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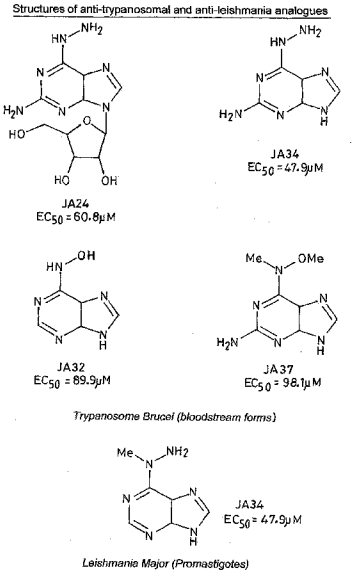
(54) Title: ANTIPARASITIC COMPOUNDS AND COMPOSITIONS



(57) Abstract: Disclosed is use of a compound having a structure according to general formula (I) defined below, in the manufacture of a medicament to treat and/or prevent a parasitic infection or infestation in a mammalian subject wherein X_1 = N or CH or C=O (X_2 = NH) or C=S (X_2 = NH) or C-OR_1 or C-halogen or C-azide; X_2 = N or CR_1 or C-halogen or $CS(O)_nR_1$ where n = 0-2 or a $(C)_m$ linker where m = 1-3 between X_2 and X_6 or C-X_5X_6 (in which case X_5X_6 at C6 (purine numbering) is replaced by H or NHR_1 or O or OR_1 or S or SR_1); X_3 = N or CH or C-NO_2; X_4 = N or CH or C-NO_2 or C-NR_1R_2 or an amidine derivative or a guanidinium derivative; X_5 = O or NR_1 or CR_1R_2; X_6 = OR_1 or O-acyl or 0-$S(O)_nR_1$ or NR_1R_2 or NH-acyl or $N(Acyl)_2$ or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or a hydrazone derivative or an oxime derivative, but if X_5 = O, X_6 cannot = O or X_5X_6 is an amidine or an *N*-substituted pyridine or substituted guanidine; Y = H or NH_2 or NR_1R_2 or -O (X_3 = NH) or OR_1 or F or Cl or Br or I or $CR_1R_2R_3$ or $S(O)_nR_1$ where n = 0-2 or azide or X_5X_6 (in which case X_5X_6 at C6 (purine numbering) is replaced by H or NHR_1 or O or OR_1 or S or SR_1); R_1, R_2, R_3 are independently selected from the group consisting of H or (optionally substituted), alkyl, alkenyl or alkynyl or aryl or aralkyl where the substituents may be selected from H, OH, NH_2,

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

halogen, N_3, CN, CHO, COOR', C0NR'$_2$, OR, NE'$_2$, SR', NR'NR'$_2$, NR'OR', NO_2 and R' is alkyl, alkenyl, alkynyl, aralkyl, acyl, sulfonyl; Z = H or substituted (alkyl or alkenyl or alkynyl or aralkyl) or a sugar derivative of general formula (II) in the β-configuration where: B is the nucleobase from Formula (I); $X_7 = CH_2$ or O or NR_1 or S; R_4 = H or OH or OR_1 or halogen or azide or a phosphate derivative; R_5 = H or F or CH_3; R_6 = H or OH or OR_1 or halogen or azide or a phosphate derivative; and R_7 = H or halogen or R_1 or a derivative of an amino acid or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

Title: Antiparasitic Compounds and Compositions

Field of the invention

The present invention relates to methods of inhibiting the replication of parasites, pharmaceutical compositions for use in inhibiting the replication of parasites, and the use of various compounds in the preparation of medicaments to inhibit parasite replication and to certain novel compounds *per se*.

Background of the invention

Diseases due to parasites represent a very large health problem worldwide. As an example, malaria (caused by infection with *Plasmodium* spp) leads to the death of 1-3 million people per annum, and the disease is endemic in several hundred million, mainly in under-developed countries. The metabolic enzymes in the parasitic organism, if sufficiently different from the host, provide general targets for chemotherapy [Fidock, D.A. *et al*, (2004), *Nature Reviews Drug Discovery*, **3**, 509]. Protozoa in general cannot synthesise purines, necessary for very many processes in the cell, including DNA and ATP synthesis. The parasite's purine requirement is supplied by the host, particularly *via* hypoxanthine from which all other purines required by the protozoa are derived. The transporters of nucleobases and nucleosides into the parasitic organism have been intensely studied in recent years. The transporters, of which there may be one or several in a particular parasite, represent a major therapeutic target [de Koning, H.P. *et al FEMS Microbiology Reviews*, (2005), **29**, 987; el Kouni, M.H. (2003), *Pharmacol. Therapeutics*, **99**, 283]. They may also, in certain cases, transport other presently used therapeutic agents, leading to an anti-parasitic effect. Rodenko *et al*, (2006 Bio-organic & Medicinal Chemistry **14**, 1618-1629) investigated the antiprotozoal activity of various di- and trisubstituted 5'-carboxamido-adenosine analogues. They found that, in that context, a small 5'-substituent such as methyl or ethyl was generally not favourable for antiprotozoal activity.

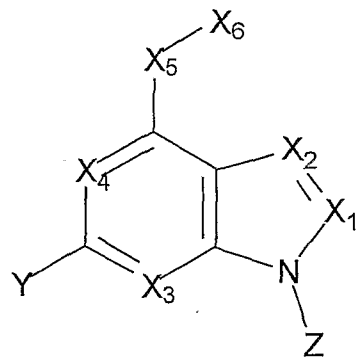
The present invention concerns, *inter alia*, purine and purine-related compounds that may act by inhibiting transporter uptake of host purines and/or by transport into the cell to interact with the protozoan metabolic systems [e.g. Klinkert, M.-Q. and Heussler, V., (2006), *Mini-Reviews in Med. Chem.*, **6**, 131; Jadhav, A.L. *et al*, (1979), *Biochem. Pharmacol.*, **28**, 1057], or possibly by as yet undiscovered mechanisms.

Mutations, over time, in the transporters or in other metabolic enzymes can lead to the development of resistance to many of the existing anti-parasitic compounds, and the need for novel compounds is great [Fidock, D.A. *et al*, (2004), *Nature Reviews Drug Discovery*, **3**, 509]. Present indications suggest that multi-drug therapy should be used, and it is intended that this should be so with compounds derived from the work described in this application.

Brief description of the invention

The present invention is directed to, *inter alia*, purine and bicyclic azole analogues, and their therapeutic uses.

In a first aspect, the invention provides for use purine analogues of general formula I, defined below, in the manufacture of a medicament to treat and/or prevent a parasitic (especially protozoal) infection or infestation in a mammalian subject;



Formula I

wherein X_1 = N or CH or C=O (X_2 = NH) or C=S (X_2 = NH) or C-OR_1 or C-halogen or C-azide;

X_2 = N or CR_1 or C-halogen or $CS(O)_nR_1$ where n = 0–2 or a $(C)_m$ linker where m = 1-3 between X_2 and X_6 or C-X_5X_6 (in which case X_5X_6 at C6 (purine numbering) is replaced by H or NHR_1 or O or OR_1 or S or SR_1);

X_3 = N or CH or C-NO_2 ;

X_4 = N or CH or C-NO_2 or C-NR_1R_2 or an amidine derivative or a guanidinium derivative;

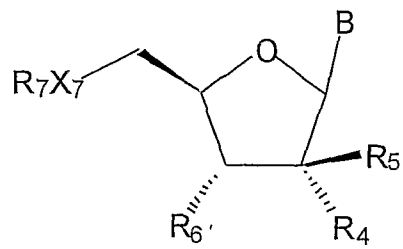
X_5 = O or NR_1 or CR_1R_2 ;

X_6 = OR_1 or O-acyl or O-$S(O)_nR_1$ or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0–2 or a hydrazone derivative or an oxime derivative but if X_5 = O X_6 cannot = O or X_5X_6 is an amidine or an *N*-substituted pyridine or substituted guanidine;

Y = H or NH_2 or NR_1R_2 or =O (X_3 = NH) or OR_1 or halogen or $CR_1R_2R_3$ or $S(O)_nR_1$ where *n* = 0–2 or azide or X_5X_6 (in which case X_5X_6 at C6 (purine numbering) is replaced by H or NHR_1 or O or OR_1 or S or SR_1);

R_1, R_2, R_3 are independently selected from the group consisting of H or (optionally substituted) alkyl or alkenyl or alkynyl or aryl or aralkyl, where the substituents may be selected from H, OH, NH_2, halogen, N_3, CN, CHO, COOR', $CONR'_2$, OR, NR'_2, SR', NR'NR'_2, NR'OR' or NO_2, where R' is alkyl, alkenyl, alkynyl, aralkyl, acyl or sulfonyl;

Z = H or (optionally substituted) alkyl or alkenyl or alkynyl or aralkyl or a β-D-linked sugar derivative of general formula II:



Formula II

B is the nucleobase from Formula I;

X_7 = CH_2 or O or NR_1 or S;

R_4 = H or OH or OR_1 or halogen or azide or a phosphate derivative;

R_5 = H or F or CH_3 ;

R_6 = H or OH or OR_1 or halogen or azide or a phosphate derivative; and

R_7 = H or halogen or R_1 or a derivative of an amino acid or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate derivative.

Note that, in formula I, if X_1 is C=O or C=S, then X_2=NH.

In general in the present invention Y_1 is preferably H or NH_2.

In general in the present invention X_1 is preferably CH.

In general in the present invention X_2 is preferably N.

In general in the present invention X_5X_6 is preferably selected from the group consisting of –NH-NH_2, -NH-OH, and –N(alkyl)-NH_2.

In general in preferred embodiments Y_1 is H or NH_2 and X_1 is CH.

In general in preferred embodiments Y_1 is H or NH_2 and X_2 is N.

In general in preferred embodiments X_1 is CH and X_2 is N.

In general in preferred embodiments Y_1 is H or NH_2, X_1 is CH and X_2 is N.

In general in preferred embodiments Y_1 is H or NH_2, X_1 is CH, X_2 is N, and X_5X_6 is selected from the group consisting of –NH-NH_2, -NH-OH and –N(alkyl)-NH_2.

In a second aspect the invention provides a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a compound as defined in the first aspect, or a pharmaceutically acceptable ester or salt thereof, preferably admixed with a further therapeutically active agent effective in the prevention or treatment of protozoal infections, with at least one pharmaceutically acceptable carrier, diluent or excipient.

In a third aspect the invention provides a method of making an anti-protozoal pharmaceutical composition for administration to a mammalian subject, the method comprising the step of mixing a therapeutically effective amount of a compound as defined in the first aspect, or a pharmaceutically acceptable ester or salt thereof, with at least one pharmaceutically acceptable carrier, diluent or excipient, and preferably also with at least one further therapeutically active agent effective in the prevent or treatment of protozoal infections.

In a fourth aspect the invention provides a method of preventing or treating a parasitic (especially protozoal) infection or infestation in a mammalian subject, the method comprising the step of administering an active agent and/or a pharmaceutical composition in accordance with the previous aspects of the invention.

The various aspects of the invention defined above relate generally to the prevention and/or treatment of protozoal infections or infestations (or compositions useful therein) in a mammalian subject. Preferably the mammalian subject is a human, but it may also be, for example, a domesticated livestock animal such as a cow, goat, sheep or the like, or a companion animal such as a dog or cat.

The invention concerns protozoal infections in general, but especially those of man, such as those caused by *Plasmodium spp.*, *Leishmania sp.* and *Trypanosomes*. The invention particularly relates to methods and compositions for the prevention and/or treatment of malaria.

Efficacy of the methods/compositions may readily be determined using, for example, an assay method along the lines described herein or by any other assay method, many of which are well-known to those skilled in the art.

Any method/composition which produces a positive response may be useful. A positive response includes, for example, a reduction in parasite numbers when active agent is administered *in vivo* or in an *in vitro* assay and/or a reduction in the amount of one or more parasite-specific antigens, which can be readily measured by standard techniques such as ELISA, ELISpot assay, FACScan etc. A "therapeutically effective amount" is an amount of antiprotozoal agent (or composition containing the agent) which elicits a detectable positive response. A "prophylactically effective amount" is an amount which results in a detectable reduction in parasite numbers and/or severity of one or more symptoms in a subject, following exposure to a parasitic protozoal organism, compared to an untreated subject exposed to the same dose of organisms.

Detailed description

Where the following terms are used in this specification, they are defined as below.

The term "nucleobase" refers to a compound that contains a ring structure containing atoms in addition to carbon, such as sulfur, oxygen or nitrogen as part of the ring. They may be either simple aromatic rings or non-aromatic rings. Positions of the ring may be optionally

substituted independently with, e.g. hydroxy, oxo, amino, imino, alkyl, bromo, chloro, cyano, azido and nitro. Included within this class of substitutents are purines and indoles (X_3, X_4 = CR).

The term "nucleoside" refers to a compound composed of any pentose or modified pentose moiety attached to a specific position of a nucleobase or to positions 7-, 8- or 9- of a purine or to the equivalent position in an analogue, and for present purposes refers, exclusively to a D-nucleoside in the β configuration, unless the context dictates otherwise. Any nucleosides having the α and/or L configuration are outside the scope of the claims of the present application.

The term "nucleotide" refers to a phosphate ester substituted on the 5'-position of a nucleoside and includes diphosphates, triphosphates, protected monophosphates and analogues thereof.

The term "purine" refers to nitrogenous bicyclic or polycyclic heterocycles containing at least one six and one five membered ring.

The term "D-nucleoside" used in the present invention describes nucleoside derivatives that have the D-ribose sugar moiety like those found in natural nucleosides such as adenosine.

The term "β" indicates the specific stereochemical configuration of a substituent at an asymmetric carbon atom in a chemical structure as drawn. In this specification it refers to the orientation of the glycosidic bond. The compounds described herein are all in the D-furanosyl configuration.

The term "protecting group" refers to a chemical group that is added to a heteroatom, such as O, S, N or P, to prevent its further reaction during the course of derivatisation of other moieties in the molecule in which the heteroatom is located. A wide variety of protecting groups are known to those skilled in the art of organic synthesis.

The term "masked phosphate derivative" is a modified phosphate group in which the negative charge(s) which would normally be present in an unmodified phosphate group are reduced or (more preferably) entirely neutralized by additional moieties. This has the benefit of facilitating transport of compounds comprising the modified phosphate group across a lipid membrane (e.g. across a cell membrane). Examples of masked phosphate derivatives are bis-POM/bis-POM PMEA (see Delaney *et al*, 2001 Antiviral Chemistry and Chemotherapy **12**, 1-35), *cyclo*Sal (Meier *et al*, Eur. J. Org. Chem. 1998, 837) and SATE (Lefebvre *et al*, J Med. Chem., 1995, **38**, 394103950). (SATE is an abbreviation of S-acyl thioethyl).

The term "tautomer" refers to functional groups that are able to undergo formal migration of a hydrogen atom accompanied by a switch of adjacent conjugated double bonds. Thus in this specification structures where X_4 = N and X_5 = NH the analogues will exist in a tautomeric equilibrium as shown in Figure 1. Thus the analogues can, depending on the constraints of the immediate environment (e.g. as an enzyme substrate or by interaction with other biomolecules), act as either a 'guanosine' or an 'adenosine' derivative.

The term "alkyl" refers to methyl, ethyl, n-propyl, isopropyl and higher carbon chains of any length, though one to six carbon atoms are preferred, and one to three carbon atoms especially preferred. The term is further exemplified to a cyclic, branched or straight chain.

The term "substituted alkyl" refers to an alkyl chain as defined above which can be further modified by a heteroatom within, where either the heteroatom is part of the chain (e.g. an ether or an amide linkage), or where the heteroatom does not form part of the chain.

The term "alkenyl" refers to carbon chains of any length, though two to six carbon atoms are preferred (and two to three carbon atoms are especially preferred), containing one or more carbon-carbon double bond. The term is further exemplified to a cyclic, branched or straight chain.

The term "substituted alkenyl" refers to an alkenyl chain as defined above which can be further modified by a heteroatom within where either the heteroatom is part of the chain (e.g. an ether or an amide linkage), or where the heteroatom does not form part of the chain.

The term "alkynyl" refers to carbon chains of any length, though two to six carbon atoms are preferred (and two to three carbon atoms especially preferred), containing one or more carbon-carbon triple bond. The term is further exemplified to a cyclic, branched or straight chain.

The term "substituted alkynyl" refers to an alkynyl chain as defined above which can be further modified by a heteroatom within where either the heteroatom is part of the chain (e.g. an ether or an amide linkage), or where the heteroatom does not form part of the chain.

The term "aryl" refers to an unsaturated aromatic carbocylic ring, which may be composed of a single ring (e.g. phenyl) or condensed rings (e.g. naphthyl), which can optionally be substituted by further substituents such as hydroxyl, chloro, cyano and nitro.

The term "alkaryl" or "arylalkyl" refers to any combination of alkyl, alkenyl or alkynyl with aryl susbstitutions, which can optionally be substituted by further substituents such as hydroxyl, chloro, cyano and nitro.

The compounds of formulae I and IA-E (below) may have multiple asymmetric centres. Accordingly they may be prepared in either optically active form or as a racemic mixture. The scope of the invention as described and claimed encompasses the individual optical isomers and non-racemic mixtures thereof as well as the racemic forms of the compounds.

A "pharmaceutically acceptable salt" may be any suitable salt derived from inorganic and organic acids and bases.

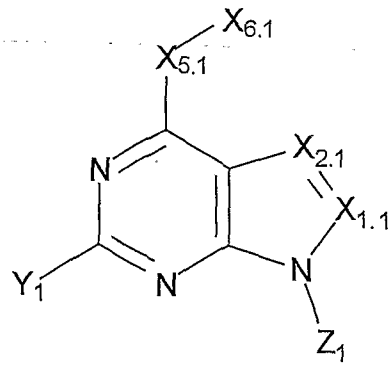
Compounds

The compounds of use in the present invention are generally described by general formula **1** above. There are, however, several subsets of compounds which are of particular interest,

including compounds according to Formulae **IA** through **IE**, each of which represent preferred embodiments of the present invention.

