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(54) Title: USE OF COMPOUNDS TO ENHANCE PROCESSIVITY OF TELOMERASE

(57) Abstract: The invention provides the use of acyclic nucleoside phosphonates and their derivatives for the preparation of a medicament for enhancing the processivity of a telomerase.

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USE OF COMPOUNDS TO ENHANCE PROCESSIVITY OF TELOMERASE

Background of the Invention

Human telomerase is a large cellular ribonucleoprotein complex (Morin GB, *Cell*, 1989, *59*, 521-9; Wenz C, et al., EMBO J 2001;20:3526-34; and Collins K, Mitchell JR., *Oncogene*, 2002, *21*, 564-79) that is responsible for adding the telomeric repeats onto the 3' ends of chromosomes (Greider CW, Blackburn EH., *Cell*, 1985, *43*, 405-13) and thus compensating for the telomere loss that accompanies chromosomal replication and cell division. Telomerase is upregulated in almost 90% of all malignancies (Kim NW, et al., *Science*, 1994, *266*, 2011-5; Shay JW, Bacchetti S, *Eur J Cancer*, 1997, *33*, 787-91; and Hiyama E, Hiyama K, *Oncogene*, 2002, *21*, 643-9). Hence, it is thought to be very promising not only as a tumor-specific marker but also as a target for anticancer treatment (Lichtsteiner SP, et al., *Ann N Y Acad Sci*, 1999, *886*, 1-11).

The identification of the hTERT component of telomerase as a functional catalytic reverse transcriptase prompted studies of inhibiting telomerase with established HIV reverse transcriptase inhibitors, such as the chain terminating 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxycytidine (ddC) (Nakamura TM, et al., *Science*, 1997, *277*, 955-9; Blackburn EH, *Annu Rev Biochem*, 1992, *61*, 113-29; and Cech TR, et al., *Biochemistry* (Moscow), 1997, *62*, 1202-5).

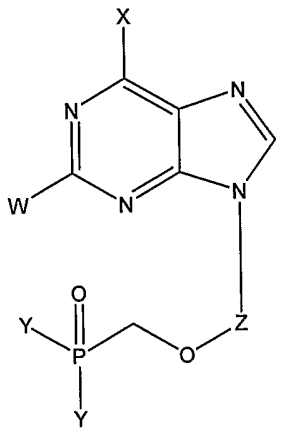
These parent nucleosides are transformed *in vivo* in the form of triphosphates, which inhibit reverse transcriptases by acting as competitive substrates for the enzyme and terminate DNA synthesis *de novo*. AZT-TP inhibits the activity of telomerase to ~50% at 30 μmol/L. Other nucleotide-based inhibitors, both purine and pyrimidine derivatives, have been evaluated against telomerase with effective IC_{50} values in the micro-molar range. In a more recent study, Fletcher reported the use of deazadeoxypurines as inhibitors of telomerase with one compound, 6-thio-7-deaza-2'-deoxyguanosine 5'-triphosphate showing an IC_{50} value of 60 nmol/L. Series of the other nucleotide analogues (ddGTP, ddATP, ddTTP, d4TTP, deazadGTP, deazadATP, thiodGTP, CBV-TP, araGTP, and FaraTTP) have previously been shown to inhibit telomerase activity (see Morin GB, *Cell*, 1989, *59*, 521-9; Strahl C, Blackburn EH., *Mol Cell Biol*, 1996, *16*, 53-65; Pai RB, et al., *Cancer Res*, 1998, *58*, 1909-13; Fletcher TM, et al., *Bioorg Chem*, 2001, *29*, 36-55; Tendian SW, Parker WB., *Mol Pharmacol*,

2000, *57*, 695-9; Strahl C, Blackburn EH., *Nucleic Acids Res*, 1994, *22*, 893-900; Fletcher TM, et al.,*Biochemistry*, 1996, *35*, 15611-7; and Raymond E, et al.,*Curr Opin Biotechnol*, 1996, *7*, 583-91).

Numerous ANPs possess excellent antiviral activities against a broad spectrum of DNA viruses and retroviruses as well as a significant antiproliferative potency. (*S*)-HPMPC (cidofovir, CDV, Vistide®), (*R*)-PMPA (tenofovir, TDV, Viread®), PMEA (adefovir, ADV, Hepsera®) were approved for treatment of cytomegalovirus retinis in AIDS patients, HIV infection, and chronic hepatitis B, respectively. In cells, ANPs are activated by conversion to their diphosphates, active antimetabolites, which inhibit viral replicases and terminate nascent DNA chain (Holý A., *Curr Pharm Des*, **2003**, *9*, 2567-92).

Summary of the Invention

The invention provides the use of an acyclic nucleoside purine of formula I:



(I)

wherein;

X is OH or $-N(R^1)_2$ wherein

R^1 independently is,

H;

C_2-C_{15} alkyl, C_3-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_3-C_{15} alkynyl, C_7-C_{15} arylalkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroalkyl or C_3-C_6 heterocycloalkyl wherein methylene in an alkyl moiety not adjacent to NH has been replaced by--O--; or

C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_6-C_{15} arylalkynyl, C_2-C_{15} alkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroaralkyl, C_4-C_6 aryl, C_3 –C_8cycloalkyl, or C_2-C_6 heterocycloalkyl;

or optionally both R^1 are joined together to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom;

wherein one of the foregoing R^1 groups can be substituted with halo, -CN or $-N_3$;

W is H or NH_2;

Y independently is, OH, $-OR^2$, $-OCH(R^3)OC(O)R^2$, a monophosphate, a diphosphate, an amino acid amidate, a polypeptide amidate, $-NHR^2$, or $-N(R^2)_2$;

R^2 independently is unsubstituted alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl; alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl wherein H is substituted by halo, carboxy, hydroxyl, cyano, nitro, N-morpholino, or amino; or alkyl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl in which a $-CH_2-$ moiety has been substituted by NH, S, or O;

Z is C_1-C_6 alkyl, optionally substituted with one or more hydroxy; and

R^3 is H or R^2;

or a prodrug, diphosphate, pharmaceutically acceptable salt, or other phosphorus-substituted derivative thereof, in the manufacture of a medicament for enhancing the processivity of a telomerase.

The invention also provides a pharmaceutical composition comprising a compound of formula I, or a prodrug or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

The invention also provides a method of increasing the number of mitotic cycles of cells continually growing in tissue cultures comprising contacting the cells with a compound of formula I, or a prodrug or a salt thereof.

The invention also provides a method of increasing mitotic cell division of non-dividing cells comprising contacting the cells *in vitro* or *in vivo* with a compound of formula I, or a prodrug or salt thereof.

The invention also provides the use of a compound of formula I, or a prodrug or pharmaceutically acceptable salt thereof to prepare a medicament for increasing the number of mitotic cycles of cells continually growing.

The invention also provides the use of a compound of formula I, or a prodrug or pharmaceutically acceptable salt thereof to prepare a medicament for increasing mitotic cell division of non-dividing cells.

The invention also provides novel compounds of formula I described herein as well as prodrugs and pharmaceutically acceptable salts thereof.

Brief Description of the Figures

Figure 1 Illustrates the structure of PME, PMEO, PMP, and HPMP purines and pyrimidines.

Figure 2 Illustrates the inhibition of HL-60 telomerase by PMEGpp as measured in Example 1.

Figure 3 Illustrates the eenhancement of telomerase processivity by 6-Me_2PMEDAPpp (A) and (*S*)-PMPApp measured in Example 1.

Figure 4 (A) Illustrates inhibition of HL-60 telomerase by guanine derivatives PMEGpp (1), (*R*)-PMPGpp (2), (*R*)-HPMPGpp (3), (*S*)-PMPGpp (4) and (B) illustrates inhibition by adenine and 2,6-diaminopurine derivatives PMEDAPpp (5), (*S*)-HPMPApp (6), PMEO-DAPypp (7) and (*R*)-6-cyprPMPDAPpp (8). The enzyme activity was measured in HL-60 cell extract in the presence of 125 μmol/L dNTPs.

Figure 5 Illustrates telomere length changes in cell line CCRF-CEM following treatment of 10 μM and 20 μM (*S*)-PMPA (see Example 2).

Detailed Description

The following abbreviations are used herein: ANP, acyclic nucleoside phosphonate; ANPpp, ANP diphosphate; TRAP, telomeric repeat amplification protocol; PMEG, 9-[2-(phosphonomethoxy)ethyl]guanine; (*R*)-PMPG, (*R*)-9-[2-(phosphonomethoxy)propyl]guanine; (*R*)-HPMPG, (*R*)-9-[(3-hydroxy-2-phosphonomethoxy)propyl]guanine; PMEDAP, 2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purine; (*S*)-PMPG, (*S*)-9-[2-(phosphonomethoxy)-propyl]guanine; (*S*)-HPMPA, (*S*)-9-[(3-hydroxy-2-phosphonomethoxy)-propyl]adenine; PMEO-DAPy, 2,4-diamino-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine; (*R*)-6-cyprPMPDAP, (*R*)-2-amino-6-(cyclopropylamino)-9-[2-(phosphonomethoxy)propyl]purine; (*R*)-PMPA, (*R*)-9-[2-(phosphonomethoxy)-propyl]adenine; (*R*)-PMPDAP, (*R*)-2,6-diamino-9-[2-(phosphonomethoxy)propyl]-purine; PMEA, 9-[2-(phosphonomethoxy)ethyl]adenine; PMEC, 1-[2-(phosphonomethoxy)ethyl]cytosine; PMET, 1-[2-(phosphonomethoxy)ethyl]thymine; (*S*)-PMPA, (*S*)-9-[2-(phosphonomethoxy)propyl]adenine; 6-Me_2PMEDAP, 2-amino-6-

(dimethylamino)-9-[2-(phosphonomethoxy)ethyl]purine; hTERT, human telomerase reverse transcriptase; AZT-TP, 3'-azido-3'-deoxythymidine 5'-triphosphate; ddC, 2',3'-dideoxycytidine; d4TTP, 2',3'-didehydro-2',3'-deoxythymidine 5'-triphosphate; deazadGTP, 7-deaza-2'-deoxyguanosine 5'-triphosphate; deazadATP, 7-deaza-2'-deoxyadenosine 5'-triphosphate; thiodGTP, 6-thio-2'-deoxyguanosine 5'-triphosphate; CBV-TP, 2',3'-didehydro-2',3'-dideoxyguanosine 5'-triphosphate; araGTP, 9-β-D-arabinofuranosylguanine 5'-triphosphate; FaraTTP, 2'-fluoro-2'-deoxy-β-arabinofuranosylthymine 5'-triphosphate; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; and TS, telomerase substrate (nontelomeric oligonucleotide).

