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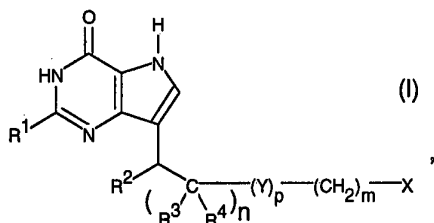
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⑤4 Virsraksts: Purīnu nukleozīdfosforilāzes inhibitori un to iegūšanas paņēmieni

⑤7 Kopsavilkums: Tiek piedāvāta viela ar formulu
2-amino-7-(R)-3H,5H-pirol[3,2-d]pirimidīn-4-ons,
kurā

R ir cikloheksenil-, cikloheksilgrupa vai -CH₂-R¹, un
R¹ ir nenoteikti aizvietota alicikliska, heterocikliska vai
piridīnigrupa. Bez tam piedāvāta viela ar formulu



kurā

R¹ ir H, -NH₂ vai -OCH₃;
R² ir neobligāti aizvietota cikliska grupa ar 5-7 oglekļa
atomiem, kas neobligāti satur vienu vai vairākus

heteroatomus;

R³ un R⁴ neatkarīgi viens no otra ir H vai
C₁₋₄-alkilgrupa;

m ir 0-4;

n ir 0-6; p ir 0-1;

X ir -CN, -CSNH₂, -PO(OH)₂, -COOH, -SO₂NH₂, -NH₂,
-OH, -C(=NH)NH₂, tetrazolil-, triazolilgrupa,
-COR⁵, kur

R⁵ ir C₁₋₄-alkilgrupa, -CF₃, -NH₂, -OC₁₋₄-alkilgrupa;

Y ir O vai -NH.

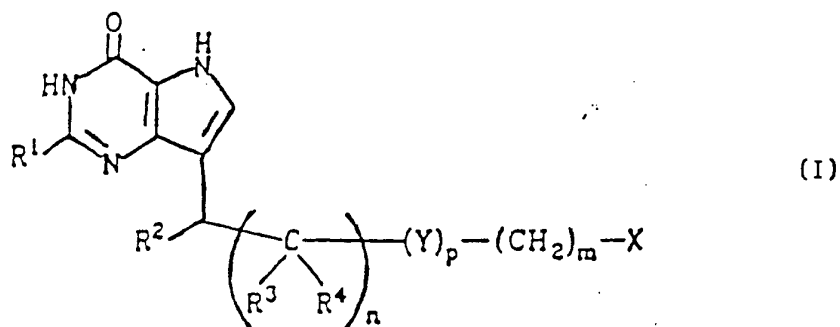
* pieteikums pieņemts ievērojot LR Ministru Padomes 1992.gada 28.februāra lēmumu Nr.72
(PSRS ir norādītā valsts PCT pieteikumā)

Patentformulas punkti

1. Savienojums 2-amino-7-(R)-3H,5H-pirololo[3,2-d]pirimidīn-4-ons, kurā R ir cikloheksenilgrupa, cikloheksilgrupa vai grupa $-\text{CH}_2-\text{R}^1$ (kur R^1 ir neobligāti nenoteikti aizvietota alicikliska, heteroalicikliska grupa vai piridīnilgrupa).
2. Savienojums pēc punkta 1, kurā R ir grupa $-\text{CH}_2-\text{R}^1$ un R^1 ir nenoteikti aizvietota 2-, 3- vai 4-piperidīnilgrupa, 2- vai 3-tetrahidrofuranilgrupa, 2- vai 3-tetrahidrotienilgrupa, 2- vai 3-pirolidīnilgrupa, 2-, 3- vai 4-tetrahidropiranilgrupa, 2-, 3- vai 4-piridīnilgrupa.
3. Savienojums pēc punkta 1, kurā R ir nenoteikti aizvietota cikloheksilgrupa vai 1-, 2- vai 3-cikloheksenilgrupa.
4. Savienojums pēc punkta 1, kurā R ir grupa $-\text{CH}_2-\text{R}^1$ un R^1 ir neobligāti aizvietota ciklopentilgrupa, cikloheksilgrupa, cikloheptilgrupa, 1- vai 2-adamantilgrupa, 1- vai 2-noradamantilgrupa, 1-norbornanilgrupa, 2-ekso-norbornanilgrupa, 1- vai 2-biciklo[2.2.2]okta-

nilgrupa, 1-, 2-, 3-, 6- vai 8-biciklo[3.2.1]oktanilgrupa, 1-, 2-, 3- vai 9-biciklo[3.3.1]nonanilgrupa, 1- vai 2-norbornenilgrupa.

5. Savienojums ar formulu (I)



kurā:

R^1 ir H, NH_2 vai OCH_3 ;

R^2 ir neobligāti aizvietota cikliska grupa ar 5 - 7 oglekļa atomiem, kas neobligāti satur vienu vai vairākus heteroatomus;

R^3 un R^4 neatkarīgi viens no otra ir ūdeņraža atoms vai C_{1-4} -alkilgrupa;

m ir 0 - 4;

n ir 0 - 6;

p ir 0 - 1;

X ir CN , $CSNH_2$, $PO(OH)_2$, $COOH$, SO_2NH_2 , NH_2 , OH , $C(=NH)NH_2$, tetrazolilgrupa, triazolilgrupa, grupa COR^5 , kurā

R^5 ir C_{1-4} -alkilgrupa, CF_3 , NH_2 , OC_{1-4} -alkilgrupa;

Y ir O vai NH.

6. Savienojums pēc punkta 5, kurā R^1 ir NH_2 , R^3 un R^4 ir H, m ir 0 un n ir 1.
7. Savienojums pēc punkta 5, kurā R^2 ir fenilgrupa.
8. Savienojums pēc punkta 5, kurā X ir CN, COOH vai CONH_2 .
9. Savienojums pēc punkta 5, kurā R^2 ir nenoteikti aizvietota 5- vai 6-locekļu aromātiska vai heteroaromātiska grupa, vai nenoteikti aizvietota alicikliska grupa vai heteroalicikliska grupa ar 5 - 9 atomiem ciklā.
10. Paņēmieni selektīvai T-limfocītu funkciju pavājināšanai zīdītāju organismā bez humorālās imunitātes pavājināšanas, kas a t š ķ i r a s ar to, ka zīdītājam ievada savienojumu pēc jebkura no punktiem 1 - 9, kas inhibē purīnu nukleozīdfosforilāzi un līdz ar to T-limfocītu veidošanos.
11. Paņēmieni ķīmiskā savienojuma iegūšanai, kas a t š ķ i r a s ar to, ka veic:
- nenoteikti aizvietota cikliskā aldehīda reakciju ar ciānetiķskābi amonija acetāta klātienē, iegūstot 3-cikloaizvietotu pentāndinitrilu;
 - 3-cikloaizvietota pentāndinitrila reakciju ar alkilformiātu bāzes klātienē, iegūstot 3-cikloaizvietotu-2-formilpentāndinitrilu;
 - 3-cikloaizvietota-2-formilpentāndinitrila reakciju ar glicīna metilestera hidrohenhlorīdu un nātrija vai amonija acetātu, ie-

gūstot N-[(3-cikloaizvietotu-2,4-diciān)-2-butenil]glicīna metil-
esteri;

d) N-[(3-cikloaizvietota-2,4-diciān)-2-butenil]glicīna metil-
estera reakciju ar hlorskudrskābes alkilesteri DBN vai DBU klātienē,
iegūstot 3-amino-4-(2-ciān-1-cikloaizvietotu-etil)-1-etil-1H-pirol-
1,2-dikarbonskābes metilesteri;

e) 3-amino-4-(2-ciān-1-cikloaizvietota-etil)-1-etil-1H-pirol-
1,2-dikarbonskābes metilestera reakciju ar bāzi, iegūstot 3-amino-4-
(2-ciān-1-cikloaizvietotu-etil)-1-etil-1H-pirol-2-karbonskābes
metilesteri.

12. Paņēmiens pēc punkta 11, kas a t š ķ i r a s ar to, ka
veic šādas papildreakcijas:

f) 3-amino-4-(2-ciān-1-cikloaizvietota-etil)-1-etil-1H-
pirol-2-karbonskābes metilestera reakciju ar benzoilizotiocianātu,
iegūstot N-benzoil-N'-[4-(2-ciān-1-cikloaizvietotu-etil)-2-metoksi-
karbonil-1H-pirol-3-il]tiourīnvielu;

g) N-benzoil-N'-[4-(2-ciān-1-cikloaizvietotas-etil)-2-metoksi-
karbonil-1H-pirol-3-il]tiourīnvielas reakciju ar alkilhalogenīdu, ie-
gūstot N-benzoil-N'-[4-(2-ciān-1-cikloaizvietotu-etil)-2-metoksikar-
bonil-1H-pirol-3-il]-S-alkiltiourīnvielu;

h) N-benzoil-N'-[4-(2-ciān-1-cikloaizvietotas-etil)-2-metoksi-
karbonil-1H-pirol-3-il]-S-alkiltiourīnvielas reakciju ar amonjaka
šķīdumu metanolā vai etanolā, iegūstot 3-cikloaizvietota-3-(2-amino-
4-okso-3H,5H-pirololo[3,2-d]pirimidin-7-il)propānnitrila un 3-cikloaiz-
vietota-3-(2-metilmerkaptio-4-okso-3H,5H-pirololo[3,2-d]pirimidin-7-il)-
propānnitrila maisījumu.

13. Paņēmiens pēc punkta 12, kas a t š ķ i r a s ar to, ka veic šādas papildreakcijas:

i) 3-cikloaizvietota-3-(2-metilmerkapt-4-okso-3H,5H-pirol- [3,2-d]pirimidin-7-il)propānnitrila reakciju ar oksidētāju, iegūstot 3-cikloaizvietotu-3-(2-metilsulfonil-4-okso-3H,5H-pirol- [3,2-d]pirimidin-7-il)propānnitrilu;

j) 3-cikloaizvietota-3-(2-metilsulfonil-4-okso-3H,5H-pirol- [3,2-d]pirimidin-7-il)propānnitrila reakciju ar nātrija alkoksīdu, iegūstot 3-cikloaizvietotu-3-(2-metoksi-4-okso-3H,5H-pirol- [3,2-d]pirimidin-7-il)propānnitrilu.

14. Paņēmiens pēc punkta 11, kas a t š ķ i r a s ar to, ka veic šādas papildreakcijas:

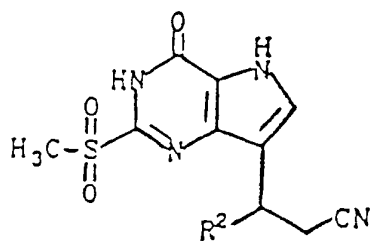
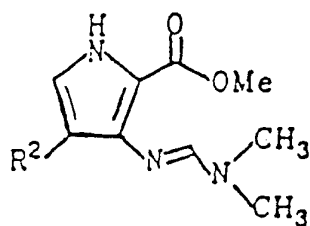
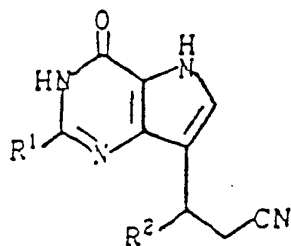
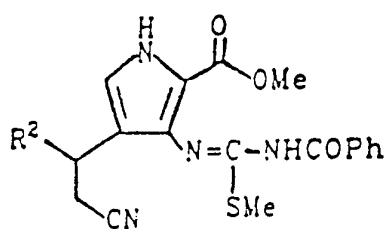
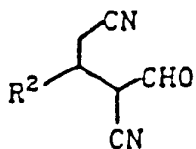
f) 3-amino-4- (2-ciān-1-cikloaizvietota-etil)-1-etil-1H-pirol- 2-karbonskābes metilestera reakciju ar dimetilformamīda dimetilacetātu, iegūstot 4-(2-ciān-1-cikloaizvietotu-etil)-3-[N-(dimetilaminometilēn)amino]-1H-pirol-2-karbonskābes metilesteri;

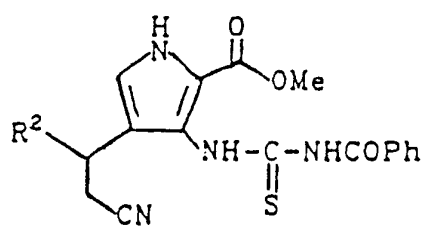
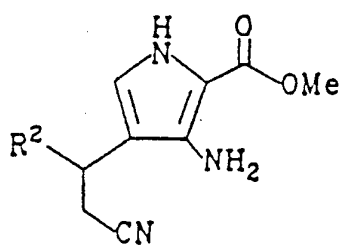
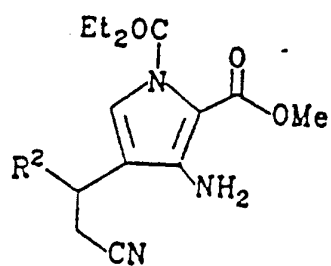
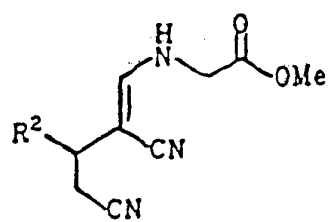
g) 4-(2-ciān-1-cikloaizvietota-etil)-3-[N-(dimetilaminometilēn)amino]-1H-pirol-2-karbonskābes metilestera reakciju ar amonjaka šķīdumu metanolā, iegūstot 3-cikloaizvietotu-3-(4-okso-3H,5H-pirol- [3,2-d]pirimidin-7-il)propānnitrilu.

15. Paņēmiens pēc punkta 11, kas a t š ķ i r a s ar to, ka cikliskais aizvietotājs ir fenilgrupa, 2- vai 3- tienilgrupa, 2- vai 3-furanilgrupa, 2-, 3- vai 4-piridinilgrupa, 2- vai 3-pirolilgrupa, 2-, 4- vai 5-tiazolilgrupa, 2- vai 3-pirazinilgrupa, 3- vai 4-piridazinilgrupa, 3-, 4- vai pirazolilgrupa, 1- vai 2-adamantilgrupa, cik-

lopentilgrupa, cikloheksilgrupa, cikloheptilgrupa vai morfolinilgrupa.

16. Savienojumi, kuros R^1 un R^2 nozīmes atbilst punktā 5 dotām:





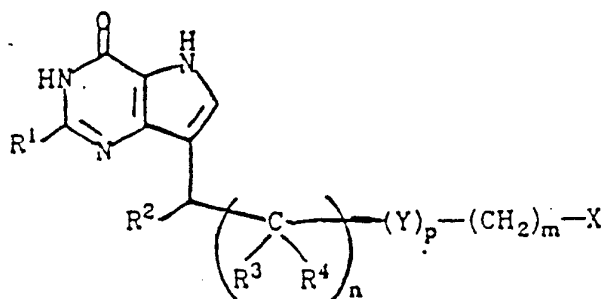
INHIBITORS OF PURINE NUCLEOSIDE PHOSPHORYLASE

The present invention relates to derivatives of 2-amino-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one and to derivatives of 4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine. It also relates to 4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidine derivatives substituted at the 7-position.

Purine nucleoside phosphorylase (PNP) catalyzes the phosphorolysis of purine nucleosides in a reversible reaction. Individuals who are deficient in PNP exhibit impaired T-cell development, resulting in lowered cell-mediated immunity, but normal B-cell development, resulting in normal humoral immunity. Accordingly, specific inhibitors of PNP that selectively inhibit T-cell development without damaging humoral immunity could be potentially effective against disorders in which activated T-cells are pathogenic.

As a PNP inhibitor, the present invention provides a 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one wherein R is optionally substituted cyclohexenyl, cyclohexyl, or -CH₂-R₁, wherein R₁ is an optionally substituted heteroalicyclic, pyridinyl or alicyclic group.

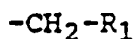
The present invention is also directed to a compound of the formula



wherein R¹ is H, NH₂, or OCH₃, R² is an optionally substituted cyclic group of 5-7 carbon atoms optionally

containing one or more heteroatoms, R^3 and R^4 are independently H or C_{1-4} alkyl, m is 0-4, n is 0-6, p is 0-1, X is CN, $CSNH_2$, $PO(OH)_2$, COOH, SO_2NH_2 , NH_2 , OH, $CNHNH_2$, tetrazole, triazole or COR^5 where R^5 is C_{1-4} alkyl, CF_3 , NH_2 , or OC_{1-4} alkyl, and Y is O or NH. The compound of the present invention is useful as a PNP inhibitor. Also contemplated according to the present invention is a pharmaceutical composition for the selective suppression of mammalian T-cell immunity comprising an pharmaceutically effective amount of the compound of the present invention and a pharmaceutically acceptable carrier or diluent and a method for the selective suppression of mammalian T-cell immunity without diminished effect on humoral immunity comprising administering to a subject a pharmaceutically effective amount of the compound of the present invention.

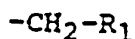
In one aspect of the invention there is provided a compound 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one (I) wherein R is



and R_1 is an optionally substituted heteroalicyclic group. Preferably, the heteroalicyclic group is a 5 or 6 membered saturated ring having oxygen, nitrogen, or sulfur as the heterocyclic atom. More preferably the heteroalicyclic group is 2- or 3-tetrahydrothienyl, 2-, 3-, or 4-piperidinyl, 2- or 3-tetrahydrofuranlyl, 2-, or 3-pyrrolidinyl, or 2-, 3-, or 4-tetrahydropyranlyl. In a preferred aspect, R_1 is unsubstituted, e.g., the compound (I) is 2-amino-7-(2-piperidinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IB), 2-amino-7-(3-piperidinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IC), 2-amino-7-(4-piperidinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (ID), 2-amino-7-(2-tetrahydrofuranlylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IE), 2-amino-7-(3-tetrahydrofuranlylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IF),

2-amino-7-(2-tetrahydrothienylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IG), 2-amino-7-(3-tetrahydrothienylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IH), 2-amino-7-(2-pyrrolidinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (II), 2-amino-7-(3-pyrrolidinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IJ), 2-amino-7-(2-tetrahydropyranylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IL), 2-amino-7-(3-tetrahydropyranylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IM), or 2-amino-7-(4-tetrahydropyranylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IN). In an alternative preferred embodiment the R_1 has one or two substituents selected from the group consisting of halogen, hydroxy, alkoxy, alkyl, or trifluoromethyl. As halogen is preferably mentioned chloro or fluoro. As alkoxy is preferably mentioned lower alkoxy, including methoxy, ethoxy, propoxy and butoxy. As alkyl is preferably mentioned lower alkyl, including methyl, ethyl, propyl and butyl.