In one embodiment compounds of use in the invention are in accordance with formula **IA**.

Formula IA compounds are 6-substituted purine derivatives having the structure:



Formula IA

where:

$X_{1.1}$ = CH or N;

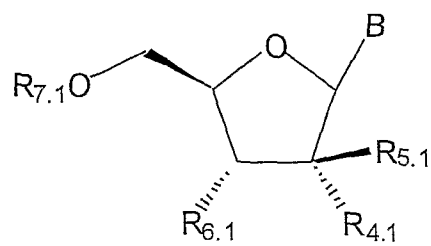
$X_{2.1}$ = CH or N or S or S-Me or C-halogen or CR_1 ;

$X_{5.1}$ = O or NH or N-R_1 ;

$X_{6.1}$ = OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or $N(Acyl)_2$ NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.1}X_{6.1}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative or a *N*-pyridinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O (R_1 and R_2, if present, are as defined previously in formula I);

Y_1 = H or NH_2 or =O (N^3 (purine numbering)= NH) or halogen or azide;

Z_1 = H or Formula **IIA** in the β-configuration



Formula IIA

where:

B is the nucleobase from Formula IA;

$R_{5.1}$ is H or F or CH_3 ;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

In preferred embodiments according to formula IA:

$X_{2.1}$ = CH or N or C-halogen or CR_1;

$X_{6.1}$ = OR_1 or O-acyl or O-$S(O)_2R_1$ or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.1}X_{6.1}$ is a hydrazone derivative or an oxime derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

Z_1 = H or Formula **IIA** in the β-configuration;

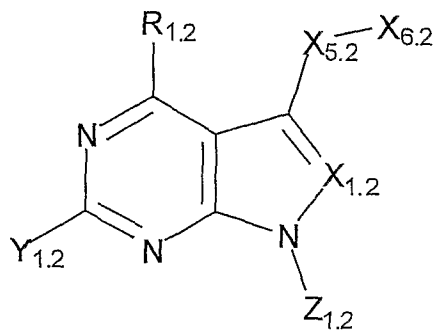
and if present,

$R_{5.1}$ is H or CH_3; and

$R_{7.1}$ = H or PO_3H_2 or $P_3O_9H_4$ or a masked phosphate.

In one embodiment, compounds of use in the invention are in accordance with formula IB.

Formula IB compounds are 7-substituted (purine numbering) purine derivatives having the structure:



Formula IB

where:

$X_{1.2}$ is N or CH;

$X_{5.2}$ is O or NH or N-R_1;

$X_{6.2}$ is OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.2}X_{6.2}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$Y_{1.2}$ = H or NH_2 or =O (N^3 = NH) or halogen or azide;

$R_{1.2}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

$Z_{1.2}$ = H or Formula **IIA** in the β-configuration; where B is the nucleobase from Formula IB;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

(R_1 and R_2, if present, are as defined previously in formula I).

In preferred embodiments according to formula IB:

$X_{6.2}$ is OR_1 or O-acyl or O-$S(O)_2R_1$ or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_2R_1$ or $X_{5.2}X_{6.2}$ is a hydrazone derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$Y_{1.2}$ = H or NH_2 or =O (N^3 (purine numbering) = NH) or halogen or azide;

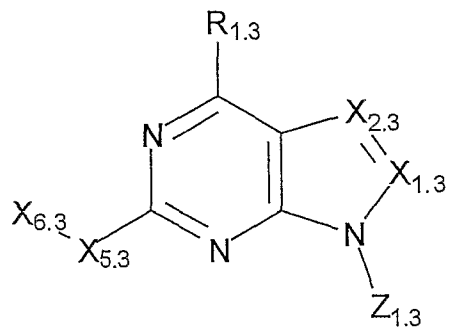
$R_{1.2}$ is O or OR_1 or NR_1R_2 or halogen;

$Z_{1.2}$ = H or Formula **IIA** in the β-configuration; and, if present,

$R_{5.1}$ is H or CH_3; and

$R_{7.1}$ = H or PO_3H_2 or $P_3O_9H_4$ or a masked phosphate.

In one embodiment, compounds of use in the invention are in accordance with formula IC. Formula IC compounds are 2-substituted purine derivatives having the structure:



Formula IC

where:

$X_{1.3}$ is CH or N;

$X_{2.3}$ is CH or N;

$X_{5.3}$ is O or NH or N-R_1;

$X_{6.3}$ is OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.3}X_{6.3}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$R_{1.3}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

$Z_{1.3}$ = H or Formula IIB in the β-configuration; where B is the nucleobase from Formula IC;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

(R_1 and R_2, if present, are as defined previously in formula I).

In preferred embodiments according to formula IC:

$X_{6.3}$ is OR_1 or O-acyl or O-$S(O)_2R_1$ or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.3}X_{6.3}$ is a hydrazone derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$R_{1.3}$ is O or OR_1 or NR_1R_2 or halogen;

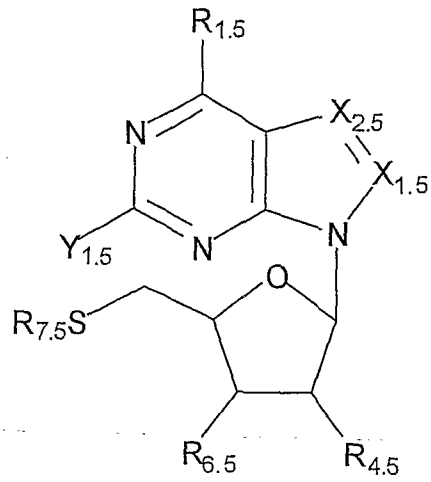
$Z_{1.3}$ = H or Formula IIB in the β-configuration; where B is the nucleobase from Formula IC

B is the nucleobase from Formula IB

$R_{5.1}$ is H or CH_3 ; and

$R_{7.1}$ = H or PO_3H_2 or $P_3O_9H_4$ or a masked phosphate.

In one embodiment compounds of use in the invention are in accordance with formula ID. Formula ID compounds are 5'-*S*-modified β-D-purine derivatives having the structure:



Formula ID

where:

$X_{1.5}$ is CH or N;

$X_{2.5}$ is CH or N;

$R_{1.5}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

$Y_{1,5}$ is H or NH_2 or =O (N^3 (purine numbering) = NH) or halogen or azide or X_5X_6;

$R_{4.5}$ is H or OH or F;

$R_{6.5}$ is H or OH or F; and

$R_{7.5}$ is acyl or alkyl or an amino acid such as homocysteine or a derivative of an amino acid such as butanoic acid. (R_1 and R_2, if present, are as defined previously in formula I).

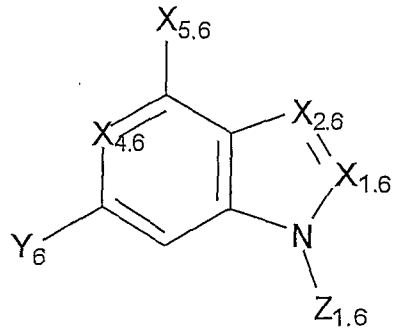
In preferred embodiments according to formula ID:

$R_{1.5}$ is O or OR_1 or NR_1R_2 or halogen;

$Y_{1,5}$ is H or NH_2 or =O (N^3 (purine numbering) = NH) or halogen or azide; and

$R_{7.5}$ is acyl or alkyl or an amino acid such as homocysteine or a derivative of an amino acid such as butanoic acid.

In one embodiment, compounds of use in the invention are in accordance with formula IE. Formula IE compounds are modified bicyclic azole derivatives having the structure:



Formula IE

where:

$X_{1.6}$ is CH or N;

$X_{2.6}$ is CH or N or CR_8;

$X_{4.6}$ is CH or C-NO_2 or C-NR_1R_2 or an amidine derivative or a guanidinium derivative;

$X_{5.6}$ is H or NH_2 or O or OR_1 or S or SR_1;

R_8, *if present, is* $CONHR_1$ *or* $CONR_1NHR_2$ or $CONR_1OR_2$;

Y_6 is H or NH_2 or NHR_1 or N_3 or halogen or O or OR_1 or S or SR_1 or $CR_1R_2R_3$;

$Z_{1.6}$ is H or R_1 or Formula IIA in the β-configuration where B is the nucleobase IE;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2). (R_1, R_2 and R_3, if present, are as defined previously in formula I).

In preferred embodiments according to general formula IE,

$X_{4.6}$ is CH or C-NO_2 or C-NR_1R_2;

$X_{5.6}$ is H or NH_2 or O or OR_1 or S;

Y_6 is H or NHR_1 or N_3 or halogen or O or OR_1;

$Z_{1.6}$ is H or R_1 or Formula IIA in the β-configuration where B is the nucleobase IE;

$R_{5.1}$ is H or CH_3; and

$R_{7.1}$ = H or PO_3H_2 or $P_3O_9H_4$ or a masked phosphate.

In yet a fifth aspect, the invention provides a novel purine nucleobase/nucleoside/nucleotide analogue, according to one of the general formulae IB, IC or IE, as defined previously. The

compound may be provided in substantially pure form (at least 50% w/w, preferably at least 75% w/w purity).

Compounds according to this aspect of the invention are believed to be novel *per se*. These compounds have, or may have, anti-protozoal activity and/or may be useful in the synthesis (*in vitro* or *in vivo*) of compounds having anti-protozoal activity. Accordingly, in a sixth aspect the invention provides a pharmaceutical composition comprising one or more compounds in accordance with the fifth aspect, in admixture with a pharmaceutically acceptable carrier, diluent or excipient. The composition may additionally comprise one or more further conventional, known antiprotozoal compounds.

In a seventh aspect the invention provides for use of a compound in accordance with the fifth aspect defined above, in the manufacture of a medicament to treat and/or prevent a parasitic, especially a protozoal, infection or infestation in a mammalian subject.

The invention also provides a method of making a pharmaceutical composition, comprising mixing one or more compounds, in accordance with the fifth aspect of the invention defined above, with a pharmaceutically acceptable carrier, diluent, or excipient (and optionally with one or more further anti-protozoal compounds); and a method of treating and/or preventing a protozoal infection or infestation in a mammalian (preferably human) subject, the method comprising the step of administering to the subject a therapeutically or prophylactically effective amount of a compound in accordance with the fifth aspect of the invention defined above.

The composition may be made, formulated and administered generally as described elsewhere in this specification.

For the avoidance of doubt it is hereby expressly stated that features described herein as "preferred", "desirable", "convenient", "advantageous", "particular" and the like may be used in the invention (and claimed) in isolation or in any combination with one or more other features so described, unless the context dictates otherwise, and the present disclosure should be interpreted accordingly.

Uses

It is contemplated that compositions containing compounds according to formula I and especially according to one of formulae IA-IE, may be used to treat a variety of conditions, and in fact any condition which responds positively to the administration of one or more of the compounds. Among these it is specifically contemplated that compounds of the invention may be used to treat an infection or an infestation of protozoal origin.

Infections which may be treated with the compounds and compositions of the present invention include, in particular, malaria, trypanosomes and Leishmania. In particular, compositions in accordance with the invention may be especially useful in treating malarial diseases caused by infection with strains of *Plasmodium* which are substantially resistant to chloroquine.

Infestations contemplated to be treated with the compounds of the present invention include protozoan infestations as well as helminth and other parasitic infestations.

Still other contemplated uses of the compounds according to the present invention include use as intermediates in the chemical synthesis of other nucleoside or nucleotide analogues which are, in turn, useful as therapeutic agents or for other purposes.

In general, the most preferred uses according to the present invention are those in which the active compounds are relatively less cytotoxic to the non-target host cells and relatively more active against those of the protozoal target.

It is contemplated that compounds and compositions according to the present invention may be administered in any appropriate formulation and under any appropriate protocol. Thus administration may take place orally, parenterally (including subcutaneous injections, intravenous injections, intramuscularly, by intrasternal injection or by fusion techniques), by inhalation spray, topically or rectally and so forth, and in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers and vehicles.

It is contemplated that compounds and compositions according to the present invention can be formulated in admixture with a pharmaceutically acceptable carrier, for example the compounds of the present invention can be administered orally as pharmacologically acceptable salts. Compounds of the present invention may also be administered intravenously in physiological saline solution (e.g. buffered to a pH of about 7.2 to 7.5, conventional buffers such as phosphates or bicarbonates or citrates could be used for this purpose). One skilled in the art may modify the formulations within the teachings of the present invention to provide alternative numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity. For example, the modification of the present compounds to render them more soluble in water or other vehicle may be easily accomplished by minor modifications, such as salt formulation or esterification etc, according to the knowledge of those skilled in the art. Those skilled in the art may also modify the route of administration in order to manage the pharmacokinetics of the compounds described in this specification for maximal beneficial effect in patients.

In certain pharmaceutical dosage forms, the pro-drug form of the compounds described in this specification, in particular acylated derivatives, pyridine esters and various salt forms of the compounds are preferred. One skilled in the art will recognise readily how to modify the present compounds to pro-drug forms to facilitate delivery of active compounds to a target site within the host organism or patient. One skilled in the art will also take advantage of favourable pharmacokinetic parameters of the pro-drug forms, where applicable, to deliver the present compounds to a targeted site within the host organism or patient to maximise the therapeutic effect of the compound.

In addition, compounds of the present invention may be administered alone or, more preferably, in combination with other agents for the treatment of the above infections or infestations or conditions. Combination therapies according to the present invention comprise the administration of at least one compound of the present invention or a functional derivative thereof with at least one other pharmaceutically active ingredient. The said at least one other pharmaceutically active ingredient may be a conventional, known anti-protozoal agent or may be a compound in accordance with the present invention. The active

ingredient(s) and pharmaceutically active agents may be administered separately or together, and when administered separately this may occur substantially simultaneously or separately in any order. The amounts of the active ingredient(s) and pharmaceutically active agent(s) and the relative timings of administration will be chosen in order to achieve the desired combined therapeutic and/or prophylactic effect. Preferably the combination therapy involves the administration of one compound of the present invention or a functional derivative thereof and one the agents mentioned herein below.

Examples of such further therapeutic agents which are effective for the treatment of protozoal infections or associated conditions include chloroquine, pyrimethamine, cycloguanil, doxycycline, mefloquine, primaquin, diminazene, isometamidium, or artemisinin or derivatives thereof. Certain compounds according to the present invention may be effective for enhancing the biological activity of certain other agents according to the present invention (or otherwise) by reducing the metabolism or inactivation of other compounds and as such may be co-administered for this intended effect.

With respect to dosage, one skilled in the art will recognize that a therapeutically and/or prophylactically effective amount will vary with the infection or condition to be treated, the degree of its severity, the treatment regimen employed, the pharmacokinetics of the agent used as well as the patient to be treated. Effective dosages may range from 1mg/kg of body weight or less to 25mg/kg of body weight or more. Generally, effective dosage of the present compound(s) ranges from less than 1mg/kg to 25mg/kg of body weight of the patient, depending upon the compound used, the condition or infection treated and the route of administration. This dosage range generally produces effective blood level concentrations of active compound ranging from 0.04 to about 100 micrograms/cc of blood in the patient. It is contemplated though that an appropriate regimen may be developed by administering a small amount, and then increasing the amount until either the side effects become unduly adverse, or the intended effect is achieved.

Administration of the active compound may range from continuous (intravenous drip) to several oral administrations per day, and may include oral, parenteral, intramuscular, intravenous, sub-cutaneous, transdermal (which may include a penetration enhancement

agent), buccal, topical and suppository administration, amongst other routes of administration.

To prepare the pharmaceutical composition according to the present invention, a therapeutically and/or prophylactically effective amount of one or more of the compounds according to the present invention is preferably intimately admixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending upon the form of the preparation desired for administration, e.g. oral or intravenous. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus for liquid oral preparations, such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavouring agents, preservatives, colouring agents and the like may be used. For solid oral preparations, such as powders, tablets, capsules and for solid preparations such as suppositories, suitable carriers and additives include sugar carrier such as dextrose, mannitol, lactose and related carriers, starches, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques. For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other agents including those which aid dispersion may be included. Where sterile water is used and to be maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared using liquid carriers, suspending agents and the like may be employed.

The invention will now be further described by way of illustrative examples, and with reference to the accompanying drawings in which:

Figure 1a shows the tautomerism of N^6-amino or N^6-hydroxy derivatives;
Figure 1b shows the conventional numbering adopted to number the atoms in the purine bycyclic structure;
Figure 2 shows the structure of various compounds displaying activity against trypanosomes or *Leishmania*;

Figure 3 shows structures of various purine nucleobase analogues according to general formula IA (Z_1 = H);

Figure 4 shows structures of various purine nucleoside analogues according to general formula IA (Z_1 = ribose);

Figure 5 shows structures of various purine nucleoside 5'-triphosphate analogues according to general formula IA (Z_1 = ribose, 5'-triphosphate);

Figure 6 shows structures of various pyrrolopyrimidine nucleoside and nucleobase analogues according to general formula IA;

Figure 7 shows structures of various pyrazolopyrimidine nucleoside and nucleobase analogues according to general formula IB;

Figure 8 shows structures of various purine nucleoside and nucleobase analogues according to general formula IC;

Figure 9 shows structures of various purine nucleoside analogues according to general formula ID;

Figure 10 shows structures of purine nucleoside analogues according to general formula IE.