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), photolysis, and/or metabolic chemical reaction(s). A prodrug is thus a covalently modified analog or latent form of a therapeutically-active compound.

"Prodrug moiety" refers to a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in A Textbook of Drug Design and Development (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Enzymes which are capable of an enzymatic activation mechanism with the phosphonate compounds include, but are not limited to, amidases, esterases, microbial enzymes, phospholipases, cholinesterases, and phosphases. Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A prodrug moiety may include an active metabolite or drug itself.

Exemplary prodrug moieties include the hydrolytically sensitive or labile acyloxymethyl esters $-CH_2OC(=O)R^9$ and acyloxymethyl carbonates $-CH_2OC(=O)OR^9$ where R^9 is C_1–C_6 alkyl, C_1–C_6 substituted alkyl, C_6–C_{20} aryl or C_6–C_{20} substituted aryl. The acyloxyalkyl ester was first used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al.

(1983) *J. Pharm. Sci.* 72: 324; also US Patent Nos. 4816570, 4968788, 5663159 and 5792756. Subsequently, the acyloxyalkyl ester was used to deliver phosphonic acids across cell membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester, the alkoxycarbonyloxyalkyl ester (carbonate), may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. An exemplary acyloxymethyl ester is pivaloyloxymethoxy, (POM) $-CH_2OC(=O)C(CH_3)_3$. An exemplary acyloxymethyl carbonate prodrug moiety is pivaloyloxymethylcarbonate (POC) $-CH_2OC(=O)OC(CH_3)_3$.

Aryl esters of phosphorus groups, especially phenyl esters, are reported to enhance oral bioavailability (De Lombaert et al. (1994) *J. Med. Chem.* 37: 498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are reported to generate the parent phosphonic acid. In some cases, substituents at the *ortho*-or *para*-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g., esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C–O bond to generate the phosphoric acid and the quinone methide intermediate. Examples of this class of prodrugs are described by Mitchell et al. (1992) *J. Chem. Soc. Perkin Trans. II* 2345; Glazier WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterification or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al. (1993) *Antiviral Res.*, 22: 155-174; Benzaria et al. (1996) *J. Med. Chem.* 39: 4958). Cyclic phosphonate esters have also been described as prodrugs of phosphorus-containing compounds (Erion et al., US Patent No. 6312662).

Examples of pharmaceutically acceptable salts include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1–C_4 alkyl). Pharmaceutically acceptable salts of a compound having an amino group include salts

of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids.

Pharmaceutically acceptable salts of a compound having a hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1–C_4 alkyl group). For therapeutic use, salts of active ingredients will typically be pharmaceutically acceptable, i.e. they will be salts derived from a pharmaceutically acceptable acid or base.

"Alkyl" is branched or unbranched hydrocarbon containing normal, secondary, or tertiary carbon atoms. Examples are methyl (Me,-CH_3), ethyl (Et,-CH_2CH_3), 1-propyl (n-Pr, n-propyl,-$CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl,-$CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl,-$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl,-$CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl,-$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl,-$C(CH_3)_3$), 1-pentyl (n-pentyl,-$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (-$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (-$CH(CH_2CH_3)_2$), 2-methyl-2-butyl-$C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl (-$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (-$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (-$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (-$CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl (-$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (-$CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl (-$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (-$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (-$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (-$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (-$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (-$C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl (-$CH(CH_3)C(CH_3)_3$.

"Alkenyl" is branched or unbranched hydrocarbon containing normal, secondary, or tertiary carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to, ethylene or vinyl (-$CH=CH_2$), allyl (-$CH_2CH=CH_2$), cyclopentenyl (-C_5H_7), 5-hexenyl (-$CH_2CH_2CH_2CH_2CH=CH_2$), and 2,5-hexadienyl (-$CH_2CH=CHCH_2CH=CH_2$).

"Alkynyl" is a branched or unbranched hydrocarbon containing normal, secondary, or tertiary carbon atoms with at least one site of unsaturation, i.e. a carbon-

carbon, *sp* triple bond. Examples include, but are not limited to, acetylenic (-C≡CH), propargyl (-CH_2C≡CH), and 2,5-hexadiynyl (-CH_2C≡CH CH_2C≡CH)

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Aralkyl" refers to an alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, , naphthylmethyl, 2-naphthylethan-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

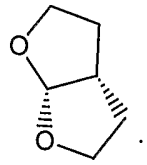
"Arylalkenyl" refers to an alkenyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical.

"Arylalkynyl" refers to an alkynyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical.

"Heterocycle" or "Heterocyclo" as used herein includes by way of example and not limitation these heterocycles described in Paquette, Leo A.; Principles of Modern Heterocyclic Chemistry (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; The Chemistry of Heterocyclic Compounds, A Series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566. In one specific embodiment of the invention "heterocycle" includes a "carbocycle" as defined herein, wherein one or more (e.g. 1, 2, 3, or 4) carbon atoms have been replaced with a heteroatom (e.g. O, N, or S).

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-

piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazoly, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, isatinoyl, and bis-tetrahydrofuranyl:



The term "treatment" or "treating," to the extent it relates to a disease or condition includes preventing the disease or condition from occurring, inhibiting the disease or condition, eliminating the disease or condition, and/or relieving one or more symptoms of the disease or condition.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or 1 meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which

may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

Protecting groups are available, commonly known and used, and are optionally used to prevent side reactions with the protected group during synthetic procedures, i.e. routes or methods to prepare the compounds. For the most part the decision as to which groups to protect, when to do so, and the nature of the chemical protecting group "PG" will be dependent upon the chemistry of the reaction to be protected against (e.g., acidic, basic, oxidative, reductive or other conditions) and the intended direction of the synthesis. The PG groups do not need to be, and generally are not, the same if the compound is substituted with multiple PG. In general, PG will be used to protect functional groups such as carboxyl, hydroxyl, thio, or amino groups and to thus prevent side reactions or to otherwise facilitate the synthetic efficiency. The order of deprotection to yield free, deprotected groups is dependent upon the intended direction of the synthesis and the reaction conditions to be encountered, and may occur in any order as determined by the artisan. A very large number of protecting groups and corresponding chemical cleavage reactions are described in Protective Groups in Organic Synthesis, Theodora W. Greene (John Wiley & Sons, Inc., New York, 1991, ISBN 0-471-62301-6) ("Greene"). See also Kocienski, Philip J.; Protecting Groups (Georg Thieme Verlag Stuttgart, New York, 1994).

Examples of suitable amino acids whose residues can be represented by Y in Formula I include the following: Glycine; Aminopolycarboxylic acids, e.g., aspartic acid, β-hydroxyaspartic acid, glutamic acid, β-hydroxyglutamic acid, β-methylaspartic acid, β-methylglutamic acid, β, β-dimethylaspartic acid, γ-hydroxyglutamic acid, β, γ-dihydroxyglutamic acid, β-phenylglutamic acid, γ-methyleneglutamic acid, 3-aminoadipic acid, 2-aminopimelic acid, 2-aminosuberic acid and 2-aminosebacic acid; Amino acid amides such as glutamine and asparagine; Polyamino- or polybasic-monocarboxylic acids such as arginine, lysine, β-aminoalanine, γ-aminobutyrine, ornithine, citruline, homoarginine, homocitrulline, hydroxylysine, allohydroxylsine and diaminobutyric acid; Other basic amino acid residues such as histidine; Diaminodicarboxylic acids such as α, α'-diaminosuccinic acid, α, α'-diaminoglutaric acid, α, α'-diaminoadipic acid, α, α'-diaminopimelic acid, α, α'-diamino-β-hydroxypimelic acid, α, α'-diaminosuberic acid, α, α'-diaminoazelaic acid, and α, α'-diaminosebacic acid; Imino acids such as proline, hydroxyproline, allohydroxyproline,

γ-methylproline, pipecolic acid, 5-hydroxypipecolic acid, and azetidine-2-carboxylic acid; A mono- or di-alkyl (typically C_1-C_8 branched or normal) amino acid such as alanine, valine, leucine, allylglycine, butyrine, norvaline, norleucine, heptyline, α-methylserine, α-amino-α-methyl-γ-hydroxyvaleric acid, α-amino- α-methyl-δ-hydroxyvaleric acid, α-amino- α-methyl-ε-hydroxycaproic acid, isovaline, α-methylglutamic acid, α-aminoisobutyric acid, α-aminodiethylacetic acid, α-aminodiisopropylacetic acid, α-aminodi-n-propylacetic acid, α-aminodiisobutylacetic acid, α-aminodi-n-butylacetic acid, α-aminoethylisopropylacetic acid, α-amino-n-propylacetic acid, α-aminodiisoamyacetic acid, α-methylaspartic acid, α-methylglutamic acid, 1-aminocyclopropane-1-carboxylic acid, isoleucine, alloisoleucine, *tert*-leucine, β-methyltryptophan and α-amino- β-ethyl-β-phenylpropionic acid; β-phenylserinyl;

Aliphatic α-amino-β-hydroxy acids such as serine, β-hydroxyleucine, β-hydroxynorleucine, β-hydroxynorvaline, and α-amino-β-hydroxystearic acid; α-Amino, α-, γ-, δ- or ε-hydroxy acids such as homoserine, δ-hydroxynorvaline, γ-hydroxynorvaline and ε-hydroxynorleucine residues; canavine and canaline; γ-hydroxyornithine; 2-hexosaminic acids such as D-glucosaminic acid or D-galactosaminic acid; α-Amino-β-thiols such as penicillamine, β-thiolnorvaline or β-thiolbutyrine; Other sulfur containing amino acid residues including cysteine; homocystine, β-phenylmethionine, methionine, S-allyl-L-cysteine sulfoxide, 2-thiolhistidine, cystathionine, and thiol ethers of cysteine or homocysteine; Phenylalanine, tryptophan and ring-substituted α-amino acids such as the phenyl or cyclohexylamino acids α-aminophenylacetic acid, α-aminocyclohexylacetic acid and α-amino-β-cyclohexylpropionic acid; phenylalanine analogues and derivatives comprising aryl, lower alkyl, hydroxy, guanidino, oxyalkylether, nitro, sulfur or halo-substituted phenyl (e.g., tyrosine, methyltyrosine and o-chloro-, p-chloro-, 3,4-dichloro, *o*-, *m*- or *p*-methyl-, 2,4,6-trimethyl-, 2-ethoxy-5-nitro-, 2-hydroxy-5-nitro- and p-nitro-phenylalanine); furyl-, thienyl-, pyridyl-, pyrimidinyl-, purinyl- or naphthyl-alanines; and tryptophan analogues and derivatives including kynurenine, 3-hydroxykynurenine, 2-hydroxytryptophan and 4-carboxytryptophan; α-Amino substituted amino acids including sarcosine (N-methylglycine), N-benzylglycine, N-methylalanine, N-benzylalanine, N-methylphenylalanine, N-benzylphenylalanine, N-

methylvaline and N-benzylvaline; and α-Hydroxy and substituted α-hydroxy amino acids including serine, threonine, allothreonine, phosphoserine and phosphothreonine.