In another aspect of the invention there is provided a compound 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (II) wherein R is

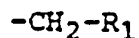


and R_1 is optionally substituted pyridinyl. In a preferred aspect, R_1 is unsubstituted, i.e., the compound (II) is 2-amino-7-(3-pyridinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IIA), 2-amino-7-(2-pyridinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IIB), or 2-amino-7-(4-pyridinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IIC). In an alternative preferred embodiment the R_1 group has one or two substituents selected from the group consisting of halogen, hydroxy, alkoxy, alkyl, or trifluoromethyl. As halogen is preferably mentioned chloro or fluoro. As alkoxy is preferably mentioned lower alkoxy, including methoxy, ethoxy, propoxy and butoxy. As alkyl is

preferably mentioned lower alkyl, including methyl, ethyl, propyl and butyl.

In another aspect of the invention there is provided a compound (III) 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one wherein the R group is unsubstituted or substituted 1-, 2-, or 3-cyclohexenyl or cyclohexyl. In a preferred aspect, R is unsubstituted, i.e., the compound (III) is 2-amino-7-(1-cyclohexenyl)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one (IIIA), 2-amino-7-(2-cyclohexenyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IIIB), 2-amino-7-(3-cyclohexenyl)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one (IIIC), or 2-amino-7-(cyclohexyl)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one (IIID). In an alternative preferred embodiment the R has at least one substituent selected from the group consisting of halogen, hydroxy, alkoxy, alkyl, or trifluoromethyl. As halogen is preferably mentioned chloro or fluoro. As alkoxy is preferably lower alkoxy, including methoxy, ethoxy, propoxy and butoxy. As alkyl is preferably mentioned lower alkyl, including methyl, ethyl, propyl and butyl.

In another aspect of the invention there is provided a compound 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one (IV) wherein R is

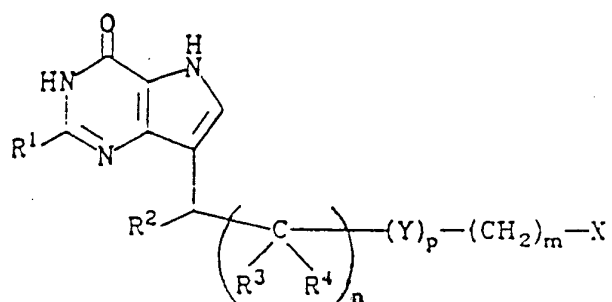


and R_1 is an optionally substituted alicyclic group. Preferable alicyclic groups include, e.g., single-ring cycloparafins such as cyclopentyl, cyclohexyl, and cycloheptyl, multi-ring cycloparafins such as 1- and 2-adamantyl, 1-norbornanyl, 2-exo-norbornanyl, 2-endo-norbornanyl, 1- and 2-bicyclo[2.2.2]-octanyl, 1-, 2-, 3-, 6-, and 8-bicyclo[3.2.1]octanyl, and 1-, 2-, and 3-bicyclo[3.3.1]nonanyl and cycloolefins such as 1- and 2-norbornenyl. Examples of the preferred compound (IV) are 2-amino-7-(2-adamantylmethyl)-3H,5H-pyrrolo[3,2-d]-

pyrimidin-4-one (IVA), 2-amino-7-(1-adamantylmethyl)-
 3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (IVB), 2-amino-7-
 (cyclopentylmethyl)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one
 (IVC), 2-amino-7-(cyclohexylmethyl)-3H,5H-pyrrolo[3,2-d]-
 5 pyrimidin-4-one (IVD), 2-amino-7-(cycloheptylmethyl)-3H,5H-
 pyrrolo[3,2-d]pyrimidin-4-one (IVE), 2-amino-7-(1-
 norbornanylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one
 (IVF), 2-amino-7-(2-exo-norbornanylmethyl)-3H,5H-
 pyrrolo[3,2-d]pyrimidin-4-one (IVG), 2-amino-7-(2-endo-
 10 norbornanylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one
 (IVH), 2-amino-7-(1-norbornenylmethyl)-3H,5H-
 pyrrolo[3,2-d]-pyrimidin-4-one (IVI), 2-amino-7-(2-
 norbornenylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one
 (IVJ), 2-amino-7-(1-bicyclo[2.2.2]-octanylmethyl)-3H,5H-
 15 pyrrolo[3,2-d]-pyrimidin-4-one (IVK), 2-amino-7-(1-bicyclo-
 [3.2.1]octanyl-methyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one
 (IVL), and 2-amino-7-(1-bicyclo[3.3.1]nonanylmethyl)-3H,5H-
 pyrrolo[3,2-d]-pyrimidin-4-one (IVM), and 2-amino-7-(1-
 noradamantyl-methyl)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one
 20 (IVN). In an alternative preferred embodiment the R₁ group
 has one or two substituents selected from the group
 consisting of halogen, hydroxy, alkoxy, alkyl, or
 trifluoromethyl. As halogen is preferably mentioned chloro
 or fluoro. As alkoxy is preferably mentioned lower alkoxy,
 25 including methoxy, ethoxy, propoxy and butoxy. As alkyl is
 preferably mentioned lower alkyl, including methyl, ethyl,
 propyl and butyl.

The present invention is also directed to a compound of
 the formula

30



wherein R^1 is H, NH_2 , or OCH_3 , R^2 is an optionally substituted cyclic group optionally containing one or more heteroatoms, R^3 and R^4 are independently H or C_{1-4} alkyl, m is 0-4, n is 0-6, p is 0-1, X is CN, $CSNH_2$, $PO(OH)_2$, COOH, SO_2NH_2 , NH_2 , OH, $CNHNH_2$, tetrazole, triazole or COR^5 where R^5 is C_{1-4} alkyl, CF_3 , NH_2 , or OC_{1-4} alkyl, and Y is O or NH.

The optionally substituted cyclic group (hereinafter referred to as cyclo) recited for the above formula includes aromatic, heteroaromatic, alicyclic, and heteroalicyclic groups preferably containing five to nine atoms. Preferred optional substituents include halogen, hydroxy, alkoxy, alkyl, and trifluoromethyl. Exemplary substituents include chloro, fluoro, methoxy, ethoxy, propoxy, butoxy, methyl, ethyl, propyl, and butyl. Preferred heteroatoms include oxygen, nitrogen, and sulfur, which can be present in combination in the same group. The preferred aromatic and heteroaromatic groups are phenyl, 2- or 3-thienyl, 2- or 3-furanyl, 2-, 3-, or 4-pyridinyl, 2- or 3-pyrrolyl, 2-, 4-, or 5-thiazolyl, 2-pyrazinyl, 3- or 4-pyridazinyl, and 3-, 4-, or 5-pyrazolyl. The preferred alicyclic and heteroalicyclic groups are 1- or 2-adamantyl, cyclohexyl, cycloheptyl, 2- or 3-tetrahydrofuranyl, 2- or 3-tetrahydrothienyl, 2- or 3-tetrahydropyranyl, 2-, 3-, or 4-piperidinyl, 3- or 4-pyrazolidinyl, 2-, 4-, or 5-thiazolidinyl, 2- or 3-piperazinyl, 2- or 3-morpholinyl, or 3- or 4-hexahydropyridazinyl.

In another aspect of the invention there is provided a method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity which comprises administering to a mammal the compound (I), whereby said compound inhibits purine nucleoside phosphorylase and thereby T-cell formation.

In a further aspect of the present invention there is provided a pharmaceutical composition for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity which comprises an effective
5 amount of the compound and a pharmaceutically acceptable diluent therefor.

The invention further relates to pharmaceutical compositions suitable for enteral, such as oral or rectal, transdermal and parenteral administration to mammals
10 including man, which are useful to inhibit purine nucleoside phosphorylase activity and for the treatment of disorders responsive thereto, comprising an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more
15 pharmaceutically acceptable carriers.

Preferred pharmaceutical compositions are tablets and gelatin capsules comprising the active ingredient together with a) diluents, e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; b)
20 lubricants, e.g., silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol; for tablets also c) binders, e.g., magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; if
25 desired d) disintegrants, e.g., starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or e) absorbents, colorants, flavors and sweeteners. Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are
30 advantageously prepared from fatty emulsions or suspensions. Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In

addition, they may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1 to 75%, preferably about 1 to 50%, of the active ingredient.

Suitable formulations for transdermal application include an effective amount of a compound of the invention with a carrier. Advantageous carriers include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. Characteristically, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.

The present invention provides a method of inhibiting purine nucleoside phosphorylase activity in mammals and treating diseases and conditions responsive thereto, e.g., autoimmune disorders, rejection of transplantation, or psoriasis, which comprises administering to a mammal in need thereof an effective amount of a compound of the invention or of a pharmaceutical composition comprising a said compound in combination with one or more pharmaceutically acceptable carriers.

A further aspect of the invention relates to a method of inhibiting the phosphorolysis and metabolic breakdown of antiviral or antitumor purine nucleosides in mammals which comprises administering in conjunction therewith to a mammal in need thereof, either separately or in combination therewith, an effective purine nucleoside phosphorylase inhibiting amount of a compound of the invention or of a said compound in combination with one or more pharmaceutically acceptable carriers. More particularly,

such relates to a method of inhibiting the phosphorolysis and metabolic breakdown of purine nucleosides known in the art, e.g., of 2'-deoxyguanosine, 2',3'-dideoxyinosine, 2',3'-dideoxyguanosine or 2',3'-dideoxyadenosine.

- 5 Furthermore, the invention thus relates to a method of potentiating the antiviral or antitumor effect of 2' or 3'-monodeoxypurine nucleosides or of 2',3'-dideoxypurine nucleosides in mammals which comprises administering in conjunction therewith to a mammal in need thereof, either
10 separately or in combination with a said nucleoside, an effective purine nucleoside phosphorylase inhibiting amount of a compound of the invention preferably in combination with one or more pharmaceutically acceptable carriers. More particularly, such relates to a method of enhancing or
15 potentiating the effect of 2',3'-dideoxypurine nucleosides known in the art, e.g., of 2',3'-dideoxyinosine, 2',3'-dideoxyguanosine or 2'-3'-dideoxyadenosine for the treatment of retrovirus infections, e.g., HIV-retrovirus infections (acquired immunodeficiency syndrome, AIDS).
20 2',3'-Dideoxypurine nucleosides are known in the art as inhibitors of HIV retrovirus infectivity and to be metabolically degraded by PNP, e.g., as described in Biochemical Pharmacology 22, 3797 (1987). Such are administered at a pharmaceutically acceptable dose which
25 is effective in inhibiting HIV-retrovirus infections. Preferably the lowest possible effective dose is used.

The pharmaceutically acceptable effective dosage of active compound of the invention to be administered is dependent on the species of warm-blooded animal (mammal),
30 the body weight, age and individual condition, and on the form of administration.

The pharmaceutical composition may be oral, parenteral, suppository or other form which delivers the compound into the bloodstream of a mammal to be treated. An oral form

has from about 1 to about 150 mg of the compound for an adult (50 to 70 kg) which is mixed together with pharmaceutically acceptable diluents such as lactose. In a typical capsule, 25 mg of the compound are mixed together
5 with 192 mg lactose, 80 mg modified starch and 3 mg magnesium stearate. Injectable forms of the compound are also contemplated for administration.

The present invention is also useful with other therapeutic agents. A daily dosage for a human weighing 50
10 to 70 kg of 1-50 mg/kg inhibits metabolic destruction of certain anticancer agents such as beta-2'-deoxy-6-thioguanosine and antiviral agents such as 2',3'-dideoxyinosine, an anti-AIDS drug. These types of agents are known to be susceptible to cleavage. Upon cleavage,
15 the agents lose effectiveness. The compounds of the present invention are capable of reducing such cleavage. This protection, therefore, enhances the efficacy of other chemotherapeutic agents.

One method of making the compound (I) of the present
20 invention uses 3-substituted propionitriles as starting materials. Such starting materials can be obtained by a variety of methods that are well documented in the literature. The compound (I) is then prepared from the starting material by an adaptation of the synthetic
25 methodology disclosed in M. I. Lim, R. S. Klein, and J. J. Fox, J. Org. Chem., 44, 3826 (1979); M.I. Lim, R.S. Klein, and J.J. Fox, Tetrahedron Lett., 21, 1013 (1980); M.I. Lim and R.S. Klein, Tetrahedron Lett., 22, 25 (1981); M. I. Lim, W. Y. Ren, B.A. Otter, and R.S. Klein, J. Org. Chem.,
30 48, 780 (1983).

A method of making the compound (II) of the present invention uses a 3-(pyridinyl)propionitrile as the starting material to make the compound (II). The appropriate 3-(pyridinyl)propionitrile can be produced by converting the

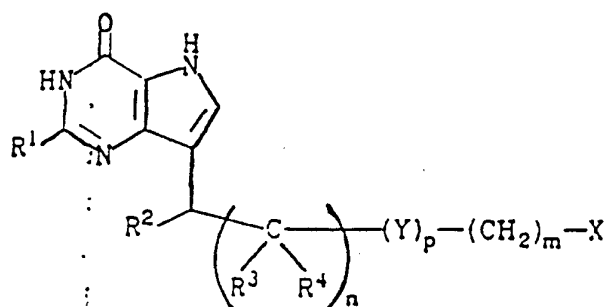
corresponding 3-(pyridinyl)propionyl chloride to the corresponding amide by ammonolysis with, e.g., ammonium hydroxide, which is then dehydrated to the desired nitrile by distillation with a dehydrating agent, such as POCl₃ or SOCl₂. Alternatively, the starting material is produced by condensation of the 3-aldehyde with cyanoacetic acid followed by decarboxylation to give the corresponding substituted acrylonitrile, which is hydrogenated to give the corresponding 3-(pyridinyl)propionitrile by either catalytic hydrogenation or magnesium metal dissolving in methanol at 0 °C, such as disclosed in Profitt, J., et al., J. Org. Chem., **40**, 127 (1975). The compound (II) is then prepared from the starting material by an adaptation of the synthetic methodology disclosed in M. I. Lim, R. S. Klein, and J. J. Fox, J. Org. Chem., **44**, 3826 (1979); M.I. Lim, R.S. Klein, and J.J. Fox, Tetrahedron Lett., **21**, 1013 (1980); M.I. Lim and R.S. Klein, Tetrahedron Lett., **22**, 25 (1981); M. I. Lim, W. Y. Ren, B.A. Otter, and R.S. Klein, J. Org. Chem., **48**, 780 (1983).

Another method of making the compound of the present invention uses a known compound, i.e., cyclohexenyl-acetonitrile, as the starting material. The compound (III) of the present invention is made by reacting the starting material in an adaptation of the synthetic methodology disclosed in M. I. Lim, R. S. Klein, and J. J. Fox, J. Org. Chem., **44**, 3826 (1979); M.I. Lim, R.S. Klein, and J.J. Fox, Tetrahedron Lett., **21**, 1013 (1980); M.I. Lim and R.S. Klein, Tetrahedron Lett., **22**, 25 (1981); M. I. Lim, W. Y. Ren, B.A. Otter, and R.S. Klein, J. Org. Chem., **48**, 780 (1983). Catalytic hydrogenation of either (IIIA), (IIIB), or (IIIC) yields the compound (IIID).

A method of making the compound (IV) of the present invention uses 3-substituted propionitriles as starting materials. Such starting materials can be obtained by a

variety of methods that are well documented in the literature. The compound (IV) is then prepared from the starting material by an adaptation of the synthetic methodology disclosed in M. I. Lim, R. S. Klein, and J. J. Fox, J. Org. Chem., **44**, 3826 (1979); M.I. Lim, R.S. Klein, and J.J. Fox, Tetrahedron Lett., **21**, 1013 (1980); M.I. Lim and R.S. Klein, Tetrahedron Lett., **22**, 25 (1981); M. I. Lim, W. Y. Ren, B.A. Otter, and R.S. Klein, J. Org. Chem., **48**, 780 (1983).

Another aspect of the present invention concerning a compound of the formula

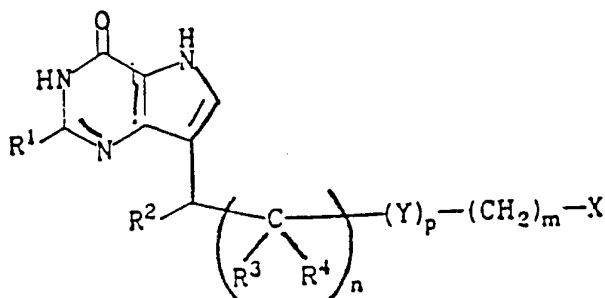


provides a method of making a 2-amino compound ($R^1 = NH_2$) and intermediates thereof. The first step of the method involves reacting an optionally substituted cyclic aldehyde with cyanoacetic acid at a molar ratio of about 1/1 to 1/5 in the presence of ammonium acetate at about reflux temperature for about 10 hours to 8 days to make a 3-cyclo-substituted pentanedinitrile as an intermediate. In the second step, the 3-cyclo-pentanedinitrile is reacted with an alkyl formate such as ethyl formate and a strong base such as the metal-containing bases sodium hydride or sodium alkoxide, e.g., sodium methoxide, at a molar ratio of about 1-2/3-6/1-3 and at a temperature of about 20-65°C for about 10 hours to 8 days to make a 3-cyclo-2-formylpentanedini-

trile as a further intermediate. The next step involves reacting the 3-cyclo-2-formylpentanedinitrile with a glycine alkyl ester hydrochloride and sodium or ammonium acetate at a molar ratio of about 1-2/1.5-4/1.5-4 and at a temperature of about 20-60°C for about 10-48 hours to make methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine as an intermediate. In the subsequent step, the methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine is reacted with an alkyl chloroformate such as ethyl chloroformate and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at a molar ratio of about 1-2/1.5-5/1.5-4 and at a temperature of about 0-50°C for about 10 hours to 10 days to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate as an intermediate. The next step involves reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate with a base such as sodium carbonate at a molar ratio of about 2/1 to 1/5 and at about room temperature for about 10-48 hours to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate as an intermediate. In the next step, the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate is reacted with benzoylisothiocyanate at a molar ratio of about 2/1 to 1/2 and at about room temperature for about 30 minutes to 3 hours to make N-benzoyl-N--[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]thiourea as an intermediate. The next step reacts the N-benzoyl-N--[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-4-3-yl]thiourea with an alkyl halide such as methyl iodide at a molar ratio of about 1/1 to 1/6 and at a temperature of about 0-30°C for about 10 minutes to 10 hours to make N-benzoyl-N--[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]S-methylthiourea as an intermediate. In the following step, the N-benzoyl-N'--[4-(2-cyano-1-cyclo-ethyl)-2-

methoxycarbonyl-1H-pyrrol-3-yl]-S-methylthiourea (about 1-2 mol) is reacted with methanolic or ethanolic ammonia at a ratio of about 1/1 to 1/20 and at a temperature of about 20-130°C for about 16-60 hours to make a mixture of a 2-amino compound of the present invention 3-cyclo-3-[2-amino-4-oxo-3H-5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile and a 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile as an intermediate in making another compound of the present invention.

