5S)-5-(ethylthiomethyl)-3,4-dihydroxypyrrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5S)-3,4-dihydroxy-5-(propylthiomethyl)pyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5S)-3,4-dihydroxy-5-(isopropylthiomethyl)pyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5S)-5-(butylthiomethyl)-3,4-dihydroxypyrrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(S)-2-amino-4-(((2S,3R,4S,5S)-3,4-dihydroxy-5-(4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-7-yl)pyrrolidin-2-yl)methylthio)butanoic acid
7-((2S,3S,4R,5R)-3,4-dihydroxy-5-methylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5R)-5-ethyl-3,4-dihydroxypropylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5R)-3,4-dihydroxy-5-propylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5R)-5-butyl-3,4-dihydroxypropylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5R)-3,4-dihydroxy-5-phenethylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5R)-cis-3,4-dihydroxy-5-styrylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2S,3S,4R,5R)-trans-3,4-dihydroxy-5-styrylpyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(S)-2-amino-6-((2R,3R,4S,5S)-3,4-dihydroxy-5-(4-oxo-4,5-dihydro-3H-pyrrolo[3,2-c]pyrimidin-7-yl)pyrrolidin-2-yl)hexanoic acid
7-((2S,3S,4R,5S)-5-(fluoromethyl)-3,4-
dihydroxypyrrolidin-2-yl)-3H-pyrrolo[3,2-d]pyrimidin-
4(5H)-one

and

2-amino-7-((2S,3S,4R,5S)-5-(fluoromethyl)-3,4-
dihydroxypyrrolidin-2-yl)-3H-pyrrolo[3,2-
d]pyrimidin-4(5H)-one

or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0044] Preferred compounds include those having the structure:

[0045] In another embodiment, as described in U.S. Patent No. 7,553,839 and in PCT
the contents of which are herein incorporated by reference, the MTIP inhibitor comprises a compound having formula (III) or (IIia):

wherein V is C¾ and W is NR¹; X is C¾; Y is selected from hydrogen, halogen and hydroxyl; Z is selected from hydrogen, halogen, SQ, OQ and Q, where Q is an optionally substituted alkyl, aralkyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain Ci-Ce alkyl; R¹ is a radical of the formula (IV)

A is selected from N, CH and CR, where R is selected from halogen or optionally substituted alkyl, aralkyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain Ci-Ce alkyl; B is OH; D is selected from OH, NH₂, SCH₃ and hydrogen; E is N; G is C¾; or a tautomer thereof or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0046] Preferably Y is H. Preferably Z is SQ. Preferably A is CH. Preferably D is H. Preferably Q is alkyl, preferably a Ci-Ce alkyl group such as methyl, ethyl, propyl, butyl, pentyl or cyclohexyl or Q is an aryl group such as a phenyl group or an aralkyl group such as a benzyl group. It is also preferred that Q is an alkyl group substituted with one or more amino and one or more carboxy groups.

[0047] Preferred compounds include those having the formula (V):
where Q is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, and straight- or branched-chain \( Ci-Ce \) alkyl; or a tautomer thereof; or an ester thereof; or a pharmaceutically acceptable salt thereof, or a prodrug thereof. Preferably, Q is optionally substituted alkyl, preferably a \( Ci-Ce \) alkyl group such as methyl, ethyl, propyl or butyl, or Q is an optionally substituted aryl group such as a phenyl group or an optionally substituted aralkyl group such as a benzyl group. Preferred compounds include those where Q is methyl or phenyl. It is also preferred that Q is an alkyl group substituted with one or more amino groups. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups.

[0048] Other preferred compounds include those having the formula (Va):

![Formula (Va)](image)

where Q is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, and straight- or branched-chain \( Ci-Ce \) alkyl; or a tautomer thereof; or an ester thereof; or a pharmaceutically acceptable salt thereof, or a prodrug thereof. Preferably, Q is optionally substituted alkyl, preferably a \( Ci-Ce \) alkyl group such as methyl, ethyl, propyl or butyl, or Q is an optionally substituted aryl group such as a phenyl group or an aralkyl group such as a benzyl group. Preferred compounds include those where Q is methyl, ethyl or phenyl. It is also preferred that Q is an alkyl group substituted with one or more amino groups. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups.

[0049] Examples of compounds of formula (III) include:
7-(((3R,4S)-3-hydroxy-4-(phenylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-((4-chlorophenylthio)methyl)-4-hydroxyoxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hydroxy-4-(methylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-(ethylthiomethyl)-4-hydroxyoxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

Compound 24 in Example 1.20, WO 04 069856
7-(((3R,4S)-3-hydroxy-4-(propylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hydroxy-4-(isopropylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

Compound 25 in Example 1.20, WO 04 069856
7-(((3S,4R)-3-(butylthiomethyl)-4-hydroxyoxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-(benzylthiomethyl)-4-hydroxyoxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(5S)-2-amino-4-(((3S,4R)-4-hydroxy-1-((4-oxo-4,5-dihydro-3H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl)pyrrolidin-3-yl)methylthiomethyl)butanoic acid
7-(((3S,4R)-3-benzyl-4-hydroxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3S,4R)-3-ethyl-4-hydroxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

Compound 28 in Example 1.24, WO 04 069856

7-(((3R,4S)-3-hydroxy-4-propylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hydroxy-4-isobutylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3S,4R)-3-butyl-4-hydroxyprpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hydroxy-4-pentylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hydroxy-4-isopentylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hydroxy-4-phenethylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one

7-(((3S,4R)-3-hexyl-4-hydroxyprpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\textit{d}]pyrimidin-4(5H)-one
(S)-2-amino-6-((3S,4R)-4-hydroxy-1-((4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)pyrroloidin-3-yl)hexanoic acid

7-(((3S,4R)-3-(benzylthio)propyl)-4-hydroxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-(cyclohexylmethyl)-4-hydroxypyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4S)-3,4-dihydroxy-3-(methylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

Preferred compounds include those having the structure:
Examples of compounds of formula (IIa) include:

7-(((3S,4R)-3,4-dihydroxy-3-(methylthiomethyl)pyrroloidin-1-yl)(methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-fluoro-4-hydroxy-3-(methylthiomethyl)pyrroloidin-1-yl)(methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one
7-((3S,4R)-3-hydroxy-4-(phenylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3R,4S)-3-((4-chlorophenylthio)methyl)-4-hydroxy.pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3S,4R)-3-hydroxy-4-(methylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3S,4R)-3-hydroxy-4-(ethylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3S,4R)-3-hydroxy-4-(propylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3S,4R)-3-hydroxy-4-(isopropylthiomethyl)pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3R,4S)-3-(butylthiomethyl)-4-hydroxy.pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

7-((3R,4S)-3-(benzylthiomethyl)-4-hydroxy.pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-\(d\)]pyrimidin-4(5H)-one

(S)-2-amino-4-(((3R,4S)-4-hydroxy-1-((4-oxo-4,5-dihydro-3H-pyrrolo[3,2-\(d\)]pyrimidin-7-yl)methyl)pyrrolidin-3-yl)methylthio)butanoic acid
7-(((3R,4S)-3-benzyl-4-hydroxy-pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-hydroxy-4-propylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4S)-3-ethyl-4-hydroxy-pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-hydroxy-4-isobutylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4S)-3-butyl-4-hydroxy-pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-hydroxy-4-pentylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4R)-3-hydroxy-4-isopentylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3R,4S)-3-hexyl-4-hydroxy-pyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-(((3S,4R)-3-hydroxy-4-phenethylpyrrolidin-1-yl)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0052] In another embodiment, as described in PCT International Patent Application Publication No. WO 2008/030119, the contents of which are herein incorporated by reference, the MTIP inhibitor comprises a compound having formula (VI):

![Chemical Structure Image](image-url)

(VI)

wherein R¹ is H or NR³R⁴; R² is H or an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR³R⁴ groups, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; provided that when R¹ is H, R² is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group which is substituted with at least one NR³R⁴ group, and optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester or nitro group; R³ and R⁴, independently of each other, are H or an
alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR³R⁴ groups, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; A is N or CH; B is OH; and D is H, OH, NH₂, or SC¾; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

Preferably, R¹ is NR³R⁴ and R² is H. It is further preferred that R¹ is NR³R⁴ and one of R³ and R⁴ is H, a lower alkyl group, e.g. a methyl group, or an aralkyl group, e.g. a benzyl group. Preferably R¹ is NR³R⁴ and one of R³ and R⁴ is H.

Preferably R³ or R⁴ is alkyl substituted by one or more hydroxy groups and/or one or more optionally substituted thiol, alkylthio, arylthio, or aralkylthio groups. For example, R³ or R⁴ may be, methylthioethyl, methylthiopropyl, methylthiohydroxypropyl, methylthiobutyl, methylthiohydroxybutyl, methylthiodihydroxybutyl, methylthiopentyl, methylthiohydroxypentyl, methylthiodihydroxypentyl, or methylthiotrihydroxypentyl.

Preferably R¹ is NR³R⁴ and one of R³ and R⁴ is H and the other is selected from the group consisting of methylthioethyl, methylthiopropyl, methylthiohydroxypropyl, methylthiobutyl, methylthiohydroxybutyl, methylthiodihydroxybutyl, methylthiopentyl, methylthiohydroxypentyl, methylthiodihydroxypentyl, and methylthiotrihydroxypentyl.

In addition, preferably R² is an optionally substituted alkyl group, more preferably an optionally substituted C1-C5 alkyl group, for example, hydroxymethyl, hydroxyethyl, hydroxypropyl, dihydroxypropyl, hydroxybutyl, dihydroxybutyl, trihydroxybutyl, hydroxypentyl, dihydroxypentyl, trihydroxypentyl, methylthioethyl, methylthiopropyl, methylthiohydroxypropyl, methylthiobutyl, methylthiohydroxybutyl, methylthiodihydroxybutyl, methylthiopentyl, methylthiohydroxypentyl, methylthiodihydroxypentyl, or methylthiotrihydroxypentyl.

Preferably A is CH. It is further preferred that D is H.

Preferred compounds include those having the formula (Via):

![Chemical Structure](image-url)
wherein \( R^i \) is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, or nitro group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

**[0059]** Other preferred compounds include those having the formula \((\text{VIb})\):

\[
\begin{align*}
\text{(VIb)} \\
\text{R}^3 \text{H} - \text{H} - \text{N} - \text{H} - \text{NH}
\end{align*}
\]

wherein \( R^3 \) is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with at least one alkoxy, thiol, alkylthio, arylthio, or aralkylthio group and optionally substituted with one or more hydroxy, halogen, carboxylic acid, carboxylate alkyl ester, or nitro group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

**[0060]** Preferably \( R^3 \) in the above formulae \((\text{VIa})\) and \((\text{VIb})\) is selected from the group consisting of, methylthioethyl, methylthiopropyl, methylthiohydroxypropyl, methylthiobutyl, methylthiohydroxybutyl, methylthiodihydroxybutyl, methylthiopentyl, methylthiohydroxypentyl, methylthiodihydroxypentyl, or methylthiotrihydroxypentyl.