Polypeptides are polymers of amino acids in which a carboxyl group of one amino acid monomer is bonded to an amino or imino group of the next amino acid monomer by an amide bond. Polypeptides include dipeptides, low molecular weight polypeptides (about 1500-5000 MW) and proteins. Proteins optionally contain 3, 5, 10, 50, 75, 100 or more residues, and suitably are substantially sequence-homologous with human, animal, plant or microbial proteins. They include enzymes (e.g., hydrogen peroxidase) as well as immunogens such as KLH, or antibodies or proteins of any type against which one wishes to raise an immune response. The nature and identity of the polypeptide may vary widely.

Peptidolytic enzymes for cleaving polypeptide conjugates are well known, and in particular include carboxypeptidases. Carboxypeptidases digest polypeptides by removing C-terminal residues, and are specific in many instances for particular C-terminal sequences. Such enzymes and their substrate requirements in general are well known. For example, a dipeptide (having a given pair of residues and a free carboxyl terminus) is covalently bonded through its α-amino group to the phosphorus or carbon atoms of the compounds herein. This peptide can be cleaved by the appropriate peptidolytic enzyme, leaving the carboxyl of the proximal amino acid residue to autocatalytically cleave the phosphonoamidate bond.

Suitable dipeptidyl groups (designated by their single letter code) include AA, AR, AN, AD, AC, AE, AQ, AG, AH, AI, AL, AK, AM, AF, AP, AS, AT, AW, AY, AV, RA, RR, RN, RD, RC, RE, RQ, RG, RH, RI, RL, RK, RM, RF, RP, RS, RT, RW, RY, RV, NA, NR, NN, ND, NC, NE, NQ, NG, NH, NI, NL, NK, NM, NF, NP, NS, NT, NW, NY, NV, DA, DR, DN, DD, DC, DE, DQ, DG, DH, DI, DL, DK, DM, DF, DP, DS, DT, DW, DY, DV, CA, CR, CN, CD, CC, CE, CQ, CG, CH, CI, CL, CK, CM, CF, CP, CS, CT, CW, CY, CV, EA, ER, EN, ED, EC, EE, EQ, EG, EH, EI, EL, EK, EM, EF, EP, ES, ET, EW, EY, EV, QA, QR, QN, QD, QC, QE, QQ, QG, QH, QI, QL, QK, QM, QF, QP, QS, QT, QW, QY, QV, GA, GR, GN, GD, GC, GE, GQ, GG, GH, GI, GL, GK, GM, GF, GP, GS, GT, GW, GY, GV, HA, HR, HN, HD, HC, HE, HQ, HG, HH, HI, HL, HK, HM, HF, HP, HS, HT, HW, HY, HV, IA, IR, IN, ID, IC, IE, IQ, IG, IH, II, IL, IK, IM, IF, IP, IS, IT, IW, IY, IV, LA, LR, LN, LD, LC, LE, LQ, LG, LH, LI, LL, LK, LM, LF, LP, LS, LT, LW, LY, LV, KA, KR, KN, KD, KC, KE, KQ, KG, KH, KI, KL, KK, KM, KF, KP, KS, KT, KW, KY, KV, MA, MR, MN, MD,

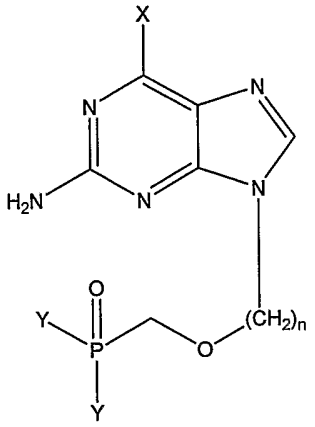
MC, ME, MQ, MG, MH, MI, ML, MK, MM, MF, MP, MS, MT, MW, MY, MV, FA, FR, FN, FD, FC, FE, FQ, FG, FH, FI, FL, FK, FM, FF, FP, FS, FT, FW, FY, FV, PA, PR, PN, PD, PC, PE, PQ, PG, PH, PI, PL, PK, PM, PF, PP, PS, PT, PW, PY, PV, SA, SR, SN, SD, SC, SE, SQ, SG, SH, SI, SL, SK, SM, SF, SP, SS, ST, SW, SY, SV, TA, TR, TN, TD, TC, TE, TQ, TG, TH, TI, TL, TK, TM, TF, TP, TS, TT, TW, TY, TV, WA, WR, WN, WD, WC, WE, WQ, WG, WH, WI, WL, WK, WM, WF, WP, WS, WT, WW, WY, WV, YA, YR, YN, YD, YC, YE, YQ, YG, YH, YI, YL, YK, YM, YF, YP, YS, YT, YW, YY, YV, VA, VR, VN, VD, VC, VE, VQ, VG, VH, VI, VL, VK, VM, VF, VP, VS, VT, VW, VY and VV. Tripeptide residues are also useful as protecting groups.

Dipeptide or tripeptide species can be selected on the basis of known transport properties and/or susceptibility to peptidases that can affect transport to intestinal mucosal or other cell types. Dipeptides and tripeptides lacking an α-amino group are transport substrates for the peptide transporter found in brush border membrane of intestinal mucosal cells (Bai, J.P.F., (1992) *Pharm Res.* 9:969-978). Transport competent peptides can thus be used to enhance bioavailability of the compounds. Di- or tripeptides having one or more amino acids in the D configuration are also compatible with peptide transport and can be utilized in the compounds. Amino acids in the D configuration can be used to reduce the susceptibility of a di- or tripeptide to hydrolysis by proteases common to the brush border such as aminopeptidase N. In addition, di- or tripeptides alternatively are selected on the basis of their relative resistance to hydrolysis by proteases found in the lumen of the intestine. For example, tripeptides or polypeptides lacking asp and/or glu are poor substrates for aminopeptidase A, di- or tripeptides lacking amino acid residues on the N-terminal side of hydrophobic amino acids (leu, tyr, phe, val, trp) are poor substrates for endopeptidase, and peptides lacking a pro residue at the penultimate position at a free carboxyl terminus are poor substrates for carboxypeptidase P. Similar considerations can also be applied to the selection of peptides that are either relatively resistant or relatively susceptible to hydrolysis by cytosolic, renal, hepatic, serum or other peptidases. Such poorly cleaved polypeptide amidates are immunogens or are useful for bonding to proteins in order to prepare immunogens.

Specific Embodiments of the Invention

Specific values described for radicals, substituents, and ranges, as well as specific embodiments of the invention described herein, are for illustration only; they do not exclude other defined values or other values within defined ranges.

A specific acyclic nucleoside purine is a compound of formula 1:



wherein;

n is 2 or 3;

X is-$N(R^1)_2$ wherein

R^1 independently is H;

C_2-C_{15} alkyl, C_3-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_3-C_{15} alkynyl, C_7-C_{15} arylalkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroalkyl or C_3-C_6 heterocycloalkyl wherein methylene in an alkyl moiety not adjacent to NH has been replaced by--O--; or

C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_6-C_{15} arylalkynyl, C_2-C_{15} alkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroaralkyl, C_4-C_6 aryl, C_2-C_6 heterocycloalkyl;

or optionally both R^1 are joined together to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom;

wherein one of the foregoing R^1 groups can be substituted with halo,-CN or-N_3, but either one or two R^1 groups are not H;

Y independently is, OH,-OR^2,-$OCH(R^3)OC(O)R^2$, a monophosphate, a diphosphate, an amino acid amidate, a polypeptide amidate,-NHR^2, or-$N(R^2)_2$;

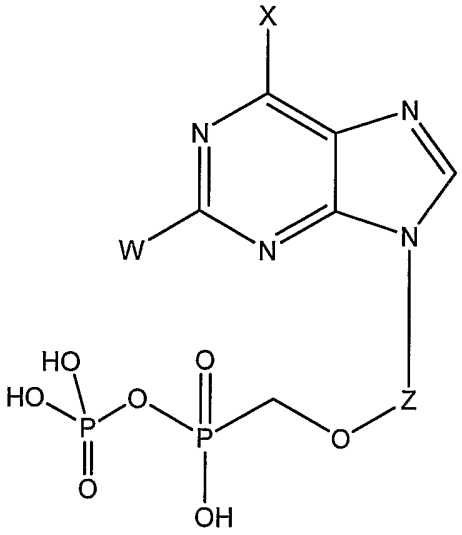
R^2 independently is unsubstituted alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl; alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or

alkenylaryl wherein H is substituted by halo, carboxy, hydroxyl, cyano, nitro, N-morpholino, or amino; or alkyl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl in which a-CH_2- moiety has been substituted by NH, S, or O;

R^3 is H or R^2;

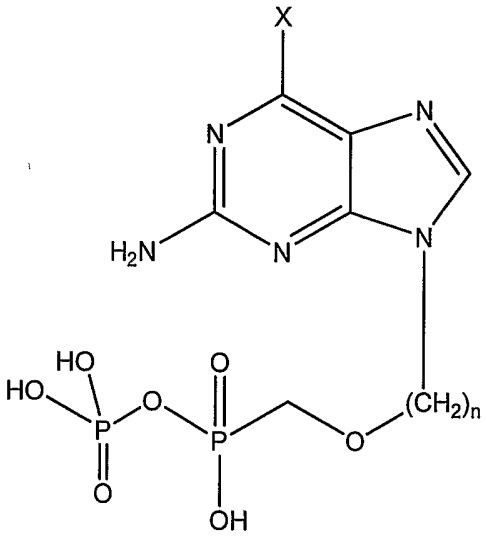
or a prodrug, diphosphate, or other phosphorus-substituted derivative thereof.

Another specific acyclic nucleoside purine of formula I is a compound of the following formula:



or a prodrug or pharmaceutically acceptable salt thereof.

Another specific acyclic nucleoside purine is a compound of the following formula:



or a prodrug thereof.

A specific value for each R^1 independently is H, C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_2-C_{15} alkynyl, or C_3–C_8cycloalkyl; or optionally both R^1 are joined together to form a

saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom.