In a further aspect concerning the compound of the formula~



there is provided a method of making a 2-methoxy compound ($R^1 = OCH_3$) and intermediates thereof. The intermediate 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile is reacted with an oxidizing agent such as permanganate or hydrogen peroxide at a molar ratio of about 1/1 to 1/10 and at a temperature of about 25-120°C for about 3-48 hours to make 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile as an intermediate. In the next step, the 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile is reacted with a sodium alkoxide such as sodium methoxide at a molar ratio of about 1/1 to 1/10 and at a temperature of about 25-100°C for

about 1-48 hours to make a 2-methoxy compound of the present invention 3-cyclo-3-[2-methoxy-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

In a further aspect, there is provided a method of making a compound wherein R¹ is hydrogen. The methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate intermediate described supra is reacted with dimethylformamide dimethyl acetal at a molar ratio of about 1/1 to 1/4 and at a temperature of about 25-100°C for about 1-10 days to make methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate as an intermediate. The next step involves reacting the methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate with methanolic or ethanolic ammonia at a molar ratio of about 1/1 to 1/20 and at a temperature of about 20-130°C for about 10-68 hours to make the compound of the present invention 3-cyclo-3-[4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

As will be apparent to the skilled artisan, variations of the aforesaid procedures are useful in making the variety of compounds of the present invention without departing from the spirit thereof. For example, compounds having different values for "n" and "m" in accordance with the formula of the present invention are obtained by either stepping up or stepping down the series by the necessary number of carbon atoms in accordance with known procedures. Also, reactions involving some intermediates require protection of nitrogen or oxygen atoms on the intermediates using known procedures.

In order to more fully describe the present invention the following non-limiting examples are provided. In the examples all parts and percentages are by weight unless

indicated otherwise. Proportions of solvent mixtures used as chromatographic eluents are by volume.

EXAMPLE 1

- 3-(3-Pyridinyl)propionitrile is prepared in this example.
- 5 A three-neck flask carrying a magnetic stir bar is fitted with a thermometer, pressure equalizing addition funnel, and a reflux condenser carrying an argon inlet. Freshly powdered potassium hydroxide (6.6 g, 0.1 mol) and anhydrous acetonitrile (150 ml) are charged into the flask and heated
- 10 at reflux while 3-pyridinecarboxaldehyde (10.7 g, 0.1 mol) in anhydrous acetonitrile (50 ml) is added dropwise over a period of about five minutes and refluxing continued for about another three minutes. The resulting hot reaction mixture is poured into an ice/water mixture (100 g), and
- 15 the resulting solution is extracted with CH_2Cl_2 (3 x 100 ml), dried with Na_2SO_4 , and evaporated to give crude 3-(3-pyridinyl)acrylonitrile, which is purified by column chromatography over silica gel using CHCl_3 as the eluent; yield 3.3 g (25.6%).
- 20 Under an argon atmosphere, a stirred solution of the acrylonitrile (2.662 g, 0.02 mol) in 99% ethanol (100 ml) is treated with a drop of 4% aqueous sodium hydroxide followed by sodium borohydride (0.378 g, 0.01 mol). Additional sodium borohydride (0.378 g) is added twice more
- 25 at four-hour intervals. The mixture is stirred at room temperature overnight, diluted with water, extracted with EtOAc and dried with Na_2SO_4 . The solvent is removed under reduced pressure and the crude product obtained is chromatographed over a column of silica using
- 30 chloroform/methanol (40/1) as the eluent to give 2.2 g (84.6%) of the product as a colorless oil.

EXAMPLE 2

3-(3-Pyridinyl)propionitrile of Example 1 is further treated in the synthesis of the present invention. Under

17

an atmosphere of dry N_2 , a mixture of 3-(3-pyridinyl)propionitrile (0.661 g, 5.0 mmole), sodium hydride (0.240 g, 10.0 mmole), and ethyl formate (1.11 g, 15.0 mmole) in anhydrous tetrahydrofuran (20 ml) is stirred
5 for 48 hours with protection from air and moisture. Volatile matter is evaporated, and a solution of the solid residue in 15 ml of cold water is adjusted at 0°C to a pH of 6 with cold 6N HCl. The resulting oily mixture is extracted with $CHCl_3$, and the extract is washed with water,
10 dried using Na_2SO_4 , and evaporated to give a dark oil, which is a mixture of 2-formyl-3-(3-pyridinyl)propionitrile and the nitrile starting material. This crude product is used in the next reaction without further purification.

EXAMPLE 3

15 Glycine methyl ester hydrochloride (0.942 g, 7.5 mmole) and anhydrous sodium acetate (0.615 g, 7.5 mmole) are added to a solution of the crude formyl compound (0.89 g) in MeOH/ H_2O (4:1, 50 ml). After 24 hours, the MeOH is evaporated in vacuo, and the mixture of water and oil is
20 extracted with $CHCl_3$. The $CHCl_3$ layer is dried (Na_2SO_4) and evaporated to give an amber oil which is applied to a silica gel column. Elution with $CHCl_3$ gave two major bands: (1) 3-(3-pyridinyl)propionitrile (used as starting material in the previous step), and (2) the desired
25 enamine.

EXAMPLE 4

Under a nitrogen atmosphere, ethyl chloroformate (0.521 g, 4.8 mmole) is added dropwise to a solution of the enamine of Example 3 (0.513 g, 3.2 mmole) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN, 1.37 g, 11.1 mmole) in
30 dry CH_2Cl_2 (15 ml) with external cooling in an ice bath. After stirring at 0 °C for one hour, the solution is allowed to a stand at room temperature overnight. After checking progress by TLC, additional $ClCO_2Et$ (0.1 ml) and

DBN (1.0 ml) are added to complete the conversion, and the solution is allowed to stand for 24 hours. Volatile matter is evaporated in vacuo and the viscous residue is purified on a short silica gel column (whose main purpose is to
5 remove the less-mobile DBN) to give an N-blocked pyrrole; which is used for the next step without further purification.

EXAMPLE 5

To a solution of the N-blocked pyrrole of Example 4
10 (0.635 g, 2.0 mmole) in MeOH (50 ml) is added solid Na_2CO_3 (0.212 g, 2.0 mmole), and the reaction mixture is stirred at room temperature for 48 hr with separation of the resultant deblocked pyrrole. The mixture is evaporated to dryness, and the residue is triturated thoroughly with H_2O
15 (25 ml) to dissolve inorganics and extracted with CHCl_3 (3 x 100 ml). The extract is dried (Na_2SO_4) and evaporated to give a viscous gum that crystallized upon drying in vacuo for use as an intermediate without further purification. More extensive purification can, however, be effected by
20 using either column chromatography employing silica gel/ CHCl_3 or recrystallization from toluene/cyclohexane (1:3).

EXAMPLE 6

Benzoyl isothiocyanate (0.232 g, 1.42 mmole) is added
25 dropwise to a solution of the unblocked pyrrole of Example 5 (0.290 g, 1.18 mmole) in dry CH_2Cl_2 (100 ml). After 1 h at room temperature, solution is evaporated, and the gummy residue is stirred in Et_2O /cyclohexane (1:1, 20 ml). The resulting suspension of yellow solid is filtered under N_2
30 pressure, and the thioureido product is dried in vacuo over P_2O_5 .

EXAMPLE 7

Methyl iodide (0.228 g, 1.61 mmole) is added to a solution of the thioureido product of Example 6 (0.383 g,

0.94 mmole) and DBN (0.140 g, 1.12 mmole) in dry CH_2Cl_2 (10 ml) at 0 °C. The solution is stirred at 0 °C for 15 min., at ambient temperature for 1 h, and then evaporated in vacuo. A solution of the residue in CHCl_3 is
5 chromatographed on a silica gel column with CHCl_3 /methanol (97:3) as eluent to give homogeneous fractions of the methylthio intermediate compound.

EXAMPLE 8

A solution of the methylthio compound of Example 7
10 (0.358 g, 0.85 mmole) in 100 ml of MeOH that has been saturated with NH_3 at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a mixture of the desired 2-amino intermediate compound,
15 benzamide, and a by-product that is a 2-methylthio derivative, as opposed to the 2-amino compound. The mixture is dissolved in methanol and the solution is evaporated with silica gel (about 5 g). The mixture is then carefully layered onto the top of a silica-gel
20 chromatography column, which is then eluted with CHCl_3 /MeOH (9:1) to give the methylthio by-product and the desired 2-amino-7-(3-pyridinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one intermediate. Further purification is obtained by recrystallization from boiling isopropyl acetate in a
25 Soxhlet apparatus.

A solution of the pyridinylmethyl intermediate in 0.1N HCl is hydrogenated with a platinum catalyst at 60 lb/in² H_2 pressure. The catalyst is generated by brief hydrogenation of PtO_2 in 0.1N HCl. When the reaction is
30 complete, the catalyst is removed by filtration under N_2 pressure, and the filtrate is evaporated. A solution of the residue in the minimum amount of ethanol is diluted slowly with a large amount of EtO_2 , and the hydrochloride

salt is obtained as a white hygroscopic solid of the compound (IC).

EXAMPLE 9

The compound (IC) of Example 8 is tested for enzyme inhibition activity. A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which PNP activity (IC_{50}) for the compound is observed, which is determined radiochemically by measuring the formation of [^{14}C]-hypoxanthine from [^{14}C]-inosine (see Biomedicine, 33, 39 (1980)) using calf spleen as the enzyme source.

EXAMPLES 10-14

The following compounds of the present invention are prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-ones wherein R is $-CH_2R_1$ in which the R_1 group is as follows:

Example 10 R_1 = 2-methyl-3-piperidinyl

Example 11 R_1 = 2-chloro-3-piperidinyl

Example 12 R_1 = 2-trifluoromethyl-3-piperidinyl

Example 13 R_1 = 2-methoxy-3-piperidinyl

Example 14 R_1 = 2-fluoro-3-piperidinyl

The compounds are prepared following the procedures set forth in Examples 1-8 using the appropriate 3-(substituted 3-pyridinyl)-propionitriles as starting materials.

EXAMPLE 15

3-(2-Pyridinyl)propionitrile is prepared in this example using the procedure of V. Boekelheide, et al., J. Am. Chem. Soc., 75, 3243 (1953). A solution of potassium cyanide (83.74 g) in water (160 ml) is added to a solution of freshly distilled 2-vinylpyridine (67.59 g) in acetic anhydride (131.30 g) with the rate of addition adjusted to maintain gentle refluxing. When the addition is complete, the resulting dark red mixture is heated for about 17 hours at 105°C in an oil bath with vigorous stirring. The cooled reaction mixture is adjusted to pH 8 with a saturated

Na₂CO₃ solution. The mixture is extracted with CHCl₃ (4 x 150 ml), washed with water, dried with Na₂SO₄, and evaporated to a dark viscous oil. The oil is fractionally distilled in vacuo through a Vigreux column. After a
5 small forerun containing 2-vinylpyridine (0.35 g), the desired 3-(2-pyridinyl)propionitrile is collected as a clear, fluorescent yellow-green, viscous oil: yield 59.8 g; 70.4%; bp 86°C at 1.0 mm Hg.

EXAMPLES 16-20

10 The following compounds of the present invention are prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-ones wherein R is -CH₂R₁ in which the R₁ group is as follows:

Example 16 R₁ = 2-piperidinyl

15 Example 17 R₁ = 4-piperidinyl

Example 18 R₁ = 3-trifluoromethyl-4-piperidinyl

Example 19 R₁ = 3-methoxy-2-piperidinyl

Example 20 R₁ = 3-fluoro-4-piperidinyl

The compounds are prepared following the procedures set
20 forth in Examples 1-8 and 15 using the appropriate 3-(2- or 4-pyridinyl)-propionitriles as starting materials.

EXAMPLE 21

A pharmaceutical composition for intraperitoneal injection is prepared for testing the compound (IC). An
25 intraperitoneal injection solution containing the compound of Example 8 is dissolved in an aqueous carrier that contains ten percent DMSO.

EXAMPLE 22.

The compound (IC) is intraperitoneally injected into
30 Lewis Rats via the test composition of Example 21 to provide 30 mg of the compound (IC), with an injection given twice per day. Controls are used, which receive only the vehicle. At specific times after administration, the animals are sacrificed and plasma samples are prepared.

The plasma is extracted with cold 0.5 N HClO₄ and neutralized with solid NH₄HCO₃. After removal of perchlorate salts, the extract is subjected to HPLC on a reversed phase column (Spherisorb ODSI). A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compound (IC).

EXAMPLES 23-33

Compounds prepared as in Examples 10-20 are each made into a pharmaceutical formulation in accordance with the preparation of Example 21 and the resultant injectable solutions are tested in accordance with the procedure of Example 22. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compounds of the present invention.

EXAMPLE 34

3-(2-Furanyl)propionitrile is prepared in this example. In a three-neck flask fitted with a condenser and drying tube, magnesium turnings (20 g) are added to a solution of 3-(2-furanyl)acrylonitrile (67.2 g) in dry methanol (2 l). Additional magnesium is added in parts as the reaction rate would allow until a total of 145 g has been added. After about five hours, the solvent is evaporated until the contents set to a solid paste, which is adjusted to a pH of about 6.5 with 6N HCl with cooling. The mixture is extracted with several portions of CHCl₃, dried with Na₂SO₄, and concentrated to a dark oil. Distillation in vacuo through a short Vigreux column gives the propionitrile as a colorless oil; yield 49.5 g (72%); bp 46.0-46.5°C at 0.5 mm.

EXAMPLE 35

3-(2-Furanyl)propionitrile of Example 34 is further treated in the synthesis of the present invention. Under an atmosphere of dry N₂, the propionitrile (48.7 g), sodium hydride (10.28 g), ethyl formate (32.74 g), and anhydrous

tetrahydrofuran (200 ml) are stirred at room temperature with protection from moisture for about 18 hours. The volatile matter is then evaporated, the resulting yellow solid is dissolved in about 20 ml of cold water with ice-bath cooling, and the solution is adjusted to a pH of 6.0 with cold 6N HCl. The resulting heavy oil precipitate is extracted into CHCl_3 , and the extract is washed with water, dried with Na_2SO_4 , and evaporated to give a thin oil which contains the crude formyl compound; yield 53.06 g.

10

EXAMPLE 36

Glycine methyl ester hydrochloride (67.05 g) and anhydrous sodium acetate (43.79 g) are added to a solution of the crude formyl compound of the previous example (53.05 g) in $\text{MeOH}/\text{H}_2\text{O}$ (4:1, 1500 ml). After 24 hours, the MeOH is evaporated in vacuo, and the mixture of water and oil is extracted with CHCl_3 . The CHCl_3 layer is dried (Na_2SO_4) and evaporated to give an amber oil (59.1 g).

EXAMPLE 37

Under a nitrogen atmosphere, ethyl chloroformate (43.68 g) is added dropwise to a solution of the amber oil of Example 36 (59.1 g) and DBN (133 g) in dry CH_2Cl_2 (400 ml) with external cooling in an ice bath. After stirring at 0 °C for one hour, the solution is allowed to stand at room temperature overnight. After checking progress by TLC, additional ClCO_2Et and DBN are added to complete the conversion, and the solution is allowed to stand for 24 hours. Volatile matter is evaporated in vacuo, the viscous residue purified on a silica gel column (whose main purpose is to remove the less-mobile DBN) to give an N-blocked pyrrole, a corresponding pyrrole without the ethoxycarbonyl blocking group on the pyrrole nitrogen, and the starting propionitrile.

25
30

EXAMPLE 38

To a solution of the N-blocked pyrrole of Example 37 (11.66 g) in MeOH (200 ml) is added solid Na_2CO_3 (4.23 g), and the reaction mixture is stirred at room temperature for 5 24 hr with separation of the resultant deblocked pyrrole. The mixture is evaporated to dryness, and the residue is triturated thoroughly with H_2O (200 ml) to dissolve inorganics and extracted with CHCl_3 (3 x 200 ml). The extract is dried (Na_2SO_4) and evaporated to give a viscous 10 gum that crystallized upon drying in vacuo for use as an intermediate without further purification. More extensive purification can, however, be effected by using either column chromatography employing silica gel/ CHCl_3 or recrystallization from toluene/cyclohexane (1:3).

15

EXAMPLE 39

Benzoyl isothiocyanate (4.58 g) is added dropwise to a solution of the unblocked pyrrole of Example 38 (5.15 g) in dry CH_2Cl_2 (75 ml). After 1 h at room temperature, solution is evaporated, and the gummy residue is dissolved 20 in Et_2O (100 ml) with almost immediate separation of the crystalline solid, which is collected by filtration. The Et_2O filtrate is heated to boiling and diluted with an equal volume of warm cyclohexane. On cooling slowly the solution gives additional thioureido product.

25

EXAMPLE 40

Methyl iodide (8.70 g) is added to a solution of the thioureido product of Example 39 (10.68 g) and DBN (4.15 g) in dry CH_2Cl_2 (250 ml) at 0 °C. The solution is stirred at 0 °C for 15 min., at ambient temperature for 1 h, and then 30 evaporated in vacuo. A solution of the residue in CHCl_3 is chromatographed on a silica gel column with CHCl_3 as eluent to give homogeneous fractions of the methylthio intermediate compound.

EXAMPLE 41

A solution of the methylthio compound of Example 40 (0.80 g, 2.0 mmole) in 25 ml of MeOH that has been saturated with NH_3 at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a mixture of the 2-amino compound, benzamide and a by-product that is a 2-methylthio derivative, as opposed to the 2-amino compound. The mixture is stirred vigorously for several minutes with 30 ml of 2:1 Et_2O /cyclohexane, and the insoluble white solid is filtered off and washed with Et_2O . The filtrate contained most of the benzamide and 2-methylthio components. A solution of the Et_2O /cyclohexane-insoluble solid (0.425 g) in MeOH is evaporated with appr. 10 g of silica gel. The powdered residue is layered evenly onto the top of a silica gel column, which is then eluted with $\text{CHCl}_3/\text{MeOH}/\text{HOAc}$ (95:5:1) to give the 2-methylthio by-product and the desired furanyl intermediate. The intermediate is recrystallized by extraction into boiling isopropyl acetate in a Soxhlet apparatus. The white crystals are collected in three crops and dried in vacuo over P_2O_5 at 110°C for 7h.