**[0061]** Examples of compounds of formula \((\text{VI})\), as described in WO 2008/0301 19, include:

- 2-amino-7-(2,3-dihydroxy-1-(2-(methylthio)ethlamino)propyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
- 7-(2,3-dihydroxy-1-(2-(methylthio)ethlamino)propyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(l-amino-2,3-dihydroxy-5-(methylthio)penty1)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(l-amino-2,3-dihydroxy-5-(methylthio)penty1)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-(2-hydroxy-l-(l-hydroxy-3-(methylthio)propan-2-ylamino)ethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(2-hydroxy-l-(l-hydroxy-3-(methylthio)propan-2-ylamino)ethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-((3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (each enantiomer);

2-amino-7-(((2-hydroxy-4-(methylthio)butyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2-hydroxy-4-(methylthio)butyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((2-hydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;  
7-((2-hydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;  

2-amino-7-((3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;  
7-((3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (DL-erythro, DL-threo);  

2-amino-7-((3-hydroxy-2-(methylthiomethyl)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;  
7-((3-hydroxy-2-(methylthiomethyl)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((3-hydroxy-2-(methylthiomethyl)propyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((3-hydroxy-2-(methylthiomethyl)propyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
5-amino-3-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
2-amino-7-((benzyl((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((benzyl((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
(Racemic mixture of above two as DL-erythro);
2-amino-7-((benzyl((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((2R,35)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-
pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((25,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-
pyrrolo[3,2-d]pyrimidin-4(5H)-one;

7-(((2R,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-
4(5H)-one;
7-(((2S,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-
4(5H)-one;
7-(((2R,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-
4(5H)-one;
7-(((2S,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-
4(5H)-one;
(Racemic mixture of above two as DL-threo);
2-amino-7-(((2R,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-
d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-
d]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-
d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-
d]pyrimidin-4(5H)-one;
7-(((2R,3R)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((2S,3S)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((2R,3S)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((2S,3R)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((2R,3S)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((2S,3R)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3S)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3S)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
3-(((2R,3R^-)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3S^-)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2R,3^-)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3^-)-3,4-dihydroxy-l-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
2-amino-7-(((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

(RS)-7-((2-hydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
(RS)-7-(((1-hydroxy-3-(methylthio)propan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

and

(RS)-7-(((1-hydroxy-3-(methylthio)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

Additional examples of compounds of formula (VI) include:
7-((2-methylthio)ethylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((3-methylthio)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((4-methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((5-methylthio)pentylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2-ethylthio)ethylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((3-ethylthio)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((4-ethylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((5-ethylthio)pentylamino)methyl)-3i-pyrrolo[3,2-i]pyrimidin-4(5i)-one
7-((2-(propylthio)ethylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((3-(propylthio)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((4-(propylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((5-(propylthio)pentylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((2-(phenylthio)ethylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((3-(phenylthio)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((4-(phenylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

7-((5-(phenylthio)pentylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one
(R)-7-((2-hydroxy-3-(methylthio)propylamino)methyl)-3//-pyrrolo[3,2-i]pyrimidin-4(5H)-one

(S)-7-((2-hydroxy-3-(phenylthio)propylamino)methyl)-3//-pyrrolo[3,2-i]pyrimidin-4(5H)-one
(5')-7-(((1-hydroxy-3-(methylthio)propan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(5')-7-(((1-hydroxy-3-(methylthio)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-(((1-hydroxy-3-(methylthio)propan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-(((1-hydroxy-3-(methylthio)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one
(5)-7-(((1-(ethylthio)-3-hydroxypropan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(S)-7-(((1-(ethylthio)-3-hydroxypropan-2-yl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-(((1-(ethylthio)-3-hydroxypropan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(RS)-7-(((1-(ethylthio)-3-hydroxypropan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(RS)-7-(((1-(ethylthio)-3-hydroxypropan-2-yl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one
(S)-7-(((1-hydroxy-3-(propylthio)propan-2-yl)(methyl)amino)methyl)-3\(H\)-pyrrolo[3,2-\(d\)]pyrimidin-4(5\(H\))-one

(R)-7-(((1-hydroxy-3-(propylthio)propan-2-yl)(methyl)amino)methyl)-3\(H\)-pyrrolo[3,2-\(d\)]pyrimidin-4(5\(H\))-one

(RS)-7-(((1-hydroxy-3-(propylthio)propan-2-yl)(methyl)amino)methyl)-3\(H\)-pyrrolo[3,2-\(d\)]pyrimidin-4(5\(H\))-one

(RS)-7-(((1-hydroxy-3-(propylthio)propan-2-yl)(methyl)amino)methyl)-3\(H\)-pyrrolo[3,2-\(i\)]pyrimidin-4(5\(H\))-one
(S)-7-(((1-hydroxy-3-(phenylthio)propan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-(((1-hydroxy-3-(phenylthio)propan-2-yl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(RS)-7-(((1-hydroxy-3-(phenylthio)propan-2-yl)(methyl)amino)methyl)-3i/-pyrrolo[3,2-i/pyrimidin-4(5H)-one

(R)-7-((1-hydroxy-3-(phenylthio)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(RS)-7-((1-hydroxy-3-(phenylthio)propan-2-ylamino)methyl)-3ii/-pyrrolo[3,2-ii]pyrimidin-4(5H)-one
(5)-7-((1-(butylthio)-3-hydroxypropan-2-ylamino)methyl)-3//-pyrrolo[3,2-<i]-pyrimidin-4(5/f)-one

(6)-7-((1-(butylthio)-3-hydroxypropan-2-ylamino)methyl)-3//-pyrrolo[3,2-<i]-pyrimidin-4(5/f)-one

(RS)-7-((1-(butylthio)-3-hydroxypropan-2-ylamino)methyl)-3//-pyrrolo[3,2-<i]-pyrimidin-4(5/f)-one

(5)-7-((1-hydroxy-3-(isopropylthio)propan-2-ylamino)methyl)-3//-pyrrolo[3,2-<i]-pyrimidin-4(5/f)-one

(Λ)-7-((1-hydroxy-3-(isopropylthio)propan-2-ylamino)methyl)-3//-pyrrolo[3,2-<i]-pyrimidin-4(5/f)-one

(RS)-7-((1-hydroxy-3-(isopropylthio)propan-2-ylamino)methyl)-3//-pyrrolo[3,2-<i]-pyrimidin-4(5/f)-one

(5*)-2-amino-4-((5)-3-hydroxy-2-((4-oxo-4,5-dihydro-3//-pyrrolo[3,2-<i]-pyrimidin-7-yl)methylamino)propylthio)butanoic acid

(5)-2-amino-4-((i?)-3-hydroxy-2-((4-oxo-4,5-dihydro-3//-pyrrolo[3,2-<i]-pyrimidin-7-yl)methylamino)propylthio)butanoic acid

(5)-2-amino-4-((RS)-3-hydroxy-2-((4-oxo-4,5-dihydro-3//-pyrrolo[3,2-<i]-pyrimidin-7-yl)methylamino)propylthio)butanoic acid
(5)-7-(((1-hydroxy-4-phenylbutan-2-ylamino)methyl)-3//-pyrrolo [3,2-i]pyrimidin-4(5 H )-one

(5)-7-(((1-hydroxybutan-2-ylamino)methyl)-3//-pyrrolo [3,2-i]pyrimidin-4(5 H )-one

(5)-7-(((1-hydroxypentan-2-ylamino)methyl)-3//-pyrrolo [3,2-i]pyrimidin-4(5 H )-one

(5)-7-(((1-hydroxyhexan-2-ylamino)methyl)-3//-pyrrolo [3,2-d]pyrimidin-4(5 H )-one

7-(((2S,3S)-1-hydroxy-3-methylpentan-2-ylamino)methyl)-3 //-pyrrolo[3,2-J]pyrimidin-4(5 H )-one

(5)-7-(((1-hydroxypropan-2-ylamino)methyl)-3//-pyrrolo [3,2-J]pyrimidin-4(5 H )-one

(5)-7-(((1-hydroxy-4-methylpentan-2-ylamino)methyl)-3//-pyrrolo [3,2-d]pyrimidin-4(5 H )-one
(R)-7-((1-hydroxy-4-phenylbutan-2-ylamino)methyl)-3//-pyrrolo[3,2-\text{i}]pyrimidin-4(5\text{H})-one

(R)-7-((1-hydroxybutan-2-ylamino)methyl)-3//-pyrrolo[3,2-\text{i}]pyrimidin-4(5\text{H})-one

(R)-7-((1-hydroxypentan-2-ylamino)methyl)-3//-pyrrolo[3,2-\text{i}]pyrimidin-4(5\text{H})-one

(R)-7-((1-hydroxyhexan-2-ylamino)methyl)-3//-pyrrolo[3,2-\text{i}]pyrimidin-4(5\text{H})-one

7-(((2\text{R},3\text{S})-1-hydroxy-3-methylpentan-2-ylamino)methyl)-3//-pyrrolo [3,2-\text{i}]pyrimidin-4(5\text{H})-one

(R)-7-((1-hydroxypropan-2-ylamino)methyl)-3//-pyrrolo[3,2-\text{i}]pyrimidin-4(5\text{H})-one

(R)-7-((1-hydroxy-4-methylpentan-2-ylamino)methyl)-3//-pyrrolo[3,2-\text{i}]pyrimidin-4(5\text{H})-one
(S)-7-((1-hydroxy-3-phenylpropan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(S)-7-((1-hydroxy-3-methylbutan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(S)-7-((2-hydroxy-1-phenylethlamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-((1-hydroxy-3-phenylpropan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-((1-hydroxy-3-methylbutan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one

(R)-7-((2-hydroxy-1-phenylethlamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one
(RS)-7-((1-hydroxy-4-phenylbutan-2-ylamino)methyl)-3H-pyrrolo[3,2-<i>l]pyrimidin-4(5H)-one

(RS)-7-((1-hydroxybutan-2-ylamino)methyl)-3H-pyrrolo[3,2-J]pyrimidin-4(5H)-one

(RS)-7-((1-hydroxypentan-2-ylamino)methyl)-3H-pyrrolo[3,2-J]pyrimidin-4(5H)-one

(RS)-7-((1-hydroxyhexan-2-ylamino)methyl)-3H-pyrrolo[3,2-<i>l]pyrimidin-4(5H)-one

7-((2RS,3S)-1-hydroxy-3-methylpentan-2-ylamino)methyl)-3H-pyrrolo[3,2-<i>l]pyrimidin-4(5H)-one

(RS)-7-((1-hydroxypropan-2-ylamino)methyl)-3H-pyrrolo[3,2-<i>l]pyrimidin-4(5H)-one

(RS)-7-((1-hydroxy-4-methylpentan-2-ylamino)methyl)-3H-pyrrolo[3,2-<i>l]pyrimidin-4(5H)-one
(S)-7-((1-hydroxyheptan-2-ylamino)methyl)-3H-pyrrolo[3,2-c]pyrimidin-4(5H)-one,
and

(S)-7-((1-hydroxyoctan-2-ylamino)methyl)-3H-pyrrolo[3,2-c]pyrimidin-4(5H)-one;

or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0063] Preferred compounds include those having the structure:
In another embodiment, the MTIP inhibitor comprises a compound having formula (VII):

![Chemical structure](image)

(VII)

wherein X is an alkyl, cycloalkyl, aralkyl, aralkenyl, alkenyl, alkynyl or aryl group, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, cycloalkyl, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR2R3 group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or X is SR1; or X is NR2R3; R1, R2 and R3 are...
independently selected from the group consisting of alkyl, alkenyl, alkynyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, thiol, alkylthio, arylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano and NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; A is N or CH; B is OH; D is H, OH, NH₂, or SCH₃; provided that X is not CH₂Z, where Z is selected from OH, hydrogen, halogen, SQ¹, OQ² and Q³, where Q¹ is an optionally substituted alkyl, aralkyl or aryl group, Q² is an optionally substituted alkyl group and Q³ is an optionally substituted alkyl group; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

[0065] Preferred compounds include those of formula (Vila):

![Formula Vila](image)

(VIIa)

wherein A, B, D and X are as defined above.

[0066] Alternatively, preferably the compound is a compound of formula (VIIb):

![Formula VIIb](image)

(VIIb)

wherein A, B, D and X are as defined above.

[0067] Preferably, in the above formulae (VII), (Vila) and (VIIb), Q¹ is an optionally substituted alkyl, aralkyl or aryl group, Q² is an optionally substituted alkyl group and Q³ is an optionally substituted alkyl group. For example, Q¹, Q² or Q³ may be optionally substituted with one or more: halogens, e.g. chlorine or fluorine; alkyl groups, e.g. methyl or cyclohexylmethyl; COOH; or NH₂.

[0068] Preferably A is CH. It is further preferred that D is H.

[0069] Preferably, X is an alkenyl or alkynyl group each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy,
alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups. X may be, for example, a lower alkenyl group, e.g. a vinyl, allyl or prop-1-en-2-yl group. X may be, for example, a lower alkynyl group, e.g. an ethynyl group or a propyn-3-yl group.

Alternatively, preferably, X is an alkyl group which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, thiol, alkylthio, arylthio, or aralkylthio, e.g. benzylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro or NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy or alkoxy groups. X may be, for example, a lower alkyl group, e.g. ethyl, propyl, butyl, isobutyl, pent-3-yl or an alkyl group substituted with a cycloalkyl group, e.g. cyclohexyl group, e.g. X may be cyclohexanemethyl. Alternatively, X may be, for example, an alkyl group which is substituted with an aralkylthio group, e.g. X may be 3-benzylthiopropyl group.

Alternatively, preferably, X is an alkyl group which is optionally substituted with one or more substituents selected from the group consisting of cycloalkyl, e.g. cycloalkyl in which one or more of the ring carbon atoms is substituted by a heteroatom chosen from nitrogen, oxygen or sulfur. In some examples, X may be cyclopropanemethyl, 2-tetrahydrofuranmethyl, 2-thietanemethyl, 3-piperidinemethyl, 2-pyrrolidinemethyl or 4-thiacyclohexanemethyl.

Alternatively, preferably, X is a cycloalkyl group which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, cycloalkyl, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy or alkoxy groups. X may be, for example, a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or adamantyl group.

Alternatively, preferably, X may be a cycloalkyl group where one or more of the ring atoms is a heteroatom, e.g. a nitrogen, sulfur or oxygen atom. X may be, for example, 2-tetrahydrofuranyl, 2-tetrahydrothienyl, 1,2-dithian-3-yl, piperidin-3-yl, thietan-2yl, 2-pyrrolidinyl or 4-thiacyclohexyl.