A specific value for each R^1 independently is H, methyl, or cyclopropyl.

A specific value for each R^1 independently is H.

A specific value for each R^1 independently is methyl.

A specific value for one R^1 is H and the other R^1 is cyclopropyl.

A specific value for W is H.

A specific value for W is NH_2.

A specific value for X is OH.

A specific value for Z is $-CH_2-CH_2-$, $-CH_2-CH(CH_3)-$, or-$CH_2-CH(CH_2OH)-$.

A specific value for n is 2.

A specific value for n is 3.

A specific value for Z is an *S*-enantiomer of $-CH_2-CH(CH_3)-$, or -$CH_2-CH(CH_2OH)-$.

A specific compound of formula I is 6-Me_2PMEDAPpp or a pharmaceutically acceptable salt or a prodrug thereof.

A specific compound of formula I is (S)-PMPApp or a pharmaceutically acceptable salt or prodrug thereof.

A specific compound of formula I is (S)-PMPA or a pharmaceutically acceptable salt or prodrug thereof.

A specific compound of formula I is 6-Me_2PMEDAPpp or a prodrug thereof.

A specific compound of formula I is (S)-PMPApp or a prodrug thereof.

A specific compound of formula I is (S)-PMPA or a prodrug thereof.

The compounds may have chiral centers, e.g., chiral carbon or phosphorus atoms. The compounds thus include racemic mixtures of all stereoisomers, including enantiomers, diastereomers, and atropisomers. In addition, the compounds include enriched or resolved optical isomers at any or all asymmetric, chiral atoms. In other words, the chiral centers apparent from the depictions are provided as the chiral isomers or racemic mixtures. Both racemic and diastereomeric mixtures, as well as the individual optical isomers isolated or synthesized, substantially free of their enantiomeric or diastereomeric partners, are all within the scope of the invention. The racemic mixtures are separated into their individual, substantially optically pure isomers through well-known techniques such as, for example, the separation of diastereomeric salts formed with optically active adjuncts, e.g., acids or bases followed

by conversion back to the optically active substances. In most instances, the desired optical isomer is synthesized by means of stereospecific reactions, beginning with the appropriate stereoisomer of the desired starting material.

The compounds can also exist as tautomeric isomers in certain cases. All though only one delocalized resonance structure may be depicted, all such forms are contemplated within the scope of the invention. For example, ene-amine tautomers can exist for purine, pyrimidine, imidazole, guanidine, amidine, and tetrazole systems and all their possible tautomeric forms are within the scope of the invention.

The invention also provides salts of compounds of Formula (I), especially pharmaceutically acceptable non-toxic salts containing, for example, Na^+, Li^+, K^+, Ca^{+2} and Mg^{+2}. Such salts may include those derived by combination of appropriate cations such as alkali and alkaline earth metal ions or ammonium and quaternary amino ions with an acid anion moiety, typically a carboxylic acid. Monovalent salts are preferred if a water soluble salt is desired.

Metal salts typically are prepared by reacting the metal hydroxide with a compound of this invention. Examples of metal salts which are prepared in this way are salts containing Li^+, Na^+, and K^+. A less soluble metal salt can be precipitated from the solution of a more soluble salt by addition of the suitable metal compound.

In addition, salts may be formed from acid addition of certain organic and inorganic acids, e.g., HCl, HBr, H_2SO_4, H_3PO_4 or organic sulfonic acids, to basic centers, typically amines, or to acidic groups. Finally, it is to be understood that the compositions herein comprise compounds in their un-ionized, as well as zwitterionic form, and combinations with stoichiometric amounts of water as in hydrates.

Also included within the scope of this invention are the salts formed with one or more amino acids. Any of the amino acids described above are suitable, especially the naturally-occurring amino acids found as protein components, although the amino acid typically is one bearing a side chain with a basic or acidic group, e.g., lysine, arginine or glutamic acid, or a neutral group such as glycine, serine, threonine, alanine, isoleucine, or leucine.

The compounds of this invention can be formulated with conventional carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, glidants, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral

administration generally will be isotonic. All formulations will optionally contain excipients such as those set forth in the Handbook of Pharmaceutical Excipients (1986). Excipients include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like. The pH of the formulations ranges from about 3 to about 11, but is ordinarily about 7 to 10.

While it is possible for the active ingredients to be administered alone it may be preferable to present them as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and physiologically innocuous to the recipient thereof.

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

A tablet can be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid

diluent. The tablets may optionally be coated or scored and optionally are formulated so as to provide slow or controlled release of the active ingredient therefrom.

For administration to the eye or other external tissues e.g., mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc.), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the invention include Tween® 60, Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. The cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-

isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils are used.

Pharmaceutical formulations according to the present invention comprise one or more compounds together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients

include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such

as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur.

Formulations suitable for administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or

tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for intrapulmonary or nasal administration have a particle size for example in the range of 0.1 to 500 microns (including particle sizes in a range between 0.1 and 500 microns in increments microns such as 0.5, 1, 30 microns, 35 microns, etc.), which is administered by rapid inhalation through the nasal passage or by inhalation through the mouth so as to reach the alveolar sacs. Suitable formulations include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol or dry powder administration may be prepared according to conventional methods and may be delivered with other therapeutic agents such as compounds heretofore used in the treatment of inflammation as described below.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

The formulations are presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally or by any other desired route.

Compounds can also be formulated to provide controlled release of the active ingredient to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of the active ingredient. Accordingly, the invention also provided compositions comprising one or more compounds formulated for sustained or controlled release.

Effective dose of active ingredient depends at least on the nature of the condition being treated, toxicity, whether the compound is being used prophylactically (lower doses) or against existing inflammation, the method of delivery, and the pharmaceutical formulation, and will be determined by the clinician using conventional dose escalation studies. It can be expected to be from about 0.0001 to about 100 mg/kg body weight per day. Typically, from about 0.01 to about 10 mg/kg body weight per day. More typically, from about .01 to about 5 mg/kg body weight per day. More typically, from about .05 to about 0.5 mg/kg body weight per day. For example, the daily candidate dose for an adult human of approximately 70 kg body weight will range from 1 mg to 1000 mg, preferably between 5 mg and 500 mg, and may take the form of single or multiple doses.

One or more compounds (herein referred to as the active ingredients) can be administered by any route appropriate to the condition to be treated. Suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural), and the like. It will be appreciated that the preferred route may vary with for example the condition of the recipient. An advantage of the compounds of this invention is that they are orally bioavailable and can be dosed orally.

Active ingredients of the invention can also be used in combination with other active ingredients. Such combinations are typically selected based on the condition to be treated, cross-reactivities of ingredients and pharmaco-properties of the combination.

It is also possible to combine any compound with one or more other active ingredients in a unitary dosage form for simultaneous or sequential administration to a patient. The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations.

The combination therapy may provide "synergy" or a "synergistic effect", i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g., in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Also falling within the scope of this invention are the *in vivo* metabolic products of the compounds described herein. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the invention includes compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radiolabelled (e.g., C^{14} or H^3) compound, administering it parenterally in a detectable dose (e.g., greater than about 0.5 mg/kg) to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours) and isolating its conversion products from the urine, blood or other biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, e.g., by MS or NMR analysis. In general, analysis of metabolites is done in the same way as conventional drug metabolism studies well-known to those skilled in the art.

The conversion products, so long as they are not otherwise found *in vivo*, are useful in diagnostic assays for therapeutic dosing of the compounds even if they possess no anti-inflammatory activity of their own.

The invention also relates to methods of making the novel compounds herein. The compounds are prepared by any of the applicable techniques of organic synthesis. Many such techniques are well known in the art. However, many of the known techniques are elaborated in Compendium of Organic Synthetic Methods (John Wiley & Sons, New York), Vol. 1, Ian T. Harrison and Shuyen Harrison, 1971; Vol. 2, Ian T. Harrison and Shuyen Harrison, 1974; Vol. 3, Louis S. Hegedus and Leroy Wade, 1977; Vol. 4, Leroy G. Wade, jr., 1980; Vol. 5, Leroy G. Wade, Jr., 1984; and Vol. 6, Michael B. Smith; as well as March, J., Advanced Organic Chemistry, Third Edition, (John Wiley & Sons, New York, 1985), Comprehensive Organic Synthesis. Selectivity, Strategy & Efficiency in Modern Organic Chemistry. In 9 Volumes, Barry M. Trost, Editor-in-Chief (Pergamon Press, New York, 1993 printing).

Generally, the reaction conditions such as temperature, reaction time, solvents, work-up procedures, and the like, will be those common in the art for the particular reaction to be performed. The cited reference material, together with material cited therein, contains detailed descriptions of such conditions. Typically the temperatures will be -100°C to 200°C, solvents will be aprotic or protic, and reaction times will be 10 seconds to 10 days. Work-up typically consists of quenching any unreacted reagents followed by partition between a water/organic layer system (extraction) and separating the layer containing the product.

A single stereoisomer, e.g., an enantiomer, substantially free of its stereoisomer may be obtained by resolution of the racemic mixture using a method such as formation of diastereomers using optically active resolving agents (Stereochemistry of Carbon Compounds, (1962) by E. L. Eliel, McGraw Hill; Lochmuller, C. H., (1975) *J. Chromatogr.*, 113:(3) 283-302). Racemic mixtures of chiral compounds can be separated and isolated by any suitable method, including: (1) formation of ionic, diastereomeric salts with chiral compounds and separation by fractional crystallization or other methods, (2) formation of diastereomeric compounds with chiral derivatizing reagents, separation of the diastereomers, and conversion to the pure stereoisomers, and (3) separation of the substantially pure or enriched stereoisomers directly under chiral conditions.

Under method (1), diastereomeric salts can be formed by reaction of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, α-methyl-β-phenylethylamine (amphetamine), and the like with asymmetric compounds bearing acidic functionality, such as carboxylic acid and sulfonic acid. The diastereomeric salts may be induced to separate by fractional crystallization or ionic chromatography. For separation of the optical isomers of amino compounds, addition of chiral carboxylic or sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts.