A solution of the intermediate (116 g, 0.5 mmole) in methanol (50 ml) is hydrogenated with 30% palladium-on-charcoal (40 mg) at 62 lb/in² H_2 pressure for about 36 hours. The catalyst is removed by filtration under N_2 pressure, and the filtrate is evaporated and reevaporated with toluene. The solid residue is recrystallized by extraction into boiling isopropyl acetate in a Soxhlet apparatus. The 2-tetrahydrofuranyl compound (IE) is obtained as a white crystalline solid, which is dried in vacuo over P_2O_5 at 110°C for about six hours; yield 73 mg (61.8%); m.p. 284-286°C dec.

EXAMPLE 42

The product of Example 41 is tested for enzyme inhibition activity according to the procedure of Example 9, and PNP activity (IC₅₀) for the compound is observed.

5

EXAMPLE 43

A pharmaceutical composition for intraperitoneal injection is prepared for testing the compound (IE). An intraperitoneal injection solution containing the compound of Example 41 is dissolved in an aqueous carrier that
10 contains ten percent DMSO.

EXAMPLE 44

Using the procedure of Example 16, the compound (IE) is intraperitoneally injected into Lewis Rats via the test composition of Example 43 to provide 30 mg of the compound
15 (IE) and the results are analyzed compared to controls. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compound (IE).

EXAMPLES 45-49

The following compounds of the present invention are
20 prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-ones wherein R is -CH₂-R₁ in which the R₁ group is as follows:

Example 45 R₁ = 3-tetrahydrofuranyl

Example 46 R₁ = 3-chloro-2-tetrahydrofuranyl

25 Example 47 R₁ = 3-trifluoromethyl-2-tetrahydrofuranyl

Example 48 R₁ = 3-methoxy-3-tetrahydrofuranyl

Example 49 R₁ = 3-fluoro-2-tetrahydrofuranyl

The compounds are prepared following the procedures set forth in Examples 34-41 using the appropriate 3-(furanyl)-
30 acrylonitriles as starting materials.

EXAMPLE 50

Under a nitrogen atmosphere, a solution of the tetrahydrofuranyl compound (IE) (500 mg) obtained from Example 41 and a crystal of phenol in 2N HBr (20 ml) is

stirred for 18 hours at 40°C. The solvent is evaporated in vacuo at low temperature, and the residue is washed with a few ml of Et₂O by decantation to remove any tribromophenol. A suspension of the residue in H₂O is adjusted to pH 7 with
5 1N NaOH to give the bromoalcohol as its free base. The solid is collected, washed with cold water, and dried at room temperature..

A solution of the bromoalcohol in anhydrous dimethylacetamide is chilled in an ice bath and treated with an equimolar portion of PBr₃. The solution is allowed to stir at room temperature for one hour, then the dimethylacetamide is evaporated in vacuo at low temperature using an air pump and dry-ice trap. A suspension of the residue in ice/water is adjusted to pH 7 using 1N NaOH to
15 give the crude reaction product containing the 1,4-dibromo compound. After filtration, washing with cold water, and drying at room temperature, the resulting product is refluxed with sodium sulfide in 50% ethanol/water, or alternatively warmed with Na₂S in N,N-dimethylacetamide
20 solution; the solvent removed under vacuum, the residue washed with water to remove NaBr, and the pH adjusted to about 7 to precipitate the 2-tetrahydrothienyl compound (IG).

EXAMPLE 51

25 The compound of Example 50 is tested for enzyme inhibition activity as in Example 9, and PNP activity (IC₅₀) for the compound is observed.

EXAMPLE 52

A pharmaceutical composition for intraperitoneal
30 injection is prepared for testing the compound (IG). An intraperitoneal injection solution is made containing the compound of Example 50 is dissolved in an aqueous carrier that contains ten percent DMSO.

EXAMPLE 53

Using the procedure of Example 16, the compound (IG) is intraperitoneally injected into Lewis Rats via the test composition of Example 52 to provide 30 mg of the compound
5 (IG) and the results are analyzed compared to controls. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compound (IG).

EXAMPLES 54-58

The following compounds of the present invention are
10 prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-ones wherein R is -CH₂-R₁ in which the R₁ group is as follows:

Example 54 R₁ = 3-tetrahydrothienyl

Example 55 R₁ = 3-chloro-2-tetrahydrothienyl

15 Example 56 R₁ = 3-trifluoromethyl-2-tetrahydrothienyl

Example 57 R₁ = 3-methoxy-2-tetrahydrothienyl

Example 58 R₁ = 3-fluoro-2-tetrahydrothienyl

The compounds are prepared following the procedures set forth in Examples 34-41 and 50 using the appropriate 3-
20 (furanyl)-acrylonitriles as starting materials.

EXAMPLE 59

Using the procedure of Example 1, 3-(2-pyrrolyl)-propionitrile is prepared using pyrrole-2-carboxaldehyde as the starting material. Following Examples 2-8, the
25 intermediate 2-amino-7-(2-pyrrolylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one is prepared from the propionitrile, from which the 2-pyrrolidinyl compound (II) is obtained by reduction of the intermediate.

EXAMPLE 60

30 Using the procedure of Example 1, 3-(3-pyrrolyl)-propionitrile is prepared using pyrrole-3-carboxaldehyde as the starting material. Following Examples 2-8, the intermediate 2-amino-7-(3-pyrrolylmethyl)-3H,5H-pyrrolo-[3,2-d]pyrimidin-4-one is prepared from the propionitrile,

from which the 3-pyrrolidinyl compound (IJ) is obtained by reduction of the intermediate.

EXAMPLE 61

Using the procedure of Example 1, 3-(2-pyranyl)
5 propionitrile is prepared using 2-pyrancarboxaldehyde as the starting material. Following Examples 2-8, the intermediate 2-amino-7-(2-pyranylmethyl)-3H,5H-pyrrolo-[3,2-d]pyrimidin-4-one is prepared from the propionitrile, from which the 2-tetrahydro-pyranyl compound (IL) is
10 obtained by reduction of the intermediate.

EXAMPLE 62

Using the procedure of Example 1, 3-(3-pyranyl)
propionitrile is prepared using 3-pyrancarboxaldehyde as the starting material. Following Examples 2-8, the
15 intermediate 2-amino-7-(3-pyranylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one is prepared from the propionitrile, from which the 3-tetrahydro-pyranyl compound (IM) is obtained by reduction of the intermediate.

EXAMPLE 63

20 Using the procedure of Example 1, 3-(4-pyranyl)
propionitrile is prepared using 4-pyrancarboxaldehyde as the starting material. Following Examples 2-8, the intermediate 2-amino-7-(4-pyranylmethyl)-3H,5H-pyrrolo-[3,2-d]pyrimidin-4-one is prepared from the propionitrile,
25 from which the 4-tetrahydro-pyranyl compound (IN) is obtained by reduction of the intermediate.

EXAMPLE 64

A solution of the methylthio compound of Example 7
(0.358 g, 0.85 mmole) in 100 ml of MeOH that has been
30 saturated with NH₃ at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a mixture of the desired 2-amino intermediate compound, benzamide, and a by-product that is a 2-methylthio

derivative, as opposed to the 2-amino compound. The mixture is dissolved in methanol and the solution is evaporated with silica gel (about 5 g). The mixture is then carefully layered onto the top of a silica-gel chromatography column, which is then eluted with $\text{CHCl}_3/\text{MeOH}$ (9:1) to give the methylthio by-product and the desired 2-amino-7-(3-pyridinylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one, compound (IIA). Further purification is obtained by recrystallization from boiling isopropyl acetate in a Soxhlet apparatus.

EXAMPLE 65

The compound (IIA) of Example 64 is tested for enzyme inhibition activity. A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which PNP activity (IC_{50}) for the compound is observed, which is determined radiochemically by measuring the formation of $[^{14}\text{C}]$ -hypoxanthine from $[^{14}\text{C}]$ -inosine (see Biomedicine, 33, 39 (1980) using calf spleen as the enzyme source.

EXAMPLES 66-68

The following compounds of the present invention are prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-ones wherein R is $-\text{CH}_2-\text{R}_1$ in which the R_1 group is as follows:

Example 66 $\text{R}_1 = 2\text{-pyridinyl}$

25 Example 67 $\text{R}_1 = 4\text{-pyridinyl}$

Example 68 $\text{R}_1 = 3\text{-chloro-2-pyridinyl}$

The compounds are prepared following the procedures set forth in Examples 1-7 and 64 using the appropriate 3-(pyridinyl)-propionitriles as starting materials.

30

EXAMPLE 69

A pharmaceutical composition for intraperitoneal injection is prepared for testing the compound (IIA). An intraperitoneal injection solution containing the compound

of Example 64 is dissolved in an aqueous carrier that contains ten percent DMSO.

EXAMPLE 70

The compound (IIA) is intraperitoneally injected into
5 Lewis Rats via the test composition of Example 69 to provide 30 mg of the compound (IIA), with an injection given twice per day. Controls are used, which receive only the vehicle. At specific times after administration, the animals are sacrificed and plasma samples are prepared.
10 The plasma is extracted with cold 0.5 N HClO_4 and neutralized with solid NH_4HCO_3 . After removal of perchlorate salts, the extract is subjected to HPLC on a reversed phase column (Spherisorb ODSI). A significant increase in plasma inosine is observed in the plasma taken
15 from animals receiving the compound (IIA).

EXAMPLES 71-73

Compounds prepared as in Examples 66-68 are each made into a pharmaceutical formulation in accordance with the preparation of Example 69 and the resultant injectable
20 solutions are tested in accordance with the procedure of Example 70. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compounds of the present invention.

EXAMPLE 74

25 1-Cyclohexenylacetonitrile is treated in the synthesis of the present invention. Under an atmosphere of dry N_2 , a solution of 1-cyclohexenylacetonitrile (9.2 g; 75.92 mmole) in anhydrous tetrahydrofuran (THF, 10 ml) is added to a stirred mixture of sodium hydride (3.18 g; 132.86 mmole)
30 and ethylformate (30.14 g; 406.93 mmole) in 50 ml THF, and the resulting mixture is stirred at room temperature for about 18 hours. Volatile matter is evaporated in vacuo at room temperature. Water (30 ml) is added to the residue at 0°C , and the solution adjusted to a pH of 6 by dropwise

addition of 6N HCl. The resulting precipitate of heavy oil is extracted into CHCl_3 . The extract is washed with water and dried with Na_2SO_4 , and the resulting organic layer evaporated to give a crude formyl compound as a red-brown
5 oil (9.6 g).

EXAMPLE 75

Glycine methyl ester hydrochloride (16.68 g, 132.85 mmole) and anhydrous sodium acetate (10.89 g, 132.85 mmole) are added to a solution of the crude formyl compound (9.6
10 g) of Example 74 without further purification in $\text{MeOH}/\text{H}_2\text{O}$ (4:1, 150 ml). After 24 hours, the MeOH is evaporated in vacuo, and the mixture of water and oil is extracted with CHCl_3 . The CHCl_3 layer is dried (Na_2SO_4) and evaporated to give an amber oil which is applied to a silica gel column.
15 Elution with CHCl_3 gave the desired enamine: yield 4.5 g.

EXAMPLE 76

Under a nitrogen atmosphere, ethyl chloroformate (3.04 g; 28.06 mmole) is added dropwise to a solution of the enamine of Example 75 (4.12 g, 18.70 mmole) and 1,5-di-
20 azabicyclo[4.3.0]-non-5-ene (DBN, 6.96 g, 56.11 mmole) in dry CH_2Cl_2 (100 ml) with external cooling in an ice bath. After stirring at 0°C for one hour, the solution is allowed to stand at room temperature overnight. After checking progress by TLC, additional ClCO_2Et (0.5 ml) and
25 DBN (3.0 ml) are added to complete the conversion, and the solution is allowed to stand for 24 hours. Volatile matter is evaporated in vacuo, the viscous residue purified on a short silica gel column (whose main purpose is to remove the less-mobile DBN) to give an N-blocked pyrrole, which is
30 used for the next step without further purification.

EXAMPLE 77

To a solution of the N-blocked pyrrole of Example 76 (3.0 g, 10.26 mmole) in MeOH (100 ml) is added solid Na_2CO_3 (2.71 g, 25.65 mmole), and the reaction mixture is stirred

at room temperature for 48 hr with separation of the resultant deblocked pyrrole. The mixture is evaporated to dryness, and the residue is triturated thoroughly with H₂O (50 ml) to dissolve inorganics and extracted with CHCl₃ (3 x 50 ml). The extract is dried (Na₂SO₄) and evaporated to give a viscous gum, which is purified on a silica gel column using CHCl₃ as the eluent; yield 2.04 g; m.p. 125°C.

EXAMPLE 78

Benzoyl isothiocyanate (0.76g, 4.65 mmole) is added dropwise to a solution of the unblocked pyrrole of Example 77 (0.91 g, 4.13 mmole) in dry CH₂Cl₂ (30 ml). After 1 h at room temperature, the solution is evaporated, and the gummy residue is triturated with methanol to give a thioureido product; yield 0.70 g; m.p. 170°C.

EXAMPLE 79

Methyl iodide (0.678 g, 4.78 mmole) is added to a solution of the thioureido product of Example 78 (0.630 g, 1.64 mmole) and DBN (0.230 g, 1.85 mmole) in dry CH₂Cl₂ (50 ml) at 0 °C. The solution is stirred at 0 °C for 15 min., at ambient temperature for 1 h, and then evaporated in vacuo. A solution of the residue in CHCl₃ is chromatographed on a silica gel column with CHCl₃ as eluent to give homogeneous fractions of the methylthio intermediate; yield 0.7 g.

EXAMPLE 80

A solution of the methylthio compound of Example 79 (0.70 g, 1.76 mmole) in 50 ml of MeOH that has been saturated with NH₃ at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a mixture of the compound (IIIA), benzamide and a by-product that is a 2-methylthio derivative, as opposed to the 2-amino compound (IIIA). The mixture is stirred vigorously for several minutes with appr. 50 ml of Et₂O, and the

insoluble white solid is filtered off and washed with Et₂O. The filtrate contained most of the benzamide and 2-methylthio components. A solution of the Et₂O-insoluble solid (0.342 g) in MeOH is evaporated with appr. 10 g of silica gel. The powdered residue is layered evenly onto the top of a silica gel column, which is then eluted with CHCl₃/MeOH/HOAc (95:5:1) to give the 2-methylthio by-product and the desired 2-amino product (IIIA). (IIIA) is recrystallized by extraction into boiling isopropyl acetate in a Soxhlet apparatus. The white crystals are collected in three crops and dried in vacuo over P₂O₅ at 110 °C for 7h; yield 44%; mp 280 °C dec., anal. calcd. for C₁₂H₁₄N₄O·0.6H₂O: C, 59.78; H, 6.35; N, 23.23. Found: C, 59.98; H, 6.46; N, 23.15.

EXAMPLE 81

The compound of Example 80 is tested for enzyme-inhibition activity. A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which PNP activity for the compound is determined radiochemically by measuring the formation of [¹⁴C]-hypoxanthine from [¹⁴C]-inosine (see Biomedicine, 33, 39 (1980) using calf spleen as the enzyme source. At 1 mM phosphate the IC₅₀ is 1.9 μM, and at 50 mM phosphate the IC₅₀ is 19 μM.

EXAMPLE 82

Following the procedures set forth in Examples 74-80, compounds (IIIB) and (IIIC) are made using 2- and 3-cyclohexenyl-acetonitrile, respectively, as starting materials. The compounds are tested as in Example 81 and significant enzyme-inhibition activity is observed.

EXAMPLES 83-87

The following compounds of the present invention are prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-ones in which the R group is as follows:

Example 83 R = 3-methyl-2-cyclohexenyl

Example 84 R = 2-chloro-3-cyclohexenyl

Example 85 R = 3-trifluoromethyl-1-cyclohexenyl

Example 86 R = 3-methoxy-1-cyclohexenyl

Example 87 R = 2-fluoro-3-cyclohexenyl

- 5 The compounds are prepared following the procedures set forth in Examples 74-81 using the appropriate substituted cyclohexenyl acetonitriles as starting materials.

EXAMPLE 88

- 10 A pharmaceutical composition for intraperitoneal injection is prepared for testing the compound (IIIA). An intraperitoneal injection solution containing the compound (IIIA) is dissolved in an aqueous carrier that contains ten percent DMSO.

EXAMPLE 89

- 15 The compound (IIIA) is intraperitoneally injected into Lewis Rats via the test composition of Example 88 to provide 30 mg of the compound (IIIA), with an injection given twice per day. Controls are used, which receive only the vehicle. At specific times after administration, the
20 animals are sacrificed and plasma samples are prepared. The plasma is extracted with cold 0.5 N HClO_4 and neutralized with solid NH_4HCO_3 . After removal of perchlorate salts, the extract is subjected to HPLC on a reversed phase column (Spherisorb ODSI). A significant
25 increase in plasma inosine is observed in the plasma taken from animals receiving the compound (IIIA).

EXAMPLES 90-94

- Compounds prepared as in Examples 83-87 are each made into a pharmaceutical formulation in accordance with the
30 preparation of Example 88 and the resultant injectable solutions are tested in accordance with the procedure of Example 89. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compounds of the present invention.

EXAMPLE 95

The compound (IIID) is prepared using 2-amino-7-(1-cyclohexenyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one as an intermediate. A solution of the intermediate (0.2 g; 0.86 mmole) in ethanol (50 ml) is hydrogenated with 10% Pd-C (50 mg) at 45 lb/in² for 16 h and filtered hot through Celite. The filtrate is evaporated to dryness, and the residue is crystallized from hot ethanol to give the compound (IIID); yield 157 mg (78%), mp >300 °C dec. anal. calcd. for C₁₂H₁₆N₄O·0. EtOH: C, 61.80; H, 7.10; N, 23.51. Found: C, 61.95; H, 7.43; N, 23.56.

