Nucleosides. 5. Synthesis of Guanine and Formycin B Derivatives as Potential Inhibitors of Purine Nucleoside Phosphorylase

Ji-Wang Chern,* Horng-Yuh Lee, and Chien-Shu Chen

Medical Laboratories and Institute of Pharmacy, National Defense Medical Center, P. O. Box 90048-512, Taipei, Taiwan, ROC(100)

Donna S. Shewach,* Peter E. Daddona,* and Leroy B. Townsend*

Departments of Pharmacology and Biological Chemistry, Medical School, Department of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109-1005

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In an effort to develop potent human purine nucleoside phosphorylase (PNP) inhibitors as immunosuppressive and chemotherapeutic agents, several 8-aminoguanine derivatives were synthesized and evaluated as potential PNP inhibitors. These studies were designed to investigate the hydrophobic effect of a substituent on the N-9 of the purine heterocycle and/or the C-5' positions.

Introduction

Purine nucleoside phosphorylase (PNP; orthophosphate ribosyltransferase; EC 2.4.2.1) is considered an essential enzyme in the purine salvage pathway, catalyzing the phosphorylation of guanosine, inosine and their 2'-deoxyribonucleoside derivatives to the respective purine bases. The discovery of T-cell-related immunodeficiency diseases associated with a lack of PNP has prompted considerable interest in developing inhibitors of this enzyme as a selective approach to cancer chemotherapy. It is well-documented that in a PNP-related immunodeficiency state, 2'-deoxyguanosine accumulates and this nucleoside is phosphorylated preferentially by immature T cells to the toxic 5'-triphosphate derivative, which results in inhibition of DNA synthesis. It has recently been reported that human PNP deficiency was caused by a single base change in the DNA genome. T-cell lymphopenia is associated with a PNP-deficient patient; therefore, it was suggested that a PNP inhibitor might be used to treat T-cell leukemias, to counter autoimmune diseases without destroying the patient's humoral immunity and to treat tissue rejection after organ transplantation. Furthermore, PNP inhibitors might provide some potential use for antimalarial treatment because the intraerythrocytic malaria parasite requires hypoxanthine and guanine formed by PNP as a source of nucleic acid synthesis and energy metabolism.

The importance of PNP inhibitory activity for lymphocyte development and function in chemotherapy has generated considerable interest in a systematic examination of the structure–activity relationships of substrates for human erythrocytic PNP. To date, several inhibitors of PNP have been identified and the majority of these compounds resemble purine bases or nucleosides. It has been established that halogen at the C-5' position of specific PNP substrates results in improved affinities but greatly decreased substrate activity, for example, 5'-deoxy-5'-idoformycin ($K_i = 7 \mu M$) illustrated that the binding to PNP by iodination at C-5' of the parent compound increased the activity by 10-fold. X-ray crystallography revealed that the 5'-iodo atom is juxtaposed over the phenylalanine residue of the active site of PNP, indicative of the enhancement of binding to PNP probably due to the charge–charge interaction of the halogen atom with the phenylalanine residue in the active site of PNP. In a previous communication, we assumed that 8-aminoguanine plays an important role in recognizing and binding to the active site of PNP, and through an attempt to investigate the bulky tolerance at the N-9 position of 8-aminoguanine, we found that 8-amino-9-benzylguanine (8-ABG) is a potent inhibitor of both human erythrocyte and parasite PNP. The intact cell experiments showed that 8-ABG acted as a competitive (with inosine) inhibitor with a $K_i$ value 0.22 $\mu M$ and could potentiate the toxicity of 2'-deoxyguanosine to MOLT-4 T-lymphoblasts in culture. We reasoned that the increased affinity of 8-ABG to PNP, more than 10-fold by a benzyl group on the N-9 position of 8-aminoguanine, is probably due to this hydrophobic interaction between the benzyl group and the phenylalanine residue (Phe$^{200}$) of the active site of PNP. Such a hydrophobic region adjacent to the active site of an enzyme has been observed on dihydrofolate reductase, guanase, and thymidine phosphorylase, and the exploitation of the hydrophobic regions in the active site for the design of reversible and irreversible inhibitors have been previously studied.
Potential Inhibitors of Purine Nucleoside Phosphorylase

Scheme I

Accordingly, we selected 8-aminoguanine and the corresponding nucleoside as our "lead compounds" for studies to investigate the hydrophobic effect of substituents on the N-9 of the purine heterocycle and C-5' positions of the carbohydrate moiety.