Alternatively, by method (2), the substrate to be resolved is reacted with one enantiomer of a chiral compound to form a diastereomeric pair (Eliel, E. and Wilen, S. (1994) Stereochemistry of Organic Compounds, John Wiley & Sons, Inc., p. 322). Diastereomeric compounds can be formed by reacting asymmetric compounds with enantiomerically pure chiral derivatizing reagents, such as menthyl derivatives, followed by separation of the diastereomers and hydrolysis to yield the free, enantiomerically enriched xanthene. A method of determining optical purity involves making chiral esters, such as a menthyl ester, e.g., (-) menthyl chloroformate in the presence of base, or Mosher ester, α-methoxy-α-(trifluoromethyl)phenyl acetate (Jacob III. (1982) *J. Org. Chem.* 47:4165), of the racemic mixture, and analyzing the NMR spectrum for the presence of the two atropisomeric diastereomers. Stable diastereomers of atropisomeric compounds can be separated and isolated by normal- and reverse-phase chromatography following methods for separation of atropisomeric naphthyl-isoquinolines (Hoye, T., WO 96/15111).

By method (3), a racemic mixture of two enantiomers can be separated by chromatography using a chiral stationary phase (Chiral Liquid Chromatography (1989) W. J. Lough, Ed. Chapman and Hall, New York; Okamoto, (1990) *J. of Chromatogr.* 513:375-378). Enriched or purified enantiomers can be distinguished by methods used to distinguish other chiral molecules with asymmetric carbon atoms, such as optical rotation and circular dichroism.

The invention will now be illustrated by the following non-limiting Examples.

Example 1

Materials and methods

The following compounds were evaluated (Fig. 1): (*S*)-HPMPApp, (*R*)-HPMPGpp, PMEApp, PMECpp, PMEGpp, PMETpp, PMEDAPpp, PMEO-DAPypp, 6-Me_2PMEDAPpp, (*R*)-6-cyprPMPDAPpp, (*R*)-PMPDAPpp, (*R*)-PMPApp, (*S*)-PMPApp, (*R*)-PMPGpp, and (*S*)-PMPGpp. (*S*)-HPMPA and (*R*)-HPMPG diphosphates were synthesized according to Otmar M, Masojídková M, Votruba I, Holý A., *Collect Czech Chem Commun*, 2001, *66*, 500-6. Other ANPs diphosphates were synthesized by the modified morpholidate method. In a typical experiment, a mixture of ANP (free acid, 1 mmol), N,N'-dicyclohexylcarbodiimide (1.3 g) and morpholine (2 mL) in 80% aqueous *tert*-butanol (20 mL) was refluxed under stirring for 6-8 hours and evaporated in vacuo. The residue in water (100 mL) was filtered over Celite®, the filtrate extracted with ether (3 x 50 mL) and the aqueous phase was taken down in vacuo. The residue was transferred into 100-mL flask, evaporated, codistilled with ethanol (2 x 20 mL) and dried overnight at 15 Pa over phosphorus pentoxide. Bis(tributylammonium) monophosphate or tris(tributylammonium) diphosphate solution in dry dimethylsulfoxide (1 mol/L, 2.5 mL) was added and the mixture was stirred at ambient temperature in a tightly closed flask for 4- 6 days. Reaction mixture was then acidified with 6 M HCl to pH 3 and an appropriate amount of activated charcoal was added. After an exhaustive washing of pelleted activated charcoal with HPLC water, desalted nucleotides were eluted by 5% NH_4OH in 50% methanol. The eluate was evaporated at 30 °C, dissolved in 0.05 M triethylammonium bicarbonate and purified by chromatography on POROS® 50HQ anion exchanger (Applied Biosystems, Foster City, CA, USA) in the linear concentration gradient of triethylammonium bicarbonate (0.05 – 0.4 mol/L). Peak corresponding to ANPpp (triethylammonium salt) was collected, evaporated in vacuo at room temperature and then converted to the ANPpp sodium salt on DOWEX™ 50X8 (Na^+) (SERVA Electrophoresis GmbH, Heidelberg, Germany).

All other chemicals and materials were commercial products, e.g. activated charcoal, N,N'-dicyclohexylcarbodiimide, morpholine, *tert*-butanol, Celite®, dimethylsulfoxide, ddGTP, streptomycin, penicillin G, CHAPS, β-mercaptoethanol, RNase A, proteinase K, PBS and RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA), fetal calf serum (PAA Laboratories GmbH, Pasching, Austria), vitamin B_{12}

(Léčiva a.s., Prague, Czech Republic), Pefabloc-SC, Protector RNase inhibitor (Roche Diagnostics GmbH, Mannheim, Germany), [γ-^{32}P]ATP (MP Biomedicals GmbH, Germany), T4 Polynucleotide Kinase Buffer and T4 Polynucleotide Kinase (TaKaRa Bio, Inc., Shiga, Japan), HEPES, deoxynucleotide triphosphates (dNTPs), Taq polymerase reaction buffer, Taq DNA polymerase (Promega, Madison, WI, USA) and TS, ACX, NT, TSNT primers (Invitrogen Ltd, Paisley, United Kingdom).

Human acute promyelocytic leukaemia HL-60 cells (ATCC CCL 240) were cultured in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, antibiotics (200 μg/mL of streptomycin and 200 units/mL of penicillin G), 10 mM β-mercaptoethanol, and vitamin B_{12} at 37 °C in a humidified atmosphere containing 5% CO_2. After harvesting in log-phase growth, cells were pelleted, washed in PBS, and frozen at-70 °C.

Extracts with telomerase activity were prepared and analyzed as described by Kim NW, et al., *Science*, 1994, *266*, 2011-5, with some modifications. Briefly, the cells were thawed and incubated on ice for 30 min in CHAPS lysis buffer containing 0.5% CHAPS, 10 mM HEPES-NaOH (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 5 mM β–mercaptoethanol, 2 mM Pefabloc-SC, 10% glycerol plus 1 μL (40 U) of Protector RNase inhibitor. 200 μL of CHAPS lysis buffer was used to lyse one million cells. In order to ensure lysis of HL-60 cells, these cells were subjected to two freeze/thaw cycles during the incubation. This additional step did not affect telomerase activity in the HL-60 cells. Cell debris was pelleted for 20 min at 16 000 x g at 4 °C. The supernatant was removed, aliquoted, frozen on dry ice, and stored at –70 °C. The protein concentration of the supernatant was determined by the Bradford assay.

Telomerase activity was determined using the TRAP assay as described by Kim NW, Wu F., *Nucleic Acids Res*, 1997, *25*, 2595-7 with the modifications described below. In the present study, the sensitivity of the TRAP assay was increased by prolongation of incubation time from 10 to 15 min and increasing the number of PCR cycles from 27 to 33. The dependence of the amount of amplified telomerase product versus number of PCR cycle was linear in the range of 30 to 35 cycles (data not shown). An aliquot of 800 pmol of TS substrate primer (5'-AATCCGTCGAGCAGAGTT-3') (SEQ ID NO:1) was labeled in 100 μL reaction mixture containing 60 μCi [γ-^{32}P]ATP (60 mCi/mL, 7000 Ci/mmol), T4 Polynucleotide kinase buffer and 40 U T4 polynucleotide kinase. After incubation for

30 min at 37 °C and then 2 min at 85 °C, an excess of unincorporated [γ-^{32}P]ATP was removed from reaction mixture on MicroSpin™ G-25 Column (Amersham Biosciences, Piscataway, NJ, USA). Forty microlitre TRAP reactions contained Taq polymerase reaction buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25 °C), 1.5 mM $MgCl_2$, 0.1% Triton X®-100), dNTPs (30, 60 and 125 μmol/L), 18 pmol of end-labeled TS substrate primer, an appropriate amount of studied ANPpp, ANPp and ANP respectively. Reaction was started by cell extract addition (0.15 μg protein). Each TRAP reaction mixture was placed in a thermocycler block preheated to 30 °C and incubated at 30 °C for 15 min and then heated at 95 °C for 2 min (for one cycle) to stop telomerase reaction. After addition of 10 μL of mixture containing 6 pmol ACX reverse primer (5'-GCGCGG[CTTACC]$_3$CTAACC-3') (SEQ ID NO:2), 3 pmol NT internal control primer, 0.01 amol TSNT internal control and 1.25 U Taq DNA polymerase, the reaction was cycled 33 times at 94 °C for 20 s, 52 °C for 30 s, 72 °C for 20 s.

To ensure that the observed telomerase activities were really dependent on telomerase itself, a number of inactivation experiments were performed. Aliquot of cell lysate was incubated with RNase A (50 μg/mL) at 37 °C for 30 min. Proteinase K and heat-inactivated cell extracts were prepared by incubating of the cell extract with proteinase K (50 μg/mL) at 37 °C for 30 min and heating 10 μL extract at 75 °C for 10 min prior to assaying 3 μL by TRAP assay respective. HL-60 cell extract showed telomerase activity with the characteristic primer extension-binding pattern on the autoradigraphs. We considered the sample as positive for telomerase activity if the signal had disappeared after RNase A treatment and if no signal was detected with the lysis buffer alone (negative control). RNase A and proteinase K treatments abolished the PCR product ladder bands and confirmed both the protein and RNA dependence of the enzyme activity.

The amplified telomerase products were analyzed on a denaturating 15% polyacrylamide-7 M urea sequencing gel at 1,900 V for 2 h with Tris-borate-EDTA. Dried gels were exposed to a PhosphorImager storage screen and the amount of reaction products was evaluated using TYPHOON™ 9410 imager\ImageQuant™ software (Molecular Dynamics, Sunnyvale, CA, USA). To compare relative telomerase activity in the presence of inhibitors, the TRAP assay signals of the telomerase ladders were normalized to the signal of the corresponding internal

standard after background substraction. The signal intensity of the bands from ANPpp, ANPp and ANP- treated samples was expressed as a percentage of the signal intensity detected in the control. Their relative intensities were calculated with the ImageQuant software. All results were expressed as mean ± SD of the four independent determinations.

Results and Discussion

The approximate IC_{50} values for the PME-derivatives are shown in Table 1, where they are listed in the order of their telomerase inhibitory potency: PMEGpp > PMEDAPpp > PMEO-DAPypp > PMEApp > PMECpp ≥ PMETpp > 6-Me_2PMEDAPpp.

Table 1

Inhibition of HL-60 telomerase by PME type of ANPpp (IC_{50})[a]

Compound	dNTPs, [μmol/L]		
	125	60	30
PMEGpp	12.7 ± 0.5	7.1 ± 1.7	2.30 ± 0.04
PMEDAPpp	76.0 ± 13.5	41.6 ± 10.1	23.3 ± 3.2
PMEO-DAPypp	133 ± 15	N.D.[b]	N.D.
PMEApp	380 ± 28	N.D.	110 ± 11
PMECpp	N.I.[c]	N.D.	106 ± 16
PMETpp	N.I.	N.D.	N.I.
6-Me_2PMEDAPpp	P.E.[d]	N.D.	N.D.