EXAMPLE 96

The compound (IIID) prepared in Example 95 is tested for enzyme-inhibition activity as in Example 81. At 1 mM phosphate the IC₅₀ is 1.3 μM, and at 50 mM phosphate the IC₅₀ is 145 μM.

EXAMPLE 97

3-(2-Adamantyl)propionitrile is prepared in this example using a modification of the procedure of M. Ohno, et al., J. Org. Chem. 53, 1285 (1988). A solution of 2-bromoadmantane (20 g; 92.96 mmole); Bu₃SnH (32.46 g; 111.5 mmole), acrylonitrile (9.86 g; 185.92 mmole), and AIBN (740 mg) in toluene (280 ml) is stirred at reflux temperature for 3 h. The reaction mixture is washed with ammonia water (0.4 M, 500 ml), the organic layer is washed with H₂O and dried over MgSO₄ and evaporated. The residue is distilled between 110-118°C (and about 0.2 mmHg); fractions are combined to give a crude sample of contaminated 3-(2-adamantyl)propionitrile with tin complexes, which is purified on silica gel column with hexanes; followed by hexanes/ethylacetate 97:3 and hexanes/ethylacetate 95:5, yield 9.4 g (53.4%); mp semi-solid.

EXAMPLE 98

3-(2-Adamantyl)propionitrile of Example 97 is further treated in the synthesis of the present invention. Under an atmosphere of dry N_2 , a mixture of 3-(2-adamantyl) propionitrile (7.0 g, 36.99 mmole), sodium hydride (1.7 g, 73.95 mmole), and anhydrous tetrahydrofuran (75 ml) is heated at 52°C in a water bath for 15 min., and a solution of ethyl formate (13.69 g, 184.89 mmole) in THF (100 ml) is added over a period of 45 min. After two hours at 50 - 55 °C, a second portion of NaH (0.8 g) and HCO_2Et (7.5 ml) are added, and the reaction mixture is stirred for about two days. A third portion of HCO_2Et (7.5 ml) and NaH (0.8 g) are added and left at room temperature for about 24 hours (unreacted nitrile is inert in the next step and can be recovered at the first purification step). The thick paste is stirred overnight and allowed to cool to room temperature. Volatile matter is evaporated under reduced pressure, and the residual pale yellow crust is dissolved in the minimum volume of cold water (appr. 150 ml) at 0 °C. The solution is adjusted to pH 6.0 by addition of 6N HCl and extracted with $CHCl_3$ (3 x 100 ml). The extract is washed with H_2O , dried over Na_2SO_4 , and evaporated in vacuo to a thick amber oil. This crude product is used in the next reaction without further purification.

EXAMPLE 99

Glycine methyl ester hydrochloride (6.96 g, 55.46 mmole) and anhydrous sodium acetate (4.55 g, 55.46 mmole) are added to a solution of the crude formyl compound (8.0 g) in $MeOH/H_2O$ (4:1, 500 ml). After 24 hours, the $MeOH$ is evaporated in vacuo, and the mixture of water and oil is extracted with $CHCl_3$. The $CHCl_3$ layer is dried (Na_2SO_4) and evaporated to give an amber oil which is applied to a silica gel column. Elution with $CHCl_3$ gave two major bands: (1) 3-(2-adamantyl)-propionitrile (used as starting

material in the previous step), and (2) the desired enamine; yield 6 g.

EXAMPLE 100

Under a nitrogen atmosphere, ethyl chloroformate (2.82 g, 5 26.0 mmole) is added dropwise to a solution of the enamine of Example 99 (5.0 g, 17.34 mmole) and 1,5-diazabicyclo-[4.3.0]non-5-ene ("DBN," 6.46 g, 52.0 mmole) in dry CH_2Cl_2 (50 ml) with external cooling in an ice bath. After stirring at 0 °C for one hour, the solution is allowed to a 10 stand at room temperature overnight. After checking progress by TLC, additional ClCO_2Et (1.81 ml) and DBN (3.23 g) are added to complete the conversion, and the solution is allowed to stand for 24 hours. Volatile matter is evaporated in vacuo, the viscous residue purified on a 15 short silica gel column (whose main purpose is to remove the less-mobile DBN) to give an N-blocked pyrrole, which is used for the next step without further purification.

EXAMPLE 101

To a solution of the crude N-blocked pyrrole of Example 100 (6.0 g, 16.59 mmole) in MeOH (100 ml) is added solid Na_2CO_3 (4.39 g, 41.49 mmole), and the reaction mixture is stirred at room temperature for 48 hr with separation of the resultant deblocked pyrrole. The mixture is evaporated to dryness, and the residue is triturated thoroughly with 25 H_2O (50 ml) to dissolve inorganics and extracted with CHCl_3 (3 x 100 ml). The extract is dried (Na_2SO_4) and evaporated to give a viscous gum, which crystallized by triturating with ether; yield 4 g; m.p. 162-163°C.

EXAMPLE 102

30 Benzoyl isothiocyanate (1.22 g, 7.47 mmole) is added dropwise to a solution of the unblocked pyrrole of Example 101 (1.91 g, 6.62 mmole) in dry CH_2Cl_2 (50 ml). After 1 h at room temperature, solution is evaporated, and the gummy residue is dissolved in Et_2O (100 ml) with almost immediate

separation of the crystalline solid. The Et₂O filtrate is heated to boiling and diluted with an equal volume of warm cyclohexane. On cooling slowly the solution gives additional thioureido product; yiled 2.81 g (95%); m.p.
5 193-194°C.

EXAMPLE 103

Methyl iodide (2.46 g, 17.39 mmole) is added to a solution of the thioureido product of Example 102 (2.7 g, 5.98 mmole) and DBN (0.82 g, 6.57 mmole) in dry CH₂Cl₂ (50
10 ml) at 0 °C. The solution is stirred at 0 °C for 15 min., at ambient temperature for 1 h, and then evaporated in vacuo to give a crude sample of the methythio intermediate compound; yield 2.78 g (crude).

EXAMPLE 104

15 A solution of the methylthio compound of Example 103 (2.78 g, 5.18 mmole) in 150 ml of MeOH that has been saturated with NH₃ at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a
20 mixture of the compound (IVA), benzamide and a by-product that is a 2-methylthio derivative, as opposed to the 2-amino compound (IVA). The mixture is stirred vigorously for several minutes with appr. 75 ml of Et₂O, and the insoluble white solid is filtered off and washed with Et₂O.
25 The filtrate contained most of the benzamide and 2-methylthio components. A solution of the Et₂O-insoluble solid (1.38 g) in MeOH is evaporated with appr. 25 g of silica gel. The powdered residue is layered evenly onto the top of a silica gel column, which is then eluted with
30 CHCl₃/MeOH/HOAc (95:5:1) to give the 2-methylthio by-product and the desired 2-amino product (IVA). (IVA) is recrystallized by extraction into boiling isopropyl acetate in a Soxhlet apparatus. The white crystals are collected in three crops and dried in vacuo over P₂O₅ at 110 °C for

7h; yield 51.6%; mp >350 °C dec.; anal. calcd. for $C_{17}H_{22}N_4O \cdot 0.21MeOH \cdot 0.22H_2O$: C, 66.88; H, 7.59; N, 18.12. Found: C, 66.86; H, 7.59; N, 18.12.

EXAMPLE 105

- 5 The compound of Example 104 is tested for enzyme inhibition activity. A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which the PNP activity (IC_{50}) for the compound is found, which is determined radiochemically by measuring the formation of [^{14}C]-
 10 hypoxanthine from [^{14}C]-inosine (see Biomedicine, 33, 39 (1980)) using calf spleen as the enzyme source. At 1 mM phosphate the IC_{50} is 0.090 μM , and at 50 mM phosphate the IC_{50} is 2.5 μM .

. EXAMPLES 106-110

- 15 The following compounds of the present invention are prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-ones wherein R is $-CH_2-R_1$ in which the R_1 group is a 2-adamantyl group as follows:

Example 106 R_1 = 2-(1-methyl)-adamantyl

- 20 Example 107 R_1 = 2-(1-chloro)-adamantyl

Example 108 R_1 = 2-(1-trifluoromethyl)-adamantyl

Example 109 R_1 = 2-(1-methoxy)-adamantyl

Example 110 R_1 = 2-(1-fluoro)-adamantyl

- 25 The compounds are prepared following the procedures set forth in Examples 98-104 using the appropriate 3-(2-adamantyl)-propio-nitriles as starting materials.

EXAMPLES 111-116

- 30 The following compounds of the present invention are prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-ones wherein R is $-CH_2-R_1$ in which the R_1 group is a 1-adamantyl group as follows:

Example 111 R_1 = 1-(2-methyl)-adamantyl

Example 112 R_1 = 1-(2-chloro)-adamantyl

Example 113 R_1 = 1-(2-trifluoromethyl)-adamantyl

Example 114 R_1 = 1-(2-methoxy)-adamantyl

Example 115 R_1 = 1-(2-fluoro)-adamantyl

Example 116 R_1 = 1-adamantyl

The compounds are prepared following the procedures set forth in Examples 98-104 using the appropriate 3-(1-adamantyl)-propio-nitriles as starting materials.

EXAMPLE 117

A pharmaceutical composition for intraperitoneal injection is prepared for testing the compound (IVA). An intraperitoneal injection solution containing the compound (IVA) is dissolved in an aqueous carrier that contains ten percent DMSO.

EXAMPLE 118

The compound (IVA) is intraperitoneally injected into Lewis Rats via the test composition of Example 117 to provide 30 mg of the compound (IVA), with an injection given twice per day. Controls are used, which receive only the vehicle. At specific times after administration, the animals are sacrificed and plasma samples are prepared. The plasma is extracted with cold 0.5 N HClO_4 and neutralized with solid NH_4HCO_3 . After removal of perchlorate salts, the extract is subjected to HPLC on a reversed phase column (Spherisorb ODSI). A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compound (IVA).

EXAMPLES 119-129

Compounds prepared as in Examples 106-116 are each made into a pharmaceutical formulation in accordance with the preparation of Example 117 and the resultant injectable solutions are tested in accordance with the procedure of Example 118. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compounds of the present invention.

EXAMPLE 130

3-Cyclopentylpropionitrile is prepared in this example. 3-Cyclopentylpropionyl chloride (57.7 g, 0.36 mole) is added dropwise to a large excess of concentrated ammonium hydroxide (400 ml) cooled in an ice/salt bath. The heavy suspension of white solid is stirred overnight, collected by filtration, washed with cold water, and recrystallized from about 2 liters of boiling water. The lustrous white plates of the amide are dried in vacuo over P_2O_5 ; yield 31.6 g (62.3%); mp 122 °C.

With protection from atmospheric moisture, a solution of the amide (23.5 g, 0.166 mole) in $POCl_3$ (150 ml) is heated at 120 °C for 1 h. The oil bath is cooled to about 70 °C, and excess $POCl_3$ is distilled off under vacuum, and the cooled residue is poured onto ice (about 300 g). The mixture is neutralized by cautious addition of solid Na_2CO_3 and extracted with several portions of Et_2O . The dried (Na_2SO_4) extract is evaporated to give a clear, pale yellow oil which is distilled in vacuo to give the desired nitrile; yield, 16.86 g (82%) bp 88.0 - 88.5 °C/8.7 mm). MS (EI): m/z 122 ($M-H$)⁺; IR (cap. film), $2245cm^{-1}$ (CN); ^1H NMR, δ 1.67 (q, 2, $-CH_2CH_2CN$), 2.36 (t, 2, $-CH_2CN$), complex multiplets centered about 1.11, 1.63, 1.86 (cyclopentyl protons).

25

EXAMPLE 131

3-Cyclopentylpropionitrile of the previous example is further treated in the synthesis of the present invention. Under an atmosphere of dry N_2 , a mixture of 3-cyclopentylpropionitrile (14.8 g, 0.12 mole), sodium hydride (5.8, 0.24 mole), and anhydrous tetrahydrofuran (300 ml) is heated at 52 °C in a water bath for 15 min., and a solution of ethyl formate (13.3 g, 0.18 mole) in THF (100 ml) is added over a period of 45 min. After two hours at 50 - 55 °C, a second portion of NaH (1.9 g) and HCO_2Et (5.0 ml) are

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added, followed in 30 min. by a third portion of HCO_2Et (unreacted nitrile is inert in the next step and can be recovered at the first purification step). The thick paste is stirred overnight and allowed to cool to room temperature. Volatile matter is evaporated under reduced pressure, and the residual pale yellow crust is dissolved in the minimum volume of cold water (about 150 ml) at 0°C . The solution is adjusted to pH 6.0 by addition of 6N HCl and extracted with CHCl_3 (3 x 100 ml). The extract is washed with H_2O , dried over Na_2SO_4 , and evaporated in vacuo to a thick amber oil. This crude product (15.6 g) is used in the next reaction without further purification.

EXAMPLE 132

Glycine methyl ester hydrochloride (19.31 g, 0.154 mole) and anhydrous sodium acetate (12.61 g, 0.154 mole) are added to a solution of the crude formyl compound of the previous example (15.6 g) in $\text{MeOH}/\text{H}_2\text{O}$ (4:1, 500 ml). After 24 hours, the MeOH is evaporated in vacuo, and the mixture of water and oil is extracted with CHCl_3 . The CHCl_3 layer is dried (Na_2SO_4) and evaporated to give an amber oil which is applied to a silica gel column. Elution with CHCl_3 gave two major bands: (1) 3-cyclopentylpropionitrile (8.22 g, 66.7 mmole or 55.6% of the nitrile used as starting material in the previous step), and (2) the desired enamine (3.45 g; 29.1% based on theoretical yield corrected for amount of nitrile present in starting material; MS (FAB): 223 ($\text{M} + \text{H}$) $^+$).

EXAMPLE 133

Under a nitrogen atmosphere, ethyl chloroformate (2.53 g, 23.3 mmole) is added dropwise to a solution of the enamine of the previous example (3.45 g, 15.5 mmole) and DBN (5.78 g, 46.6 mmole) in dry CH_2Cl_2 (50 ml) with external cooling in an ice bath. After stirring at 0°C for one hour, the solution is allowed to stand at room temperature

overnight. After checking progress by TLC, additional ClCO_2Et (0.5 ml) and DBN (3.0 ml) are added to complete the conversion, and the solution is allowed to stand for 24 hours. Volatile matter is evaporated in vacuo, the viscous
5 residue purified on a short silica gel column (whose main purpose is to remove the less-mobile DBN) to give an N-blocked pyrrole (4.50 g; 98%), which is used for the next step without further purification.

EXAMPLE 134

10 To a solution of the N-blocked pyrrole of the previous example (4.50 g, 15.3 mmole) in MeOH (100 ml) is added solid Na_2CO_3 (1.62 g, 15.3 mmole), and the reaction mixture is stirred at room temperature for 48 hr with separation of the resultant deblocked pyrrole. The mixture is evaporated
15 to dryness, and the residue is triturated thoroughly with H_2O (50 ml) to dissolve the inorganics and extracted with CHCl_3 (3 x 100 ml). The extract is dried (Na_2SO_4) and evaporated to give a viscous gum that crystallized upon drying in vacuo; yield 2.97 g (87.4%) of material suitable
20 for use as an intermediate without further purification. More extensive purification can, however, be effected by using either column chromatography employing silica gel/ CHCl_3 or recrystallization from toluene/cyclohexane (1:3).

25

EXAMPLE 135

Benzoyl isothiocyanate (2.62 g, 16.03 mmole) is added dropwise to a solution of the unblocked pyrrole of Example 134 (2.97 g, 13.36 mmole) in dry CH_2Cl_2 (100 ml). After 1 h at room temperature, solution is evaporated, and the
30 gummy residue is dissolved in Et_2O (100 ml) with almost immediate separation of crystalline solid; yield 1.75 g. The Et_2O filtrate is heated to boiling and diluted with an equal volume of warm cyclohexane. On cooling slowly the solution gave an additional 1.58 g of thioureido product;

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total yield 3.33 g (64.6%). A small amount of the thioureido product is recrystallized from warm Et₂O/cyclohexane (15 ml each); mp 123 - 125 °C. MS (FAB): 386 (M + H)⁺. Anal. Calcd, for C₂₀H₂₃N₃O₃S·0.45C₆H₁₂: C, 64.40; H, 6.76; N, 9.93. Found: C, 64.51; H, 7.10; N, 9.93.

EXAMPLE 136

Methyl iodide (2.60 g, 18.32 mmole) is added to a solution of the thioureido product of Example 135 (3.21 g, 8.33 mmole) and DBN (1.24 g, 9.99 mmole) in dry CH₂Cl₂ (80 ml) at 0 °C. The solution is stirred at 0 °C for 15 min., at ambient temperature for 1 h, and then evaporated in vacuo. A solution of the residue in CHCl₃ is chromatographed on a silica gel column with CHCl₃ as eluent to give homogeneous fractions of the methylthio intermediate compound; yield, 2.46 g (74%).

EXAMPLE 137

A solution of the methylthio compound of Example 136 (2.07 g, 5.18 mmole) in 150 ml of MeOH that has been saturated with NH₃ at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a mixture of the compound (IVC), benzamide and a by-product that is a 2-methylthio derivative, as opposed to the 2-amino compound (IVC). The mixture is stirred vigorously for several minutes with appr. 75 ml of Et₂O, and the insoluble white solid is filtered off and washed with Et₂O. The filtrate contained most of the benzamide and 2-methylthio components. A solution of the Et₂O-insoluble solid (1.13 g) in MeOH is evaporated with appr. 10 g of silica gel. The powdered residue is layered evenly onto the top of a silica gel column, which is then eluted with CHCl₃/MeOH/HOAc (95:5:1) to give the 2-methylthio by-product (252 mg; MS (FAB): 264 (M + H)⁺) and the desired

2-amino product (IVC) (679 mg, 56.4%). (IVC) is recrystallized by extraction into boiling isopropyl acetate in a Soxhlet apparatus. The white crystals are collected in three crops and dried in vacuo over P_2O_5 at 110 °C for 5 7h; yield 540 mg (44.9%); mp 324 - 326 °C dec.; MS (FAB): 233 ($M + H$)⁺; anal. calcd. for $C_{12}H_{16}N_4O$: C, 62.05; H, 6.94; N, 24.12. Found: C, 62.04; H, 7.11; N, 24.48.

EXAMPLE 138

The compound of Example 137 is tested for enzyme- 10 inhibition activity in accordance with the procedure of Example 9. At 1 mM phosphate the IC_{50} is 0.029 μM , and at 50 mM phosphate the IC_{50} is 1.8 μM .