Alternatively, preferably, X is an aryl group which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy,
cycloalkyl, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR₃ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups. The aryl group may be a heteroaryl group, where one or more of the ring carbon atoms is a heteroatom, e.g. a nitrogen, sulfur or oxygen atom. X may be, for example, a phenyl group or an optionally substituted triazole group. Where X is an optionally substituted triazole group the triazole ring may optionally be substituted with one or more substituents selected from the group consisting of aryl group, e.g. phenyl; alkyl group, e.g. a lower alkyl group, e.g. a propyl group which may optionally be substituted with one or more substituents selected from aryl, hydroxyl, or alkoxy; aralkyl group, e.g. benzyl; or cycloalkyl group. Where X is an optionally substituted triazole group, the triazole ring may be attached to the pyrrolidine ring via either a triazole ring nitrogen or a triazole ring carbon atom.

[0075] Alternatively, preferably, X is SR, where R is alkyl, alkenyl, alkynyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, cycloalkyl, thiol, alkylthio, arylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR₃ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy or alkoxy groups. For example, X may be phenylthio, 4-chlorophenylthio, 4-fluorophenylthio, 3-fluorophenylthio, 4-methylphenylthio, ethylthio, propylthio, butylthio, pentylthio, 3-fluoropropylthio, 2,3-dihydroxypropylthio, 3-hydroxypropylthio, 2-hydroxyethylthio, allylthio or 4-chlorobutylthio.

[0076] Alternatively, preferably, X is NR₃, where R₂ and R₃ are independently selected from alkyl, alkenyl, alkynyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, cycloalkyl, thiol, alkylthio, arylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR₃ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups. For example, X may be diethylamino, ethylamino, propylamino, butylamino, 2-hydroxyethylamino, 3-hydroxypropylamino, 2,3-dihydroxypropylamino, 3-fluoroethylamino, trifluoroethylamino, bis(2-hydroxyethyl)amino, 3-butenylamino, benzylamino, 4-fluorobenzylamino, 4-chlorobenzylamino, or N-methyl-benzylamino.

[0077] Examples of compounds of formula (VII) include:
(±)-α-S-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-phenylpyrrolidine;
(-)-ra<sup>s</sup>-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-vinylpyrrolidine;
(-)-ra<sup>s</sup>-l-[(9-Deazahypoxanthin-9-yl)methyl]-4-ethynyl-3-hydroxy-4-vinylpyrrolidine;
(-)-ra<sup>s</sup>-4-Butyl-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(prop-1-en-2-yl)-pyrrolidine;
(-)-ra<sup>s</sup>-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(pent-3-yl)-pyrrolidine;
(-)-ra<sup>s</sup>-4-Cyclopentyl-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(pent-3-yl)-pyrrolidine;
(-)-ra<sup>s</sup>-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine;
(-)-ra<sup>s</sup>-4-Butyl-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine;
(-)-ra<sup>s</sup>-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine;
(±)-ra<sup>s</sup>-4-(1-Benzyl-1H-1,2,3-triazol-4-yl)-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine;
(±)-\text{ra-as-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(tetrazol-5-yl)-} \text{pyrrolidine; (3R,4R)-l-[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(tetrazol-5-yl)-} \text{pyrrolidine; and (3S,4R)-l-[(9-Deaza-adenin-9-yl)methyl]-4-ethyl-3-hydroxypyrrolidine; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester thereof.}

[0078] Preferably, the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for human purine nucleoside phosphorylase (PNP), as determined by the method described in Bantia, et al., Immunopharmacology 35, p. 54, paragraph 2.1 (1996) (52). Preferably, the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for 5'-methylthioadenosine nucleosidase (MTAN). Preferably, the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for 5'-methylthioadenosine phosphorylase (MTAP). "IC50" means the molar concentration of an inhibitor needed to reduce the rate of product formation by 50% from the uninhibited reaction in an assay mixture containing a target enzyme.

[0079] The active compounds may be administered to a subject, such as a human, by a variety of routes, including orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally or via an implanted reservoir. The specific dosage required for any particular patient will depend upon a variety of factors, including the patient's age and body weight.

[0080] For oral administration the compounds can be formulated into solid or liquid preparations, for example tablets, capsules, powders, solutions, suspensions and dispersions. Such preparations are well known in the art as are other oral dosage regimes not listed here. In the tablet form the compounds may be tableted with conventional tablet bases such as lactose, sucrose and corn starch, together with a binder, a disintegration agent and a lubricant. The binder may be, for example, corn starch or gelatin, the disintegrating agent may be potato starch or alginic acid, and the lubricant may be magnesium stearate. For oral administration in the form of capsules, diluents such as lactose and dried cornstarch may be employed. Other components such as colourings, sweeteners or flavourings may be added.

[0081] When aqueous suspensions are required for oral use, the active ingredient may be combined with carriers such as water and ethanol, and emulsifying agents, suspending agents and/or surfactants may be used. Colourings, sweeteners or flavourings may also be added.

[0082] The compounds may also be administered by injection in a physiologically acceptable diluent such as water or saline. The diluent may comprise one or more other
ingredients such as ethanol, propylene glycol, an oil or a pharmaceutically acceptable surfactant.

The compounds may also be administered topically. Carriers for topical administration of the compounds of include mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. The compounds may be present as ingredients in lotions or creams, for topical administration to skin or mucous membranes. Such creams may contain the active compounds suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds may further be administered by means of sustained release systems. For example, they may be incorporated into a slowly dissolving tablet or capsule.

The compounds can be administered to plants, for example, by spraying the plant surfaces with the compound and a carrier of the type normally used in agricultural applications, such as, for example, water or an oil. Typical concentrations can be, for example, 1 - 100 grams of inhibitor per 1000 gallons of plant spray.

The present invention also provides for the use of a subgrowth inhibiting amount of an MTIP inhibitor for treating bacterial infections in a subject. The present invention further provides for the use of a subgrowth inhibiting amount of an MTIP inhibitor for the preparation of a composition for treating bacterial infections in a subject.

The invention also provides a method for determining whether or not a compound is a candidate for treating an infection caused by bacteria that use 5'-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway, the method comprising determining whether or not the compound inhibits MTIP, wherein a compound that inhibits MTIP is a candidate for treating an infection caused by bacteria that use MTIP in a quorum sensing pathway and wherein a compound that does not inhibit MTIP is not a candidate for treating an infection caused by bacteria that use MTIP in a quorum sensing pathway.

Preferably, the MTIP inhibitor has an IC50 value less than 50 nanomolar for MTIP. Preferably, the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for human purine nucleoside phosphorylase (PNP), as determined by the method described in Bantia, *et al.*, *Immunopharmacology* 35, p. 54, paragraph 2.1 (1996) (52). Preferably, the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for
5′-methylthioadenosine nucleosidase (MTAN). Preferably, the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for 5′-methylthioadenosine phosphorylase (MTAP). "IC50" means the molar concentration of an inhibitor needed to reduce the rate of product formation by 50% from the uninhibited reaction in an assay mixture containing a target enzyme.

An "inhibitor" is a substance that when added to an assay mixture for a target enzyme causes a decrease in the rate of product formation by interaction with the enzyme.

The bacteria can be, for example, Pseudomonas aeruginosa, Pseudomonas syringae or Xanthomonas campestris.

Enzymatic assays can be carried out, for example, as described herein in Experimental Details.

The invention also provides a chemical compound of formula (I)

![Chemical Structure](attachment:structure.png)

wherein A is CH or N; B is OH; D is chosen from H, OH, NH₂, or SCH₃; X is OH; Y is H; and Z is Q, where Q is a methyl group which is substituted with one or more substituents selected from the group consisting of methoxy, amino and carboxy, or Q is an optionally substituted, alkenyl, aralkyl, aralkenyl, aryl group or C2-C₁₀ alkyl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C₁-C₆ alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

Preferably, D is H. It is also preferred that A is CH. It is further preferred that D is H and A is CH.

Preferably, Q is an optionally substituted alkyl group, e.g. a C2-C6 alkyl group such as an ethyl, propyl or butyl group. It is also preferred that Q is an alkyl group substituted with one or more amino groups. Preferably Q is an alkyl group substituted with one or more amino and one or more carboxy groups, e.g. -(CH₂)₄CH(NH₂)COOH.
Alternatively, preferably, Q is an optionally substituted aralkyl group, e.g. a phenylethyl or benzyl group. In still other preferred embodiments, Q is an optionally substituted aralkenyl group, e.g. a phenylethenyl group, e.g. a cis-phenylethenyl group or a trans-phenylethenyl group.

Preferably, the compound has an IC50 value greater than or equal to 50 nanomolar for human purine nucleoside phosphorylase (PNP), as determined by the method described in Bantia, et al, Immunopharmacology 35, p. 54, paragraph 2.1 (1996) (52). Preferably, the compound has an IC50 value greater than or equal to 50 nanomolar for 5'-methylthioadenosine nucleosidase (MTAN). Preferably, the compound has an IC50 value greater than or equal to 50 nanomolar for 5'-methylthioadenosine phosphorylase (MTAP).

The invention also provides a compound selected from the group consisting of
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0098] The invention further provides a compound selected from the group consisting of
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[0099] The invention still further provides a compound selected from the group consisting of:
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

The invention also provides a pharmaceutical composition comprising any one or more of the compounds disclosed herein and a pharmaceutically acceptable salt.

The term "pharmaceutically acceptable salt" is intended to apply to non-toxic salts derived from inorganic or organic acids, including, for example, the following acid salts: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate,
hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, p-toluene sulfonate, salicylate, succinate, sulfate, tartrate, thiocyanate, and undecanoate.

[00102] The invention also provides a pharmaceutical composition or an agrochemical composition comprising one or more of the compounds disclosed herein and a pharmaceutically or agrochemically acceptable carrier. The compound can be present in a concentration that is a sub-bacterial-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor.

[00103] The invention also provides a pharmaceutical composition or an agrochemical composition comprising any one or more of the compounds disclosed herein and one or more additional compounds, such as 5'-methylthioinosine or 5'-methylthioadenosine.

[00104] The invention also provides methods for treating infections caused by bacteria that use 5'-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway comprising coadministering to a subject having the infection a sub-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor, and 5'-methylthioinosine and/or 5'-methylthioadenosine.

[00105] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

Introduction

[00106] P. aeruginosa is an unusual bacterium as it possesses a putative 5'-methylthioadenosine phosphorylase (MTAP: PA3004 gene) instead of MTAN. MTAP is rare in bacteria and common in mammals, while MTAN is not found in mammals. The action of MTAP on MTA would be functionally similar to that of MTAN by relieving MTA product inhibition of AHL synthase and SAM recycling in P. aeruginosa (18). The PA3004 gene in P. aeruginosa PA01 was originally annotated from sequence homology, followed by metabolic studies in PA3004 mutants that mistakenly supported the MTAP assignment (19, 20). The present study establishes the PA3004 encoded protein to be 5'-methylthioinosine phosphorylase (MTIP) rather than MTA phosphorylase (MTAP). Examination of MTA
metabolism in *P. aeruginosa* using [8-14C]MTA confirmed that the pathway involves MTIP. Transition state analogue inhibitors with picomolar $K_i$ values for $P_a$MTIP are described.

**Materials and Methods**

[00107] **Chemicals.** MT-ImmH, PhT-ImmH, and MT-DADMe-ImmH were synthesized by the Carbohydrate Chemistry Team of Industrial Research Ltd, Lower Hutt, New Zealand (Figure 1). [8-14C]MTA was synthesized as described previously (21). All other chemicals and reagents were obtained from Sigma or Fisher Scientific, and were of reagent grade.

[00108] **Plasmid construction.** A synthetic gene was designed based on the predicted protein sequence of NP_25 1694.1 in NCBI, annotated as MTAP of *Pseudomonas aeruginosa PAO1*. The synthetic gene was obtained in a pJexpress414 expression vector from DNA2.0 Inc. This construct encodes an additional 14 amino acids at the N-terminus which includes a His$_8$ tag and a TEV cleavage site.