[a] Values are means ± SD of the four independent determinations; [b] not determined; [c] no inhibition; [d] processivity enhancement.

The guanine derivative PMEGpp is the most potent telomerase inhibitor among all acyclic nucleotide analogues studied with the IC_{50} 12.7 ± 0.5 μmol/L at 125 μM dNTPs (Table 1, Fig. 2). Its inhibitory potency towards telomerase is comparable to that of ddGTP (IC_{50} 8.1 ± 0.4 μmol/L at 125 μM dNTPs), which is known to be one of the most effective nucleotide analogue based telomerase inhibitors. PMEGpp inhibits telomerase activity by 50% when it is present in the range 0.07 to 0.11 of the dGTP concentration only, depending on the dNTPs concentration. The PMEG

monophosphate and PMEG itself do not show at 125 μM dNTPs any effect on telomerase activity up to the concentration of 300 μM PMEG and/or PMEGp, respectively.

PMEDAPpp, which selectively inhibits DNA polymerase δ (Holý A., *Curr Pharm Des*, **2003**, *9*, 2567-92) and exerts significant cytostatic effects, inhibits the activity of telomerase with IC_{50} 76 ± 13.5 μmol/L (at 125 μM dNTPs). Surprisingly, N^6- dimethyl derivative 6-Me_2PMEDAPpp increases processivity of the enzyme (Fig. 3A). Nonphosphorylated form 6-Me_2PMEDAP has no effect on telomerase ladder pattern.

PMEApp with pyrimidine derivatives PMETpp and PMECpp, which inhibit retroviral reverse transcriptases (Holý A., *Curr Pharm Des*, **2003**, *9*, 2567-92), show no significant inhibitory potency towards telomerase. The inhibition is observed with PMEO-DAPypp; this open ring ANP is considered to be PMEDAPpp analogue (Fig. 1).

Inhibitory potency of PMEGpp and PMEDAPpp towards telomerase is consistent with the capability to induce apoptosis, strong cytostatic efficiency, and anticancer activity of their parental compounds PMEG and PMEDAP (Holý A., *Curr Pharm Des*, **2003**, *9*, 2567-92).

The approximate IC_{50} values for the diphosphates of PMP-derivatives, listed in the order of their efficacy as telomerase inhibitors, are shown in Table 2: (*R*)-PMPGpp > (*S*)-PMPGpp > (*R*)-6-cyprPMPDAPpp > (*R*)-PMPApp > (*R*)-PMPDAPpp ≥ (*S*)-PMPApp.

Table 2

Inhibition of HL-60 telomerase by PMP type of ANPpp (IC_{50})[a]

	dNTPs, [μmol/L]		
Compound	125	60	30
(*R*)-PMPGpp	17.3 ± 2.8	11.3 ± 1.6	3.6 ± 1.0
(*S*)-PMPGpp	81.7 ± 19.6	N.D.[b]	N.D.
(*R*)-6-cyprPMPDAPpp	152 ± 3	N.D.	N.D.
(*R*)-PMPApp	224 ± 30	125 ± 24	51.3 ± 6.7
(*R*)-PMPDAPpp	376 ± 24	N.D.	N.D.

(*S*)-PMPApp	P.E.[c]	N.D.	N.D.

[a] Values are means ± SD of the four independent determinations; [b] not determined; [c] processivity enhancement.

The most potent inhibitor among the PMP type analogues is the guanine derivative (*R*)-PMPGpp, which inhibits the enzyme activity with the IC_{50} at 5 to 8 times lower concentration compared to that of the natural substrate dGTP. (*S*)-PMPGpp is dramatically less inhibitory than its (*R*)-enantiomer: its IC_{50} exceeds almost 5 times that of (*R*)-PMPGpp. This indicates that absolute configuration plays a significant role in inhibition of telomerase and that the enzyme can distinguish between the (*R*)- and (*S*)-enantiomers. Alike, (*S*)-PMPApp increases processivity of the enzyme (Fig. 3B), while no significant inhibition of telomerase activity is found for (*R*)-PMPApp (IC_{50} 224 ± 30 μmol/L at 125 μM dNTPs), which is very efficient as a chain-terminating inhibitor of retroviral reverse transcriptases (Holý A., *Curr Pharm Des*, **2003**, *9*, 2567-92). These results are consistent with the observation of Pai RB, et al., *Cancer Res*, **1998**, *58*, 1909-13 on discrimination between the D and L enantiomers of FaraTTP, and Tendian SW, Parker WB., *Mol Pharmacol*, **2000**, *57*, 695-9, concerning inhibition efficiency difference in the pair of D and L enantiomers of CBV-TP. Structure-activity relationship study also indicates enantioselectivity of some of these ANP-type inhibitors of human telomerase. In general, (*R*)-enantiomers of the PMP-derivatives possess stronger affinity towards the enzyme than (*S*)-enantiomers.

The approximate IC_{50} values of the diphosphates of purine HPMP derivatives are shown in Table 3. In accordance with the PME and PMP series, the adenine derivative (*S*)-HPMPApp is less inhibitory than the guanine derivative (*R*)-HPMPGpp.

Table 3

Inhibition of HL-60 telomerase by HPMP type of ANPpp (IC_{50})[a]

	dNTPs, [μmol/L]		
Compound	125	60	30
(*R*)-HPMPGpp	43.6 ± 5.9	28.3 ± 9.5	23.3 ± 5.9
(*S*)-HPMPApp	104 ± 15	61 ± 25	N.D.[b]

[a] Values are means ± SD of the four independent determinations; [b] not determined.

The previously reported inhibitory effect of ddGTP on telomerase activity (see Pai RB, et al., *Cancer Res*, **1998**, *58*, 1909-13) is comparable with inhibitory potency of the most efficient ANP based compounds PMEGpp and (*R*)-PMPGpp. (*R*)-HPMPGpp, PMEDAPpp, (*S*)-PMPGpp, (*R*)-6-cyprPMPDAPpp, PMEO-DAPypp and (*S*)-HPMPApp have been shown as moderate inhibitors with IC_{50} values comparable to the concentrations of natural substrates. Although the telomerase active site has been shown to be related to that of other reverse transcriptases (Nugent CI, Lundblad V., *Genes Dev*, **1998**, *12*, 1073-85), (*R*)-PMPApp and PMEApp, nucleotide analogues known to be chain-terminating inhibitors of retroviral reverse transcriptases (Holý A., *Curr Pharm Des*, **2003**, *9*, 2567-92), do not inhibit telomerase activity.

The data in Table 1, 2 and 3 show that the IC_{50} values for inhibition of telomerase activity by all guanine derivatives studied [PMEGpp, (*R*)-PMPGpp, (*S*)-PMPGpp and (*R*)-HPMPGpp] are from 2 to 10 times lower than the dGTP concentration in the assay. Thus, the affinities of these nucleotide analogues towards telomerase are considerably higher than that for the natural substrate dGTP (Fig. 4A). In accordance with human telomeric sequence, adenine and/or 2,6-diaminopurine ANPpps are less efficient inhibitors (Fig. 4B).

Interestingly, two compounds from the studied series differ very substantially from the other ANPpp molecules: (*S*)-PMPApp and 6-Me_2PMEDAPpp do not inhibit telomerase, however, they increase the processivity of telomerase at 125 μM dNTPs concentration (Fig. 3). We can speculate that both (*S*)-PMPApp and 6-Me_2PMEDAPpp might interfere in some way with telomerase reaction cycle similarly to dGTP, which is supposed to facilitate telomerase RNA template and DNA strand separation and increase repeat addition processivity (Hammond PW, Cech TR., *Biochemistry*, **1998**, *37*, 5162-72; and Hardy CD, et al.,*J Biol Chem*, **2001**, *276*, 4863-71) in cooperating two-subunits of telomerase complex (see Wenz C, et al., *EMBO J*, **2001**, *20*, 3526-34).

According to the "anchor site model", the RNA template of one subunit could be used primarily for substrate binding, while the second template would be copied during telomere repeats addition (Kelleher C, et al., *Trends Biochem Sci*, **2002**, *27*, 572-9). So-called DNA anchor site, which is distinct from the catalytic site, could be

affected by both (*S*)-PMPApp and/or 6-Me_2PMEDAPpp and might thereby facilitate processivity during the synthesis of telomere repeats.

Example 2

Using a procedure similar to that described above, (*S*)-PMPA was found to increase telomere length in CCRF-CEM cells *in vivo* for about 1 kb when supplied in the growth medium for 5 weeks at concentrations 10 and 20 μM (see Figure 5). This data is consistent with the fact that (*S*)-PMPA diphosphate does not inhibit telomerase activity and was shown to increase the processivity of telomerase *in vitro* at 125 μM dNTPs.

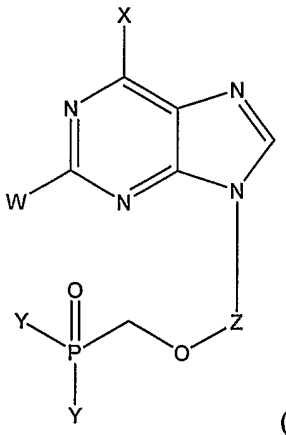
Example 3

Using a procedure similar to that described above, (*S*)-PMPA was also shown to enhance the processivity of telomerase in CCRF-CEM T lymphoblastoid cell extracts (human acute lymphoblastic leukemia, ATCC CCL 119), in human cervix carcinoma HeLa S3 (ATCC CCL 2.2) cell extracts, in mouse lymphocytic leukemia L1210 cells and in T-cell spontaneous leukemia/lymphoma (inbred Sprague-Dawley rat strain – see Otová B, et al., *Folia Biol* (Praha), **2002**, *48*, 213-26).

All literature and patent citations herein are hereby expressly incorporated by reference at the locations of their citation. Specifically cited sections or pages of the above cited works are incorporated by reference with specificity. The invention has been described in detail sufficient to allow one of ordinary skill in the art to make and use the subject matter of the following claims. It is apparent that certain modifications of the methods and compositions of the following claims can be made within the scope and spirit of the invention.