EXAMPLES 139-142

The following compounds of the present invention are 15 prepared that are 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-ones wherein R is $-CH_2-R_1$ in which the R_1 group is as follows:

Example 139 R_1 = 3-methylcyclopentyl

Example 140 R_1 = 2-chlorocyclopentyl

20 Example 141 R_1 = 3-trifluoromethylcyclopentyl

Example 142 R_1 = 3-methoxycyclopentyl

The compounds are prepared following the procedures set 25 forth in Examples 130-137 using the appropriate 3-(substituted cyclopentyl)-propionitriles as starting materials.

EXAMPLE 143

A pharmaceutical composition for intraperitoneal injection is prepared for testing the compound (IVC). An intraperitoneal injection solution is prepared containing 30 the compound (IVC) is dissolved in an aqueous carrier that contains ten percent DMSO.

EXAMPLE 144

Using the procedure of Example 112, the compound (IVC) is intraperitoneally injected into Lewis Rats via the test composition of Example 143 and the results compared with
5 controls. A significant increase in plasma inosine is observed in the plasma taken from animals receiving the compound (IVC).

EXAMPLE 145

3-Cyclohexylpropionitrile is prepared in this example. A
10 solution of cyclohexanepropionic acid (50 g; 0.32 mole) and thionyl chloride (152 g; 1.28 mole) in 100 ml benzene is allowed to stand overnight and is then evaporated to an oily residue. The residue is added in portions to 28% aqueous ammonia (270 ml) at 25°C and the mixture stirred
15 for about two hours. The resulting product is collected by filtration, washed with cold water, and recrystallized from about 2 liters of boiling water. The lustrous white plates of the amide are dried in vacuo over P₂O₅; yield 45.5 g.

With protection from atmospheric moisture, a solution of
20 the amide (45.5 g, 0.293 mole) in SOCl₂ (200.3 g; 1.68 mole) refluxed for about six hours. The oil bath is cooled to about 70°C, and excess SOCl₂ is distilled off under vacuum, and the cooled residue is poured onto ice (about 300 g). The mixture is neutralized by cautious addition of
25 solid Na₂CO₃ and extracted with several portions of Et₂O. The dried (Na₂SO₄) extract is evaporated to give a clear, pale yellow oil which is distilled in vacuo to give the desired nitrile; yield 42.0 g.

EXAMPLE 146

30 3-Cyclohexylpropionitrile of Example 145 is further treated in the synthesis of the present invention. Under an atmosphere of dry N₂, a mixture of 3-cyclohexylpropionitrile (22.3 g, 0.16 mole), sodium hydride (5.38, 0.224 mole), and anhydrous tetrahydrofuran (120 ml) is

heated at 52 °C in a water bath for 15 min., and a solution of ethyl formate (55.4 g, 0.75 mole) in THF (50 ml) is added over a period of 45 min. After two hours at 50 - 55 °C, a second portion of NaH (2.0 g) and HCO₂Et (5.0 ml) are added (unreacted nitrile is inert in the next step and can be recovered at the first purification step), and the reaction mixture is stirred for about three days at 55 °C and then allowed to cool to room temperature. Volatile matter is evaporated under reduced pressure, and the residual pale yellow crust is dissolved in the minimum volume of cold water (about 75 ml) at 0 °C. The solution is adjusted to pH 6.0 by addition of 6N HCl and extracted with CHCl₃ (3 x 100 ml). The extract is washed with H₂O, dried over Na₂SO₄, and evaporated in vacuo to a thick amber oil. This crude product is used in the next reaction without further purification.

EXAMPLE 147

Glycine methyl ester hydrochloride (30.60 g, 0.24 mole) and anhydrous sodium acetate (19.99 g, 0.24 mole) are added to a solution of the crude formyl compound of the previous example (25.22 g) in MeOH/H₂O (4:1, 500 ml). After 24 hours, the MeOH is evaporated in vacuo, and the mixture of water and oil is extracted with CHCl₃. The CHCl₃ layer is dried (Na₂SO₄) and evaporated to give an amber oil which is applied to a silica gel column. Elution with CHCl₃ gave two major bands: (1) 3-cyclohexylpropionitrile (used as starting material in the previous step), and (2) the desired enamine; yield 16 g.

EXAMPLE 148

Under a nitrogen atmosphere, ethyl chloroformate (1.38 g, 12.7 mmole) is added dropwise to a solution of the enamine of Example 147 (2.0 g, 8.46 mmole) and DBN (2.1 g, 16.9 mmole) in dry CH₂Cl₂ (50 ml) with external cooling in an ice bath. After stirring at 0 °C for one hour, the

solution is allowed to stand at room temperature overnight. After checking progress by TLC, additional ClCO_2Et (0.5 ml) and DBN (1.5 ml) are added to complete the conversion, and the solution is allowed to stand for 24
5 hours. Volatile matter is evaporated in vacuo, the viscous residue purified on a short silica gel column (whose main purpose is to remove the less-mobile DBN) to give an N-blocked pyrrole, which is used for the next step without further purification.

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EXAMPLE 149

To a solution of the N-blocked pyrrole of Example 148 (2.6 g, 8.43 mmole) in MeOH (100 ml) is added solid Na_2CO_3 (2.23 g, 21.07 mmole), and the reaction mixture is stirred at room temperature for 48 hr with separation of the
15 resultant deblocked pyrrole. The mixture is evaporated to dryness, and the residue is triturated thoroughly with H_2O (50 ml) to dissolve inorganics and extracted with CHCl_3 (3 x 100 ml). The extract is dried (Na_2SO_4) and evaporated to give a viscous gum, which is purified on a silica gel
20 column using CHCl_3 as the eluent; yield 1.67 g (84%); m.p. 73-74°C.

EXAMPLE 150

Benzoyl isothiocyanate (0.74 g, 4.02 mmole) is added dropwise to a solution of the unblocked pyrrole of Example
25 149 (0.95 g) in dry CH_2Cl_2 (20 ml). After one hour at room temperature, the solution is evaporated, and the gummy residue is dissolved in Et_2O (100 ml) with almost immediate separation of crystalline solid. The Et_2O filtrate is heated to boiling and diluted with an equal volume of warm
30 cyclohexane. On cooling slowly the solution gives additional thioureido product; total yield 1.41 g (88%); m.p. 156-157°C.

EXAMPLE 151

Methyl iodide (1.1 g, 7.6 mmole) is added to a solution of the thioureido product of Example 150 (0.96 g, 2.61 mmole) and 1,5-diazabicyclo[4.3.0]non-5-ene (0.38 g, 3.0 mmole) in dry CH_2Cl_2 (20 ml) at 0°C. The solution is stirred at 0°C for 15 min., at ambient temperature for 1 h, and then evaporated in vacuo. A solution of the residue in CHCl_3 is chromatographed on a silica gel column with CHCl_3 as eluent to give homogeneous fractions of the methylthio intermediate compound; yield 0.92 g.

EXAMPLE 152

A solution of the methylthio compound of Example 151 (0.8 g, 1.93 mmole) in 50 ml of MeOH that has been saturated with NH_3 at 0 °C is heated at 90-95 °C for 24 hours in a glass-lined stainless steel bomb. The contents of the chilled bomb are evaporated in vacuo to give a mixture of the compound (IVD), benzamide and a by-product that is a 2-methylthio derivative, as opposed to the 2-amino compound (IVD). The mixture is stirred vigorously for several minutes with appr. 75 ml of Et_2O , and the insoluble white solid is filtered off and washed with Et_2O . The filtrate contained most of the benzamide and 2-methylthio components. A solution of the Et_2O -insoluble solid (0.390 g) in MeOH is evaporated with appr. 10 g of silica gel. The powdered residue is layered evenly onto the top of a silica gel column, which is then eluted with $\text{CHCl}_3/\text{MeOH}/\text{HOAc}$ (95:5:1) to give the 2-methylthio by-product and the desired 2-amino product (IVD). (IVD) is recrystallized by extraction into boiling isopropyl acetate in a Soxhlet apparatus. The white crystals are collected in three crops and dried in vacuo over P_2O_5 at 110 °C for 7h; yield 49%, mp >300 °C; anal. calcd. for $\text{C}_{13}\text{H}_{18}\text{N}_4\text{O}$: C, 63.39, H, 7.36; N, 22.74. Found: C, 63.50; H, 7.74; N, 22.67.

EXAMPLE 153

The compound of Example 152 is tested for enzyme inhibition activity as in Example 105. At 1 mM phosphate the IC_{50} is $0.037 \mu M$, and at 50 mM phosphate the IC_{50} is 2.2 μM .

EXAMPLE 154

The compound of Example 152 is tested to determine its effectiveness in potentiation of the toxicity of 2'-deoxyguanosine (d-Guo) (see D. A. Schewach et al., Cancer Res., 46, 519 (1986), and J. C. Sircar et al., Agents and Actions, 21, 253 (1987)). CCRF-CEM cells are grown in RPMI-1640 medium. To a suspension cultures of these cells, d-Guo at a fixed concentration ($5.62 \mu M$) and the compound at varied concentrations are added and the number of cells are determined in a Coulter counter 24, 48, and 72 hours thereafter. From these data, the IC_{50} is calculated to be $2.0 \mu M$ as the concentration of the compound required to reduce the increase in cell number between 0 and 72 hours to 50% of that of control cultures.

EXAMPLE 155

The compound 2-amino-7-(3-methylcyclohexylmethyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one is prepared. First, using the procedures set forth in Examples 146-152 above, but with 3-(3-methylbenzyl)-propionitrile as the starting material, the aryl derivative 2-amino-7-(3-methylbenzyl)-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one is made. A solution of the aryl derivative (0.2 g, 0.78 mmole) in trifluoroacetic acid (TFA) (20 ml) is hydrogenated with PtO_2 at 60 lb/in² for 24 h. Catalyst is filtered off through a Celite bed, and the filtrate is evaporated. The residue is triturated with methanol and left in the refrigerator overnight. The resulting crystallized trifluoroacetate salt precipitates from the solution and is collected by filtration. The TFA salt is suspended in 8 ml

of H₂O, adjusted to pH8 by conc. NH₄OH and sonicated. The pure product is collected, washed with H₂O and dried: yield 165 mg (81%); mp 282 °C. Anal: Calcd. for C₁₄H₂₀N₄O: C, 64.60; H, 7.74; N, 21.52. Found: C, 64.24; H, 7.96; N, 21.51%.

EXAMPLE 156

The procedure described in Example 155 is repeated to prepare 2-amino-7-(3-trifluoromethylcyclohexyl-methyl)-3H,5H-pyrrolo-[3,2-d]pyrimidin-4-one using 3-(3-trifluoromethylbenzyl)-propionitrile as the starting compound: yield 69%; mp 165 °C. Anal. calcd. for C₁₄H₁₇N₄OF₃·0.6H₂O: C, 51.72; H, 5.64; N, 17.23. Found: C, 51.82; H, 5.71; N, 16.81%.

EXAMPLE 157

The compound prepared in Example 155 is tested for enzyme-inhibition activity as in Example 105. At 1 mM phosphate the IC₅₀ is 0.025 μM, and at 50 mM phosphate the IC₅₀ is 0.0.820 μM.

EXAMPLE 158

The compound prepared in Example 156 is tested for enzyme-inhibition activity as in Example 105. At 1 mM phosphate the IC₅₀ is 0.020 μM, and at 50 mM phosphate the IC₅₀ is 0.740 μM.

EXAMPLE 159

3-Cycloheptylpropionitrile is prepared in this example according to the procedure of Example 97 using a solution of 2-bromocycloheptane (25.57 g; 144.38 mmole); Bu₃SnH (50.42 g; 173.26 mmole), acrylonitrile (15.32 g; 288.77 mmole), and AIBN (1.13 g) in toluene (300 ml). Yield is 16 g; mp oil.

EXAMPLE 160

3-Cycloheptylpropionitrile of Example 159 is further treated in the synthesis of the present invention. Under an atmosphere of dry N₂, a mixture of 3-cycloheptyl-

propionitrile (8.5 g, 56.19 mmole), sodium hydride (2.6 g, 112.39 mmole), and anhydrous tetrahydrofuran (100 ml) is heated at 52 °C in a water bath for 15 min., and a solution of ethyl formate (20.81 g, 280.99 mmole) in THF (100 ml) is added over a period of 45 min. After two hours at 50-55°C, a second portion of NaH (1.35 g) and HCO₂Et (10.4 g) are added, followed in 30 min. by a third portion of HCO₂Et (unreacted nitrile is inert in the next step and can be recovered at the first purification step). The thick paste is stirred overnight and allowed to cool to room temperature. Volatile matter is evaporated under reduced pressure, and the residual pale yellow crust is dissolved in the minimum volume of cold water (about 150 ml) at 0°C. The solution is adjusted to pH 6.0 by addition of 6N HCl and extracted with CHCl₃ (3 x 100 ml). The extract is washed with H₂O, dried over Na₂SO₄, and evaporated in vacuo to a thick amber oil. This crude product is used in the next reaction without further purification.

EXAMPLE 161

Glycine methyl ester hydrochloride (9.35 g, 74.47 mmole) and anhydrous sodium acetate (6.10 g, 74.47 mmole) are added to a solution of the crude formyl compound of Example 160 (8.9 g; 49.65 mmole) in MeOH/H₂O (4:1, 250 ml). After 24 hours, the MeOH is evaporated in vacuo, and the mixture of water and oil is extracted with CHCl₃. The CHCl₃ layer is dried (Na₂SO₄) and evaporated to give an amber oil which is applied to a silica gel column. Elution with CHCl₃ gave two major bands: (1) 3-cycloheptylpropionitrile (used as starting material in the previous step), and (2) the desired enamine, which is recrystallized from a CHCl₃/Et₂O mixture; yield 6.18 g; m.p. 57-58°C.

EXAMPLE 162

Under a nitrogen atmosphere, ethyl chloroformate (4.01 g, 37.03 mmole) is added dropwise to a solution of the enamine

of Example 161 (6.18 g, 24.69 mmole) and DBN (9.19 g, 74.04 mmole) in dry CH_2Cl_2 (100 ml) with external cooling in an ice bath. After stirring at 0 °C for one hour, the solution is allowed to stand at room temperature overnight. After checking progress by TLC, additional ClCO_2Et (0.5 ml) and DBN (3.0 ml) are added to complete the conversion, and the solution is allowed to stand for 24 hours. Volatile matter is evaporated in vacuo, the viscous residue purified on a short silica gel column (whose main purpose is to remove the less-mobile DBN) to give an N-blocked pyrrole, which is used for the next step without further purification.

EXAMPLE 163

To a solution of the N-blocked pyrrole of Example 162 (7.8 g, 24.19 mmole) in MeOH (100 ml) is added solid Na_2CO_3 (6.41 g, 60.48 mmole), and the reaction mixture is stirred at room temperature for 48 hr with separation of the resultant deblocked pyrrole. The mixture is evaporated to dryness, and the residue is triturated thoroughly with H_2O (50 ml) to dissolve inorganics and extracted with CHCl_3 (3 x 100 ml). The extract is dried (Na_2SO_4) and evaporated to give a viscous gum, which was purified by column chromatography employing silica gel/ CHCl_3 ; yield 4 g; m.p. 88-89°C.

EXAMPLE 164

Benzoyl isothiocyanate (1.5 g, 8.96 mmole) is added dropwise to a solution of the unblocked pyrrole of Example 163 (1.99 g, 7.95 mmole) in dry CH_2Cl_2 (50 ml). After 1 h at room temperature, solution is evaporated, and the gummy residue is dissolved in Et_2O (100 ml) with almost immediate separation of the crystalline solid. The Et_2O filtrate is heated to boiling and diluted with an equal volume of warm cyclohexane. On cooling slowly the solution gives

55

additional thioureido product; yield 2.89 g (88%); m.p. 158-159°C.

EXAMPLE 165

Methyl iodide (1.7 g, 11.96 mmole) is added to a solution
5 of the thioureido product of Example 164 (1.7 g, 4.1 mmole)
and DBN (0.56 g, 4.52 mmole) in dry CH_2Cl_2 (80 ml) at 0 °C.
The solution is stirred at 0°C for 15 min., at ambient
temperature for 1 h, and then evaporated in vacuo. A
solution of the residue in CHCl_3 is chromatographed on a
10 silica gel column with CHCl_3 as eluent to give homogeneous
fractions of the methylthio intermediate compound.

EXAMPLE 166

A solution of the methylthio compound of Example 165
(1.72 g, 4.02 mmole) in 50 ml of MeOH that has been
15 saturated with NH_3 at 0 °C is heated at 90-95 °C for 24
hours in a glass-lined stainless steel bomb. The contents
of the chilled bomb are evaporated in vacuo to give a
mixture of the compound (IVE), benzamide and a by-product
that is a 2-methylthio derivative, as opposed to the 2-
20 amino compound (IVE). The mixture is stirred vigorously
for several minutes with appr. 75 ml of Et_2O , and the
insoluble white solid is filtered off and washed with Et_2O .
The filtrate contained most of the benzamide and 2-
methylthio components. A solution of the Et_2O -insoluble
25 solid (0.850 g) in MeOH is evaporated with appr. 10 g of
silica gel. The powdered residue is layered evenly onto
the top of a silica gel column, which is then eluted with
 $\text{CHCl}_3/\text{MeOH}/\text{HOAc}$ (95:5:1) to give the 2-methylthio by-
product and the desired 2-amino product (IVE). (IVE) is
30 recrystallized by extraction into boiling isopropyl acetate
in a Soxhlet apparatus. The white crystals are collected
in three crops and dried in vacuo over P_2O_5 at 110 °C for
7h; yield 54%, mp >300 °C dec.; anal. calcd. for $\text{C}_{14}\text{H}_{20}\text{N}_4\text{O}$:

C, 64.60; H, 7.74; N, 21.52. Found: C, 64.78; H, 8.01; N, 21.61.

EXAMPLE 167

The compound of Example 166 is tested for enzyme
5 inhibition activity as in Example 105. At 1 mM phosphate the IC_{50} is 0.030 μ M, and at 50 mM phosphate the IC_{50} is 0.840 μ M.

EXAMPLE 168

Using the procedure of Example 97, 3-(1-norbornanyl)-
10 propionitrile is made from 1-bromonorbornane, and 3-(2-norbornanyl)-propionitrile (mixture of 2-exo and 2-endo) is made from 2-bromonorbornane. Following Examples 98-104, the propionitriles are converted to the compounds (IVF), IV(G), and (IVH).