[00109] **Enzyme purification and preparation.** BL21-CodonPlus(DE3)-RIPL *E. coli* were transformed with the above plasmid and grown overnight at 37 °C in 50 mL of LB medium with 100 μg/mL Ampicillin. The culture was transferred into 1 L of LB/Ampicillin medium and growth continued at 37 °C to an O.D.$_{600}$ of 0.7. Expression was induced by addition of 1 mM IPTG. After 4 hours at 37 °C, the cells were harvested by centrifugation at 4500 g for 30 min. The cell pellet was suspended in 20 mL of 15 mM imidazole, 300 mM NaCl, and 50 mM phosphate, pH 8.0 (lysis buffer), with 2 tablets of EDTA-free protease inhibitor (from Roche Diagnostics) and lysozyme (from chicken egg) added to the mixture. Cells were disrupted twice with a French Press and centrifuged at 20,000 g for 30 min. The supernatant was loaded onto a 4 mL column of Ni-NTA Superflow resin previously equilibrated with 5 columns of lysis buffer. The column was washed with 5 volumes of 80 mM imidazole, 300 mM NaCl, and 50 mM phosphate, pH 8.0 (wash buffer), and enzyme was eluted with 3 volumes of 250 mM imidazole, 300 mM NaCl, and 50 mM phosphate, pH 8.0 (elution buffer). The purified enzyme (> 95% purity on the basis of SDS-PAGE) was dialyzed against 50 mM Hepes, pH 7.4 and concentrated to 8 mg/ml. Enzyme was stored at -80 °C. The extinction coefficient of target protein is 20.4 mM$^{-1}$cm$^{-1}$ at 280 nm, determined by ProtParam program from ExPASy.

[00110] **Enzymatic assays.** Product formation was monitored by conversion of hypoxanthine to uric acid by xanthine oxidase (22). The extinction coefficient of this assay was 12.9 mM$^{-1}$cm$^{-1}$ at 293 nm. Enzyme activity with adenosine or MTA as substrates was
determined by conversion of adenine to 2,8-dihydroxyadenine using the same coupling enzyme (23). Its extinction coefficient at 293 nm was 15.2 mM^-1 cm^-1. Reactions were carried out at 25 °C in 1 cm cuvettes, 1 mL volumes of 100 mM Hepes, pH 7.4, 100 mM phosphate, pH 7.4, variable concentrations of nucleoside substrate, 0.5 unit of xanthine oxidase, 5 mM DTT, and appropriate amounts of purified MTIP. Reactions were initiated by addition of enzyme and the initial rates were monitored with a CARY 300 UV-Visable spectrophotometer. Control rates (no PaMTIP) were subtracted from initial rates. $K_m$ and $k_{cat}$ values for PaMTIP were obtained by fitting initial rates to the Michaelis-Menten equation using GraFit 5 (Erithacus Software). Phosphate was found to be near saturation when present at 100 mM.

**Inhibition assays.** Assays for slow-onset inhibitors were carried out by adding 1 nM PaMTIP into reaction mixtures at 25 °C containing 100 mM Hepes, pH 7.4, 100 mM phosphate, pH 7.4, 2 mM MTI, 5 mM DTT, 0.5 unit of xanthine oxidase and variable inhibitor concentration. Inhibitors were present at $\geq 10$ times the enzyme concentration, required to simplify data analysis (24). Assays for MTA inhibition used 200 μM MTI. Controls having no enzyme and no inhibitor were included in all of the inhibition assays. Inhibition constants were obtained by fitting initial rates with variable inhibitor concentrations to equation (1) using GraFit 5 (Erithacus Software):

\[ \frac{v_i}{v_0} = \frac{[S]}{K_m + [S]} + \frac{K_m[M]}{K_i} \]

where $v_i$ is the initial rate in the presence of inhibitor, $v_0$ is the initial rate in the absence of inhibitor, $K_m$ is the Michaelis constant for MTI, $[S]$ and $[I]$ are MTI and inhibitor concentrations, respectively, and $K_i$ is the inhibition constant. Slow-onset inhibitors display a second phase of tighter binding after reaching a thermodynamic equilibrium with the enzyme. The equilibrium constant for the second binding phase is indicated as $K_i^*$. This constant was obtained by fitting initial final rates and inhibitor concentrations to equation (1) using GraFit 5 (22).

**Protein crystallization and data collection.** Recombinant PaMTIP (9 mg/ml) in 50 mM HEPES, pH 7.4 crystallized in 30% polyethylene glycol monomethyl ether 2000, and 0.1 M potassium thiocyanate in the presence of 5 mM MTI and 5 mM sulfate by sitting-drop vapor diffusion. Crystals were transferred to a fresh drop of crystallization solution supplemented with 20% glycerol and flash-cooled in liquid nitrogen. X-ray diffraction data
were collected at Beamline X29A, Brookhaven National Laboratory and processed with the HKL2000 program suite (Table 1).

Structure determination and refinement. The crystal structure of PaMTIP was determined by molecular replacement with Molrep using the published structure of Sulfolobus tokodaii MTAP (PDB:1V4N) as the search model (25). A model without catalytic site ligands was built by Phenix (26), followed by iterative rounds of manual model building and refinement in COOT and REFMAC5 (27, 28). Although PaMTIP was co-crystallized in the presence of sulfate and MTI to mimic the Michaelis complex of PaMTIP, based on ligand-omitted F_o-F_c maps (contoured at 3 σ) electron density was consistent with the presence of only a purine ring in the active site. PaMTIP was later confirmed to hydrolyze MTI under these conditions. Hypoxanthine was modeled in the active site of PaMTIP (Table 1).

MTA catabolism using [8-14C]MTA. P. aeruginosa PAO1 (ATCC number: 15692) was grown at 37 °C in LB medium for 16 hours. Cells were collected by centrifugation at 16100 g and washed twice with 100 mM phosphate, pH 7.4. Washed cells were lysed using BugBuster (Novagen). Cleared lysate (53 μL) was incubated with [8-14C]MTA (10 μL containing approximately 0.1 μCi 14C) in 100 mM phosphate, pH 7.4, for 10 and 25 min. Reaction mixtures were quenched with perchloric acid (1.8 M final concentration) and neutralized with potassium hydroxide. Precipitates were removed by centrifugation and carrier hypoxanthine, adenine, MTI, and MTA were added to the cleared supernatant. Metabolites were resolved on a C_{18} Luna HPLC column (Phenomenex) with a gradient of 5-52.8% acetonitrile in 20 mM ammonium acetate, pH 5.2. UV absorbance was detected at 260 nm and the retention times were 5.1 min for hypoxanthine, 7.5 min for adenine, 20.4 min for MTI, and 21.9 min for MTA. Fractions were collected in scintillation vials, dried, reconstituted in 200 μL deionized water prior to addition of 10 mL ULTIMA GOLD LSC-Cocktail and 14C was counted for three cycles at 20 minutes per cycle using a Tri-Carb 2910TR liquid scintillation analyzer. Control experiments replaced cell lysate by lysis buffer in reaction mixtures.

Results and Discussion

Annotation of PA3004 as a MTAP. The PA3004 gene of P. aeruginosa PAO1 encodes a protein (NCBI ID of NP_251694.1) annotated as a "probable nucleoside phosphorylase" in PseudoCAP (29). It was later proposed to be an MTAP based on catabolism studies in mutant strains of P. aeruginosa (19, 20). PAO503 is a P. aeruginosa
methionine-auxotroph. A new strain (PA06422) was created by inactivating the PA3004 gene of PAO503. While PAO503 was complemented for growth on minimum medium with methionine, homocysteine or MTA, PAO6422 responded to methionine and homocysteine but was not complemented by MTA (20). These results supported an MTAP activity for the PA3004 encoded protein. The results also support a pathway of MTA → MTI → hypoxanthine + MTR-l-P → methionine, but this was not considered.

[00116] **PA3004 encodes a MTI phosphorylase.** Here, the recombinant protein from PA3004 was purified and tested for substrate specificity (Table 2). The recombinant protein could not utilize MTA in the presence or absence of phosphate. The most favorable reaction was phosphorolysis of MTI with a $k_{cat}$ of 4.8 s$^{-1}$, $K_m$ of 2.6 µM ($k_{cat}/K_m$ of 1.8 x 10$^6$ M$^{-1}$s$^{-1}$). Enzyme was less active with inosine with a $k_{cat}$ of 0.57 s$^{-1}$ and a $K_m$ of 90 µM ($k_{cat}/K_m$ of 6.3 x 10$^3$ M$^{-1}$s$^{-1}$) and depended on phosphate for significant activity. The methylthio- group of MTI is important for both substrate binding and catalysis. Adenosine is a weak substrate with a $k_{cat}$ of 0.0549 s$^{-1}$ and a $K_m$ of 23 µM ($k_{cat}/K_m$ of 2.4 x 10$^3$ M$^{-1}$s$^{-1}$). Thus the protein encoded by PA3004 is a relatively specific MTI phosphorylase. The catalytic efficiency ($k_{cat}/K_m$) for MTI is 290 times larger than for inosine, the second best substrate. For comparison, the MTI phosphorylase activity of $P_f$PnP (see below) has a $k_{cat}/K_m$ of 9.4 x 10$^4$ M$^{-1}$s$^{-1}$ for MTI and a $k_{cat}/K_m$ of 3.6 x 10$^5$ M$^{-1}$s$^{-1}$ for inosine.

[00117] MTI phosphorylase activity has been documented in *Caldeririella acidophilan* MTAP, human MTAP, human PNP, and *P.falciparum* PNP, but in these, MTI is a relatively poor substrate (30-33). MTIP of *P. aeruginosa* is the only reported MTIP with high specificity for MTI. MTA is not a substrate (less than the detection level of 10$^{-4}$ $k_{cat}$) but is a competitive inhibitor of MTI, with a $K_i$ value of 70 µM, three times greater than the $K_m$ of adenosine. Thus, MTA binds to the active site of the enzyme but is not catalytically competent.

[00118] Recombinant MTIP was expressed with a 14 amino acid extension at the N-terminus. Incubation with TEV protease removed 13 of these, leaving one additional glycine at the N-terminus. This construct exhibited identical substrate specificity as the original recombinant protein. The crystal structure (see below) shows the N-terminal extension to be remote from the active site. Unchanged substrate specificity with or without the 13 amino acid extension supports activity of the native enzyme to be $Pa$MTIP.

[00119] **Crystal structure of PaMTIP: hypoxanthine.** The crystal structure of $Pa$MTIP in complex with hypoxanthine was determined to 2.0 Å resolution with two homotrimers in
the asymmetric unit. Residues 2 to 54 and 60 to 243 of each PaMTIP monomer are ordered in the electron density map. The N terminal His$_6$ tag and TEV protease site are disordered and distant from the active site. The PaMTIP monomer exhibits an $\alpha$$\beta$-fold consisting of a 7-stranded $\beta$-sheet and 5 a-helices. The active sites of PaMTIP are located near the interfaces formed between monomers in the trimer. Each trimeric PaMTIP forms three active sites. Although PaMTIP was co-crystallized with MTI and sulfate (5 mM) to mimic the Michaelis complex, the ligand-omitted difference Fourier map showed only the presence of hypoxanthine. Kinetic experiments demonstrated slow hydrolysis of MTI (3.8 x 10$^{-5}$ s$^{-1}$) to generate hypoxanthine and 5-methylthioribose. Crystallization attempts with apo-PoMTIP or with PaMTIP in complex with hypoxanthine and phosphate failed to yield crystals. In the crystal structure, hypoxanthine is wedged between the backbone of a 5-stranded $\beta$ sheet and the side chains of Leu$_{180}$ and Met$_{190}$. Structure-based sequence alignment revealed that Met$_{190}$ is conserved and Leu$_{180}$ is replaced by Phe in human PNP and human MTAP (Fig. 2). Hypoxanthine N7 and O6 form hydrogen bond with the side chain of Asn$_{223}$ in PaMTIP and N1 forms a hydrogen bond with the side chain of Asp$_{181}$.

**Comparison with Other Purine N-Ribosylphosphorylases.** PaMTIP, PNP, and MTAPs have similar functions in the phosphorolysis of nucleosides, but show different substrate specificities. The structural architecture of PaMTIP is similar to the four MTAPs and seven trimeric PNP$s$ in the Protein Data Bank (r.m.s.d is in the range of 0.8 to 1.2 Å for the monomers). Structure-based sequence alignments with PaMTIP show a 28-40% identity with the four MTAPs and 20-32% with the seven trimeric PNP$s$ (Fig. 2). However, PaMTIP shows no significant similarity in amino acid sequence or quaternary structures with the hexameric PNP$s$ including that from *Plasmodium* species. Because of this structural difference, *Plasmodium* PNP$s$ are not included in the following analysis even though most *P. falciparum* PNP$s$ also catalyze the phosphorolysis of MTI (34). The active sites of PaMTIP can be compared to those of PNP and MTAP by considering three distinct regions corresponding to the purine-, (methylthio)ribose- and phosphate-binding sites. The Glu$_{201}$ and Asn$_{243}$ (human PNP numbering) are conserved in the purine binding site of PaMTIP and PNP$s$ (Fig. 2), and have an important role in 6-oxopurine specificity by hydrogen bonding to N1, O6 and N7 of the 6-oxopurine (35). Human PNP exhibits a 350,000-fold catalytic preference for 6-oxopurines compared to 6-aminopurines (36). The Asn$_{243}$Asp mutant and Glu$_{201}$Gln:Asn$_{243}$Asp double mutant in human PNP is known to shift the substrate preference in favor of adenosine, a 6-aminopurine substrate (36). In contrast, MTAP$s$ prefer 6-aminopurine (31). Human MTAP Ser$_{178}$ (via a water molecule), Asp$_{220}$
and Asp222 are conserved for the MTAPs, forming hydrogen bonds to N1, O6 and N7 of 6-aminopurine, respectively (37).