Claims

1. The use of an acyclic nucleoside purine of formula I:



(I)

wherein;

X is OH or $-N(R^1)_2$ wherein

R^1 independently is,

H;

C_2-C_{15} alkyl, C_3-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_3-C_{15} alkynyl, C_7-C_{15} arylalkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroalkyl or C_3-C_6 heterocycloalkyl wherein methylene in an alkyl moiety not adjacent to NH has been replaced by--O--; or

C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_6-C_{15} arylalkynyl, C_2-C_{15} alkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroaralkyl, C_4-C_6 aryl, C_3 –C_8cycloalkyl, or C_2-C_6 heterocycloalkyl;

or optionally both R^1 are joined together to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom;

wherein one of the foregoing R^1 groups can be substituted with halo,-CN or-N_3;

W is H or NH_2;

Y independently is, OH,$-OR^2$,$-OCH(R^3)OC(O)R^2$, a monophosphate, a diphosphate, an amino acid amidate, a polypeptide amidate,$-NHR^2$, or-$N(R^2)_2$;

R^2 independently is unsubstituted alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl; alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or

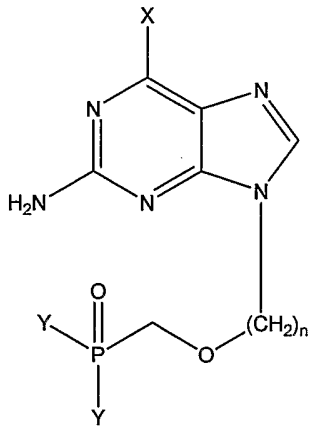
alkenylaryl wherein H is substituted by halo, carboxy, hydroxyl, cyano, nitro, N-morpholino, or amino; or alkyl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl in which a-CH_2- moiety has been substituted by NH, S, or O;

Z is C_1-C_6 alkyl, optionally substituted with one or more hydroxy; and

R^3 is H or R^2;

or a prodrug, diphosphate, pharmaceutically acceptable salt, or other phosphorus-substituted derivative thereof in the manufacture of a medicament for enhancing the processivity of a telomerase.

2. The use of claim 1 wherein the acyclic nucleoside purine is a compound of formula I:



wherein;

n is 2 or 3;

X is-$N(R^1)_2$ wherein

R^1 independently is H;

C_2-C_{15} alkyl, C_3-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_3-C_{15} alkynyl, C_7-C_{15} arylalkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroalkyl or C_3-C_6 heterocycloalkyl wherein methylene in an alkyl moiety not adjacent to NH has been replaced by--O--; or

C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_6-C_{15} arylalkynyl, C_2-C_{15} alkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroaralkyl, C_4-C_6 aryl, C_2-C_6 heterocycloalkyl;

or optionally both R^1 are joined together to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom;

wherein one of the foregoing R^1 groups can be substituted with halo,-CN or-N_3, but either one or two R^1 groups are not H;

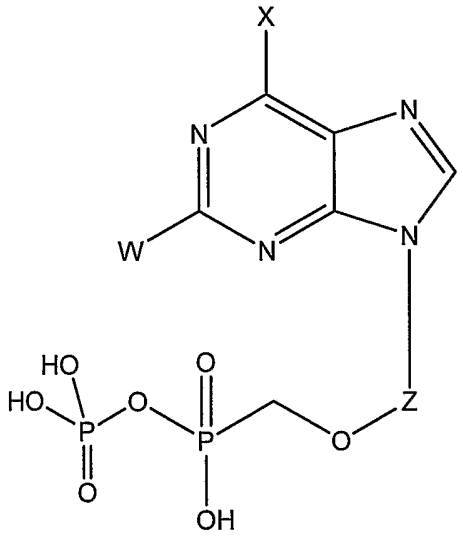
Y independently is, OH,-OR^2,-$OCH(R^3)OC(O)R^2$, a monophosphate, a diphosphate, an amino acid amidate, a polypeptide amidate,-NHR^2, or-$N(R^2)_2$;

R^2 independently is unsubstituted alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl; alkyl, aryl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl wherein H is substituted by halo, carboxy, hydroxyl, cyano, nitro, N-morpholino, or amino; or alkyl, alkenyl, alkynyl, alkaryl, alkynylaryl or alkenylaryl in which a-CH_2- moiety has been substituted by NH, S, or O;

R^3 is H or R^2;

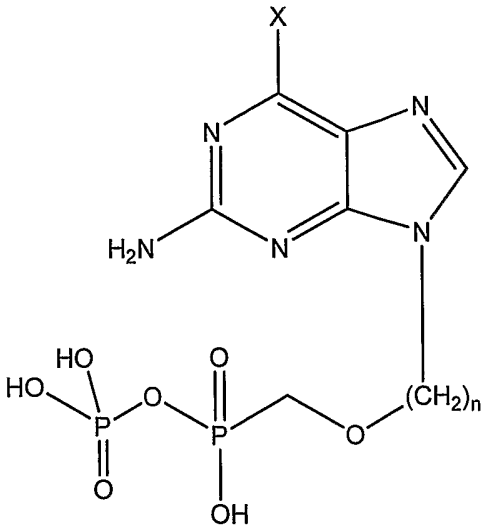
or a prodrug, diphosphate, or other phosphorus-substituted derivative thereof in the manufacture of a medicament for enhancing the processivity of a telomerase or inhibiting an enzyme that causes neoplasia in an animal.

3. The use of claim 1 wherein the acyclic nucleoside purine of formula I is a compound of the following formula:



or a prodrug or pharmaceutically acceptable salt thereof.

4. The use of claim 2 wherein the acyclic nucleoside purine is a compound of the following formula:



or a prodrug thereof.

5. The use of any one of claims 1-4 wherein each R^1 independently is H, C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_2-C_{15} alkynyl, or C_3–C_8cycloalkyl; or optionally both R^1 are joined together to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom.

6. The use of any one of claims 1-4 wherein each R^1 independently is H, methyl, or cyclopropyl.

7. The use of any one of claims 1-4 wherein each R^1 independently is H.

8. The use of any one of claims 1-4 wherein each R^1 independently is methyl.

9. The use of any one of claims 1-4 wherein one R^1 is H and the other R^1 is cyclopropyl.

10. The use of claim 1 or 3 wherein W is H.

11. The use of claim 1 or 3 wherein W is NH_2.

12. The use of claim 1, 3, or 10 wherein Z is $-CH_2-CH_2-$, $-CH_2-CH(CH_3)-$, or-$CH_2-CH(CH_2OH)-$.

13. The use of claim 2 or 4 wherein n is 2.

14. The use of claim 2 or 4 n is 3.

15. The use of claim 1, 3, or 10 wherein the compound of formula I is an *S*-enantiomer.

16. The use of claim 1 wherein the compound of formula I is 6-Me_2PMEDAPpp or a prodrug thereof.

17. The use of claim 1 wherein the compound of formula I is (S)-PMPApp or a prodrug thereof.

18. The use of claim 1 wherein the compound of formula I is (*S*)-PMPA or a prodrug thereof.

19. A pharmaceutical composition comprising a compound as described in any one of claims 1-18 and a pharmaceutically acceptable excipient.

20. A method of increasing the number of mitotic cycles of cells continually growing in tissue cultures comprising contacting the cells with a compound as described in any one of claims 1-18.

21. A method of increasing mitotic cell division of non-dividing cells comprising contacting the cells *in vitro* or *in vivo* with a compound as described in any one of claims 1-18.

22. The use of a compound as described in any one of claims 1-18 to prepare a medicament for increasing the number of mitotic cycles of cells continually growing.

23. The use of a compound as described in any one of claims 1-18 to prepare a medicament for increasing mitotic cell division of non-dividing cells.

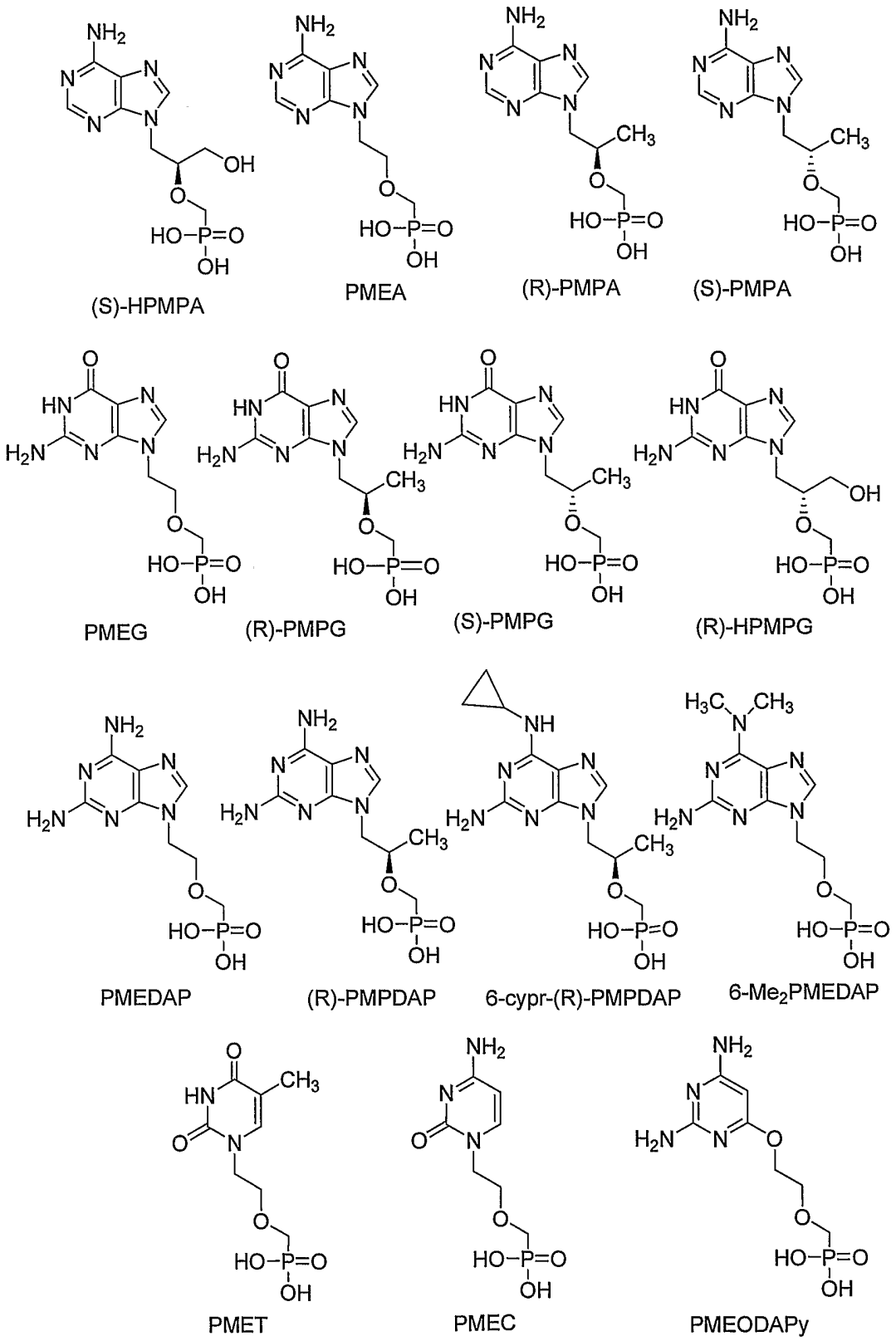


Fig. 1. Structure of PME, PMEO, PMP, and HPMP purines and pyrimidines.