EXAMPLES

Example 1

***In* vitro and *in* vivo anti-malarial testing**

***In vitro* parasite growth inhibition assays and *in vitro* drug-drug interactions.**

In vitro parasite growth inhibition was assessed by the incorporation of [^{3}H] hypoxanthine based on the method used by Desjardins (R. E. Desjardins *et al*, *Antimicrob. Agents Chemother.*, 1979, **16**, 710) and modified as described by Vivas and co-workers (L. Vivas *et al*, *Exp. Parasitol.*, 2005, **111**, 105). All assays included chloroquine diphosphate as a standard and control wells with untreated infected and uninfected erythrocytes. The compounds were dissolved in 100% dimethylsulfoxide (Sigma) and serial dilutions were made in assay medium. Fifty microlitres of *P. falciparum* (65–75% ring stage) culture at 0.5% parasitaemia or uninfected erythrocytes were added to each well reaching a final volume of 100 µl per well, a final haematocrit of 2.5% and final dimethylsulfoxide concentrations □ 0.01%. Plates were incubated at 37°C in 5% CO_2, 95% air mixture for 24 h, at which point 20 µl (0.1 µCi/well) of [^{3}H] hypoxanthine (Perkin Elmer, Hounslow, United

Kingdom) was added to each well and returned to the incubator for an additional 24 h incubation period at which point, the experiment was terminated by placing the plates in a -80°C freezer. Plates were thawed and harvested onto glass fibre filter mats using a 96-well cell harvester (Harvester 96 ™, Tomtec, Oxon, UK) and left to dry. After the addition of MeltiLex™ solid scintillant (PerkinElmer, Hounslow, United Kingdom) the incorporated radioactivity was counted using a Wallac® 1450 Betalux scintillation counter (Wallac®). Data acquired by the Wallac® BetaLux scintillation counter were exported into a MICROSOFT® EXCEL spreadsheet (Microsoft Corp.), and the IC_{50}/IC_{90} values of each drug were calculated by using XLFit® (ID Business Solutions Ltd., UK) line fitting software.

Three strains of *P. falciparum* were used:

1. 3D7 variant of NF54 which is known to be sensitive to all anti-malarials.
2. K1 strain originating from Thailand that is resistant to chloroquine and pyrimethamine, but sensitive to mefloquine.
3. VS1 strain originating from Vietnam that is highly chloroquine, pyrimethamine and cycloguanil resistant.

Full suppressive 4-day Peters' test. *In vivo* tests were performed under the Home Office Animals (Scientific Procedures) Act 1986. CD-1 outbred 20g male mice (Charles Rivers, UK), were kept in specific pathogen-free conditions and fed *ad libitum*. For subcutaneous administration, the compounds were dissolved in 10% dimethylsulfoxide (DMSO) 0.05% Tween 80 (Sigma, Dorset, UK) in distilled water. For oral administration, compounds were dissolved in standard suspending formula (SSV) [0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, 0.9% NaCl (all Sigma)]. Mice were infected intravenously with 2×10^6 *P. chabaudi* AS parasitized red cells and treated subcutaneously (s.c.) or orally (p.o.) with 0.2 ml of a solution of the test compounds two hours (day 0) and on days 1, 2, 3 and 4 post-infection, at a dose of 30mg test compound per Kg body weight. Parasitaemia was determined by microscopic examination of Giemsa stained blood films taken on day 5 post infection. Microscopic counts of blood films from each mouse were processed using MICROSOFT® EXCEL (Microsoft Corp.) and expressed as percentages of

inhibition from the arithmetic mean parasitaemias of each group in relation to the untreated group. Dose response curves were obtained and ED_{50} and ED_{90} values calculated.

Results for *in vitro* testing are shown in Table 1, for *in vivo* testing in Table 2 below.

Table 1 Anti-plasmodial *in vitro* activity of purine analogues ([^{3}H] hypoxanthine assay)

COMPOUND	IC_{50}[μM]			CC_{50}[μM]	Selectivity Index (CC_{50}/IC_{50})		
	3D7	K1	VS1	KB cells (cytotox)	3D7	K1	VS1
JA23	2.77	3.60	0.29	1476	533	410	5008
JA24	10.16	10.00	12.22	1364.00	134	136	112
JA25	13.26	7.48	7.92	2.26	<1	<1	<1
JA26	11.42	20.57	9.87	6.16	<1	<1	<1
JA27	72.71	35.40	22.47	101.00	1.4	2.9	4.5
JA28	11.61	6.90	8.56	44.30	3.8	6.4	5.2
JA30	16.03	10.83	13.33	280.7	17.5	25.9	21.1
JA31	231.94	184.45	224.78	784.16	3.4	4.3	3.5
JA32	7.48	10.21	5.57	1485.8	199	146	267
JA33	23.08	22.08	18.77	49.28	2.1	2.2	2.6
JA34	188.95	108.87	89.32	1000.74	5.3	9.2	11.2
JA35	507.89	478.01	418.51	n.d.	n.d.	n.d.	n.d.
JA36	>558	>558	>558	n.d.	n.d.	n.d.	n.d.
JA37	>613	>613	>613	n.d.	n.d.	n.d.	n.d.
JA38	428.06	435.35	>555	n.d.	n.d.	n.d.	n.d.
JA39	98.90	353.61	>606	1257.6	12.7	3.6	2.1
JA40	>306	>306	>306	n.d.	n.d.	n.d.	n.d.
JA41	27.77	9.03	16.79	45.01	1.6	5.0	2.7
JA43	21.81	12.48	25.22	15.34	<1	1.2	<1
JA54	400.48	-	351.63	n.d.	-	-	-
JA55	19.33	-	19.82	167	8.6	-	8.4
JA56	61.84	-	36.57	n.d.	-	-	-
JA57	14.2	-	10.96	109	7.7	-	10.0
JA58	33.31	-	34	n.d.	-	-	-
JA59	13.15	-	14.2	889	67.6	-	62.6
JA60	183.37	-	126.06	n.d.	-	-	-
Chloroquine	0.0024	0.32	0.86	187	77917	584	217
podophylotoxin	n/a	n/a	n/a	0.048	-	-	-

3D7: drug sensitive

K1: chloroquine and pyrimethamine resistant

VS1: highly chloroquine, pyrimethamine and cycloguanil resistant

n.d.: not determined

Table 2 *in vivo* activity of purine analogues in the *P. berghei* ANKA model (Peters' 4-day test

Route of administration	Percentage of inhibition of parasitaemia at 30mg/kg x 4 d (once daily)			
	JA23	JA24	JA32	Pyrimethamine
s.c.	23.4%	14.0%	0%	100%
p.o.	12.3%	22.3%	3.9%	100%

s.c.: subcutaneously
p.o. : oral

Referring to Table 1, it is apparent that those compounds with the greatest selectivity index (i.e. those compounds with the least cytotoxicity for the host and greatest toxicity for protozoa) were the nucleoside analogues JA23 and JA24, and the nucleobase analogue JA32. These compounds, (and JA23 in particular) were especially promising in respect of strain VS1, which is highly resistant to many conventional anti-malarials. This promise was confirmed by *in vivo* data, which showed that all three compounds (but especially JA23 and JA24) inhibited parasitaemia when given orally, and JA23 and JA24 also inhibited parasitaemia when administered sub-cutaneously.

Nevertheless, from the *in vitro* data, it was expected that JA23, 24 and 32 would show rather more activity *in vivo* than was in fact found.

There are two possible reasons to account for the difference between the *in vitro* and the *in vivo* data. One explanation is that *P. falciparum* (human malaria) does not grow in or infect rodents so there is a need to use rodent malaria models. Additionally, rodent malaria strains cannot be maintained in *in vitro* cultures, although they can be cultured for a short period to do uptake experiments.

Thus, the *in vitro* and *in vivo* experiments were necessarily conducted using different strains of *Plasmodium*.

A second explanation is that the analogues, being highly polar, may be rapidly excreted from the mice with very little actual uptake of drug. This is a well-known phenomenon for polar materials. To counteract this effect the analogues may be modified to facilitate greater bioavailability. Thus the modifications to X6 (O-acyl or O-S(O)nR_1 or NR_1R_2 or NH-acyl or NH-OS(O)2R_1 or NH-S(O)nR_1 where n = 0-2 or a hydrazone derivative or an oxime derivative) are used to enhance the *in vivo* bioavailability of analogues. These modifications represent preferred embodiments of the invention, both in general and in particular relation to JA23, 24 and 32.

Example 2

***In vitro* anti-trypanosomal testing**

***Trypanosoma cruzi*: *in vitro* assay**

Parasite and cell cultures

The *Trypanosoma cruzi* (MHOM/CL/00/Tulahuen) transfected with β-galactosidase (Lac Z) gene, was used (F. S. Buckner *et al, Antimicrob. Agents Chemother.*, 1996, **40**, 2592).
The strain was maintained on an L-6 rat skeletal myoblast cell line (obtained from European Collection of Animal Cell Cultures, ECACC, Salisbury, UK) cell-layer in RPMI 1640 medium, supplemented with 10% heat inactivated foetal calf serum (FCS). All cultures and assays were conducted at 37°C under an atmosphere of 5% $C0_2$ /95% air mixture.

Primary peritoneal mouse (CD1) macrophages were collected by lavage, two days after induction with i.p. injection of 2ml 2% soluble starch. All cultures and assays were conducted at 37°C under an atmosphere of 5% CO_2/95% air mixture.

Drug sensitivity assays

Stock drug solutions were prepared in 100% DMSO (dimethylsulfoxide) unless otherwise suggested by the supplier at 20 mg/ml. The stocks were kept at 4°C (unless otherwise advised) for up to 2-3 weeks in the dark. For the assays, compounds were further diluted to the appropriate concentration, normally in the range of 30μg/ml to 0.1 μg/ml, in RPMI 1640 medium without phenol red, plus 10% FCS.

Assays were performed in sterile 96-well microtiter plates, each well containing 100 µl of $4x10^5$ mouse macrophages/ml (4×10^4/well) in RPMI 1640 medium without phenol red plus 10% FCS. After 24 h, 100 µl of a suspension containing 2×10^6 trypomastigotes/ml, (2×10^5/well) from culture are added to the wells. 24 h later, the medium was removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls, toxicity to host cells and presence or absence of trypomastigotes in the overlay. Benznidazole was used as the standard drug over the concentration range as above.

At 72 h, the substrate CPRG/ Nonidet (50 µl) was added to all wells; medium was not removed prior to this. A colour reaction became visible within 2-6 h, which was read photometrically at 540nm on a spectrophotometer. The results were expressed as % reduction in β-galactosidase activity compared to control wells, normalised with uninfected macrophages. This is related to a previously established parasite number to β-galactosidase signal slope. Data were transferred into a graphic programme, dose – response inhibition curves are determined using MSExcelfit and IC_{50} values were calculated.

Primary screen

The compounds were tested, in triplicate, at 4 concentrations (30 - 10 - 3 - 1 µg/ml). Benznidazole® (Roche) was included as the reference drug and has an IC_{50} value in the range of 0.5 – 1.5 µg/ml.

Human African trypanosomiasis: in vitro screening model

Parasite cultures

Trypanosoma brucei rhodesiense STIB 900

The bloodstream form trypomastigotes were maintained in MEM medium with Earle's salts supplemented with 25 mM HEPES, 1g/l additional glucose, 10ml/l MEM non-essential aminoacids (100x), 0.2 mM 2-mercaptoethanol, 2mM Na-pyruvate, 0.1mM hypoxanthine, 0.05mM bathocuprionedisulphonic acid, 0.15mM L-cysteine and 15% heat inactivated, foetal calf serum. All cultures and assays were conducted at 37°C under an atmosphere of 5% CO_2 /95% air mixture.

Drug sensitivity assays

Stock drug solutions were prepared in 100% DMSO (dimethylsulfoxide) unless otherwise suggested by the supplier at 20 mg/ml, and ball milled or sonicated if necessary. The stocks were kept at 4°C. For the assays, the compound was further diluted to the appropriate concentration using complete medium.

Assays were performed in sterile 96-well microtiter plates, each well containing 100 µl of parasite culture (1 x 10^4 bloodstream forms) with or without serial drug dilutions at 37°C for 72 h in 5% CO_2. The highest concentration for the test compounds was 30 µg/ml. Each drug was tested in triplicate. A 3-fold serial dilution was performed down to a suitable concentration to obtain an IC_{50} value. Initial testing was conducted at 30, 10, 3 and 0.1µg/ml. The positive control drug was Pentamidine, which was diluted down to 0.0001µg/ml (12 dilutions). Negative control wells were without drug, blanks were medium only. After 72h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and to determine the minimum inhibitory concentration (MIC): this was the lowest drug concentration at which no trypanosomes with normal morphology and motility as compared to the control wells, were seen.

20µl of Alamar Blue were added to each well and the plates incubated for another 2-4h. Then the plates were read on a Gemini Plate Reader (Molecular Devices) using an excitation wave length of 530 nm and an emission wave length of 580 nm (cut off 550nm).

Primary screen

A preliminary screen used the *Trypanosoma brucei rhodesiense* STIB 900 strain. The compounds were tested at 4 concentrations (drug concentration range from 30 µg/ml to 1 µg/ml in 3-fold dilutions). In this assay pentamidine had an ED_{50} value of 0.1 to 0.02ng/ml.

Results for anti-trypanosomal (*T. Brucei*) (bloodstream form) testing are shown in Table 3, below.

Table 3 *In vitro* antiprotozoal activity of purine analogues

Compound ID	*Parasite*	IC_{50}(μg/ml)	95% C.I. (μg/ml)	Toxicity IC_{50} (μg/ml)
JA23	*L. donovani HU3*	>30		<0.3
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA24	*L. donovani HU3*	>30		>300
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA25	*L. donovani HU3*	1.39	0.0002400-8036	3.5
	T. cruzi Tulahuan	6.75	4.77 to 9.56	
	T.b.rhodesiense STIB900	>30		
JA26	*L. donovani HU3*	>30		204.13
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA27	*L. donovani HU3*	>30		7.94
	T. cruzi Tulahuan	9.942	4.163 to 23.74	
	T.b.rhodesiense STIB900	20.22	16.87 – 39.72	
JA28	*L. donovani HU3*	>30		8.87
	T. cruzi Tulahuan	28.19	13.55 to 58.65	
	T.b.rhodesiense STIB900	>30		
JA30	*L. donovani HU3*	>30		21.28
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA31	*L. donovani HU3*	>30		>300
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA32	*L. donovani HU3*	>30		>300
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA33	*L. donovani HU3*	>30		>300
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		
JA34	*L. donovani HU3*	>30		>300
	T. cruzi Tulahuan	29.18	7.868 to 108.3	
	T.b.rhodesiense STIB900	>30		
JA35	*L. donovani HU3*	>30		200.76
	T. cruzi Tulahuan	>30		
	T.b.rhodesiense STIB900	>30		

JA36	*L. donovani HU3*	**>30**		221.85
	T. cruzi Tulahuan	**>30**		
	T.b.rhodesiense STIB900	**>30**		
JA37	*L. donovani HU3*	**>30**		>300
	T. cruzi Tulahuan	**>30**		
	T.b.rhodesiense STIB900	**>30**		
JA38	*L. donovani HU3*	**>30**		>300
	T. cruzi Tulahuan	**>30**		
	T.b.rhodesiense STIB900	**>30**		
JA39	*L. donovani HU3*	**>30**		>300
	T. cruzi Tulahuan	**>30**		
	T.b.rhodesiense STIB900	**>30**		
JA40	*L. donovani HU3*	**>30**		>300
	T. cruzi Tulahuan	**>30**		
	T.b.rhodesiense STIB900	**>30**		
JA41	*L. donovani HU3*	**>30**		7.41
	T. cruzi Tulahuan	**>30**		
	T.b.rhodesiense STIB900	**>30**		
JA43	*L. donovani HU3*	**>30**		10.12
	T. cruzi Tulahuan	**17.63**	6.730 to 46.17	
	T.b.rhodesiense STIB900	**>30**		
Pentastam	*L. donovani HU3*	**>90***		
Pentamidine	*T.b.rhodesiense STIB900*	**0.006**		
Benznidazole	*T. cruzi Lac Z*	**0.76**	0.52 to 1.13	
Podophyllotoxin	KB cell toxicity			0.003, 0.0001

*Parasite burden was heavy which affected the ED_{50} of the Pentastam.

Example 3

***In vitro* anti-Leishmania testing**

***Leishmania donovani*: *in vitro* assay**

Parasite and cell cultures:

Leishmania donovani MHOM/ET/67/HU3 strain (also known as LV9 or L82) was used. The strain was maintained in the Syrian Hamster *(Mesocricetus auratus)*. Amastigotes were collected from the spleen of an infected hamster and spleen parasite burden was assessed using the Stauber technique and by Thoma™ haemocytometer. Primary peritoneal mouse (CD1) macrophages were collected by lavage, two days after induction with i.p. injection of 2ml 2% soluble starch. All cultures and assays were conducted at 37°C under an atmosphere of 5% CO_2/95% air mixture.

Drug sensitivity assays

Assays were performed in sterile 16-well tissue culture slides. 100 µl of RPMI1640 medium, supplemented with 10% heat inactivated fetal calf serum containing $4x10^5$/ml peritoneal macrophages were added/well and left for 24 hours at 37°C in 5% CO_2/95% air mixture. After this period 100µl of amastigotes, suspended in the same medium, were added at a given ratio of 7 amastigotes: 1 macrophage. After a further 24 h, prior to the addition of drug, one slide was methanol fixed and Giemsa stained to determine a suitable infection level.

20 mg/ml compound stock solutions were prepared as advised by the supplier, or dissolved in 100% DMSO. Stock solutions were kept at 4°C (unless otherwise advised) for 2-3 weeks in the dark. The compounds were diluted to 30 µg/ml in RPMI 1640 +10% heat inactivated fetal calf serum prior to addition to the assay and a three-fold series of dilutions made. 72 h after the addition of compound, the medium was replaced with fresh drug containing medium.

After 5 days of incubation with the compounds, parasite growth was microscopically assessed after methanol fixation and staining with a 10% Giemsa solution. The level of infection/well was evaluated by counting the number of infected macrophages per 100

macrophages. Parasite growth was compared to untreated control wells (100% parasite growth). The results were expressed as % reduction in parasite burden compared to control wells. Data were analysed by Microsoft xl/fit and ED_{50}/ED_{90} (with 95% confidence limits) values determined.

Primary screen

The compounds were tested in quadruplicate at 4 concentrations (30 - 10 - 3 - 1 μg/ml). Pentostam® (sodium stibogluconate) or another pentavalent antimonial was included as the reference drug. Sodium stibogluconate normally gives an ED_{50} value of between 5-10 μg Sb^{V}/ml.

The results for anti-leishmania (promastigotes) testing are shown in Table 3 below.