EXAMPLE 169

Using the procedure of Example 97, 3-(1-bicyclo-
[3.2.1]octanyl)-propionitrile, 3-(2-bicyclo-
[3.2.1]octanyl)-propionitrile, 3-(3-bicyclo-
[3.2.1]octanyl)-propionitrile, and 3-(8-bicyclo-
20 [3.2.1]octanyl)-propionitrile are made respectively from 1-bromo-bicyclo[3.2.1]octane, 2-bromo-bicyclo[3.2.1]octane, 3-bromo-bicyclo[3.2.1]octane, and 8-bromo-bicyclo[3.2.1]octane. Following Examples 98-104, the propionitriles are converted to the compound (IVL) and the
25 related 2-bicyclo[3.2.1]octanyl, 3-bicyclo[3.2.1]octanyl, and 8-bicyclo[3.2.1]octanyl derivatives.

EXAMPLE 170

Using a modification of the procedure disclosed in D. Farcasiu, Synthesis, 615 (1972), 6-bicyclo[3.2.1]octane-
30 carboxaldehyde is prepared by reacting bicyclo[3.2.1]octan-6-one with trimethylsulfoxonium iodide, giving an intermediate epoxide, which is then converted to the aldehyde by treatment with boron trifluoride etherate. Following the procedure of Netherlands Pat. 6,610,204, the

aldehyde is condensed with cyanoacetic acid by refluxing in a pyridene/toluene solution with a catalytic quantity of ammonium acetate for 48-72 hours to give the corresponding acrylonitrile. The acrylonitrile is then hydrogenated
5 using a palladium-on-carbon catalyst in methanol as taught in Profitt, et al, J. Org. Chem., 40, 127 (1975) to give 3-(6-bicyclo-[3.2.1]octanyl)-propionitrile. Following Examples 98-104, the propionitrile is converted to the 6-bicyclo[3.2.1]octanyl derivative related to the compound
.10 (IVL).

EXAMPLE 171

Using the procedure of Example 97, 3-(1-bicyclo[3.3.1]nonanyl)-propionitrile and 3-(3-bicyclo-[3.3.1]nonanyl)-propionitrile are respectively made from 1-bromo-
15 bicyclo[3.3.1]nonane and 3-bromo-bicyclo[3.3.1]nonane. Following Examples 98-104, the propionitriles are converted to the compound (IVM) and the related 3-bicyclo[3.3.1]-nonanyl derivative.

EXAMPLE 172

20 Following the procedure of Example 171, bicyclo[3.3.1]-nonane-9-one is reacted to form the corresponding aldehyde, from which is made the corresponding 3-substituted propionitrile, which is then converted into the 9-bicyclo[3.3.1]nonanyl derivative related to the compound
25 (IVM).

EXAMPLE 173

Following the procedure of Example 170, 2-bicyclo[3.3.1]-nonanecarboxaldehyde is reacted to form the corresponding 3-substituted propionitrile, which is then converted into
30 the 2-bicyclo[3.3.1]nonanyl derivative related to the compound (IVM).

EXAMPLE 174

Using the procedure of Example 97, 3-(1-noradamantyl)-propionitrile is made from 1-bromonoradamantane, and 3-(2-

noradamantyl)-propionitrile is made from 2-bromonoradamantane. Following Examples 98-104, the propionitrile is converted to the final compound (IVN).

EXAMPLE 175

5 Following the procedure of Example 170, 3-noradamantane-carboxaldehyde is reacted to form the corresponding 3-substituted propionitrile, which is then converted into the 3-noradamantyl derivative related to the compound (IVN).

EXAMPLE 176

10 Following the procedure of Example 170, noradamantane-7-one is reacted to form the corresponding aldehyde, from which is made the corresponding 3-substituted propionitrile, which is then converted into the 7-noradamantyl derivative related to the compound (IVN).

15

EXAMPLE 177

Using the procedure of Example 97, 3-(1-bicyclo[2.2.2]octanyl)propionitrile is made from 1-bromobicyclo[2.2.2]octane and 3-(2-bicyclo[2.2.2]octanyl)propionitrile is made from 2-bromobicyclo[2.2.2]octane. Following Examples 98-
20 104, the propionitriles are converted to the compound (IVK) and the related 2-bicyclo[2.2.2]octanyl derivative.

EXAMPLE 178

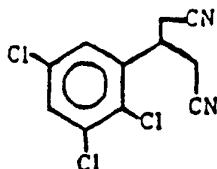
Using the procedure of Example 97, 3-(1-norbornenyl)-propionitrile is made from 1-bromonorbornene. Following
25 Examples 98-104, the propionitrile is converted to the compound (IVI).

EXAMPLE 179

Following the procedure of Example 170, 5-norborene-2-carboxaldehyde (a mixture of 2-endo and 2-exo) is reacted
30 to form the corresponding 3-substituted propionitrile, which is then converted into the compound (IVJ).

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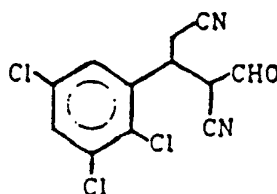
EXAMPLE 180



5 The above intermediate compound is prepared in this
10 Example by the modification of the procedure of Schiemenz,
G. P.; Engelhard, H. (Chem. Ber., 1962, 95, 195).

A mixture of cyanoacetic acid (25.38 g, 298.38 mmol),
2,3,5-trichlorobenzaldehyde (25.0 g, 119.35 mmol), ammonium
acetate (500 mg), toluene (120 ml), and pyridine (65 ml) is
15 heated at reflux for 16 h in a flask fitted with Dean-Stark
trap and condenser. The solvents are evaporated in vacuo,
residue is extracted with CHCl₃, which is washed with H₂O,
dried (Na₂SO₄), and evaporated to give the crude product,
which is purified by silica gel column chromatography using
20 hexane-EtOAc mixture as the eluent. Yield 23.69 g (73%);
mp 90-91 °C.

: EXAMPLE 181

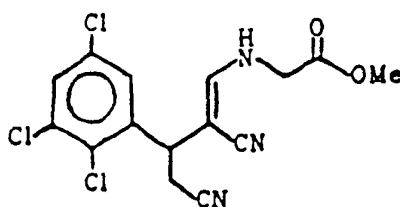


25

30 The above intermediate compound is prepared in this
Example. To a stirred mixture of NaH (1.56 g, 65.05 mmol)
and ethyl formate (14.78 g, 199.51 mmol) in THF (100 ml) is
added substituted pentanedinitrile of Example 180 (10.17 g,
37.17 mmol) at room temperature under a nitrogen

atmosphere, and the resulting reaction mixture is stirred for 24 h. Volatile matter is evaporated in vacuo at room temperature. Water (50 ml) is added to the residue at 0-5 °C, and the solution is adjusted to pH 5-6 by 20% conc. HCl (v/v). The heavy oil is extracted into ethyl acetate, washed with H₂O (1 x 100 ml) and dried (MgSO₄). The ethyl acetate layer is evaporated to give a red-brown oil (11.0 g) that is used in the next step without further purification.

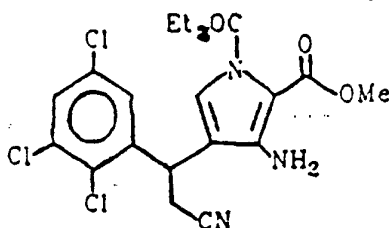
EXAMPLE 182



The above intermediate compound is prepared in this Example. Glycine methyl ester hydrochloride (8.17 g, 65.06 mmol) and sodium acetate (5.33 g, 65.06 mmol) are added to a solution of the crude formyl compound of Example 181 (11.0 g) in a mixture of MeOH (80 ml) and H₂O (20 ml), and the resulting solution is stirred at room temperature for 22 h. After evaporation of solvent at room temperature, the residue is extracted with ethyl acetate. The washed (H₂O) and dried (MgSO₄) organic layer is evaporated to give an oil. Flash column chromatography (silica gel) using CHCl₃ as eluent gave the pure desired enamine as a mixture of cis-trans isomers which is recrystallized from MeOH, yield 10.41 g (75%), mp 142-143 °C.

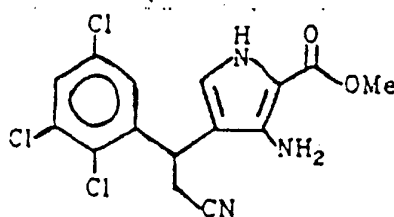
61

EXAMPLE 183



5 The above intermediate compound is prepared in this
 Example. A solution of enamine of Example 182 (10.0 g,
 26.84 mmol) in dry CH_2Cl_2 (100 ml) is cooled to 0 °C and
 10 treated with 1,5-diazabicyclo[4.3.0]non-5-ene (10.53 g,
 84.79 mmol) under a nitrogen atmosphere followed by ethyl
 chloroformate (6.90 g, 63.57 mmol). The solution is
 stirred at 0 °C for 1 h and then at room temperature for 48
 h. Volatiles are evaporated in vacuo to give a viscous
 15 dark gum which is purified by flash column chromatography
 over silica gel using CHCl_3 as the eluent. All the
 fractions containing the desired N-protected pyrrole are
 pooled and evaporated to give a foamy light pale yellow
 material which is stirred in MeOH (100 ml) to give the
 20 crystalline material which is recrystallized from CHCl_3 -
 MeOH, yield 8.92 g (74.7%), mp 160-161 °C.

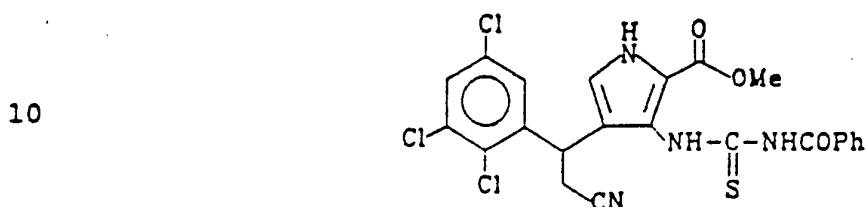
EXAMPLE 184



25 The above intermediate compound is prepared in this
 30 Example. A suspension of N-protected pyrrole of Example
 183 (8.6 g, 19.34 mmol) in MeOH (300 ml) is treated with
 Na_2CO_3 (5.12 g, 48.34 mmol) and the reaction mixture is
 stirred at room temperature for 17 h with separation of the
 deblocked pyrrole during the first hour. Solid sodium

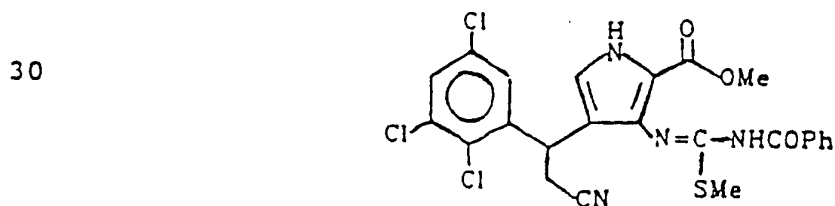
carbonate is removed by filtration and washed well with MeOH. The filtrate is reduced to a volume of 25 ml and kept in a refrigerator for 1 h to give 5.23 g of crystalline product. Further concentration of the mother liquor gave an additional 0.14 g of pure product; total yield 6.45 g (89.5 %), mp 178-181 °C.

EXAMPLE 185



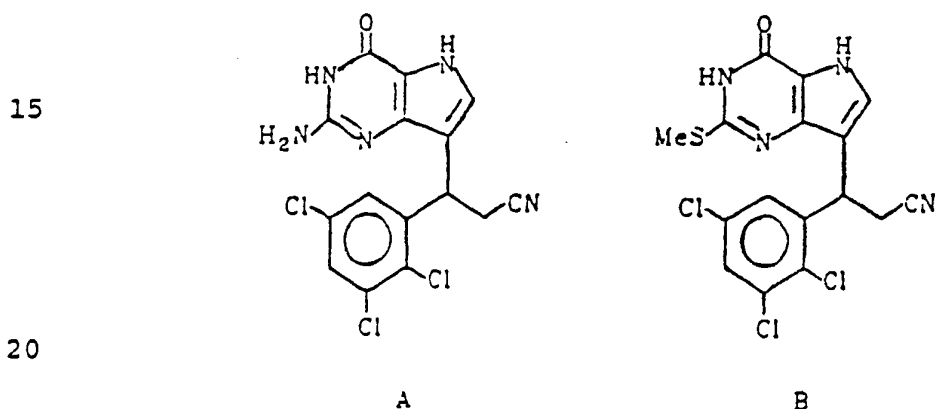
The above intermediate compound is prepared in this Example. To a suspension of pyrrole of Example 184 (5.83 g, 15.64 mmol) in dichloromethane (100 ml) is added benzoylisothiocyanate (2.88 g, 17.64 mmol) at room temperature under nitrogen. The reaction mixture is stirred for 30 min with the separation of the desired thioureido compound. Additional benzoyl isothiocyanate (0.5 ml) is added to it and again stirred for 30 min. The solvent is evaporated to dryness, and the light yellow residue is triturated with methanol. The white crystalline material is isolated by filtration and recrystallized from a chloroform-ether mixture to give the required thioureido compound as an analytically pure sample, yield 7.71 g, (92%), mp 210-211 °C.

EXAMPLE 186



The above intermediate compound is prepared in this Example. A solution of thioureido compound of Example 185 (6.75 g, 12.6 mmol) and 1,5-diazabicyclo[4.3.0]non-5-ene (1.76 g, 14.20 mmol) in dry CH_2Cl_2 (200 ml) is cooled to 0 °C and treated with methyl iodide (5.20 g, 36.65 mmol). The reaction mixture is stirred at 0 °C for 10 min and then for 1 h at room temperature. The solvent is evaporated at room temperature, and the residue is extracted with CHCl_3 , washed with H_2O (2 x 50 ml), dried (Na_2SO_4) and evaporated to give a glassy foam (6.95 g) which is used in the next step without purification.

EXAMPLE 187

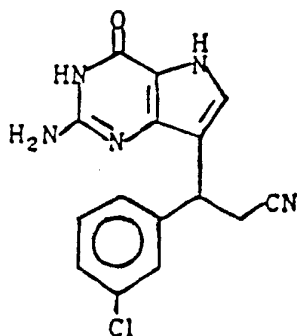


The above compounds A and B are prepared in this Example. The compound A is a compound of the present invention and the compound B is an intermediate. A solution of the methylthio intermediate of Example 186 (6.90 g, 12.54 mmol) in MeOH (200 ml) is saturated at 0 °C with ammonia and heated at 100 °C for 20 h in a glass-lined stainless steel bomb. The reaction mixture is brought to room temperature and the solvent is evaporated at room temperature. Purification of the crude mixture by flash column chromatography over silica gel using CHCl_3 as eluent gave 8B (1.1 g, 21%), mp 290-291 °C then CHCl_3 -MeOH (95:5) gave pure 8A (2.76 g, 57.5%), mp 284-285 °C.

EXAMPLE 188

The compound of the present invention of Example 187 is tested for enzyme inhibition activity. A purine nucleoside phosphorylase (PNP) enzyme assay is performed in which the PNP activity (IC_{50}) for the compound (8A) is found, which is determined radiochemically by measuring the formation of $[^{14}C]$ -hypoxanthine from $[^{14}C]$ -inosine (see Biomedicine, 1980, 33, 39) using calf spleen as the enzyme source. At 1 mM phosphate the IC_{50} is 0.64 μM and at 50 mM phosphate the IC_{50} is 10 μM .

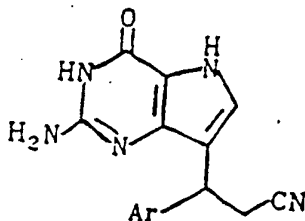
EXAMPLE 189

IC

Following the procedure set forth in Examples 180-187, 3-(3-chlorophenyl)-3-(2-amino-4-oxo-3,4-dihydropyrrolo[3,2-d]pyrimidin-7-yl)propanenitrile is prepared using 3-(3-chlorophenyl)-pentanedinitrile as the starting material, yield 54.5%, mp 157-158 °C.

EXAMPLE 190

Following the procedure set forth in Examples 180-187, the following compounds are also prepared (1-9).

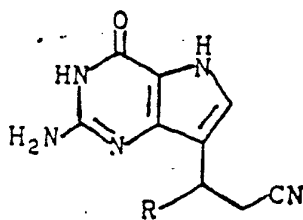


3-Aryl-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]-
pyrimidin-7-yl)propanenitrile

Where Ar is each of the following: (1) phenyl, 2,3-dichlorophenyl, 3-methylphenyl, and 3-methoxyphenyl, (2) thienyl (2- and 3-), (3) furanyl (2- and 3-), (4) pyridinyl (2-, 3-, and 4-), (5) pyrrolyl (2- and 3-), (6) thiazolyl (2-, 4-, and 5-), (7) 2-pyrazinyl, (8) pyridazinyl (3- and 4-), and (9) pyrazolyl.

EXAMPLE 191

Following the procedure set forth in Examples 180-187, the following compounds 10-14 and 21 are prepared starting from the appropriately substituted pentanedinitrile. Compounds 15-20, and 22 are prepared from the corresponding unsaturated Ar analogues in Example 190. In this procedure, the nitrile group of the unsaturated analogue is first converted to an amide group by acid- or base-catalyzed hydrolysis, then the unsaturated Ar group is converted to the saturated R² group by known catalytic hydrogenation, followed by reconvertng the amide back to the nitrile by known dehydration procedures.

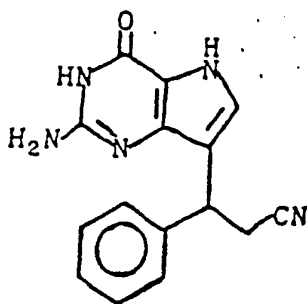


3-(Substituted)-3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]-
pyrimidin-7-yl)propanenitrile

Where R² is each of: 10) 1-adamantyl, 11) 2-adamantyl, 12) cyclohexyl, 13) cycloheptyl, 14) cyclopentyl, 15) tetrahydrofuranyl, 16) tetrahydrothienyl, 17) tetrahydropyranyl, 18) pyrazolidinyl, 19) thiazolidinyl,

20) piperaziny1, 21) morpholiny1, and 22) hexahydropyridaziny1.