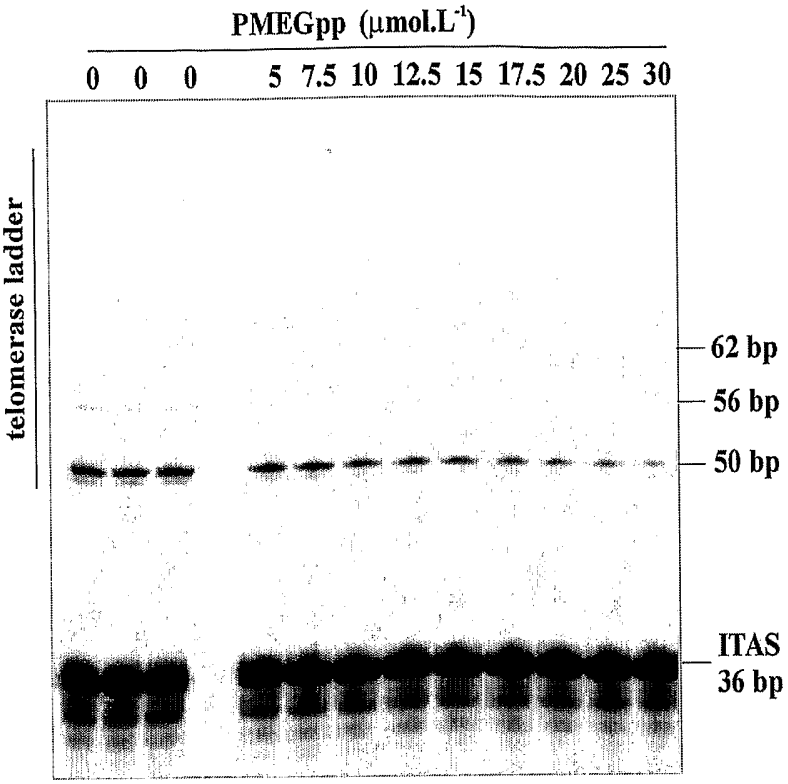


Fig. 2. Inhibition of HL-60 telomerase by PMEGpp. Telomerase activity was measured in HL-60 cell extract in the presence of 125 μmol.L^{-1} dNTPs and increasing concentrations of PMEGpp (0 - 30 μmol.L^{-1}). ITAS – internal standard.

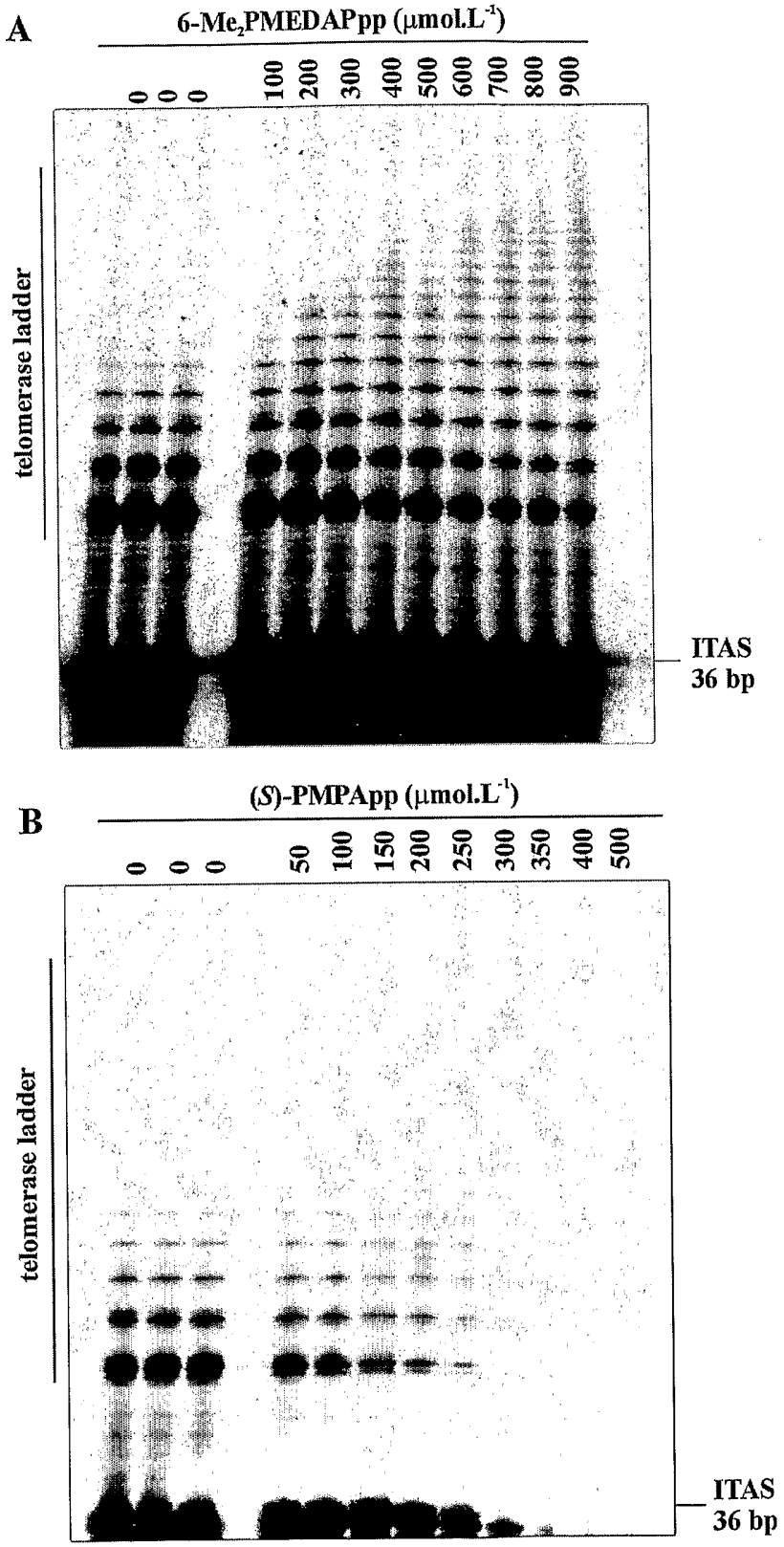


Fig. 3. Enhancement of telomerase processivity by 6-Me_2PMEDAPpp (A) and (*S*)-PMPApp (B). Telomerase activity was measured in HL-60 cell extract in the presence of 125 μmol.L^{-1} dNTPs and increasing concentrations of 6-Me_2PMEDAPpp (0 - 900 μmol.L^{-1}) and (*S*)-PMPApp (0 - 500 μmol.L^{-1}). ITAS – internal standard.

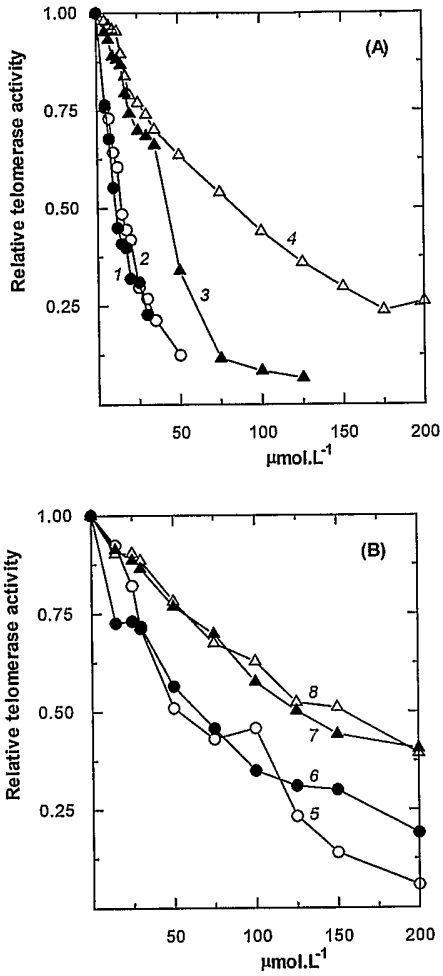


Fig. 4. (A) Inhibition of HL-60 telomerase by guanine derivatives PMEGpp (1), (*R*)-PMPGpp (2), (*R*)-HPMPGpp (3), (*S*)-PMPGpp (4) and (B) by adenine and 2,6-diaminopurine derivatives PMEDAPpp (5), (*S*)-HPMPApp (6), PMEO-DAPypp (7) and (*R*)-6-cyprPMPDAPpp (8). The enzyme activity was measured in HL-60 cell extract in the presence of 125 µmol.L^{-1} dNTPs.

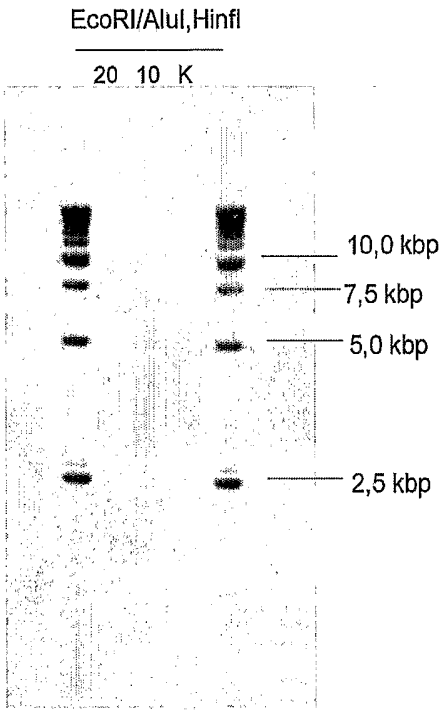


Fig 5. **Telomere length changes in cell line CCRF-CEM.** Terminal restriction fragments were measured after 5-weeks ANP treatment by the method of Southern blotting of genomic DNA digested by restriction enzymes (*Eco*RI, *Alu*I, *Hin*fI). Treatment of 10 μM and 20 μM (*S*)-PMPA resulted in telomere lengthening. K - control, 10 - 10 μM (*S*)-PMPA, 20 - 20 μM (*S*)-PMPA.