Example 4

Synthesis

In general, compounds according to the present invention were synthesised by reaction of an appropriate hydroxylamine derivative or hydrazine derivative with a halogeno-substituted purine or purine analogue. High resolution mass spectra were recorded on a Bio-Apex II FT-ICR spectrometer. ^{1}H-Nuclear Magnetic Resonance (NMR) spectra were recorded at 300 MHz on a Bruker DRX300 instrument whilst ^{13}C-NMR spectra were recorded at 125 MHz on a Bruker DRX500 instrument. Unless stated otherwise, deuterated MeOH was used as the n.m.r. solvent with tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are given in parts per million (p.p.m.) downfield from TMS. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets and app. dt = apparent doublet of triplets. All coupling constants are given in Hertz (Hz). Thin-layer chromatography (t.l.c.) plates with plastic backing coated with Merck Kieselgel PF_{254} were used. Flash chromatography used Merck Kieselgel 60 (230 – 400 mesh) with an eluent flow rate of ca. 5 mL/min being maintained by air pressure. All commercially available reagents were used as received and where appropriate anhydrous quality material was purchased. The term ether refers to diethyl ether. All compounds are

named according to the IUPAC system and were obtained using the ACD/ILAB web service (http://www.acdlabs.com).

Example 5

Synthesis of purine nucleobase analogues according to general Formula IA (Z_1 = H). (See Figure 3)

General Procedure for the synthesis of purine analogues

To a solution of 6-chloro purine or 2-amino 6-chloropurine (1.0 eq.) in EtOH : H_2O - 1 : 1, in a sealed tube, nucleophile (10 eq.) was added. The reaction mixture was stirred at 40°C until the reaction was complete (monitoring by TLC). The crude reaction mixture was then evaporated under reduced pressure and then recrystallised in a mixture of dioxane, diethylether and methanol. After filtration the white solid crystals were collected and characterised.

***N*^{6}-Hydroxy-9*H*-purin-6-amine (JA32)** (A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, 1958, **80**, 3932)

Prepared by general procedure from 6-chloropurine (0.25 g, 1.61 mmol) and hydroxylamine (50% in water) (3 mL) in water (5 mL) heated at 60°C for 0.5 h to give product (0.168 g, 87 %) as a white solid; δ_H (DMSO) 12.45 (1H, br. s, OH), 10.91 (1H, br. s, NH), 9.44 (1H, br. s, NH), 8.09 (1H, s, ArH), 7.96 (1H, s, ArH).

6-Hydrazino-9*H*-purine (JA33) (J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, 1957, **79**, 2185; J. A. Montgomery and C. Temple, *J. Am. Chem. Soc.*, 1961, **83**, 630)

δ_H (DMSO) 12.77 (1H, br. s, NH), 8.69 (1H, br. s, NH), 8.18 (1H, s, ArH), 8.09 (1H, s, ArH), 4.60 (2H, s, NH_2).

6-Hydrazino-9*H*-purin-2-amine (JA34) (J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, 1957, **79**, 2185)

δ_H (DMSO) 12.06 (1H, br. s, NH), 8.22 (1H, s, ArH), 7.73 (1H, s, ArH), 5.73 (2H, s, NH_2).

6-(1-Methylhydrazino)-9*H*-purine (JA35) (A. Giner-Sorolla *et al*, *J. Med. Chem.*, 1968, **11**, 521)

Prepared by general procedure from 6-chloropurine (0.20 g, 1.29 mmol) and *N*-methylhydrazine (1 mL) in water (5 mL) heated at 90°C for 12 h to give product (0.213 g, 87 %) as a white solid; δ_H (DMSO) 8.18 (1H, s, ArH), 8.09 (1H, s, ArH), 3.41 (3H, s, NMe).

6-(1-Methylhydrazino)-9*H*-purin-2-amine (JA36) (A. Giner-Sorolla *et al*, *J. Med. Chem.*, 1968, **11**, 521)

δ_H (DMSO) 7.88 (1H, s, ArH), 6.67 (2H, s, NH_2), 3.41 (3H, s, NMe).

N^6-Methoxy-N^6-methyl-9*H*-purin-6-amine (JA37) (T. Fujii *et al*, *Chem. Pharm. Bull.*, 1983, **31**, 3149)

Prepared by general procedure from 6-chloropurine (1.08 g, 7.0 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (2.73 g, 28.0 mmol) with triethylamine (3.54 g, 35 mmol) in 1-butanol (14 mL) heated under reflux for 4 h to give product (1.07 g, 86 %) as a pale yellow solid; δ_H (DMSO)

N^6-Methoxy-9*H*-purine-2,6-diamine (JA38)

Prepared by general procedure from 2-amino-6-chloropurine (0.47 g, 2.79 mmol) and methoxylamine (1.31 g, 27.9 mmol) in EtOH:H_2O (1:1) (10 mL) heated at 60°C for 24 h to give product (0.45 g, 90 %) as a pale greyish solid; δ_H (DMSO) 8.25 (1H, s, ArH), 7.31 (2H, s, NH_2), 3.80 (3H, s, OMe); m/z (HRMS) Found: $(M+Na)^+$, 203.0664, $C_6H_8N_6O$ requires $(M+Na)^+$ 203.0657, deviation 3.4 ppm.

N^6-Methoxy-9*H*-purin-6-amine (JA39) (A. Giner-Sorolla *et al*, *J. Med. Chem.*, 1968, **11**, 521)

Prepared by general procedure from 6-chloropurine (1.00 g, 6.47 mmol) and methoxylamine (6.00 g, 127 mmol) in 1-butanol (100 mL) heated at 70-80°C for 12 h to give product (0.91 g, 85 %) as a pale yellow solid; δ_H (DMSO) 8.11 (1H, s, ArH), 7.75 (1H, s, ArH), 3.77 (3H, s, OMe); UV λ_{max} (nm) (10% MeOH in H_2O) 273 (ε = 13100). pH 1, λ_{max} 275 (ε = 13000). pH 7, λ_{max} 271.

N^6-Hydroxy-9*H*-purine-2,6-diamine (JA41)

Prepared by general procedure from 2-amino-6-chloropurine (0.50 g, 2.98 mmol) and hydroxylamine (50% in water) (0.91 mL, 29.8 mmol) in EtOH:H_2O (1:1) (10 mL) heated at 60°C for 24 h to give product (0.39 g, 78 %) as a white solid; δ_H (DMSO) 9.54 (1H, s, NH), 7.65 (1H, s, ArH), 6.41 (2H, s, NH_2); m/z (HRMS) Found: $(M+H)^+$, 167.0687, $C_5H_6N_6O$ requires $(M+H)^+$ 167.0681, deviation 3.2 ppm.

N^6-Methoxy-N^6-methyl-9*H*-purine-2,6-diamine (JA42)

Prepared by general procedure from 2-amino-6-chloropurine (0.58 g, 3.56 mmol) and *N,O*-dimethylhydroxylamine (2.17 g, 35.6 mmol) in EtOH:H_2O (1:1) (10 mL) heated at 60°C for 24 h to give product (0.16 g, 24 %) as a white solid; δ_H (DMSO) 7.96 (1H, s, ArH), 3.86 (3H, S, OMe), 3.45 (3H, s, NMe); m/z (HRMS) Found: $(M+H)^+$, 195.0990, $C_7H_{10}N_6O$ requires $(M+H)^+$ 195.0994, deviation –2.0 ppm.

N^6-benzyloxyadenine (JA54)

Prepared by general procedure from 6-chloropurine (0.99 g, 6.41 mmol) and *O*-benzylhydroxylamine hydrochloride (5.57 g, 34.9 mmol) in EtOH:H_2O (1:1) (20 mL) and diisopropylethylamine (6.0 mL, 34.6 mmol), heated at 60°C for 24 h to give product (0.75 g, 48 %) as a white solid; m/z (HRMS) Found: $(M+Na)^+$, 264.0858, $C_{12}H_{11}N_5ONa$ requires $(M+Na)^+$ 264.0861, deviation –1.3 ppm.

Example 6: Synthesis of purine nucleoside analogues according to general Formula IA (Z_1 = ribose). (See Figure 4)

General Procedure for the synthesis of purine analogues

To a solution of 6-chloro purine riboside or 2-amino 6-chloropurine riboside (1.0 eq.) in EtOH : H_2O - 1 : 1, in a sealed tube, nucleophile (10 eq.) was added. The reaction was allowed to stir at 40°C until reaction was complete (monitoring by TLC). The crude reaction mixture was then evaporated under reduced pressure and then recrystallised in a mixture of dioxane, diethylether and methanol. After filtration the white solid crystals were collected and characterised.

2-Amino-N^6-amino-N^6-methyladenosine (JA23) (T. Naito *et al, Chem. Pharm. Bull.*, 1964, **12**, 951)

Prepared by general procedure from 2-amino-6-chloropurine riboside (0.50 g, 1.66 mmol) and *N*-methyl hydrazine (0.89 mL, 16.6 mmol) in EtOH : H_2O - 1 : 1 (10 mL) to give product (0.43 g, 83 %) as a white solid; δ_H (DMSO) 7.95 (1H, s, ArH), 5.88 (2H, br. s, NH_2), 5.74 (1H, d, *J* 6.1, 1'-H), 5.39 - 5.33 (2H, m, 2'-OH, 5'-OH), 5.12 (1H, d, *J*4.6, 3'-OH), 4.46 (1H, dd, *J* 11.4, 5.9, 2'-H), 4.08 (1H, m, 3'-H), 3.88 (1H, m, 4'-H), 3.62 (1H, dt, *J* 12.0, 4.2, 5'-H_a), 3.55 - 3.41 (4H, m, 5'-H_b and NMe); δ_C (DMSO) 159.7, 154.4, 152.6, 135.5, 113.2, 87.2, 85.8, 73.6, 71.1, 62.0, 49.0; m/z (HRMS) Found: $(M+H)^+$, 312.1422, $C_{11}H_{17}N_7O_4$ requires $(M+H)^+$ 312.1420, deviation 0.6 ppm. UV λ_{max} (nm) (10% MeOH in H_2O) 288 (ε = 12100), λ_{min} 250. pH 1, λ_{max} 297 (ε = 12200), 256 (ε = 11200), λ_{min} 274, 237. pH 12, λ_{max} 287 (ε = 13400), λ_{min} 251. ε260 (M) 8500.

2-Amino-N^6-amino-adenosine (JA24) (T. Naito *et al*, *Chem. Pharm. Bull.*, 1964, **12**, 951)
Prepared by general procedure from 2-amino-6-chloropurine riboside (0.56 g, 1.85 mmol) and hydrazine monohydrate (0.90 mL, 16.6 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.49 g, 90 %) as a white solid; δ_H (DMSO) 8.55 (1H, br. s, OH), 7.93 (1H, s, ArH), 5.90 (2H, s, NH_2), 5.75 (1H, d, *J* 6.3, 1'-H), 5.43 (1H, dd, *J* 6.6, 4.9, 5'-OH), 5.39 (1H, d, *J* 6.3, 2'-OH), 5.13 (1H, d, *J* 4.5, 3'-OH), 4.52 (1H, dd, *J* 11.3, 6.1, 2'-H), 4.46 (2H, br. s, NH_2), 4.11 (1H, dd, *J* 7.7, 4.6, 3'-H), 3.92 (1H, dd, *J* 6.7, 3.5, 4'-H), 3.66 (1H, dt, *J* 12.2, 4.2, 5'-H_a), 3.54 (1H, ddd, *J* 12.2, 6.6, 4.0, 5'-H_b); δ_C (DMSO) 160.3, 156.3, 151.4, 136.3, 112.9, 87.3, 85.9, 73.7, 71.1, 62.1; m/z (HRMS) Found: $(M+H)^+$, 298.1259, $C_{10}H_{15}N_7O_4$ requires $(M+H)^+$ 298.1264, deviation –1.7 ppm. UV λ_{max} (nm) (10% MeOH in H_2O) 282 (ε = 12300), 260 (ε = 9200), λ_{min} 265, 242. pH 1, λ_{max} 290 (ε = 10700), 254 (ε = 9600), λ_{min} 271, 236. pH 12, λ_{max} 282 (ε = 11600), λ_{min} 243. ε260 (M) 9200.

N^6-Aminoadenosine (JA25) (R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, 1957, **79**, 6401; J. A. J. Johnson *et al*, *J. Am. Chem. Soc.*, 1958, **80**, 699)
Prepared by general procedure from 6-chloropurine riboside (0.69 g, 2.41 mmol) and hydrazine monohydrate (1.17 mL, 24.1 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.57 g, 84 %) as a white solid; δ_H (DMSO) 9.03 (1H, br. s, NH), 8.34 (1H, s, ArH), 8.23 (1H, s, ArH), 5.88 (1H, d, *J* 6.2, 1'-H), 5.45 (1H, d, *J* 6.3, 2'-OH), 5.40 (1H, dd, *J* 7.0, 2.3, 5'-OH), 5.20 (1H, d, *J*4.6, 3'-OH), 4.58 (1H, app t, *J* 5.4, 2'-H), 4.13 (1H, dd, *J* 7.8, 4.6, 3'-H), 3.95 (1H, dd, *J* 6.4, 3.3, 4'-H), 3.66 (1H, m, 2, 5'-H_a), 3.53 (1H, m, 5'-H_b), 3.34 (2H, s,

NH_2); δ_C (DMSO) 155.9, 152.7, 148.7, 140.1, 118.9, 88.3, 86.3, 73.9, 71.0, 62.0; m/z (HRMS) Found: $(M+H)^+$, 283.1154, $C_{10}H_{15}N_6O_4$ requires $(M+H)^+$ 283.1155, deviation –0.3 ppm.

N^6-Methoxyadenosine (JA26) (A. Giner-Sorolla *et al*, *J. Med. Chem.*, 1968, **11**, 521; T. Fujii *et al*, *Chem. Pharm. Bull.*, 1973, **21**, 1676; T. Fujii *et al*, *Chem. Pharm. Bull.*, 1987, **35**, 4482)

Prepared by general procedure from 6-chloropurine riboside (0.57 g, 2.00 mmol) and methoxylamine (0.93 g, 20.0 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.33 g, 55 %) as a white solid; δ_H (DMSO, D_2O shake) 8.21 (1H, s, ArH), 7.76 (1H, s, ArH), 5.78 (1H, d, *J* 5.8, 1'-H), 4.45 (1H, app t, *J* 5.4, 2'-H), 4.09 (1H, app t, *J* 4.2, 3'-H), 3.91 (1H, app q, *J* 3.6, 4'-H), 3.75 (3H, s, OMe), 3.62 (1H, dd, *J* 12.0, 3.8, 5'-H_a), 3.51 (1H, dd, *J* 12.0, 3.9, 5'-H_b); δ_C (DMSO) 164.6, 153.4, 146.4, 138.6, 118.5, 87.9, 86.0, 74.4, 70.7, 62.1, 61.8; m/z (HRMS) Found: $(M+H)^+$, 298.1171, $C_{11}H_{15}N_5O_5$ requires $(M+H)^+$ 298.1165, deviation 2.1 ppm; UV λ_{max} (nm) (10% MeOH in H_2O) 268 (ε = 13200), λ_{min} 236. pH 1, λ_{max} 267 (ε = 18300), λ_{min} 235. pH 12, λ_{max} 280 (ε = 14700), λ_{min} 241. ε260 (M) 11700.

N^6-Amino-N^6-methyladenosine (JA27) (A. Giner-Sorolla *et al*, *J. Med. Chem.*, 1968, **11**, 521)

Prepared by general procedure from 6-chloropurine riboside (0.53 g, 1.85 mmol) and *N*-methylhydrazine (1.0 mL, 18.5 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.46 g, 85 %) as a white solid; δ_H (DMSO) 8.37 (1H, s, ArH), 8.19 (1H, s, ArH), 5.89 (1H, d, *J* 6.0, 1'-H), 5.56 (2H, br. s, NH_2), 5.46 (1H, d, *J* 6.2, 2'-OH), 5.38 (1H, dd, *J* 6.8, 4.7, 5'-OH), 5.20 (1H, d, *J* 4.7, 3'-OH), 4.57 (1H, dd, *J* 11.2, 5.9, 2'-H), 4.13 (1H, dd, *J* 8.0, 4.6, 3'-H), 3.95 (1H, m, 4'-H), 3.70 – 3.50 (5H, m, 5'-H_a, 5'-H_b, NMe); δ_C (DMSO) 153.9, 152.2, 149.9, 139.0, 119.0, 88.3, 86.2, 74.0, 71.0, 62.0, 37.5; m/z (HRMS) Found: $(M+H)^+$, 297.1315, $C_{11}H_{16}N_6O_4$ requires $(M+H)^+$ 297.1311, deviation 1.3 ppm. UV λ_{max} (nm) (10% MeOH in H_2O) 275 (ε = 14300), λ_{min} 236. pH 1, λ_{max} 267 (ε = 15500), λ_{min} 234. pH 12, λ_{max} 276 (ε = 13000), λ_{min} 238. ε260 (M) 9500.

N^6-Hydroxyadenosine (JA28) (A. Giner-Sorolla *et al*, *J. Med. Chem.*, 1968, **11**, 521; J.-L. G. Montero *et al*, *J. Het. Chem.*, 1977, **14**, 483)

Prepared by general procedure from 6-chloropurine riboside (0.56 g, 1.94 mmol) and *N*-hydroxylamine (50% solution in water) (1.2 mL, 19.4 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.43 g, 77 %) as a white solid; δ_C (DMSO) 157.0, 148.6, 146.3, 139.2, 124.9, 87.9, 86.1, 74.2, 70.9, 62.0; m/z (HRMS) Found: $(M+H)^+$, 284.0995, $C_{10}H_{13}N_5O_5$ requires $(M+H)^+$ 284.0995, deviation 0.0 ppm. UV λ_{max} (nm) (10% MeOH in H_2O) 265 (ε = 11900), λ_{min} 231. pH 1, λ_{max} 265 (ε = 17700), λ_{min} 232. pH 12, λ_{max} 295 (ε = 10100), λ_{min} 241. ε260 (M) 12300.