**[00121]**  Human PNP is efficient for phosphorolysis of purine nucleosides with a 5'-hydroxyl group but not for purine nucleosides with a 5'-methylthio group. The conserved His257 and Phel59 (from the adjacent monomer) of human PNP are important in binding the 5'-hydroxyl group, whereas in MTAP and PaMTIP, a small hydrophobic amino acid corresponding to His257 and a His corresponding to Phel59 (human PNP numbering), provides space to accommodate the 5'-methylthio group. Consistent with these observations, the His257Gly mutant of human PNP binds 5'-methylthio- inhibitors tighter than the corresponding 5'-hydroxyl- inhibitors (38). Superposition of the liganded human MTAP and PNP structures reveals a 1 Å shift of phosphate toward the purine in human MTAP. Phosphate forms favorable hydrogen bonds with the 2'- and 3'- hydroxyl groups of (5'-methylthio)ribose, and thereby anchors (5'-methylthio)ribose to the active site. The spatial shift of the phosphate of human MTAP relative to that of human PNP places the 5'-methylthioribose closer to the purine binding site and this shift provides more room to accommodate the 5'-methylthio group in the active site of human MTAP. Structure-based sequence alignment shows that PaMTIP and MTAP share key residues in the phosphate and (methylthio)ribose- binding sites; however, PaMTIP has a purine binding site more similar to the PNP, consistent with its preference for 6-oxypurinines.

**[00122]**  *P. aeruginosa* Catabolism [8-14C]MTA. Substrate specificity and structural data of PA3004 support a physiological function as MTIP. But this role requires production of MTI in *P. aeruginosa*; however, there is no previous report of MTI as a metabolite in *P. aeruginosa* and the published genetic approach supported MTAP activity (20). Catabolism of [8-14C]MTA in lysates of *P. aeruginosa* was followed by analysis of hypoxanthine, adenine, MTI, and MTA. If MTA is first deaminated to MTI followed by PaMTIP action, the sequential conversion to [8-14C]MTI and hypoxanthine would occur without adenine formation. After 10 min with lysate, 77% of the MTA was converted to MTI (52%) and hypoxanthine (25%) without significant formation of adenine (Fig. 3). As 98% of the total 14C-label was recovered, the results establish MTA conversion to MTI and hypoxanthine but not to adenine. At 25 min incubation, over 97% of the [8-14C] MTA was recovered as MTI (45%) and hypoxanthine (53%). Continuous conversion of MTA → MTI → hypoxanthine without significant MTAP or MTAN activity is supported by these results, highlighting the requirement for an MTA deaminase to catalyze the conversion of MTA to MTI. There is no significant MTA phosphorylase or MTA nucleosidase activity in *P. aeruginosa* abstracts.
MTI Formation in P. falciparum. A similar pathway of MTA catabolism is found in Plasmodium species (34). The adenosine deaminase of P. falciparum (PfADA) deaminates adenosine and MTA as substrates with similar $k_{cat}/K_m$ values (6.2 x 10$^4$ and 8.8 x 10$^4$, M$^{-1}$s$^{-1}$ respectively) (34). Its purine nucleoside phosphorylase (PfPNP) also degrades inosine and MTA to hypoxanthine with similar catalytic efficiency (3.6 x 10$^5$ and 9.4 x 10$^4$, M$^{-1}$V$^{-1}$ respectively) (33). Human PNP is a poor catalyst for MTI phosphorolysis and mammalian ADA does not deaminate MTA (34). The substrate specificities of the P. falciparum enzymes permit conversion of MTA to hypoxanthine via MTI. This process was reconstituted in vitro with purified PfADA and PfPNP (34). Metabolic and genomic studies have thus established that neither P. aeruginosa nor P. falciparum encode MTAN or MTAP activities.

MTI in Other Organisms. Although MTI has been used as an MTA analogue, its function as a metabolite has been documented only in Plasmodium (30,31,39-42). Metabolism studies on Saccharomyces cereviceae suggested in vivo conversion of MTA to MTI, but the corresponding enzyme(s) and fate of MTI have not been determined (43). In humans, MTAP catalyzes the conversion of MTA to adenine and 5-methylthioribose-a-D-1-phosphate. And no other fate is known for MTA although it appears in human urine (44). Excess MTA added to cultured human cells is known to form some MTI through the reverse reaction of PNP using hypoxanthine and MTR-1-P, but it is not physiologically significant (45-46). The physiological fate of [8-$^{14}$C]MTI in P. falciparum has been established by following its incorporation into nucleic acids, where it was shown to be equivalent to inosine as a nucleic acid precursor (34).

The surprising discovery of MTIP catalytic activity in Psuedomonas aeruginosa led to a search of the genome data bases for other microbial species that could be treated in the same manner as P. aeruginosa (Table 4). Of particular interest are Pseudomonas syringae and Xanthomonas campestris, which are important plant pathogens. P. syringae produces ice nucleation-active (Ina) proteins that cause water to freeze at fairly high temperatures resulting in surface frost damage to plants (51). It has been estimated that frost accounts for approximately $1$ billion in crop damage each year in the United States alone. Xanthomonas campestris causes a variety of plant diseases.

Picomolar inhibitors of PaMTIP. Immucillins are transition-state analogues developed for N-ribosyl transferases that have ribocation character in their transition states (47). ImmH is a first generation Immucillin, representing early transition states exemplified
by bovine PNP (Fig. 1). DADMe-ImmH is a second generation Immucillin and mimics the fully dissociated transition states of human and \textit{P. falciparum} PNP (48). 5'-Alkylthio- and arylthio- derivatives of two generations of Immucillins (MT-ImmH, PhT-ImmH, and MT-DADMe-ImmH) have been synthesized to target the transition-state features of P/PNP with MTI as the substrate (34). The inhibitors exhibited slow-onset inhibition of PoxMTTP, suggesting a slow conversion of initial enzyme-inhibitor complex to a more stable conformation.

**[00127]** MT-ImmH inhibited PoxMTTP with a \(K_i^*\) value of 76 pM, binding 4-fold more tightly than MT-DADMe-ImmH, suggesting that the first generation Immucillins more closely resemble the transition state (Table 3). PhT-ImmH was the most tightly bound inhibitor with a \(K^*\) value of 35 pM and demonstrating the importance of hydrophobic interactions at the 5'-position. Increasing the lipophilicity at the equivalent position in the second generation inhibitors by extending the alkyl chain length in going from MT-DADMe-ImmH to PrT-DADMe-ImmH similarly enhanced potency, in this instance by a factor of 4, confirming that both first and second generation Immucillins can provide highly effective inhibitors. Introduction of the 4'-C-hydroxy group (4'-HO-MT-DADMe-ImmH) provides a nanomolar inhibitor of PoxMTTP. The acyclic Immucillin, MT-SerMe-ImmH, inhibited PoxMTTP with a \(K_i^*\) value of 96 pM, confirming that this class of Immucillin is also able to inhibit MTIP enzymes effectively. The MT-TrisMe-ImmH analogue, has a \(K_i\) value of 19 nM. The \(K_{MT}/K^*\) values were 34,000 for MT-ImmH, 7,600 for MT-DADMe-ImmH, and 74,000 for PhT-ImmH, emphasizing the potency of these transition state analogue inhibitors. Inhibitor specificity can be compared to that for P/PNP, which catalyzes the same reaction. Thus, MT-ImmH, PhT-ImmH, and MT-DADMe-ImmH bind more weakly to P/PNP with dissociation constants of 2.7 nM, 150 nM, and 0.9 nM, respectively (Table 3) (33, 49). Binding of the bulky, hydrophobic 5'-PhT-group is preferred by PoxMTIP where \(K^* = 35\) pM.

This inhibitor induces slow-onset, tight-binding inhibition. In contrast, the same inhibitor has \(K = 150\) nM with P/PNP, where it binds 4,300-fold more weakly and does not induce slow-onset inhibition. The \(K^*\) values for certain MTIP inhibitors are indicated below.
(A) $Z = \text{SMe} \quad K_i^* = 76 \text{ pM}$

(B) $Z = \text{SPh} \quad K_i^* = 35 \text{ pM}$

(C) $Z = \text{SMe} \quad K_i^* = 340 \text{ pM}$

(D) $Z = \text{SMe} \quad K_i^* = 96 \text{ pM}$
MT-ImmH
$K_i^* = 76 \pm 5 \text{ pM}$
$k_i = 840 \pm 50 \text{ pM}$

PT-ImmH
$K_i^* = 35 \pm 3 \text{ pM}$
$k_i = 660 \pm 90 \text{ pM}$

MT-DADMe-ImmH
$K_i^* = 340 \pm 20 \text{ pM}$
$k_i = 800 \pm 100 \text{ pM}$

PrT-DADMe-ImmH
$K_i^* = 72 \pm 2 \text{ pM}$
$k_i = 380 \pm 40 \text{ pM}$

4'-HO-MT-DADMe-ImmH
$K = 1.21 \pm 0.01 \text{ nM}$

MT-SerMe-ImmH
$k_i^* = 96 \pm 2 \text{ pM}$
$K_i = 720 \pm 30 \text{ pM}$

MT-TrisMe-ImmH
$K = 19 \pm 1 \text{ nM}$

[00128] The Nature of the PaMTIP Transition State. PfPNP has a fully-dissociated ribocation transition state with approximately 3 Å between the CI' ribocation and N9 and a similar separation between CI' and the attacking phosphate oxygen. Thus, it prefers to bind
MT-DADMe-ImmH rather than MT-ImmH (50). PaMTIP shows the opposite pattern, consistent with an early, dissociative transition state.

[00129] **Implications for Quorum Sensing.** Inhibition studies of PaMTIP have identified inhibitors with $K_{1}^{*}$ values in the picomolar range. These are candidates for blocking PaMTIP activities. In most bacteria, MTAN inhibition blocks quorum sensing, but the lack of MTAN in *P. aeruginosa* indicates that PaMTIP becomes an equivalent target. This study has revealed the pathway of MTA metabolism in *P. aeruginosa* and provided new tools to explore this unusual bacterial pathway.
Table 1. Data collection and refinement statistics.

<table>
<thead>
<tr>
<th>Data collection</th>
<th></th>
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<tbody>
<tr>
<td>PDB</td>
<td>30ZB</td>
</tr>
<tr>
<td>Space group</td>
<td>P4i2i2</td>
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<tr>
<td>Cell dimension</td>
<td></td>
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<tr>
<td>a, b, c (Å)</td>
<td>99.5, 99.5, 334.9</td>
</tr>
<tr>
<td>α, β, γ (°)</td>
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<td>Resolutions (Å)</td>
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<tr>
<td></td>
<td>(2.9-2.8)</td>
</tr>
<tr>
<td>( R_{\text{sym}} ) (%)</td>
<td>17.2 (95.5)</td>
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<tr>
<td>( I/\sigma I )</td>
<td>9.6 (1.9)</td>
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<tr>
<td>Completeness (%)</td>
<td>100.0 (100.0)</td>
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<tr>
<td>Redundancy</td>
<td>7.0 (7.2)</td>
</tr>
</tbody>
</table>

| Refinement | |
| Resolution (Å) | 20.00-2.0 |
| No. reflections | 39915 |
| \( R_{\text{work}} / R_{\text{free}} \) (%) | 20.1 / 26.2 |

| B-factors (Å²) | |
| Protein (main chain) | 39.9 |
| (side chain) | 40.9 |
| Water | 25.2 |
| Ligand | 46.0 |

| No. of Atoms | |
| Protein | 10800 |
| Water | 68 |
| Ligand | 60 |

| R.m.s deviations | |
| Bond lengths (Å) | 0.012 |
| Bond angles (°) | 1.46 |

| Ramachran analysis | |
| allowed region | 99.3% |
| disallowed region | 0.7% |

Numbers in parentheses are for the highest-resolution shell. One crystal was used for each data set.
Table 2. Substrate specificity of PαMTIP.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$\gamma_{\text{cat}}$ (s$^{-1}$)</th>
<th>$K_m$ (μM)</th>
<th>$K_{\text{cat}}/K_m$ (x 10$^3$ M$^{-1}$s$^{-1}$)</th>
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<tbody>
<tr>
<td>MTI</td>
<td>4.8 ± 0.2</td>
<td>2.6 ± 0.4</td>
<td>1800 ± 300</td>
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<tr>
<td>MTA $^a$</td>
<td>N/A</td>
<td>70 ± 20</td>
<td>N/A</td>
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<tr>
<td>inosine</td>
<td>0.57 ± 0.04</td>
<td>90 ± 20</td>
<td>6 ± 1 ($^{100}$) b</td>
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<tr>
<td>adenosine</td>
<td>0.0549 ± 0.0005</td>
<td>23 ± 1</td>
<td>2.4 ± 0.1 ($^{750}$) b</td>
</tr>
</tbody>
</table>

$^a$ MTA is not a substrate of PαMTIP. $K_i$ is used instead of $K_m$ for MTA.