EXAMPLE 192

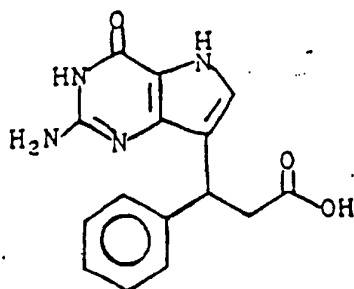


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanenitrile, is prepared in this Example. A solution of the compound A obtained in Example 187 (2.0 g, 5.22 mmol) in warm ethanol (250 ml) and dimethylformamide (DMF) (150 ml) is hydrogenated over 30% Pd/C catalyst (1.0 g) in the presence of triethylamine (2.64 g, 5.0 equivalent) at atmospheric pressure. After 5 h, the reaction is complete, and the catalyst is filtered off under N₂ pressure. The solid obtained by evaporation of the filtrate is triturated and sonicated with H₂O and dried, yield 1.28 g (88%), mp 168-170 °C.

EXAMPLE 193

The compound prepared in Example 192 is tested for enzyme inhibition activity as in Example 188. At 1 mM phosphate the IC₅₀ is 0.023 μM and at 50 mM phosphate the IC₅₀ is 4.7 μM.

EXAMPLE 194

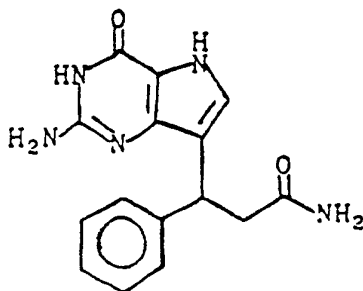


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanoic acid, is prepared in this example. A solution of the compound obtained in Example 192 (0.200 g, 0.72 mmol) in 6N HCl (3.0 ml) is heated at reflux for 18 h. The solvent is evaporated in vacuo and the residue is triturated with H₂O (6 ml), adjusted to pH 10 by conc. ammonium hydroxide. Insoluble material is collected by filtration and the filtrate is readjusted to pH 6.8. White material which is precipitated out is collected, washed with water and dried, yield 0.19 g (89%), mp 290 °C dec.

EXAMPLE 195

The compound prepared in Example 194 is tested for enzyme inhibition activity as in Example 188. At 1 mM phosphate the IC₅₀ is 0.012 μM and at 50 mM phosphate the IC₅₀ is 0.19 μM.

EXAMPLE 196

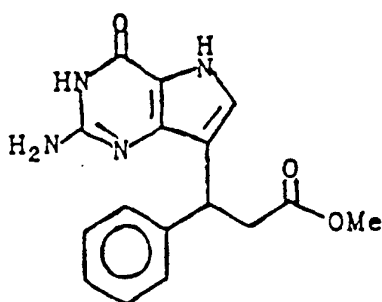


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanamide, is prepared in this example. A solution of the compound obtained in Example 192 (0.200 g, 0.72 mmol) in conc. H₂SO₄ (0.5 ml) is stirred at room temperature for 20 h and then poured onto crushed ice (5.0 g) and adjusted to pH 6.8 by conc. NH₄OH. The precipitated solid is collected, washed with H₂O and dried, yield 0.180 g, mp 199-201 °C dec.

EXAMPLE 197

The compound prepared in Example 196 is tested for enzyme inhibition activity as in Example 188. At 1 mM phosphate the IC_{50} is 0.20 μ M and at 50 mM phosphate the IC_{50} is 6.6 μ M.

EXAMPLE 198

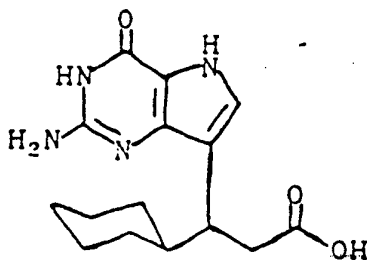


The above compound, 3-(2-amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-phenylpropanoic acid, methyl ester, is prepared in this example. Thionyl chloride (0.2 g, 0.17 mmol) is added to stirred methanol (4.0 ml) at 0 °C. The compound obtained in Example 194 (0.2 g, 0.67 mmol) is added and the mixture is stirred at room temperature for 18 h. The solvent is removed on a water aspirator (30°C) and vacuum pump (lyophilize) to give a semisolid mass which is purified on a silica gel column using $CHCl_3$ -MeOH as the eluent, yield 0.1 g.

EXAMPLE 199

The compound prepared in Example 198 is tested for enzyme inhibition activity. Significant activity (IC_{50}) is found.

EXAMPLE 200



3-(2-Amino-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl)-3-cyclohexylpropanoic acid is prepared in this example. A solution of the compound obtained in Example 194 (83 mg, 0.28 mmol) in trifluoroacetic acid (TFA) (15 ml) is hydrogenated with PtO_2 (83 mg) at 60 lb/in² for 24 h. The catalyst is filtered off through a Celite bed, and the filtrate is evaporated at 25 °C. The residue is suspended in H_2O (8 ml), and adjusted to pH 8.5 by conc. NH_4OH and filtered through a Whatman filter paper to remove brown colored impurities. The colorless filtrate is adjusted to pH 6.8, and the precipitated compound is filtered, washed with H_2O , and dried, yield 65 mg (77%), mp >300 °C.

EXAMPLE 201

The compound prepared in Example 200 is tested for enzyme inhibition activity as in Example 188. At 1 mM phosphate the IC_{50} is 0.097 μM and at 50 mM phosphate the IC_{50} is 1.0 μM .

EXAMPLE 202

A compound of the present invention is prepared wherein X is $\text{PO}(\text{OH})_2$. The nitrile group of the compound of Example 192 is converted to the corresponding amide by treatment with sulfuric acid. Using a Hoffman degradation reaction, the amide is converted to the corresponding amine, which is then converted to the corresponding pyridinium salt using a pyrillium salt. Conversion of the salt to the corresponding halide is accomplished using sodium bromide, which is then converted to the phosphonic ester using triethyl phosphite. Hydrolysis of the ester using trimethylsilylbromide yields the corresponding phosphonic acid wherein "n" is 1 and "m" is 0.

EXAMPLE 203

This Example makes a compound of the present invention by stepping up the number of carbon atoms from "m" is 0 to "m" is 1. The nitrile group of the compound of Example 192 is

reduced to the corresponding aldehyde, which is then converted to the corresponding alcohol. Using phosphorous tribromide the alcohol is converted to the corresponding alkyl bromide, which is then converted to the nitrile
5 compound of the present invention wherein m is 1 using potassium cyanide.

EXAMPLE 204

In this example a compound of the present invention is prepared wherein "p" is 1 and "Y" is oxygen. The alcohol
10 prepared as an intermediate in the previous example is converted to the corresponding diethyl phosphonomethyl ether using diethylchloromethyl phosphonate. Removal of the ethyl groups of the ester is accomplished using trimethylsilylbromide to give the phosphonic acid.

15

EXAMPLE 205

In this example a compound of the present invention is made wherein "Y" is NH and "X" is SO_2NH_2 . The nitrile group of the compound of Example 192 is reduced to the amine using standard catalytic hydrogenation with palladium
20 in acidic media (usually 0.01 N to 1 N HCl), which is then converted to the sulfamide using sulphamoyl chloride.

EXAMPLE 206

In this example a compound of the present invention is prepared wherein "X" is COOH and "Y" is NH by reacting the
25 methyl amine intermediate prepared in the previous example with chloroacetic acid.

EXAMPLE 207

In this example a compound of the present invention is prepared wherein "X" is $\text{PO}(\text{OH})_2$ and "Y" is NH by reacting
30 the methyl amine intermediate prepared in Example 206 with diethylchloromethyl phosphonate, and reacting the resulting product with trimethylsilylbromide.

EXAMPLE 208

In this example a compound of the present invention is prepared wherein "X" is SO_2NH_2 and "Y" is oxygen by reacting the alcohol intermediate prepared in Example 203 with sulphamoyl chloride.

EXAMPLE 209

In this example a compound of the present invention is prepared wherein R^1 is H, R^2 is phenyl, R^3 and R^4 are hydrogen, m is 0, n is 1, p is 0, and X is CN. A modification of the procedure disclosed in Mu-Ill Lim, et al., J. Org. Chem., Vol. 44, No. 22, 3826 (1979) is used. A mixture of the compound of Example 184 and dimethylformamide dimethyl acetal is reacted at room temperature for two days and then evaporated to dryness in vacuo. The residue is crystallized to give the pure N-(dimethylamino)methylene derivative, which is cyclized with saturated methanolic ammonia to give the desired end product.

EXAMPLE 210

In this example a compound of the present invention is prepared wherein R^1 is OCH_3 , R^2 is phenyl, R^3 and R^4 are hydrogen, m is 0, n is 1, p is 0, and X is CN. Using the compound B of Example 187, the S-methyl group is oxidized to methylsulfone, which then is converted to the final methoxy compound by treatment with sodium methoxide in methanol.

EXAMPLE 211

In this example a compound of the present invention is prepared wherein X is tetrazole. The compound of Example 192 is treated with lithium azide in the presence of ammonium chloride as a catalyst in dimethylformamide (DMF) at 100 degrees C to give the desired tetrazole.

EXAMPLE 212

In this example a compound of the present invention is prepared wherein X is triazole. The compound of Example 198 is treated with hydrazine hydrate to give the
5 corresponding hydrazide, which is then treated with imino ether to give the desired triazole.

EXAMPLE 213

The compound prepared in Example 189 is tested for enzyme inhibition activity as in Example 188. At 1 mM phosphate
10 the IC_{50} is 0.012 μM and at 50 mM phosphate the IC_{50} is 2.0 μM .

EXAMPLE 214

In this example an amidine compound of the present invention is prepared, i.e., wherein X in the recited
15 generic formula is $CNHNH_2$. The compound A from Example 187 is reacted with sodium methoxide in methanol at room temperature for about 2 days to give a methyl-imidate intermediate. The intermediate is then reacted with ammonia in methanol to give the amidine product.

WHAT IS CLAIMED IS:

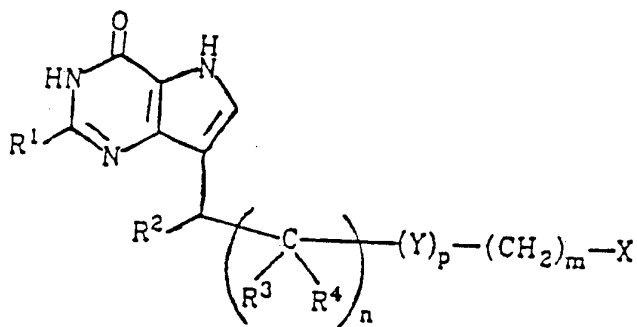
1. A compound 2-amino-7-(R)-3H,5H-pyrrolo[3,2-d]-pyrimidin-4-one wherein R is cyclohexenyl, cyclohexyl, or $\text{CH}_2\text{-R}_1$, and wherein R_1 is an optionally substituted heteroalicyclic, pyridinyl or alicyclic group.

2. The compound of claim 1 wherein R is $\text{-CH}_2\text{-R}_1$ and R_1 is optionally substituted 2-, 3-, or 4-piperidinyl, 2- or 3-tetrahydrofuranyl, 2- or 3-tetrahydrothienyl, 2- or 3-pyrrolidinyl, 2-, 3-, or 4-tetrahydropyranyl, or 2-, 3-, 4-pyridinyl.

3. The compound of claim 1 wherein R is optionally substituted cyclohexyl or 1-, 2-, or 3-cyclohexenyl.

4. The compound of claim 1 wherein R is $\text{-CH}_2\text{-R}_1$ and R_1 is optionally substituted cyclopentyl, cyclohexyl, cycloheptyl, 1- or 2-adamantyl, 1- or 2-noradamantyl, 1-norbornanyl, 2-exo-norbornanyl, 2-endo-norbornanyl, 1- or 2-bicyclo[2.2.2]octanyl, 1-, 2-, 3-, 6-, or 8-bicyclo[3.2.1]octanyl, 1-, 2-, 3-, or 9-bicyclo[3.3.1]nonanyl, or 1- or 2-norbornenyl.

5. A compound of the formula



wherein R^1 is H, NH_2 , or OCH_3 , R^2 is an optionally substituted cyclic group of 5-7 carbon atoms optionally containing one or more heteroatoms, R^3 and R^4 are independently H or C_{1-4} alkyl, m is 0-4, n is 0-6, p is 0-1, X is CN, CSNH_2 , PO(OH)_2 , COOH , SO_2NH_2 , NH_2 , OH, CNHNH_2 ,

tetrazole, or triazole, COR⁵ where R⁵ is C₁₋₄ alkyl, CF₃, NH₂, or OC₁₋₄ alkyl, and Y is O or NH.

6. The compound of claim 5 wherein R¹ is NH₂, R³ and R⁴ are H, m is 0 and n is 1.

5 7. The compound of claim 5 wherein R² is phenyl.

8. The compound of claim 5 wherein X is CN, COOH, or CONH₂.

9. The compound of claim 5 wherein R² is an optionally substituted 5- or 6-membered aromatic or heteroaromatic
10 group or an optionally substituted alicyclic group or heteroalicyclic group of 5-9 members.

10. A method for the selective suppression of mammalian T-cell function without diminished effect on humoral immunity which comprises administering to a mammal the
15 compound of any of claims 1-8 or 9 whereby said compound inhibits purine nucleoside phosphorylase and thereby T-cell formation.

11. A method for making a chemical compound comprising the steps of:

20 a) reacting an optionally substituted cyclic aldehyde with cyanoacetic acid in the presence of ammonium acetate to make a 3-cyclo-substituted pentanedinitrile;

b) reacting the 3-cyclo-pentanedinitrile with an alkyl formate and a base to make a 3-cyclo-2-
25 formylpentanedinitrile;

c) reacting the 3-cyclo-2-formylpentanedinitrile with glycine methyl ester hydrochloride and sodium or ammonium acetate to make methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine;

30 d) reacting the methyl N-[(3-cyclo-2,4-dicyano)-2-butenyl]glycine with an alkyl chloroformate and DBN or DBU to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate; and

e) reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1-ethyl-1H-pyrrole-1,2-dicarboxylate with a base to make methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate.

5 12. The method of claim 11 further comprising the steps of:

f) reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate with benzoylisothiocyanate to make N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-10 1H-pyrrol-3-yl]thiourea;

g) reacting N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]thiourea with an alkyl halide to make N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]S-methylthiourea; and

15 h) reacting N-benzoyl-N'-[4-(2-cyano-1-cyclo-ethyl)-2-methoxycarbonyl-1H-pyrrol-3-yl]-S-methylthiourea with methanolic or ethanolic ammonia to make a mixture of 3-cyclo-3-[2-amino-4-oxo-3H-5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile and 3-cyclo-3-[2-methylmercapto-4-oxo-20 3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

13. The method of claim 12 further comprising the steps of:

i) reacting the 3-cyclo-3-[2-methylmercapto-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile with an 25 oxidizing agent to make 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile; and

j) reacting the 3-cyclo-3-[2-methylsulfonyl-4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile with sodium alkoxide to make 3-cyclo-3-[2-methoxy-4-oxo-3H,5H-30 pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

14. The method of claim 11 further comprising the steps of:

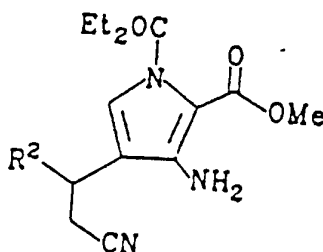
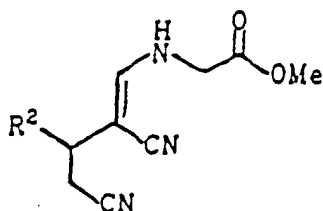
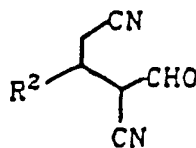
f) reacting the methyl 3-amino-4-(2-cyano-1-cyclo-ethyl)-1H-pyrrole-2-carboxylate with dimethylformamide dimethyl

acetal to make methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate; and

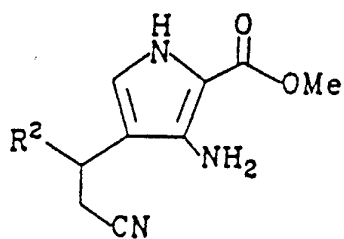
g) reacting the methyl 4-(2-cyano-1-cyclo-ethyl)-3-[N-(dimethylaminomethylene)amino]-1H-pyrrole-2-carboxylate with methanolic ammonia to make 3-cyclo-3-[4-oxo-3H,5H-pyrrolo[3,2-d]pyrimidin-7-yl]propanenitrile.

15. The method of claim 11 wherein the cyclic substituent is phenyl, 2- or 3-thienyl, 2- or 3-furanyl, 2-, 3-, or 4-pyridinyl, 2- or 3-pyrrolyl, 2-, 4-, or 5-thiazolyl, 2- or 3-pyrazinyl, 3- or 4-pyridazinyl, 3-, 4-, or 5-pyrazolyl 1- or 2-adamantyl, cyclopentyl, cyclohexyl, cycloheptyl, or morpholinyl.

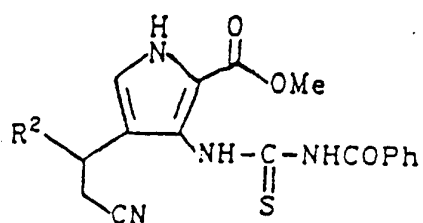
16. A compound of the formula



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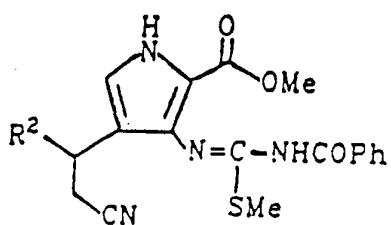


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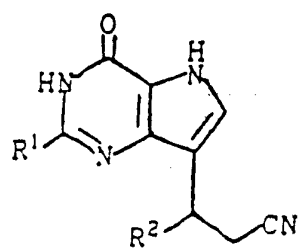


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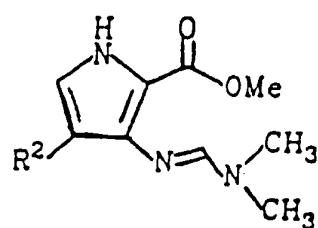


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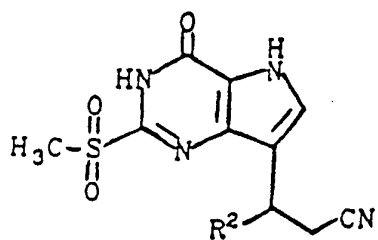
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or

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wherein R¹ and R² are as defined claim 5 .