2-Amino-N^6-hydroxyadenosine (JA30) (T. Naito *et al*, *Chem. Pharm. Bull.*, 1964, **12**, 951)
Prepared by general procedure from 2-amino-6-chloropurine riboside (0.64 g, 2.11 mmol) and *N*-hydroxylamine (1.3 mL, 21.1 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.43 g, 69 %) as a white solid; δ_H (DMSO) 10.00 (1H, br. s, NH), 7.98 (1H, s, ArH), 6.82 (1H, br. s, OH), 5.67 (1H, d, *J* 5.9, 1'-H), 5.41 (1H, d, *J* 6.1, 2'-OH), 5.14 (1H, br. s, 3'-OH), 4.39 (1H, app. t, *J* 5.4, 2'-H), 4.07 (1H, app. t, *J* 4.1, 3'-H), 3.87 (1H, dd, *J* 7.3, 3.7, 4'-H), 3.60 (1H, dd, *J* 12.0, 3.9, 5'-H_a), 3.50 (1H, dd, *J* 12.0, 3.9, 5'-H_b), 3.34 (2H, br. s, NH_2); δ_C (DMSO) 163.0, 153.5, 148.0, 136.4, 110.7, 87.1, 85.7, 74.1, 70.8, 61.8; m/z (HRMS) Found: $(M+H)^+$, 297.1103, $C_{10}H_{14}N_6O_5$ requires $(M+H)^+$ 299.1104, deviation –0.3 ppm. UV λ_{max} (nm) (10% MeOH in H_2O) 281 (ε = 10900), λ_{min} 242. pH 1, λ_{max} 294 (ε = 10900), 257 (ε = 11200), λ_{min} 274, 237. pH 12, λ_{max} 296 (ε = 10800), λ_{min} 261. ε260 (M) 9200.

2-Amino-N^6-methoxyadenosine (JA31) (K. Miura *et al*, *Chem. Pharm. Bull.*, 1975, **23**, 464; T. Ueda *et al*, *Chem. Pharm. Bull.*, 1978, **26**, 2122)
Prepared by general procedure from 2-amino-6-chloropurine riboside (0.52 g, 1.71 mmol) and methoxylamine (0.80 g, 17.1 mmol) in EtOH : H_2O- 1:1 (10 mL) to give product (0.34 g, 63 %) as a white solid; δ_H (DMSO) 8.31 (1H, s, ArH), 7.04 (2H, s, NH_2), 5.70 (1H, d, *J* 5.2, 1'-H), 5.40 (3H, br. s, 3× OH), 4.37 (1H, app. t, *J* 5.0, 2'-H), 4.09 (1H, app. t, *J* 4.4, 3'-H), 3.89 (1H, dd, *J* 7.3, 3.6, 4'-H), 3.78 (3H, s, OMe), 3.63 (1H, dd, *J* 12.0, 3.6, 5'-H_a), 3.53 (1H, dd, *J* 12.0, 3.7, 5'-H_b); δ_C (DMSO) 164.7, 153.3, 147.0, 135.7, 108.3, 87.5, 85.7, 74.3, 70.4, 62.7, 61.4; m/z (HRMS) Found: $(M+H)^+$, 313.1266, $C_{11}H_{16}N_6O_5$ requires $(M+H)^+$ 313.1260, deviation 1.8 ppm. UV λ_{max} (nm) (10% MeOH in H_2O) 280 (ε = 14800), λ_{min} 242.

pH 1, λ_{max} 297 (ε = 13100), 256 (ε = 10900), λ_{min} 273, 239. pH 12, λ_{max} 288 (ε = 19200), λ_{min} 253. ε260 (M) 10500.

2-Amino-*N*-methoxy-*N*-methyladenosine (JA40)

Prepared by general procedure from 2-amino-6-chloropurine riboside (0.30 g, 0.98 mmol) and *N,O*-dimethylhydroxylamine (0.60 g, 9.8 mmol) in EtOH:H_2O (1:1) (10 mL) heated at 60°C for 24 h to give product (0.16 g, 50 %) as a white solid; δ_H (DMSO, D_2O wash) 8.49 (1H, s, ArH), 5.78 (1H, d, *J* 5.3, 1'-H), 4.40 (1H, app t, *J* 5.1, 2'-H), 4.12 (1H, app t, *J* 4.4, 3'-H), 3.92 (1H, m, 4'-H), 3.87 (3H, s, OMe), 3.80 (3H, s, NMe), 3.65 (1H, dd, *J* 12.0 and 3.8, 5'-H_a), 3.54 (1H, dd, *J* 12.0 and 3.7, 5'-H_b); m/z (HRMS) Found: $(M+H)^+$, 327.1418, $C_{12}H_{18}N_6O_5$ requires $(M+H)^+$ 327.1417, deviation 0.2 ppm.

N^6-Methoxy-N^6-methyladenosine (JA43) (T. Fujii and T. Saito, *Chem. Pharm. Bull.*, 1990, **38**, 1886)

Prepared by general procedure from 6-chloropurine riboside (0.80 g, 2.82 mmol) and *N,O*-dimethylhydroxylamine (1.75 g, 28.2 mmol) in EtOH:H_2O (1:1) (10 mL) heated at 60°C for 24 h to give product (0.63 g, 72 %) as a white solid; δ_H (DMSO) 8.85 (1H, s, OH), 8.61 (1H, s, ArH), 8.51 (1H, s, ArH), 5.93 (1H, d, *J* 7.0, 1'-H), 5.46 (1H, s, OH), 5.20 (1H, s, OH), 4.56 (1H, m, 2'-H), 4.14 (1H, m, 3'-H), 3.95 (1H, m, 4'-H), 3.85 (3H, S, OMe), 3.68-3.33 (5H, m, NMe, 5'-H_a and 5'-H_b); m/z (HRMS) Found: $(M+H)^+$, 312.1316, $C_{12}H_{17}N_5O_5$ requires $(M+H)^+$ 312.1308, deviation 2.7 ppm.

N^6-Phenylamino-adenosine (JA57)

Prepared by general procedure from 6-chloropurine riboside (0.50 g, 1.73 mmol) and phenylhydrazine (1.7 mL, 17.3 mmol) in EtOH: H_2O (1:1) (10 mL), heated at 60°C for 24 h to give a mixture of regioisomers (JA57, 0.39g, 63%) and its corresponding isomer (30%) as a yellow solid; m/z (HRMS) Found: $(M+H)^+$, 359.1465, $C_{16}H_{18}N_6O_4$ requires $(M+H)^+$ 359.1468, deviation –0.8 ppm.

N^6-allyloxyadenosine (JA58)

Prepared by general procedure from 6-chloropurine riboside (1.1324 g, 3.95 mmol) and *O*-allylhydroxylamine hydrochloride (2.60 g, 23.7 mmol) in EtOH (10 mL) and

diisopropylethylamine (5.5 mL, 31.6 mmol), heated at 60°C for 24 h to give product (0.67 g, 53 %) as a white solid; m/z (HRMS) Found: $(M+H)^+$, 324.1307, $C_{13}H_{18}N_5O_5$ requires $(M+H)^+$ 324.1308, deviation –0.4 ppm.

N^6-benzyloxyadenosine (JA59)

Prepared by general procedure from 6-chloropurine riboside (0.43 g, 1.5 mmol) and *O*-benzylhydroxylamine hydrochloride (1.0 g, 6.3 mmol) in EtOH:H_2O (1:1) (10 mL) and triethylamine (1.5 mL, 10.5 mmol), heated at 60°C for 24 h to give product (0.26 g, 46 %) as a white solid; m/z (HRMS) Found: $(M+Na)^+$, 396.1276, $C_{17}H_{19}N_5O_5Na$ requires $(M+Na)^+$ 312.1284, deviation –1.9 ppm.

2-Amino-N^6-benzyloxyadenosine (JA60)

Prepared by general procedure from 2-amino-6-chloropurine riboside (0.59 g, 1.95 mmol) and *O*-benzylhydroxylamine hydrochloride (1.86 g, 11.7 mmol) in EtOH: H_2O (1:1) (10 mL) and diisopropylethylamine (2.71 mL, 15.6 mmol), heated at 60°C for 24 h to give product (0.68 g, 90 %) as a white solid; m/z (HRMS) Found: $(M+H)^+$, 389.1574, $C_{17}H_{21}N_6O_5$ requires $(M+H)^+$ 389.1573, deviation 0.1 ppm.

Example 7: Synthesis of purine nucleoside 5'-triphosphates analogues according to general Formula IA (Z_1 = ribose, 5'-triphosphate). (See Figure 5)

General Procedure for the synthesis of purine analogues

2-Amino-6-chloropurine riboside-5'-triphosphate

To an ice-cold solution of 2-Amino-6-chloropurine riboside (0.481 g, 1.59 mmol) and proton sponge (0.511 g, 2.38 mmol) in trimethyl phosphate (8 mL) was added phosphoryl chloride (178 μL, 1.91 mmol) and the solution stirred at 0°C for 5 hours. To this was added simultaneously tributylamine (1.5 mL) and tetrabutylammonium pyrophosphate solution (0.5 M in DMF, 6.36 mL), and the solution stirred for a further 30 minutes. The reaction was then quenched by the addition of 0.5 M TEAB buffer (10 mL), and stored at 4°C overnight. The solution was evaporated to dryness and re-dissolved in water (20 mL) and applied to a Sephadex A25 column in 0.05 M TEAB buffer. The column was eluted with a linear gradient of 0.05-1.0 M TEAB. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 27 %). HPLC (Phenomenex Luna 10μ C-18 reverse

phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 25% to 100% buffer B over 45 minutes at 8 mL/min.) showed the product to be pure. δ_P (D_2O) γ-P –9.1 (d); α-P –10.3, (d); β-P -22.1, (t).

6-Chloropurine riboside-5'-triphosphate

To an ice-cold solution of 6-chloro purine riboside (0.503 g, 1.75 mmol) and proton sponge (0.562 g, 2.62 mmol) in trimethyl phosphate (9 mL) was added phosphoryl chloride (196 µL, 2.10 mmol) and the solution stirred at 0°C for 5 hours. To this was added simultaneously tributylamine (1.7mL) and tetrabutylammonium pyrophosphate solution (0.5 M in DMF, 7 mL), and the solution stirred for a further 30 minutes. The reaction was then quenched by the addition of 0.5 M TEAB buffer (10 mL), and stored at 4°C overnight. The solution was evaporated to dryness and re-dissolved in water (20 mL) and applied to a Sephadex A25 column in 0.05 M TEAB buffer. The column was eluted with a linear gradient of 0.05-1.0 M TEAB. Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 26 %). HPLC (Phenomenex Luna 10µ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 25% to 100% buffer B over 45 minutes at 8 mL/min.) showed the product to be pure. δ_P (D_2O) γ-P –5.4, (d); α-P –10.3, (d); β-P -21.5, (t).

N^6-Hydroxyadenosine 5' -triphosphate (JA44)

To a solution of 6- chloropurine riboside 5' -triphosphate (90.6 µmol) in water (2 mL), hydroxylamine (50% w/v in water, 100 µL) was added and resulting mixture was heated at 40°C for 3 h. The crude product was then purified by HPLC (Phenomenex Luna 10µ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 58 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P –8.0, (d); α-P –10.0, (d); β-P -21.3, (t).

N^6-methoxyadenosine 5'-triphosphate (JA45)

To a solution of 6- chloropurine riboside 5' -triphosphate (45.3 µmol) in water (1 mL), methoxyamine (100 µL) was added and resulting mixture was heated at 40°C for 16 h. The

crude product was then purified by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 26 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P – 7.3, (d); α-P –11.8, (d); β-P -23.1, (t).

N^6-Amino-N^6-methyladenosine 5' -triphosphate (JA46)

To a solution of 6- chloropurine riboside 5' -triphosphate (94.0 μmol) in water (2 mL), methylhydrazine (100 μL) was added and resulting mixture was heated at 40°C for 16 h. The crude product was then purified by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 48 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P – 7.1, (d); α-P –9.9, (d); β-P -21.0, (t).

2-Amino-*N*^{6}-hydroxyadenosine 5' -triphosphate (JA47)

To a solution of 2-amino 6- chloropurine riboside 5' -triphosphate (85.2 μmol) in water (3 mL), hydroxylamine (50% w/v in water, 100 μL) was added and resulting mixture was heated at 40°C for 3 h. The crude product was then purified by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 52 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P –8.7, (d); α-P –10.1, (d); β-P -21.6, (t).

2-Amino-*N*^{6}-methoxyadenosine 5' -triphosphate (JA48)

To a solution of 2-amino 6- chloropurine riboside 5' -triphosphate (86.4 μmol) in water (2 mL), methoxyamine (100 μL) was added and resulting mixture was heated at 40°C for 16 h. The crude product was then purified by HPLC (Phenomenex Luna 10μ C-18 reverse phase

column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 32 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P –6.2, (d); α-P –9.8, (d); β-P -20.8, (t).

2-Amino-N^6-amino-adenosine 5' -triphosphate (JA49)

To a solution of 2-amino 6- chloropurine riboside 5' -triphosphate (85.2 μmol) in water (3 mL), hydrazine monohydrate (100 μL) was added and resulting mixture was heated at 40°C for 3 h. The crude product was then purified by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 35 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P –8.8, (d); α-P –10.0, (d); β-P -21.6, (t).

2-Amino-N^6-amino-N^6-methyladenosine 5' -triphosphate (JA50)

To a solution of 2-amino 6- chloropurine riboside 5' -triphosphate (85.2 μmol) in water (3 mL), methylhydrazine (100 μL) was added and resulting mixture was heated at 40°C for 16 h. The crude product was then purified by HPLC (Phenomenex Luna 10μ C-18 reverse phase column, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0 % to 40% buffer B over 45 minutes at 8 mL/min.). Appropriate fractions were pooled and evaporated to dryness to give desired product as a white solid (yield 83 %). The title compound was converted into its sodium salt by passage through a Dowex 50WX4-200 resin (Na^+ form). δ_P (D_2O) γ-P –7.1, (d); α-P –9.9, (d); β-P -21.0, (t).

Example 8: Synthesis of pyrrolopyrimidine and purine nucleoside analogues according to general Formula IBA. (See Figure 6)

General synthesis of pyrrolopyrimidine analogues is well described in the literature. Treatement of C-6 chloro derivatives with hydroxylamine and hydrazine derivatives leads to compounds of general Formula IA.

Ex 9.1 is prepared from 2-methylthio-6-chloropyrrolopyrimidine with hydroxylamine as described above.

Ex 9.2 is prepared 2-methylsulfonyl-6-chloropyrrolopyrimidine with hydroxylamine as described above.

Ex 9.3 is prepared from Ex 9.1 by treatment with phenylsulfonyl chloride.

Ex 9.4 is the 2'-deoxynucleoside derivative of JA28. It is prepared by the action of hydroxylamine on 6-chloropurine-2'-deoxyriboside.

Ex 9.5 where R is H or methyl or ribose is prepared according to the general method described by Adamiak (R.W. Adamiak *et al*, (1985), Nucleic Acids Res., **13**, 2989).

Example 9: Synthesis of purine nucleoside analogues according to general Formula IB. (See Figure 7)

General synthesis of analogues is as described above, *viz* reaction of a halogeno-modified nucleobase or nucleoside with hydroxylamine or hydrazine derivatives.

Ex 10.1-10.3 are prepared from the corresponding 7-iodo derivatives (purine numbering) by the action of hydroxylamine.

Ex 10.4-10.6 are prepared from the corresponding 7-iodo derivatives (purine numbering) by the action of hydrazine.

Ex 10.7 is prepared by the action of methyl hydrazine on the corresponding 7-iodo derivative (purine numbering) of 2'-deoxypyrazolopyrimidine.

Example 10: Synthesis of purine nucleoside analogues according to general Formula IC. (See Figure 8)

General synthesis of analogues is as described above, *viz* reaction of a C2-halogeno-modified nucleobase or nucleoside with hydroxylamine or hydrazine derivatives.

Ex 11.1 is prepared by the action of methyl hydrazine on 2'-deoxy-2-chloroadenoisne.

Ex 11.2 is prepared by the action of methylhydroxylamine on 2'-deoxy-2-chloroadenoisne.

Ex 11.3 is prepared by the action of methyl hydrazine on 2'-deoxy-2-chloroinosine.

Ex 11.4 is prepared by the action of methylhydroxylamine on 2'-deoxy-2- chloroinosine.

Example 11: Synthesis of purine nucleoside analogues according to general Formula ID. (See Figure 9)

Compounds were prepared as described by Cohen (H.M. Cohen *et al*, (2005), *Org. Biomol. Chem.* **3**, 152).

Example 12: Synthesis of purine nucleoside analogues according to general Formula IE. (See Figure 10)

Methyl-1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-nitroindole-3-carboxylate (Ex 13.2)

To a solution of 5-nitroindole (3 g, 18.5 mmol) in ether (100 cm^3) at 0°C was added oxalyl chloride (8 cm^3, 92 mmol) dropwise, and stirring continued at 0°C for 24 hr. The solid was filtered and dried. Yield 4.1 g, 88%. The acid chloride was suspended in water (100 cm^3) and potassium hydroxide (1.1g, 19.6 mmol) added and the solution heated at 90°C for 1 h. The solution was cooled, acidified and the yellow solid filtered. The solid was resuspended in hydrogen peroxide solution (30%, 50 cm^3) and the solution heated at reflux for 3 hours. After cooling the solid was filtered, dried and recrystallised from aqueous ethanol to give a greenish-yellow solid. Yield 2.45 g, 64%. mp 285-287°C. δ_H 7.64 (1H, d, *J* 9, H7), 8.07 (1H, dd, J_1 9, J_2 2.2, H6), 8.27 (1H, s, H2), 8.88 (1H, d, *J* 2.2, H4), 12.44 (2H, br. s, NH, CO_2H).