$^b$ Numbers in [] are fold decreases of $\gamma_{\text{cat}}/K_m$ in comparison with those of MTI.

$^c$ Values are ± S.E.

Table 3. Summary of Ki values for PaMTIP and PfPnP.

<table>
<thead>
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<th>Inhibitors</th>
<th>PaMTIP</th>
<th>PfPnP$^a$</th>
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<tbody>
<tr>
<td></td>
<td>$K_i$ (pM)</td>
<td>$K_i^*$ (pM)</td>
</tr>
<tr>
<td>MT-ImmH</td>
<td>840 ± 50</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>PhT-ImmH</td>
<td>660 ± 90</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>MT-DADMe-ImmH</td>
<td>800 ± 100</td>
<td>340 ± 20</td>
</tr>
</tbody>
</table>

$^a$ Inhibition constants of PfPnP were from Lewandowicz et al. (49).

$^b$ Values are ± S.E.

Table 4. Microbial species searched for possible MTIP catalytic activity.

<table>
<thead>
<tr>
<th>Feature</th>
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<td>Descr</td>
<td>Species</td>
<td></td>
</tr>
<tr>
<td>31909191</td>
<td>VBIAcFer09666_1307</td>
<td>MTAP</td>
<td>Acidaminococcus fermentans DSM 20731</td>
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Description of Chemical Compounds

[00130] In one embodiment, as described in part in U.S. Patent No. 5,985,848 and in PCT International Patent Application Publication No. WO 99/19338, the contents of which are herein incorporated by reference, the MTIP inhibitor comprises a compound having formula (I):

![Chemical Structure](image)

wherein A is CH or N; B is OH; D is chosen from H, OH, NH₂, or SCH₃; and X and Y are independently selected from H, OH or halogen, except that when one of X and Y is hydroxy or halogen, the other is hydrogen; Z is selected from CH₂SQ, CH₂OQ or Q, where Q is an optionally substituted alkyl, aralkyl, aralkenyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino,
carboxy, or straight- or branched-chain C1-Ce alkyl; provided that Z is not CH2OH; or a
tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

[00131] Methods to make compounds of formula (I) can be found in WO 99/19338
and in U.S. Patent No. 5,985,848.

[00132] Methods to make compounds of formula (I) in which X = OH and Y = H can
be found in PCT International Patent Application Publication No. WO 00/061783 and in U.S.
Patent No. 6,693,193, the contents of which are herein incorporated by reference.

[00133] 1,4-Dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol can be converted into
compounds of formula (i) below. Compounds of formula (i) can be converted into
compounds of formula (I) by the methods provided in WO 00/061783 and in U.S. Patent No.
6,693,193.

![Formula (i)](attachment:image)

[00134] The compound of Formula (ii) below can be converted into compounds of
formula (I) where Z = Q by oxidation at C-5', Witting reaction with the resulting aldehyde,
optionally hydrogenating the double bond so formed, then removing the protecting groups by
acid hydrolysis or a combination of acid hydrogenolysis and acid hydrolysis. Compound (ii)
can be prepared by selectively removing the 5'-O-tert-butylidemethylsilyl protecting group of
compound 40 in Evans et al, J. Org. Chem., 66 (2001) 5723, e.g. by the use of
tetrabutylammonium fluoride.

![Formula (ii)](attachment:image)
A method to make the compound can be found in WO 99/19338 and in U.S. Patent No. 5,985,848, where it was referred to as (1S)-1,4-dideoxy-1-C-(4-hydroxy-3,2-dipyrrrolo[3,2-d]pyrimidin-7-yl)-1,4-imino-5-methylthio-\textit{D}-ribitol. Subsequently, the compound was reported as an inhibitor of the PNP of \textit{Plasmodium falciparum} and its method of synthesis was described (W. Shi et al., \textit{J. Biol. Chem.}, 279 (2004) 18103) (33).

The compound is reported in connection with its inhibition of the human and \textit{Plasmodium falciparum} PNP and a method of synthesis was described (A. Lewandowicz et al, \textit{J. Biol. Chem.}, 280 (2005) 30320) (49).

where Z = H is disclosed in A. Lewandowicz et al, \textit{J. Biol. Chem.}, 280 (2005) 30320 (49) as an inhibitor of PNP.

the contents of which are herein incorporated by reference, the MTIP inhibitor comprises a compound having formula (III) or (Ilia):

\[
\begin{align*}
Z & \quad \text{CH}_2 \\
\text{V} & \quad \text{OH} \\
\text{W} & \quad \text{X} \\
\end{align*}
\]

(III) or

\[
\begin{align*}
Z & \quad \text{OH} \\
\text{Y} & \quad \text{V} \\
\text{W} & \quad \text{X} \\
\end{align*}
\]

(IIIa)

wherein \( V \) is \( C\) and \( W \) is \( NR^1 \); \( X \) is \( C\); \( Y \) is selected from hydrogen, halogen and hydroxyl; \( Z \) is selected from hydrogen, halogen, \( SQ\), \( OQ\) and \( Q\), where \( Q \) is an optionally substituted alkyl, aralkyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain \( Ci-Ce \) alkyl; \( R^1 \) is a radical of the formula (IV)

\[
\begin{align*}
\text{A} & \quad \text{B} \\
\text{G} & \quad \text{D} \\
\text{E} & \quad \text{H} \\
\text{N} & \quad \text{E} \\
\end{align*}
\]

(IV)

\( A \) is selected from \( N\), \( CH \) and \( CR \), where \( R \) is selected from halogen or optionally substituted alkyl, aralkyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain \( Ci-Ce \) alkyl; \( B \) is \( OH \); \( D \) is selected from \( OH\), \( NH_2\), \( SCH_3 \) and hydrogen; \( E \) is \( N \); \( G \) is \( C\); or a tautomer thereof or a pharmaceutically acceptable salt thereof; or an ester thereof.

[00139] Methods to make compounds of formula (III) and (Ilia) are provided in U.S. Patent No. 7,553,839 and in WO 04/018496, and in WO 2007/069924, and also in US 7,655,795 and WO 2004/069856, the contents of all of which are herein incorporated by reference.

[00140] The compound
is disclosed in WO 2004/018496 and in U.S. Patent No. 7,553,839 as "(3R,4S)-1-[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(methylthiomethyl)pyrrolidine", exemplified as compound 37 (Example 29) and shown to have PNP inhibitory properties. It is disclosed as (3R,4S)-1-[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(methylthiomethyl)pyrrolidine in WO 2004/069856 and in US 7,655,795 as a compound that can be prepared by the process disclosed and claimed therein. The compound is also reported as an inhibitor of human and Plasmodium falciparum PNP and a method of synthesis is described (A. Lewandowicz et al, J. Biol. Chem., 280 (2005) 30320) (49).

The compound

is disclosed in in WO 2004/018496 and in U.S. Patent No. 7,553,839, as "(3S,4R)-1-[(9-deazahypoxanthin-9-yl)methyl]-3,4-dihydroxy-4-methylthiomethylpyrrolidine", exemplified as compound 33 (Example 28) and shown to have PNP inhibitory properties. This is an example where Y = OH. It is also reported in connection with its inhibition of the human and Plasmodium falciparum PNP and a method of synthesis is described (A. Lewandowicz et al, J. Biol. Chem., 280 (2005) 30320) (49).

The compound

The compound is disclosed in US 7,655,795 and WO 2004/069856 as "(3R,4S)-1-[(9-deazahypoxanthin-9-yl)-3-hydroxy-4-(propylthiomethyl)-pyrrolidine" and exemplified as compound 24 in Example 1.20 as a compound that can be prepared by the process disclosed and claimed therein. It is reported in connection with its inhibition of human and Plasmodium falciparum PNP and a method of synthesis was described (A. Lewandowicz et al, J. Biol. Chem., 280 (2005) 30320) (49).

The compound:


The compound:

This compound can also be prepared according to the following procedure:

(3R,4S)-tert-Butyl 3-(tert-butyldimethylsilyloxy)-4-formylpyrrolidine-1-carboxylate (A.1). This compound is prepared as described in Longshaw, Alistair I.; Adanitsch, Florian; Gutierrez, Jemy A.; Evans, Gary B.; Tyler, Peter C.; Schramm, Vern L. Design and Synthesis of Potent "Sulfur-Free" Transition State Analogue Inhibitors of 5'-Methylthioadenosine Nucleosidase and 5'-Methylthioadenosine Phosphorylase. Journal of Medicinal Chemistry (2010), 53(18), 6730-6746.

(3R,4S)-tert-Butyl 3-(tert-butyldimethylsilyloxy)-4-(propen-2-yl)pyrrolidine-1-carboxylate (A.2). n-Butyllithium (1.95 mL, 2.7 mmol, 1.4 M solution in hexanes) was added drop-wise to a stirred suspension of ethyltriphenylphosphonium bromide (2.03 g, 5.5 mmol) in THF (10 mL) at 0 °C. After 30 min a solution of A.1 (977 mg, 3.0 mmol) in THF (10 mL) was added. The reaction mixture was stirred overnight and allowed to warm to room temperature. The reaction was quenched with water (30 mL) and extracted with ethyl acetate (100 mL). The combined organic phases were washed with saturated aqueous sodium hydrogen carbonate solution (50 mL) and brine (50 mL) and then dried (MgSC^), filtered and concentrated in vacuo. Flash chromatography of the residue (1 : 9, EtOAc : Petrol) afforded
A.2 as a colorless oil (250 mg, 40%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta =$ 5.59 (m, 1H), 5.17 (m, 2H), 3.95 (m, 1H), 3.66 - 3.48 (m, 2H), 3.17 - 2.32 (m, 2H), 1.67 (d, $J = 6.6$ Hz, 2H), 1.46 (s, 9H), 0.87 (s, 9H) and 0.04 ppm (s, 6H).

[00149] 3-(tert-butyldimethylsilyloxy)-4-propylpyrrolidine-1-carboxylate (A.3).
Perlman's catalyst (75 mg, 0.9 mmol, 20 wt% on carbon) was added to a solution of A.2 (221 mg, 0.65 mmol) in ethanol (5 mL) under a argon atmosphere. The reaction mixture was placed under a hydrogen atmosphere and stirred overnight and then filtered through Celite® and concentrated under reduced pressure to afford the title compound A.3 (220 mg, 99%). NMR confirmed that no olefinic protons remained and that no further purification was necessary.

[00150] (3R,4S)-4-Propyl-3-hydroxypyrroline (A.4). Concentrated HCl (1 ml, 12 mmol) was added to a solution of A.3 (22 g, 0.62 mmol) in methanol (5 ml) and concentrated in vacuo. The resulting residue was dissolved in additional concentrated HCl (1 ml, 12 mmol) and concentrated in vacuo. The residue was dissolved in water (10 ml) and washed with CHCl$_3$ (2 x 20 ml) and the water layer concentrated in vacuo to afford A.4.

[00151] (3R,4S)-l-[(9-Deazahypoxanthin-9-yl)methyl]-4-propyl-3-hydroxypyrroline (A.5). 9-Deazahypoxanthine (0.147 g, 1.09 mmol) was added to a solution of A.4 (0.09 g, 0.54 mmol) and sodium acetate (0.089 g, 1.09 mmol) in water (2 ml). After 5 min aqueous formaldehyde (0.084 ml, 1.087 mmol) was added and the resulting suspension warmed to 95 °C bath temperature and the resulting mixture stirred overnight. The reaction mixture was then cooled to room temperature and absorbed onto silica gel. The resulting residue was purified by chromatography eluting with 25% 7N NH$_3$ in MeOH:CHCl$_3$ to afford A.5 (90 mg, 60%) as a white solid. $^{13}$C NMR (125 MHz, D$_2$O): $\delta =$ 163.5, 151.7, 145.4, 127.7, 118.8, 109.7, 76.2, 59.9, 57.1, 47.3, 46.1, 34.6, 20.6, and 13.4 ppm.

[00152] The compound:

\[\text{The compound:} \]

is prepared according to the following procedure:
To a solution of B.1 (1.3 g, 1.985 mmol) in THF (30 mL) was added acetic acid (0.114 mL, 1.985 mmol) and then TBAF (tetrabutyl ammonium fluoride 1M THF) (1.730 ml, 5.96 mmol). The solution was stirred at ~ 50-60 °C for 16 h. After evaporation, toluene was added and the solution was washed water, dried (MgSC\(^{\alpha}\)) and concentrated. Chromatography (25-50% EtOAc/Hex) gave B.2 as a foam (1.0 g, 1.85 mmol, 93%).

To a stirred solution of B.2 (100 mg, 185 µmol) in dry DCM (3 mL) was added Dess-Martin periodinane (157 mg, 370 µmol). After 16 h the mixture was concentrated to dryness. Chromatography (20-60% EtOAc/Hex) gave B.3 as a syrupy product (58 mg, 108 µmol, 58%).

A 1M solution of lithium bis(trimethylsilyl)amide in THF (204 µL, 204 µmol) was added to a suspension of benzyl triphenylphosphonium bromide (97 mg, 225 µmol) in dry THF (3 mL) at -75 °C and then the mixture was allowed to warm to 0 °C. After 30 mins at 0 °C a solution of B.3 (55 mg, 102 µmol) in THF (1 mL) was added and then the mixture
was stirred at RT. After 30 mins chloroform was added and the mixture was washed with water, dried (MgSO\textsubscript{4}) and concentrated. Chromatography of the residue (15-40% EtOAc/Hex) gave B.4 as a syrup (59 mg 96 \mu\textmu\textmu\textmu\textmu\textmu\textmu, 94%).

[00156] To a solution of B.4 (55 mg, 90 \mu\textmu\textmu\textmu\textmu\textmu\textmu) in EtOH and some NH\textsubscript{3}/MeOH was added 10\% Pd/C and the mixture was stirred under H\textsubscript{2}. After 2 days the mixture was filtered and the filtrate concentrated. Chromatography of the residue (20-60\% EtOAc/Hex) gave B.5 as a syrup (25 mg, 50 \mu\textmu\textmu\textmu\textmu\textmu\textmu, 56%).

[00157] A solution of B.5 (25 mg, 50.5 \mu\textmu\textmu\textmu\textmu\textmu\textmu) in MeOH (4 mL) and cone aq HCl (4 mL) was heated under reflux for 3 h and then concentrated to dryness. The residue in water was lyophilized to a white powder of B.6 (18 mg, 48 \mu\textmu\textmu\textmu\textmu\textmu\textmu, 94\%). \textsuperscript{1}H nmr (d\textperiodcenteredMeOH with a little DC\textsubscript{1}/D\textsubscript{2}O) \delta 9.1 (1H, s), 8.1 (1H, s), 7.31 (4H, m), 7.20 (1H, m), 4.96 (1H, d), 4.73 (1H, dd), 4.30 (1H, t), 3.68 (1H, m), 2.84 (2H, m), 2.27 (2H, m). \textsuperscript{13}C nmr δ 152.5, 146.7, 141.7, 132.5, 131.9, 129.7, 127.4, 119.8, 106.5, 74.1, 73.8, 66.6, 57.3, 34.0, 33.5. HRMS calc for MH\textsuperscript{+} C\textsubscript{18}H\textsubscript{15}N\textsubscript{2}O\textsubscript{3} 341.1614; found 341.1618.

[00158] In another embodiment, as described in PCT International Patent Application Publication No. WO 2008/0301 19, the contents of which are herein incorporated by reference, the MTIP inhibitor comprises a compound having formula (VI):

![Diagram](image-url)

(VI)

wherein \( R^1 \) is H or NR\textsuperscript{3}R\textsuperscript{4}; \( R^2 \) is H or an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR\textsuperscript{3}R\textsuperscript{4} groups, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; provided that when \( R^1 \) is H, \( R^2 \) is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group which is substituted with at least one NR\textsuperscript{3}R\textsuperscript{4} group, and optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid,
carboxylate alkyl ester or nitro group; R³ and R⁴, independently of each other, are H or an alkyl, alkenyl, alkylnyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR³R⁴ groups, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; A is N or CH; B is OH; and D is H, OH, NH₂, or SC³⁄₂; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

Methods to make compounds of formula (VI) are provided in WO 08 0301 19. Certain of the amino-alcohols required for use in the synthesis of the compounds of formula (VI) can be obtained through reduction of amino-acids to the corresponding amino-alcohols.

In another embodiment, the MTIP inhibitor comprises a compound having formula (VII):

![Diagram](VII)

wherein X is an alkyl, cycloalkyl, aralkyl, aralkenyl, alkenyl, alkynyl or aryl group, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, cycloalkyl, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or X is SR¹; or X is NR²R³; R¹, R² and R³ are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano and NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; A is N or CH; B is OH; D is H, OH, NH₂, or SCH³⁄₂; provided that X is not CH₂Z, where Z is selected from OH, hydrogen, halogen, SQ¹, OQ² and Q³, where Q¹ is an optionally substituted alkyl, aralkyl or aryl group, Q² is an optionally substituted alkyl group.
and Q is an optionally substituted alkyl group; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

[00161] Methods to make compounds of formula (VII) are provided in U.S. Patent No. 7,553,839 and in WO 04/018496, the contents of all of which are herein incorporated by reference, where a 3-hydroxypyrrolidine is coupled to 9-deaza9-formylpurine derivative by reductive amination, and in US Patent No. 7,655,795 and WO 2004/069856, the contents of all of which are herein incorporated by reference, where a 3-hydroxypyrrolidine is coupled to 9-deazapurine and formaldehyde in a Mannich reaction. Suitable 3-hydroxypyrrolidines may be prepared by methods detailed in PCT/NZ2010/000148 and in A. Longshaw et al, J. Med. Chem., 53 (2010) 6730.

REFERENCES


What is claimed is:

1. A method for treating an infection caused by bacteria that use 5'-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway comprising administering to a subject having the infection a sub-growth inhibiting amount of a 5'-methylthioinosine phosphorylase (MTIP) inhibitor.

2. The method of Claim 1, wherein the bacteria is *Pseudomonas aeruginosa, Pseudomonas syringae* or *Xanthomonas campestris*.

3. The method of Claim 1 or 2, wherein the subject is an animal

4. The method of Claim 3, wherein the subject is a human.

5. The method of Claim 1 or 2, wherein the subject is a plant.

6. The method of Claim 1, wherein the bacteria is *Pseudomonas aeruginosa* and the subject is a human.

7. The method of Claim 1, wherein the bacteria is *Pseudomonas syringae* or *Xanthomonas campestris* and the subject is a plant.

8. The method of any of Claims 1-7, wherein the MTIP inhibitor comprises a compound having formula (I):

   ![Chemical Structure](image)

wherein

A is CH or N;
B is OH;
D is chosen from H, OH, NH₂, or SCH₃;
X and Y are independently selected from H, OH or halogen, except that when one of X and Y is hydroxy or halogen, the other is hydrogen;
Z is selected from CH₂SQ, CH₂OQ or Q, where Q is an optionally substituted alkyl, aralkyl, aralkenyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C₁-C₆ alkyl; provided that Q is not CH₂OH;
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof; or a prodrug thereof.

9. The method of Claim 8, wherein A is CH.

10. The method of Claims 8 or 9, wherein D is H.

11. The method of any of Claims 8-10, wherein X is OH and Y is H.

12. The method of any of Claims 8-11, wherein Z is CH₂SQ or Q.

13. The method of any of Claims 8-12, where Z is CH₂SQ, and Q is an optionally substituted Ci-Ce alkyl group, aryl group or aralkyl group.

14. The method of any of Claims 8-12, where Z is Q, and Q is an optionally substituted Ci-Ce alkyl group, aralkyl group, or aralkenyl group.

15. The method of any of Claims 8-11, wherein Z is phenylthiomethyl, p-chlorophenylthiomethyl, methylthiomethyl, ethylthiomethyl, propylthiomethyl, n-propylthiomethyl or isopropylthiomethyl, butylthiomethyl, -CH₂S(CH₂)₂CHNH₂COOH, methyl, ethyl, propyl, butyl, benzyl, phenylethenyl or -CH₂(CH₂)₃CH(NH₂)COOH.

16. The method of Claim 8, wherein A is CH or N; B is OH; D is chosen from H, OH, NH₂, or SCH₃; and X is OH; Y is H; Z is Q, where Q is an optionally substituted alkyl,
alkenyl, aralkyl or aryl group, each of which is optionally substituted with one or more
substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or
branched-chain \textit{Ci-Ce} alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt
thereof; or an ester thereof.

17. The method of Claim 8, wherein the MTIP inhibitor comprises a compound having
formula (II):

\[
\begin{array}{c}
\text{O-S-} \\
\text{H} \\
\text{H} \\
\text{H}
\end{array}
\]

(II)

wherein \( Q \) is an optionally substituted alkyl, aralkyl or aryl group, each of which is optionally
substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino,
carboxy, or straight- or branched-chain \textit{Ci-Ce} alkyl;

or a tautomer thereof; or a pharmaceutically acceptable salt thereof, or an ester thereof; or a
prodrug thereof.

18. The method of Claim 17, wherein \( Q \) is a \textit{Ci-Ce} alkyl group or an aryl group.

19. The method of Claim 17, wherein \( Q \) is methyl.

20. The method of Claim 17, wherein \( Q \) is phenyl.

21. The method of Claim 8, wherein wherein the MTIP inhibitor comprises a compound
having formula (Ila):

\[
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{H}
\end{array}
\]

(Ila)
wherein Q is alkyl, alkenyl, aralkyl, aralkenyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C1-C6 alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt thereof, or an ester thereof.

22. The method of Claim 8, wherein the MTIP inhibitor comprises a compound having the structure selected from the group consisting of:

- [Chemical structures]

- [Chemical structures]
a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

23. The method of Claim 8, wherein the MTIP inhibitor comprises a compound having the structure:
24. The method of any of Claims 1-7, wherein the MTIP inhibitor comprises a compound having formula (III) or formula (Ilia):

wherein
V is CH₂;
W is NR₁;
X is CH₂;
Y is selected from hydrogen, halogen and hydroxyl;
Z is selected from hydrogen, halogen, SQ, OQ and Q, where Q is an optionally substituted alkyl, aralkyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C₁-C₆ alkyl;
R₁ is a radical of the formula (IV)
A is selected from N, CH and CR, where R is selected from halogen or optionally substituted alkyl, aralkyl or aryl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C1-Ce alkyl;
B is OH;
D is selected from OH, NH2, SCH3 and hydrogen;
E is N;
G is CH2;
or a tautomer thereof or a pharmaceutically acceptable salt thereof; or an ester thereof.

25. The method of Claim 24, wherein Y is H.

26. The method of Claim 24 or 25, wherein A is CH.

27. The method of any of Claims 24-26, wherein D is H.

28. The method of any of Claims 24-27, wherein Z is SQ.

29. The method of any of Claims 24-28, wherein Q is an optionally substituted aryl group or aralkyl group, or Q is an optionally substituted C1-Ce alkyl group.

30. The method of any of Claims 24-29, wherein Q is methyl.

31. The method of any of Claims 24-29, wherein Q is phenyl.

32. The method of Claim 24, wherein the MTIP inhibitor comprises a compound having formula (V):
where Q is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, and straight- or branched-chain \( Ci-Ce \) alkyl;
or a tautomer thereof; or an ester thereof; or a pharmaceutically acceptable salt thereof.

33. The method of Claim 32, wherein Q is an optionally substituted aryl group or aralkyl group, or Q is an optionally substituted \( Ci-Ce \) alkyl group.

34. The method of Claim 33, wherein Q is methyl.

35. The method of Claim 33, wherein Q is phenyl.

36. The method of Claim 24, wherein the MTIP inhibitor comprises a compound having formula (Va):

where Q is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, and straight- or branched-chain \( Ci-Ce \) alkyl; or a tautomer thereof; or an ester thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof.