To a solution of 5-nitroindole-3-carboxylic acid (2.45 g, 12 mmol) in methanol (50 cm^3) was added sulphuric acid (1 cm^3) and the solution stirred at reflux for 6 hours. The solution was allowed to cool and then poured onto ice-water, neutralised with sodium bicarbonate and the product filtered to give a yellow solid, which was recrystallised from methanol as an off-white solid. Yield 1.76 g, 67%. mp 282-284°C. δ_H 3.85 (3H, s, OCH_3), 7.65 (1H, d, *J* 9, H7), 8.08 (1H, dd, J_1 9, J_2 2.3, H6), 8.35 (1H, s, H2), 8.83 (1H, d, *J* 2.3, H4), 8.5 (1H, s, NH). uv λmax/nm 320 (15000), 251 (32450), λmin/nm 284, 213; pH 12 λmax/nm 366 (13100), 271 (31500), 213 (48100). m/z 243.1 $(M+Na)^+$. Accurate mass measurement on $(M+Na)^+$ $C_{10}H_8N_2O_4Na$ 243.0396, deviation –5.73 ppm.

To a solution of methyl-5-nitroindole-3-carboxylate (1.22 g, 5.5 mmol) in acetonitrile (50 cm^3) was added sodium hydride (60%, 0.27 g, 7 mmol) and the solution stirred at room temperature for 30 mins. To this was then added α-3,5-di-*O*-*p*-toluoyl-2-deoxyribofuranosyl chloride (2.6 g, 6.7 mmol) and stirring continued for 2 hours. The solvent was evaporated and the product

worked up to give a brown foam, which was chromatographed (CH_2Cl_2/0-2% MeOH) to give a pale yellow foam. Yield 2.25g, 73%. δ_H 2.36 (3H, s, toluoyl-CH_3), 2.40 (3H, s, toluoyl-CH_3), 2.80-2.87 (1H, m, H2'), 2.99-3.08 (1H, m, H2''), 3.82 (3H, s, OCH_3), 4.44-4.67 (3H, m, H4', H5', H5''), 5.71-5.73 (1H, m, H3'), 6.72 (1H, t, *J* 6.7, H1'), 7.26-7.39 (4H, m, toluoyl-CH), 7.78-8.03 (6H, m, 4 x toluoyl-CH, H7, H6), 8.57 (1H, s, H2), 8.81 (1H, d, *J* 1.9, H4). uv λmax/nm 316 (9600), 246 (32300), λmin/nm 302, 222; pH 1 λmax/nm 274 (28400), 253 (29800), λmin/nm 268, 225; pH 12 λmax/nm 316 (13700), 229 (34900), λmin/nm 306, 232, 209. m/z 595.1 $(M+Na)^+$. Accurate mass measurement on $C_{31}H_{28}N_2O_9Na$ 595.16750, deviation -2.99ppm.

A solution of methyl-1-(3,5-di-O-p-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-nitroindole-3-carboxylate (2.3 g, 4 mmol) in methanol (100 cm^3) containing triethylamine (5 cm^3) was heated at reflux overnight. The solution was evaporated and the product chromatographed (CH_2Cl_2/0-5% MeOH) to give a pale yellow solid, which recrystallised from ethanol to give off-white needles. Yield 1.22 g, 90%. mp 162-164°C. (Found: C, 53.41; H, 4.83; N, 8.27%; $C_{15}H_{16}N_2O_7$ requires C, 53.57; H, 4.80; N, 8.33%). δ_H 2.31-2.39 (1H, m, H2'), 2.48-2.56 (1H, m, H2''), 3.51-3.63 (2H, m, H5', H5''), 3.30-3.38 (1H, m, H4'), 3.33 (3H, s, CH_3), 4.35-4.40 (1H, m, H3'), 5.04 (1H, t, *J* 5.1, 5'-OH), 5.36 (1H, d, *J* 4.1, 3'-OH), 6.50 (1H, t, *J* 6.2, H1'), 7.96 (1H, d, *J* 9.2, H7), 8.13 (1H, dd, J_1 9.1, J_2 2.3, H6), 8.62 (1H, s, H2), 8.84 (1H, d, *J* 2.2, H4). uv λmax/nm 318 (9100), 266 (24300), λmin/nm 290, 216. m/z 359.1 $(M+Na)^+$. Accurate mass measurement on $C_{15}H_{16}N_2O_7Na$ 359.08480, deviation -2.09ppm.

1-(2-Deoxy-β-D-ribofuranosyl)-5-nitroindole-3-carboxamide (Ex 13.1)

A solution of the above ester (300 mg, 0.89 mmol) in 0.880 ammonia solution (10 cm^3) and the solution stirred at 50°C overnight. The solution was evaporated and the product crystallised from ethanol to give a pale green solid. Yield 133 mg, 46%. mp 220-222°C. (Found: C, 52.15; H, 4.75; N, 12.87%; $C_{15}H_{16}N_3O_7$ requires C, 52.34; H, 4.71; N, 13.08%). δ_H 2.34-2.49 (2H, m, H2', H2''), 3.46-3.59 (2H, m, H5', H5''), 3.85-3.89 (1H, m, H4'), 4.39 (1H, br. s, H3'), 4.89 (1H, t, *J* 5.3, 5'-OH), 5.38 (1H, d, *J* 4.2, 3'-OH), 6.47 (1H, t, H1'), 7.16, 7.70 (2 x br. s, $CONH_2$), 7.86 (1H, d, *J* 9, H7), 8.08 (1H, dd, J_1 9, J_2 2, H6), 8.48 (1H, s, H2), 9.06 (1H, d, *J* 2, H4). uv λmax/nm 320 (9700), 267 (24600), λmax/nm 291, 223. ε_{260} (μM) = 22.7. m/z 344.1 $(M+Na)^+$. Accurate mass measurement on $C_{14}H_{15}N_3O_6Na$ 344.08470, deviation -3.45ppm.

1-(2-Deoxy-β-D-ribofuranosyl)-5-nitroindole-3-methylcarboxamide (Ex 13.3)

A solution of the methyl ester (200 mg, 0.59 mmol) in 40% aqueous methylamine solution (10 cm^3) and the solution stirred at 50°C overnight. The solution was evaporated and the product crystallised from ethanol to give a yellow solid. Yield 96 mg, 48%. mp 248-250°C. (Found: C, 52.51; H, 5.07; N, 12.03%; $C_{15}H_{17}N_3O_7.0.5H_2O$ requires C, 52.32; H, 5.26; N, 12.20%). δ_H 2.33-2.46 (2H, m, H2', H2''), 2.79 (3H, d, *J* 4.4, $NHCH_3$), 3.46-3.57 (2H, m, H5', H5''), 3.85-3.89 (1H, m, H4'), 4.39 (1H, br. s, H3'), 4.91 (1H, br. s, 5'-OH), 5.39 (1H, d, *J* 3, 3'-OH), 6.47 (1H, t, *J* 6.5, H1'), 7.86 (1H, d, *J* 9, H7), 8.08 (1H, dd, J_1 9.1, J_2 2.2, H6), 8.19 (1H, br, NH), 8.41 (1H, s, H2), 9.06 (1H, d, *J* 2, H4). uv λmax/nm, λmax/nm 321 (8300), 267 (20700), 203 (25800), λmin/nm 291, 222. ε_{260} (μM) = 19.2. m/z 358.1 $(M+Na)^+$. Accurate mass measurement on $C_{15}H_{17}N_3O_6Na$ 358.10120, deviation -0.94ppm.

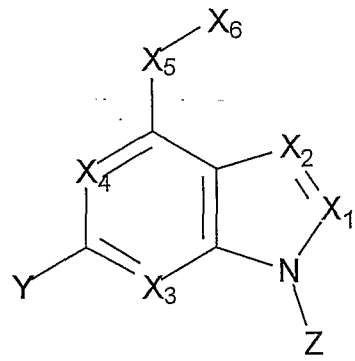
Ex 13.4 was prepared by the action of hydrazine on Ex 13.2.

Ex 13.5 was prepared by the action of methyl hydrazine on Ex 13.2.

Ex 13.6 was prepared from the corresponding bromoindole by the action of hydroxylamine using Pd catalysts.

Claims

1. Use of a compound having a structure according to general formula 1 defined below, in the manufacture of a medicament to treat and/or prevent a parasitic infection or infestation in a mammalian subject



Formula I

wherein X_1 = N or CH or C=O (X_2 = NH) or C=S (X_2 = NH) or C-OR_1 or C-halogen or C-azide;

X_2 = N or CR_1 or C-halogen or $CS(O)_nR_1$ where n = 0–2 or a $(C)_m$ linker where m = 1-3 between X_2 and X_6 or C-X_5X_6 (in which case X_5X_6 at C6 (purine numbering) is replaced by H or NHR_1 or O or OR_1 or S or SR_1);

X_3 = N or CH or C-NO_2;

X_4 = N or CH or C-NO_2 or C-NR_1R_2 or an amidine derivative or a guanidinium derivative;

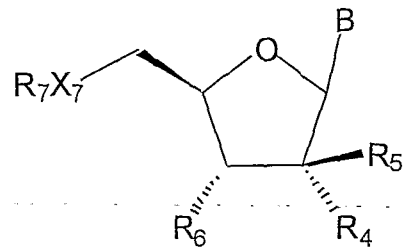
X_5 = O or NR_1 or CR_1R_2;

X_6 = OR_1 or O-acyl or O-$S(O)_nR_1$ or NR_1R_2 or NH-acyl or or $N(Acyl)_2$ or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0–2 or a hydrazone derivative or an oxime derivative, but if X_5 = O X_6 cannot = O or X_5X_6 is an amidine or an *N*-substituted pyridine or substituted guanidine;

Y = H or NH_2 or NR_1R_2 or =O (X_3 = NH) or OR_1 or F or Cl or Br or I or $CR_1R_2R_3$ or $S(O)_nR_1$ where n = 0–2 or azide or X_5X_6 (in which case X_5X_6 at C6 (purine numbering) is replaced by H or NHR_1 or O or OR_1 or S or SR_1);

R_1, R_2, R_3 are independently selected from the group consisting of H or (optionally substituted), alkyl, alkenyl or alkynyl or aryl or aralkyl where the substituents may be selected from H, OH, NH_2, halogen, N_3, CN, CHO, COOR', $CONR'_2$, OR, NR'_2, SR', NR'NR'_2, NR'OR', NO_2 and R' is alkyl, alkenyl, alkynyl, aralkyl, acyl, sulfonyl;

Z = H or (optionally substituted) alkyl or alkenyl or alkynyl or aralkyl or a β-D-linked sugar derivative of general formula II in the β-configuration



Formula II

where:

B is the nucleobase from Formula I;

X_7 = CH_2 or O or NR_1 or S;

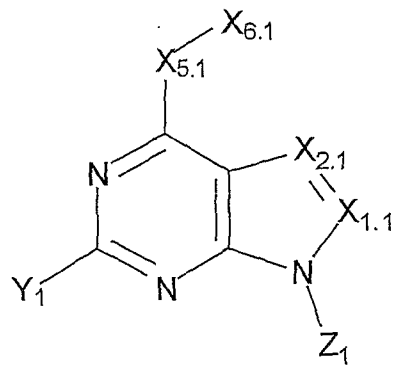
R_4 = H or OH or OR_1 or halogen or azide or a phosphate derivative;

R_5 = H or F or CH_3;

R_6 = H or OH or OR_1 or halogen or azide or a phosphate derivative; and

R_7 = H or halogen or R_1 or a derivative of an amino acid or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

2. A use according to claim 1, wherein the active agent has a structure according to Formula IA below



Formula IA

where:

$X_{1.1}$ = CH or N

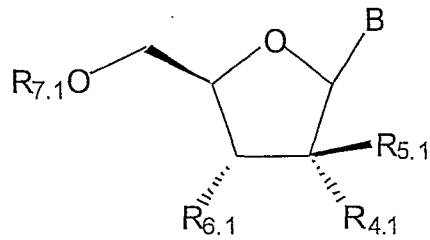
$X_{2.1}$ = CH or N or S or S-Me or C-halogen or CR_1;

$X_{5.1}$ = O or NH or N-R_1;

$X_{6.1}$ = OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or $N(Acyl)_2$ NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.1}X_{6.1}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative or a *N*-pyridinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

Y_1 = H or NH_2 or =O (N^3 (purine numbering)= NH) or halogen or azide;

Z_1 = H or Formula **IIA** in the β-configuration



Formula IIA

where:

B is the nucleobase from Formula IA;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F;

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

3. A use according to claim 2, wherein the active agent has a structure according to formula IA, wherein

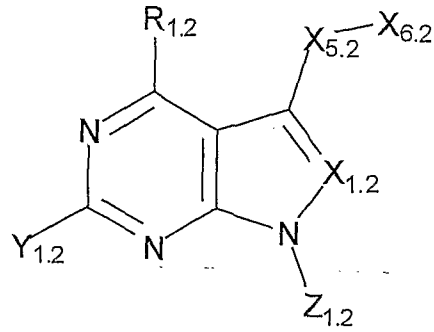
B is the nucleobase from Formula IA;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F;

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

4. A use according to claim 1, wherein the active agent has a structure according to Formula IB below:



Formula IB

where:

$X_{1.2}$ is N or CH;

$X_{5.2}$ is O or NH or N-R_1;

$X_{6.2}$ is OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.2}X_{6.2}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$Y_{1.2}$ = H or NH_2 or =O (N^3 = NH) or halogen or azide;

$R_{1.2}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

$Z_{1.2}$ = H or Formula **IIA** in the β-configuration where B is the nucleobase from Formula IB;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2);

5. A use according to claim 4, wherein the active agent has a structure according to formula IB wherein:

$X_{1.2}$ is N or CH;

$X_{5.2}$ is O or NH or N-R_1;

$X_{6.2}$ is OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.2}X_{6.2}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$Y_{1.2}$ = H or NH_2 or =O (N^3 = NH) or halogen or azide;

$R_{1.2}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

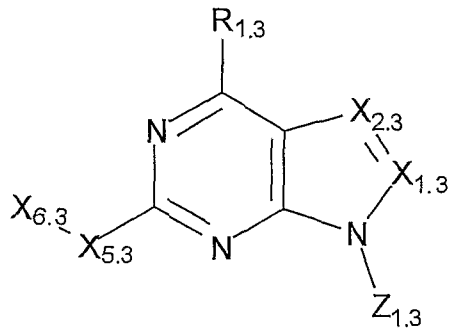
$Z_{1.2}$ = H or Formula **IIA** in the β-configuration where B is the nucleobase from Formula IB;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2);

6. A use according to claim 1, wherein the active agent has a structure according to Formula IC below:



Formula IC

where:

$X_{1.3}$ is CH or N;

$X_{2.3}$ is CH or N;

$X_{5.3}$ is O or NH or N-R_1;

$X_{6.3}$ is OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.3}X_{6.3}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$R_{1.3}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

$Z_{1.3}$ = H or Formula IIB in the β-configuration where B is the nucleobase from Formula IC;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F;

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2);

7. A use according to claim 6, wherein the active agent has a structure according to formula IC wherein

$X_{1.3}$ is CH or N;

$X_{2.3}$ is CH or N;

$X_{5.3}$ is O or NH or N-R_1;

$X_{6.3}$ is OR_1 or O-acyl or O-$S(O)_nR_1$ where n = 0-2 or NR_1R_2 or NH-acyl or NH-$OS(O)_2R_1$ or NH-$S(O)_nR_1$ where n = 0-2 or $X_{5.3}X_{6.3}$ is a hydrazone derivative or an oxime derivative or an amidine derivative or a guanidinium derivative but $X_{5.1}$ and $X_{6.1}$ cannot both be O;

$R_{1.3}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

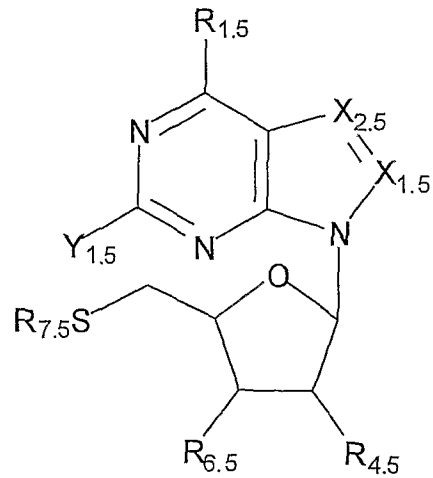
$Z_{1.3}$ = H or Formula IIB in the β-configuration where B is the nucleobase from Formula IC;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F;

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2);

8. A use according to claim 1, wherein the active agent has a structure according to Formula ID below:



Formula ID

where:

$X_{1.5}$ is CH or N;

$X_{2.5}$ is CH or N;

$R_{1.5}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

$Y_{1,5}$ is H or NH_2 or =O (N^3 (purine numbering) = NH) or halogen or azide or X_5X_6;

$R_{4.5}$ is H or OH or F;

$R_{6.5}$ is H or OH or F; and

$R_{7.5}$ is acyl or alkyl or an amino acid such as homocysteine or a derivative of an amino acid such as butanoic acid.

9. A use according to claim 8, wherein the active agent has a structure according to general formula ID, wherein

$X_{1.5}$ is CH or N;

$X_{2.5}$ is CH or N;

$R_{1.5}$ is O or OR_1 or S or SR_1 or NR_1R_2 or halogen;

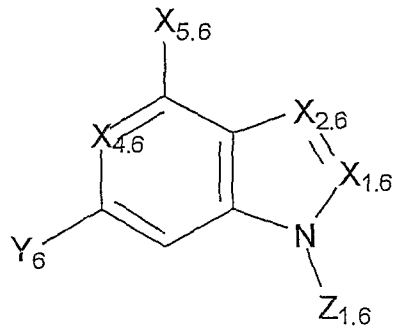
$Y_{1,5}$ is H or NH_2 or =O (N^3 (purine numbering) = NH) or halogen or azide or X_5X_6;

$R_{4.5}$ is H or OH or F;

$R_{6.5}$ is H or OH or F; and

$R_{7.5}$ is acyl or alkyl or an amino acid such as homocysteine or a derivative of an amino acid such as butanoic acid.