37. The method of Claim 24, wherein the MTIP inhibitor comprises a compound having the structure selected from the group consisting of:
and
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

38. The method of Claim 24, wherein the MTIP inhibitor comprises a compound having the structure:

39. The method of Claim 24, wherein the MTIP inhibitor comprises a compound having the structure selected from the group consisting of:
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

40. The method of any of Claims 1-7, wherein the MTIP inhibitor comprises a compound having formula (VI):

and
wherein

R\textsuperscript{1} is H or NR\textsubscript{3}R\textsubscript{4};

R\textsuperscript{2} is H or an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR\textsuperscript{3}R\textsuperscript{4} groups, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups;

provided that when R\textsuperscript{1} is H, R\textsuperscript{2} is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group which is substituted with at least one NR\textsuperscript{3}R\textsuperscript{4} group, and optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester or nitro group;

R\textsuperscript{3} and R\textsuperscript{4}, independently of each other, are H or an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, or NR\textsuperscript{3}R\textsuperscript{4} groups, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups;

A is N or CH;

B is OH; and

D is H, OH, NH\textsubscript{2}, or SCH\textsubscript{3};

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

41. The method of Claim 40, wherein A is CH.
42. The method of Claim 40 or 41, wherein \( D \) is H.

43. The method of any of Claims 40-4, wherein \( R^1 \) is \( NR^2 R^4 \).

44. The method of any of Claims 40-43, wherein \( R^2 \) is H.

45. The method of Claim 40, wherein the MTIP inhibitor comprises a compound having; formula (Vla):

\[
\begin{align*}
\text{H} & \\
\text{N} & \\
\text{R}^2 & \\
\text{N} & \\
\text{H} & \\
\text{C} & \\
\text{NC} & \\
\text{NH} & \\
\end{align*}
\]

\[(Vla)\]

wherein \( R^3 \) is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is optionally substituted with one or more hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, or nitro group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

46. The method of Claim 40, wherein the MTIP inhibitor comprises a compound having; formula (Vlb):

\[
\begin{align*}
\text{H} & \\
\text{N} & \\
\text{R}^2 & \\
\text{N} & \\
\text{H} & \\
\text{C} & \\
\text{NC} & \\
\text{NH} & \\
\end{align*}
\]

\[(Vlb)\]

wherein \( R^3 \) is an alkyl, alkenyl, alkynyl, aralkyl, aralkenyl, aralkynyl, or aryl group, each of which is substituted with at least one alkoxy, thiol, alkylthio, arylthio, or aralkylthio group and optionally substituted with one or more hydroxy, halogen, carboxylic acid, carboxylate alkyl ester, or nitro group, where each alkylthio, arylthio and aralkylthio group is optionally
substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

47. The method of Claim 40, wherein the MTIP inhibitor comprises a compound selected from the group consisting of:

2-amino-7-(2,3-dihydroxy-1-(2-(methylthio)ethylamino)propyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(2,3-dihydroxy-1-(2-(methylthio)ethylamino)propyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-(1-amino-2,3-dihydroxy-5-(methylthio)pentyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(1-amino-2,3-dihydroxy-5-(methylthio)pentyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-(2-hydroxy-1-(1-hydroxy-3-(methylthio)propan-2-ylamino)ethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(2-hydroxy-1-(1-hydroxy-3-(methylthio)propan-2-ylamino)ethyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-((3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-((2-hydroxy-4-(methylthio)butyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((2-hydroxy-4-(methylthio)butyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-((2-hydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((2-hydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

2-amino-7-((3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((3-hydroxy-2-(methylthiomethyl)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((3-hydroxy-2-(methylthiomethyl)propylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
5-amino-3-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
7-((3-hydroxy-2-(methylthiomethyl)propyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((3-hydroxy-2-(methylthiomethyl)propyl)(methyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
5-amino-3-((1,3-dihydroxy-2-(methylthiomethyl)propan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
7-((benzyl((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((benzyl((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((benzyl((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-((benzyl((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-((benzyl((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butyl)amino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2R,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2R,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3S)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3R)-2,3-dihydroxy-4-(methylthio)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;

\[
\begin{align*}
\text{MeS} & \quad \text{HO} \\
\text{OH} & \quad \text{NH} \quad \text{NH} \\
\text{C} & \quad \text{O} \\
\end{align*}
\]

7-(((2R,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((25,35)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((2R,35)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
7-(((25,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((25',3S)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3S)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
2-amino-7-(((25',3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-3 H-pyrrolo[3,2-i]pyrimidin-4(5H)-one;
3-(((2R,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3S)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2R,3S)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3R)-3,4-dihydroxy-1-(methylthio)butan-2-ylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;

3-(((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;
3-(((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one;

2-amino-7-(((2R,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
2-amino-7-(((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2R,3S)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one;
7-(((2S,3R)-3,4-dihydroxy-2-(methylthiomethyl)butylamino)methyl)-3H-pyrrolo[3,2-d]pyrimidin-4(5H)-one; or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

48. The method of Claim 40, wherein the MTIP inhibitor comprises a compound selected from the group consisting of:
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.
49. The method of Claim 40, wherein the compound has the structure

![Chemical Structure Diagram]
50. The method of any of Claims 1-7, wherein the MTIP inhibitor comprises a compound having formula (VII):

![Chemical Structure](image)

(VII)

wherein

X is an alkyl, cycloalkyl, aralkyl, aralkenyl, alkenyl, alkynyl or aryl group, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, cycloalkyl, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano, thiazole and NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups; or X is SR¹; or X is NR²R³;

R¹, R² and R³ are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from the group consisting of hydroxy, alkoxy, thiol, alkylthio, arylthio, aralkylthio, halogen, carboxylic acid, carboxylate alkyl ester, nitro, cyano and NR²R³ group, where each alkylthio, arylthio and aralkylthio group is optionally substituted with one or more alkyl, halogen, amino, hydroxy, or alkoxy groups;

A is N or CH;

B is OH;

D is H, OH, NH₂, or SCH₃;

provided that X is not CH₂Z, where Z is selected from OH, hydrogen, halogen, SQ¹, OQ² and Q³, where Q¹ is an optionally substituted alkyl, aralkyl or aryl group, Q² is an optionally substituted alkyl group and Q³ is an optionally substituted alkyl group;

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester prodrug form thereof.

51. The method of Claim 50, wherein the MTIP inhibitor comprises a compound having formula (Vila):
52. The method of Claim 50, wherein the MTIP inhibitor comprises a compound having formula (VIIb):

(VIIb).

53. The method of any of Claims 50-52, wherein Q₁ is an optionally substituted alkyl, aralkyl or aryl group, Q₂ is an optionally substituted alkyl group and Q₃ is an optionally substituted alkyl group.

54. The method of any of Claims 50-53, wherein A is CH.

55. The method of any of Claims 50-54, wherein D is H.

56. The method of Claim 50, wherein the compound is selected from the group consisting of:

(±)-4-Allyl-l-[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-pyrrolidine;
(±)-4-Cyclopropyl-l-[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-pyrrolidine;
(±)-4-Cyclohexyl-l-[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-pyrrolidine;
(±)-4-Cyclohexylmethyl-l-[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-pyrrolidine;
(±)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(prop-1-en-2-yl)-pyrrolidine; 
(±)-4-Butyl-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(±)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine; 
(±)-4-Cyclopentyl-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(±)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(1H-1,2,3-triazol-1-yl)pyrrolidine; 
(±)-4-(1-Benzyl-1H-1,2,3-triazol-4-yl)-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(±)-5-4-(3-Benzylthiopropyl)-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(3R,4S)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-4-ethyl-3-hydroxypyrrolidine; 
(3R,4S)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-phenylpyrrolidine; 
(3R,4S)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-vinylpyrrolidine; 
(3R,4S)-4-Allyl-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(3R,4S)-4-Cyclopentyl-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(3R,4S)-4-Cyclohexyl-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(3R,4S)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(prop-1-en-2-yl)-pyrrolidine; 
(3R,4S)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(pent-3-yl)-pyrrolidine; 
(3R,4S)-4-Cyclopentyl-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(3S,4S)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(1H-1,2,3-triazol-1-yl)pyrrolidine; 
(3R,4R)-4-(1-Benzyl-1H-1,2,3-triazol-4-yl)-l-{[(9-deazahypoxanthin-9-yl)methyl]-3-hydroxypyrrolidine; 
(±)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(2-methylpropyl)pyrrolidine; 
(±)-4-Butyl-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine; 
(±)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine; 
(±)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(thiazol-2-yl)-pyrrolidine; 
(3R,4R)-l-{[(9-Deazahypoxanthin-9-yl)methyl]-3-hydroxy-4-(tetrazol-5-yl)-pyrrolidine; 
(3S,4R)-l-{[(9-Deaza-adenin-9-yl)methyl]-4-ethyl-3-hydroxypyrrolidine; 

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester thereof.
57. The method of any of Claims 1-56, wherein the sub-growth inhibiting amount of the MTIP inhibitor inhibits quorum sensing in the bacteria.

58. A pharmaceutical composition or an agrochemical composition comprising a sub-bacterial-growth inhibiting amount of a 5′-methylthioinosine phosphorylase (MTIP) inhibitor and a pharmaceutically acceptable carrier or an agrochemically acceptable carrier.

59. The pharmaceutical composition of Claim 58, wherein the sub-bacterial-growth inhibiting amount of the MTAN inhibitor inhibits quorum sensing in bacteria.

60. The pharmaceutical composition of Claim 58 or 59, wherein the bacteria is *Pseudomonas aeruginosa, Pseudomonas syringae* or *Xanthomonas campestris*.

61. The pharmaceutical composition of any one of Claims 58-60, wherein the pharmaceutical composition is formulated in dosage form.

62. A method for determining whether or not a compound is a candidate for treating an infection caused by bacteria that use 5′-methylthioinosine phosphorylase (MTIP) in a quorum sensing pathway, the method comprising determining whether or not the compound inhibits MTIP, wherein a compound that inhibits MTIP is a candidate for treating an infection caused by bacteria that use MTIP in a quorum sensing pathway and wherein a compound that does not inhibit MTIP is not a candidate for treating an infection caused by bacteria that use MTIP in a quorum sensing pathway.

63. The method of Claim 62, wherein the bacteria is *Pseudomonas aeruginosa, Pseudomonas syringae* or *Xanthomonas campestris*.

64. The method of any of Claims 1-57 or 62-63, or the composition of any of Claims 58-61, wherein the MTIP inhibitor has an IC50 value less than 50 nanomolar for MTIP.

65. The method of any of Claims 1-57 or 62-64, or the composition of any of Claims 58-61 or 64, wherein the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for human purine nucleoside phosphorylase (PNP).
66. The method of any of Claims 1-57 or 62-65, or the composition of any of Claims 58-61 or 64-65, wherein the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for 5'-methylthioadenosine nucleosidase (MTAN).

67. The method of any of Claims 1-57 or 62-66, or the composition of any of Claims 58-61 or 64-66, wherein the MTIP inhibitor has an IC50 value greater than or equal to 50 nanomolar for 5'-methylthioadenosine phosphorylase (MTAP).

68. A compound having the formula:

\[ \text{Formula Image} \]

wherein A is CH or N; B is OH; D is chosen from H, OH, NH₂, or SCH₃; X is OH; Y is H; and Z is Q, where Q is a methyl group which is substituted with one or more substituents selected from the group consisting of methoxy, amino and carboxy, or Q is an optionally substituted, alkenyl, aralkyl, aralkenyl, aryl group or C₂-C₁₀ alkyl group, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, or straight- or branched-chain C₁-Cₑ alkyl; or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

69. The compound of Claim 68, wherein A is CH.

70. The compound of Claim 69 or 70, wherein D is H.

71. A compound selected from the group consisting of:
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

72. A compound selected from the group consisting of:
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

73. A compound selected from the group consisting of:
or a tautomer thereof; or a pharmaceutically acceptable salt thereof; or an ester thereof.

74. A pharmaceutical composition or an agrochemical composition comprising the compound of any of Claims 68-73 and a pharmaceutically or an agrochemically acceptable carrier.

75. The composition of any of Claims 58-61 or 74, comprising 5′-methylthioinosine and/or 5′-methylthioadenosine.
76. The method of any of Claims 1-57, comprising coadministering the 5'-methylthioinosine phosphorylase (MTIP) inhibitor with 5'-methyhiinosine and/or 5'-methylthioadenosine.
FIGURE 1
FIGURE 3
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/7076; A61K 31/41 (2012.01)
USPC - 514/46, 514/359, 536/27.8, 424/94.5

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/46, 514/359, 536/27.8, 424/94.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/94.1; 435/183, 193-194; 514/18.7, 27.21, 27.2, 27.13, 27.1, 22.1, 43, 45, 183, 536/27.6 (see search terms below)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>Y</td>
<td>US 6,562,785 B1 (SHAPIRO) 13 May 2003 (13.05.2003) Title; col 2, In 40-45; col 9, In 5-20</td>
</tr>
<tr>
<td>Y</td>
<td>US 2008/0070860 A1 (SNYDER et al.) 20 March 2008 (20.03.2008) para [0003], [0005], [0008], [0010], [0006], [0074], [0144], [0172]</td>
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</table>

Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search 9 March 2012 (09.03.2012)

Date of mailing of the international search report 23 APR 2012

Authorized officer: Lee W. Young
PCT HelperWeb: PCT/US 09/63300
PCT OSP: PCT/US 09/63300

Form PCT/ISA/2 10 (second sheet) (July 2009)
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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</table>
**INTERNATIONAL SEARCH REPORT**

### Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ✓ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ✓ Claims Nos.: 8-57, 61, 64-70, 74-76
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ✓ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ✓ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  

**Remark on Protest**

- □ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- □ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- □ No protest accompanied the payment of additional search fees.