10. A use according to claim 1, wherein the active agent has a structure according to Formula IE below:



Formula IE

where:

$X_{1.6}$ is CH or N;

$X_{2.6}$ is CH or N or CR_8;

$X_{4.6}$ is CH or C-NO_2 or C-NR_1R_2 or an amidine derivative or a guanidinium derivative;

$X_{5.6}$ is H or NH_2 or O or OR_1 or S or SR_1;

R_8, if present, is $CONHR_1$ or $CONR_1NHR_2$ or $CONR_1OR_2$;

Y_6 is H or NH_2 or NHR_1 or N_3 or halogen or O or OR_1 or S or SR_1 or $CR_1R_2R_3$

$Z_{1.6}$ is H or R_1 or Formula IIA in the β-configuration, where B is the nucleobase IE;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

11. A use according to claim 10, wherein the active agent has a structure according to general formula IE, wherein

$X_{1.6}$ is CH or N;

$X_{2.6}$ is CH or N or CR_8;

$X_{4.6}$ is CH or C-NO_2 or C-NR_1R_2 or an amidine derivative or a guanidinium derivative;

$X_{5.6}$ is H or NH_2 or O or OR_1 or S or SR_1;

R_8, if present, is $CONHR_1$ or $CONR_1NHR_2$ or $CONR_1OR_2$;

Y_6 is H or NH_2 or NHR_1 or N_3 or halogen or O or OR_1 or S or SR_1 or $CR_1R_2R_3$

$Z_{1.6}$ is H or R_1 or Formula IIA in the β-configuration, where B is the nucleobase IE;

$R_{5.1}$ is H or F or CH_3;

$R_{4.1}$ and $R_{6.1}$ are independently selected from H or OH or F; and

$R_{7.1}$ = H or PO_3H_2 or $P_2O_6H_3$ or $P_3O_9H_4$ or a methylene derivative of $P_2O_6H_3$ or $P_3O_9H_4$ or a masked phosphate or a phosphonate derivative (5'-O replaced with CH_2).

12. A use according to any one of the preceding claims wherein Y, Y_1, $Y_{1.2}$, $Y_{1.5}$ or Y_6, as appropriate, is H or NH_2.

13. A use according to any one of the preceding claims, wherein X_1, $X_{1.1}$, $X_{1.2}$, $X_{1.3}$, $X_{1.5}$ or $X_{1.6}$, as appropriate, is CH.

14. A use according to any one of the preceding claims, wherein X_2, $X_{2.1}$, $X_{2.3}$, $X_{2.5}$ or $X_{2.6}$, as appropriate, is N.

15. A use according to any one of the preceding claims, wherein X_5X_6, $X_{5.1}X_{6.1}$, or $X_{5.2}X_{6.2}$, if present, is selected from the group consisting of –NH-NH_2, -NH-OH and –N(alkyl)-NH_2.

16. A use according to claim 1, wherein Y is H or NH_2, X_1 is CH, X_2 is N, and X_5X_6 is selected from the group consisting of –NH-NH_2, -NH-OH and –N(alkyl)-NH_2.

17. A purine nucleobase, nucleoside or nucleotide analogue according to general formula IB as set forth in claim 4.

18. A purine nucleoside, nucleoside or nucleotide analogue according to general formula IC as set forth in claim 6.

19. A purine nucleobase, nucleoside or nucleotide analogue according to general formula IE as set forth in claim 10.

20. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of an active agent as defined in any one of the preceding claims, or a pharmaceutically acceptable ester or salt thereof, admixed with at least one pharmaceutically acceptable carrier, diluent or excipient.

21. A pharmaceutical composition according to claim 20, comprising a plurality of different active agents, each active agent being as defined in accordance with any one of claims 1-19.

22. Use of a compound according to any one of claims 17, 18 or 19, in the manufacture of a pharmaceutical composition.

23. A pharmaceutical composition comprising one or more compounds in accordance with any one of claims 17, 18 or 19, or a pharmaceutically acceptable ester or salt thereof, in admixture with a pharmaceutically acceptable carrier, diluent or excipient.

24. A pharmaceutical composition according to any one of claims 20, 21 or 23 further comprising one or more conventional anti-protozoal agents.

25. A pharmaceutical composition according to claim 24, wherein the conventional anti-protozoal agent is selected from chloroquine, pyrimethamine, cycloguanil, doxycycline, mefloquine, primaquin, diminazene, isometamidium, or artemisinin or derivatives thereof.

26. A method of treating or preventing a protozoal infection or infestation in a mammalian subject, the method comprising administering to the subject a therapeutically or prophylactically effective dose of a pharmaceutical composition according to any one of claims 20-21 or 23-25.

27. A use according to any one of claims 1-19, or a method according to claim 26, wherein the subject is a human.

28. A use according to any one of claims 1-19, or a method according to claim 26, wherein the infection or infestation is caused by *Plasmodium spp.*, Trypanosomes or *Leishmania spp.*

29. A use according to any one of claims 1-19, or a method according to claim 26, wherein the infection is malaria caused by a chloroquine resistant strain of *Plasmodium spp.*

30. A compound, according to the definition in any one of claims 1-16, for use in the prevention and/or treatment of a protozoal infection or infestation in a mammalian subject.

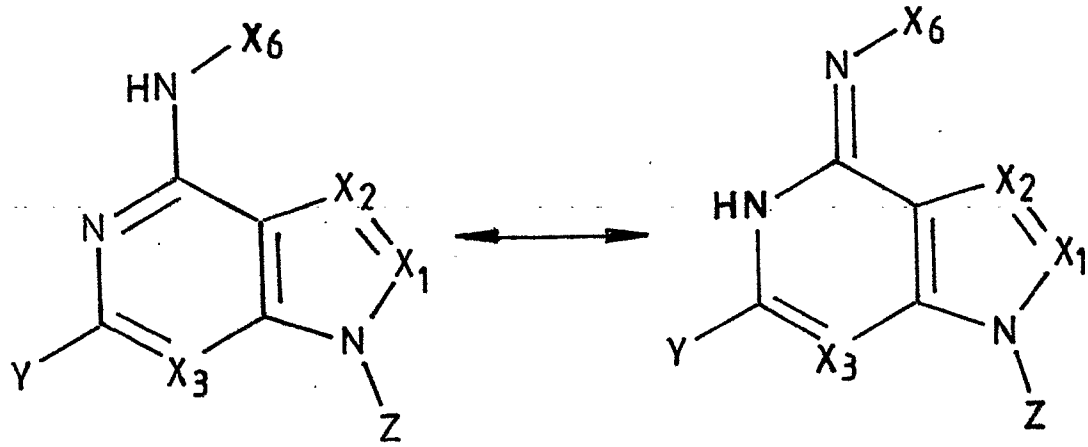

X6
HN
X2
N
X1
Y
X3
N
Z
X6
N
HN
X2
X1
Y
X3
N
Z

Fig. 1a

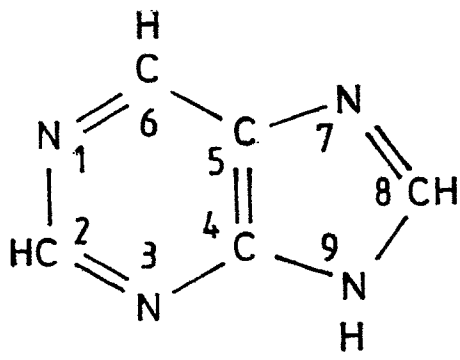
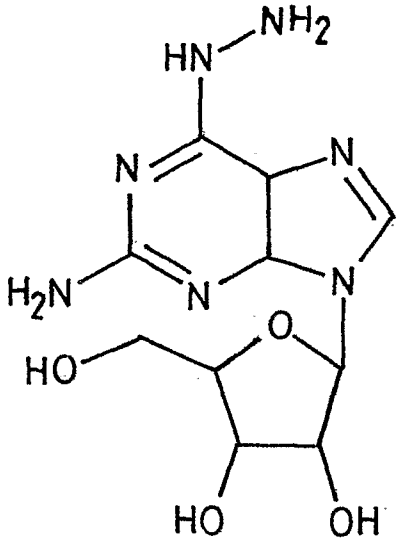
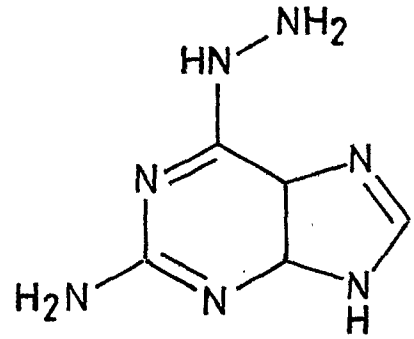

H
C
N
6
C
7
N
1
5
8
CH
HC
2
3
4
C
9
N
N
H

Fig. 1b

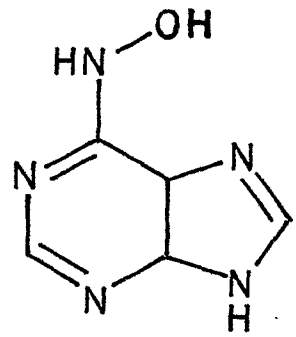
Structures of anti-trypanosomal and anti-leishmania analogues



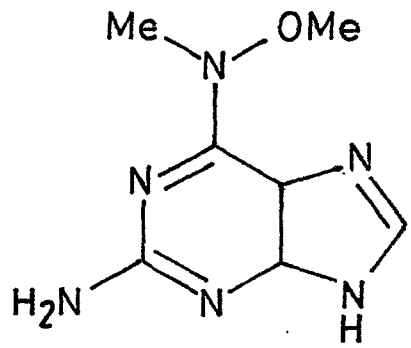
JA24
EC_{50} = 60.8μM



JA34
EC_{50} = 47.9μM

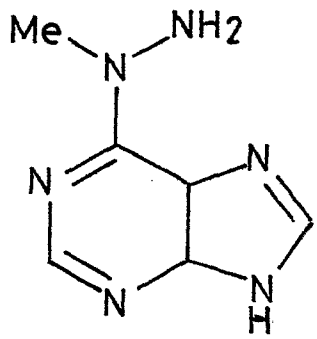


JA32
EC_{50} = 89.9μM



JA37
EC_{50} = 98.1μM

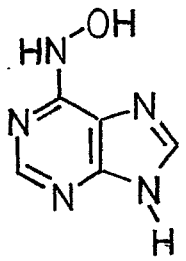
Trypanosome Brucei (bloodstream forms)



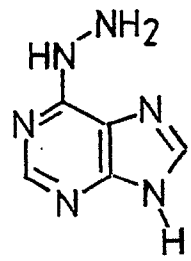
JA34
EC_{50} = 47.9μM

Leishmania Major (Promastigotes)

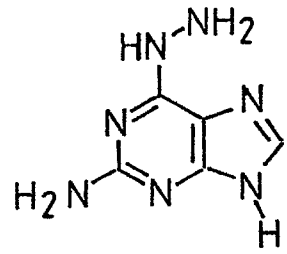
Fig. 2



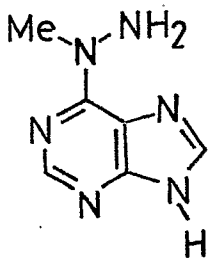
JA32



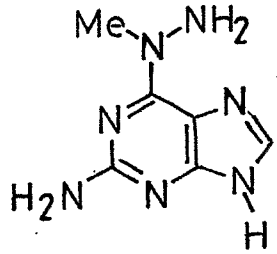
JA33



JA34



JA35



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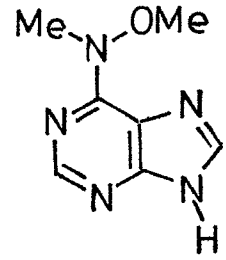

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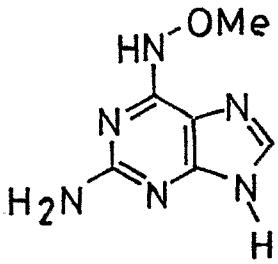
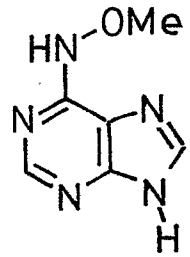


JA38



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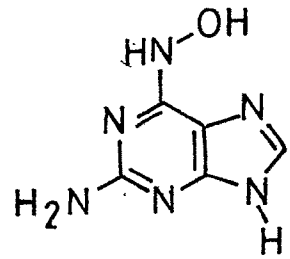

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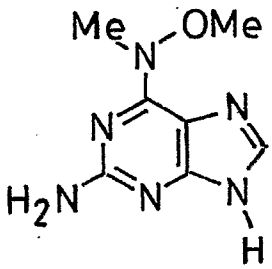

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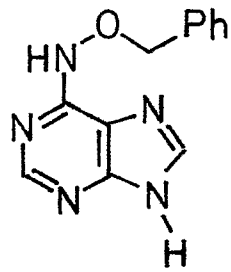
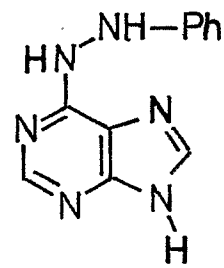
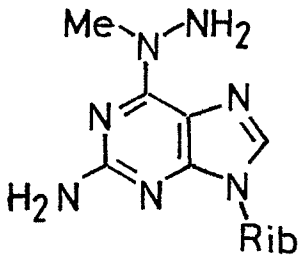


JA54



JA55

Fig. 3



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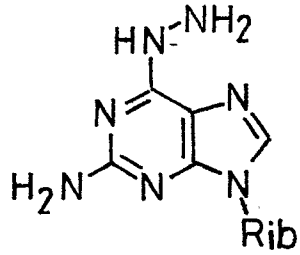

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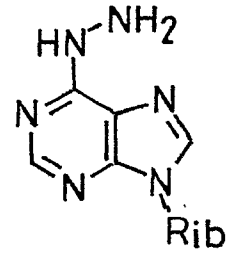

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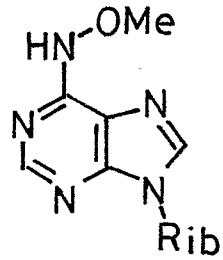
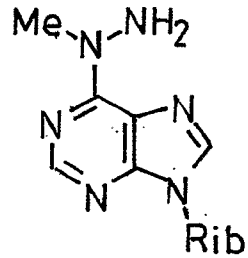


JA26



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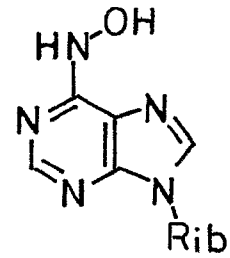

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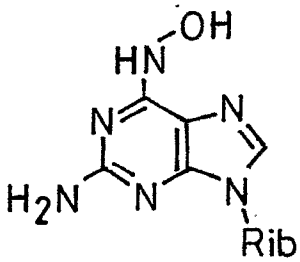
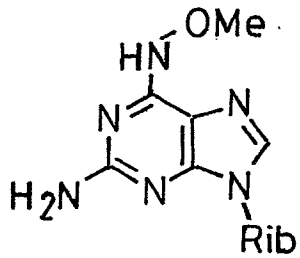


JA30



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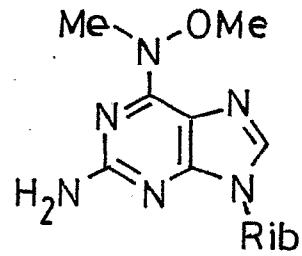

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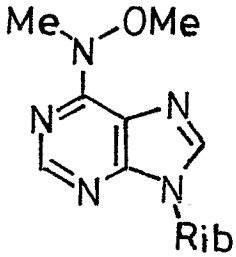
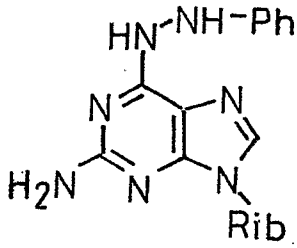


JA43

Fig. 4 Sheet 1



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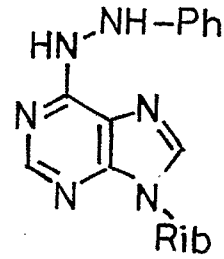

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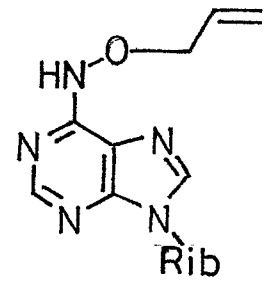

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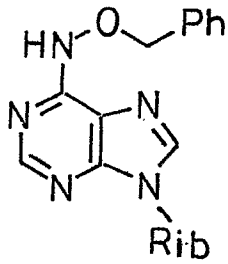
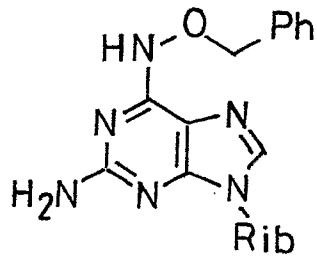
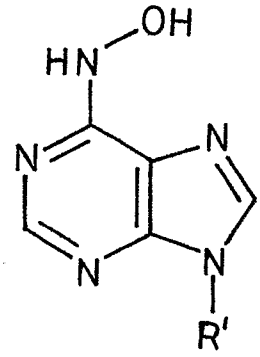


JA59



JA60

Fig. 4 Sheet 2



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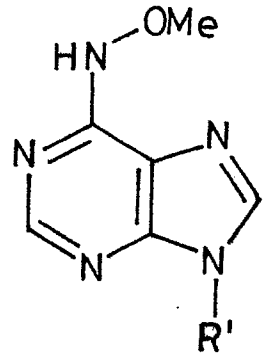
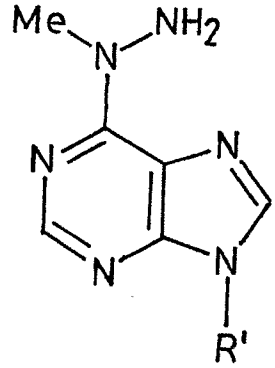
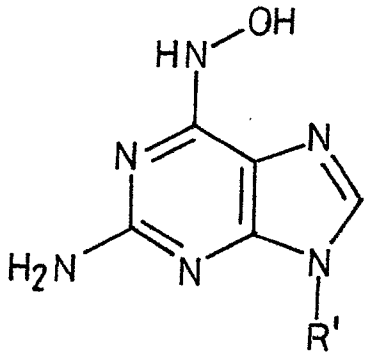


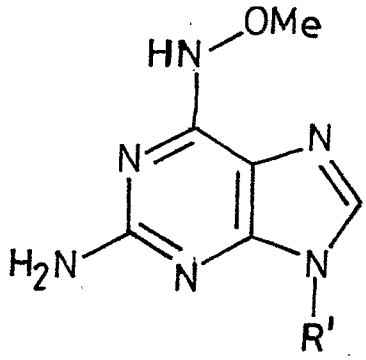
JA45



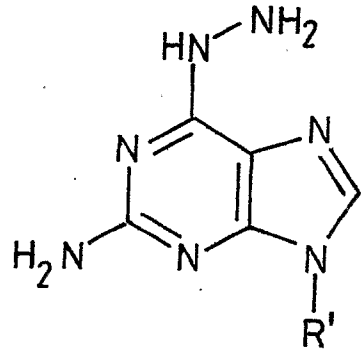
JA46



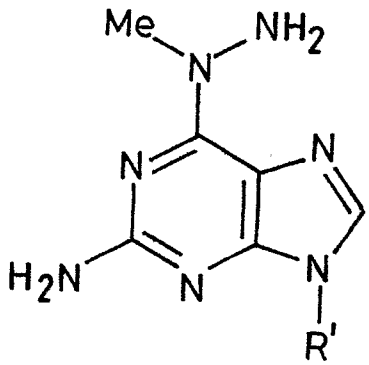
JA47



JA48



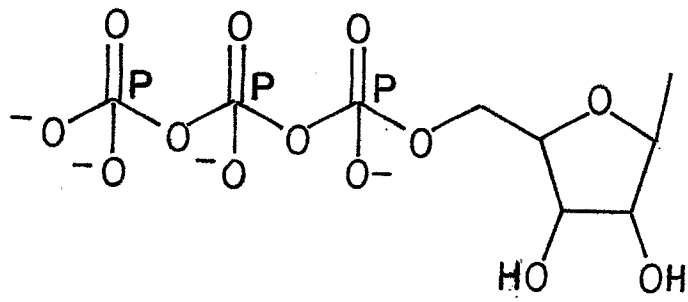
JA49

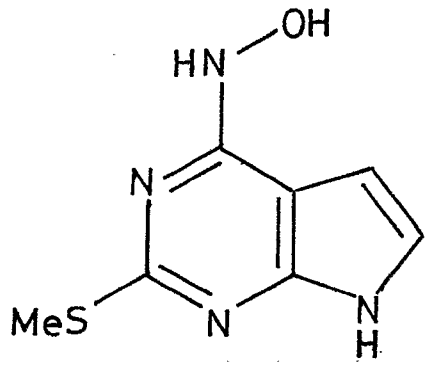


JA50

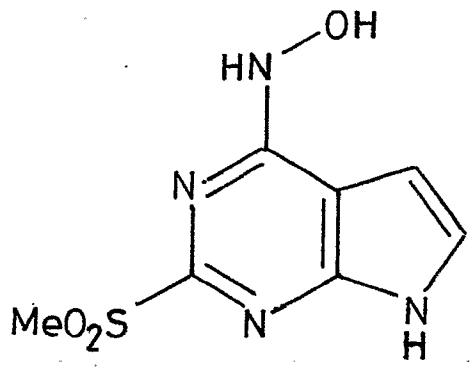
Fig. 5

R' =

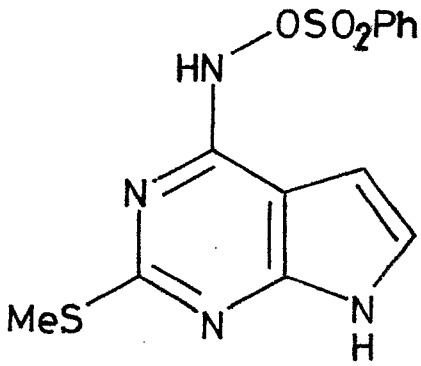




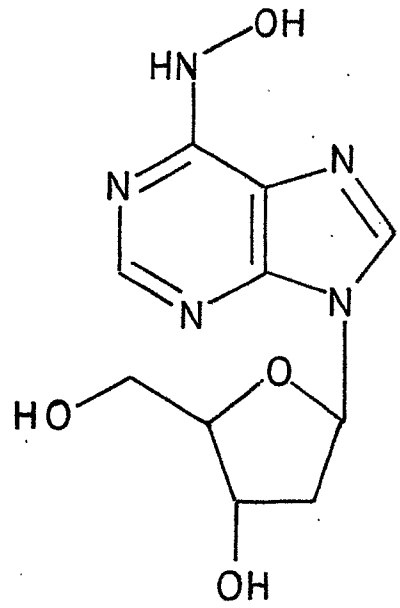
Ex 9.1



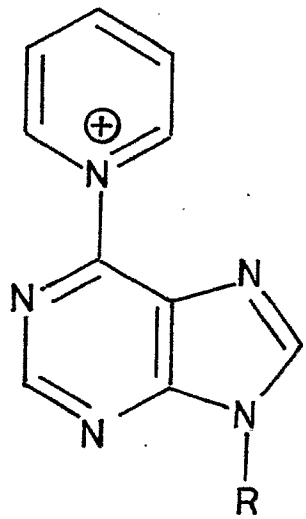
Ex 9.2



Ex 9.3

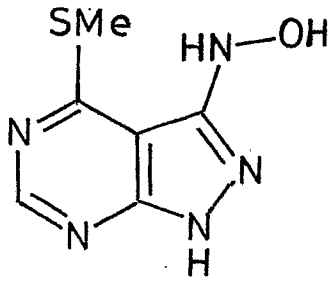


Ex 9.4

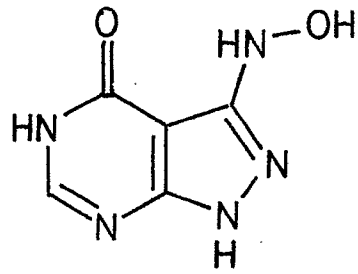


Ex 9.5

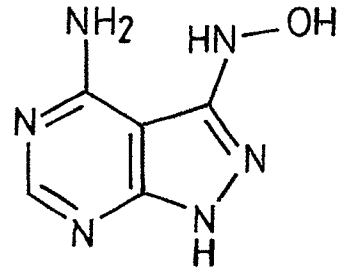
Fig. 6



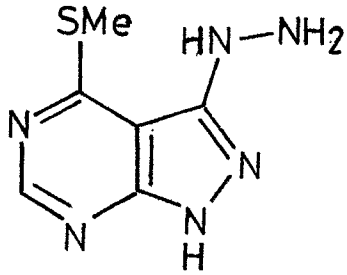
Ex 10.1



Ex 10.2



Ex10.3



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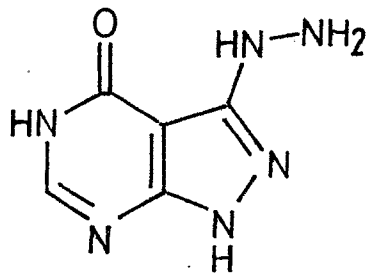
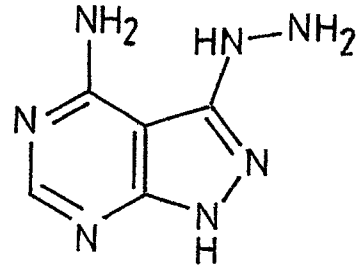


Ex 10.5



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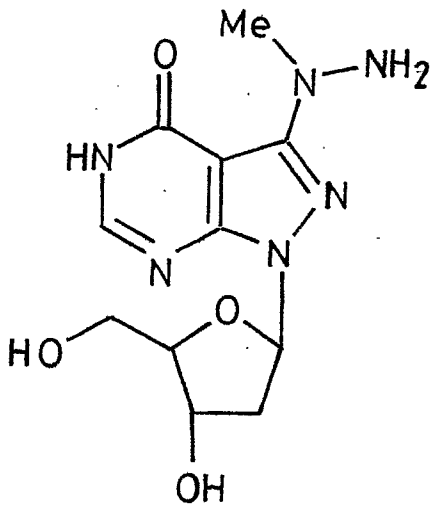
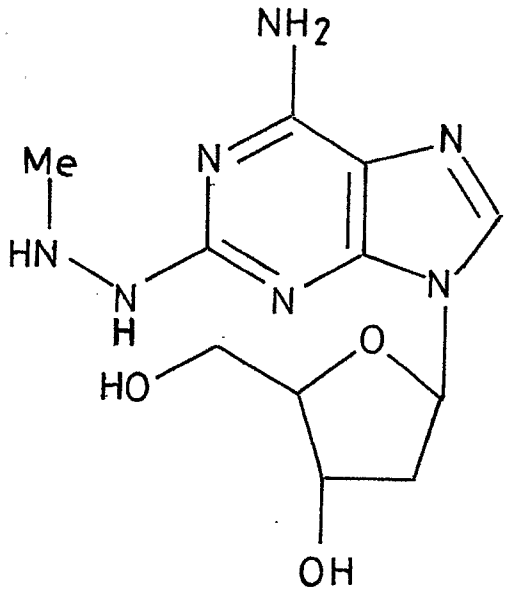
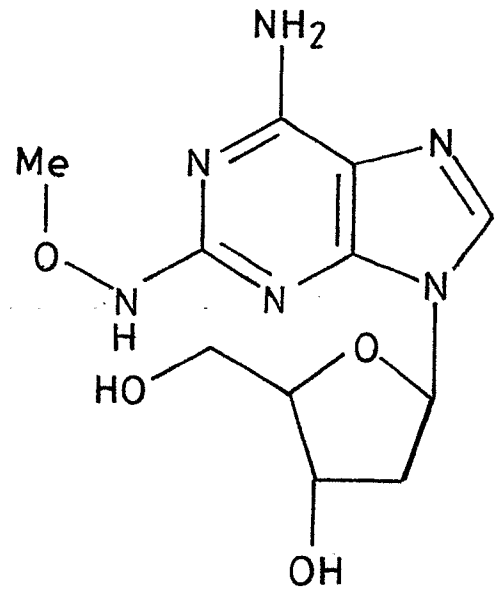


Ex 10.7

Fig. 7



Ex 11.1



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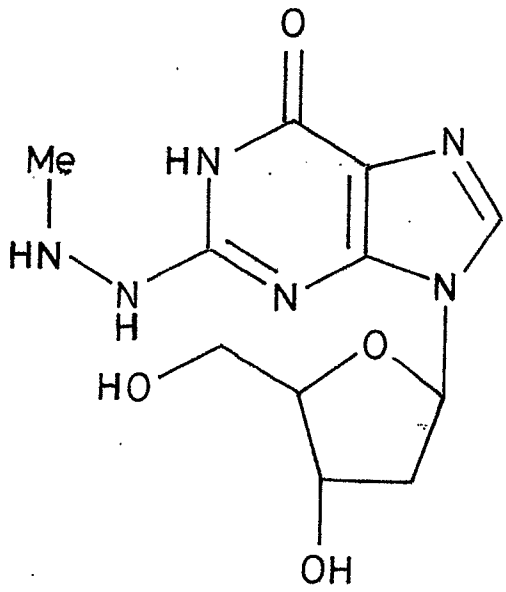

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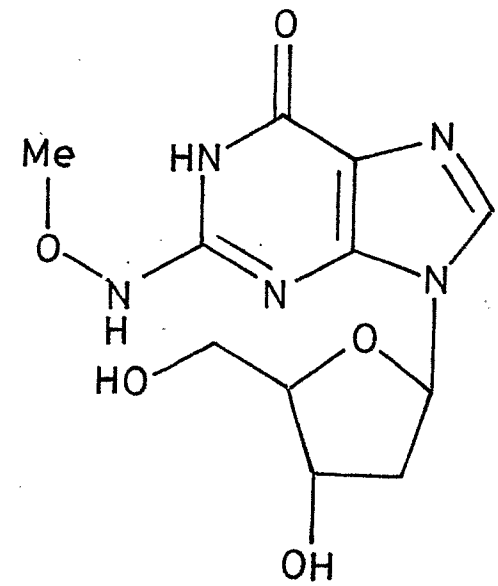
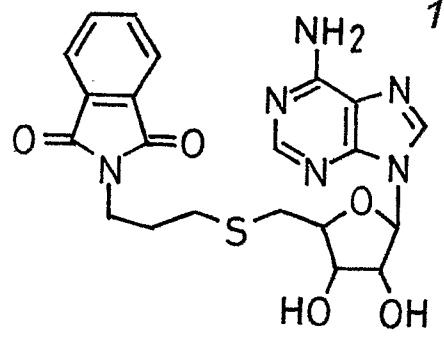
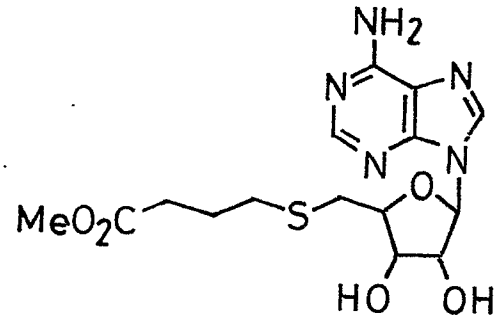


Ex 11.4

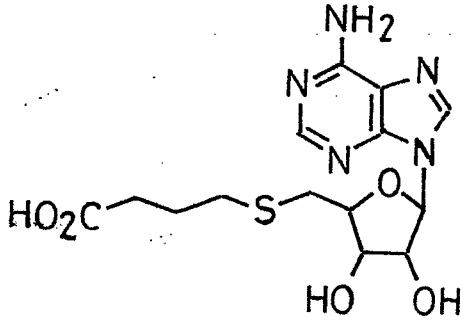
Fig. 8



JA70



JA71



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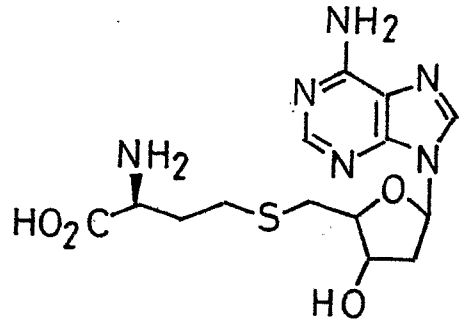

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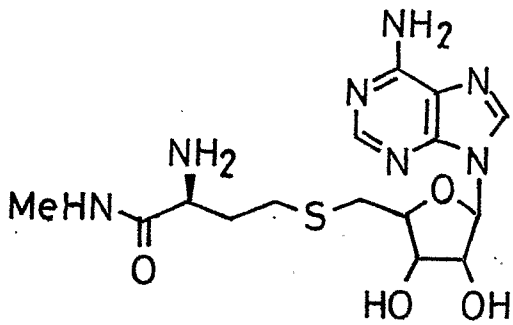

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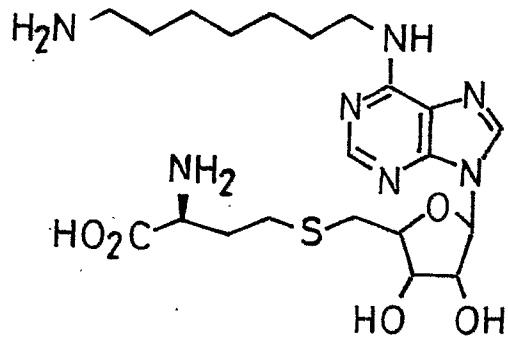
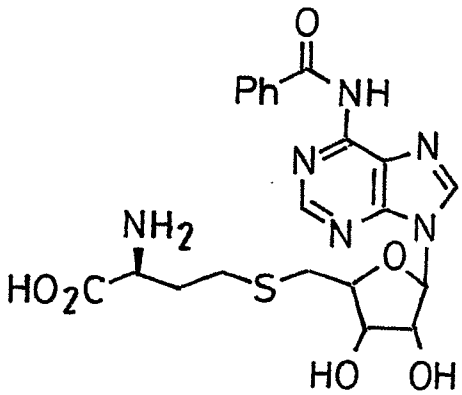
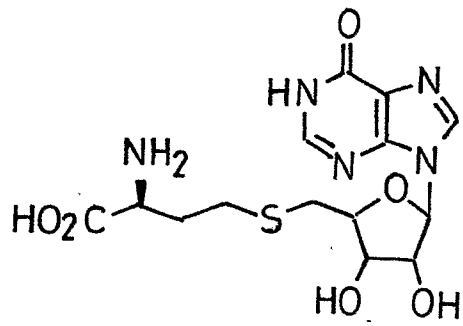


JA75

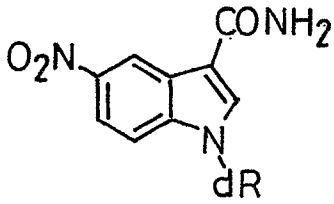


JA76

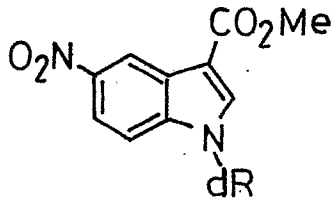


JA77

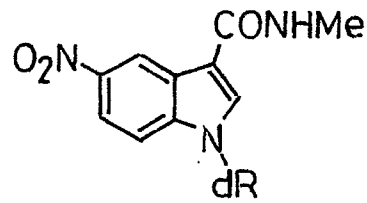
Fig. 9



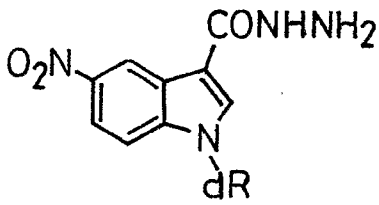
EX 13.1



EX13.2



EX13.3



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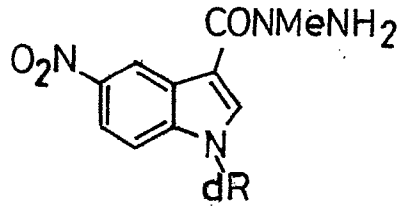
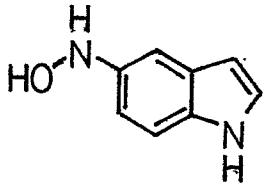


EX13.5



EX13.6

Fig. 10