Results and Discussion

Chemistry. 8-Amino-9-substituted-guanines (9a-e) were synthesized (Scheme I) by a method previously published from our laboratory. This involves the initial synthesis of the key intermediates, 2-amino-4-(substituted-amino)pyrimidin-6-ones (3a-e) which were subjected to nitrosation with sodium nitrite and subsequent reduction with sodium dithionite to give the 2,5-diamino-4-(substituted-amino)pyrimidin-6-ones (5a-e). Compounds 5a-e were readily oxidized in the air; therefore, they were condensed with methoxycarbonyl isothiocyanate at once without further purification. Then cyclodesulfurization and ring annulation of the resulting thiourea derivatives 6-e with dicyclohexylcarbodiimide, followed by a ring transformation of the oxazol-[5,4-d]pyrimidines 7a-e led to the formation of the imidazo[4,5-dlpyrimidines 8a-e. Subsequent hydrolysis of 8a-e with a 10% sodium hydroxide solution afforded the target compounds.

In the 8-aminoacyclovir derivatives series (Scheme II), 8-aminoacyclovir (15) was synthesized by a previous method. Bromination of acyclovir (13) was effected using saturated bromine water to give 8-bromoacyclovir (14) in 84% yield. Treatment of the bromo compound with 50% aqueous hydrazine at reflux afforded compound 15. The iodination of acyclovir and 8-aminoacyclovir, with triphenylphosphine and iodine in N,N-dimethylformamide using Moffat's procedure furnished 9-[2-(iodoethoxy)methyl]guanine (16) and 8-amino-9-[2-(iodoethoxy)methyl]guanine (17), respectively.

8-Amino-5'-deoxy-5'-[p-(fluorosulfonyl)guanosine (19) was prepared (Scheme III) in 59% yield by a simple treatment of 8-aminoguanosine (12) with diphenyl disulfide in the presence of tributylphosphine. 8'-[p-(Fluorosulfonyl)benzoyl]guanosine (21) was previously prepared for a ligand study by a direct acylation of the 5'-OH group of guanosine hydrochloride with p-(fluorosulfonyl)benzoyl chloride in HMPA. For our studies, 21 was synthesized by a modification of this procedure by a direct acylation of the 5'-OH of guanosine with p-(fluorosulfonyl)benzoyl chloride, instead of the hydrochloride salt. The structure of this compound was determined on the basis of its 1H-NMR spectrum.

However, the treatment of 8-aminoguanosine with p-(fluorosulfonyl)benzoyl chloride in HMPA resulted in a nonselective acylation. The treatment of 8-aminoguanosine dihydrochloride with p-(fluorosulfonyl)benzoyl chloride in HMPA afforded the dihydrochloride salt of the desired compound. The neutralization of 8-aminoguanosine dihydrochloride with p-(fluorosulfonyl)benzoyl chloride, instead of the hydrochloride salt. The structure of this compound was determined on the basis of its 1H-NMR spectrum.

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with OH, SCH₂, and propyl groups substituted at C-8 show little or no PNP inhibitory activity. In a previous study, we found that by adding an amino group at the 8 position of acyclovir, to obtain compound 15 led to an increase of more than a 10-fold affinity to PNP.

A recent report on a metabolite of the antitherapeutic drug acyclovir, acyclovir diprophosphate, revealed this compound was the most potent inhibitor of PNP to date with a \( K_i = 0.0087 \mu M \) (determined at a low inorganic phosphate concentration). The \( K_i \) value increased to 0.51 \( \mu M \) when the phosphate concentration was increased to 50 \( \mu M \), suggesting that there is a positive charge adjacent to the active site which is binding to an anionic charge on the terminal phosphate ester moiety of acyclovir diprophosphate. Accordingly, metabolically stable "multisubstrate" acyclic nucleotide analogues containing a purine and phosphate-like moiety such as 9-(phosphonoacetyl)hyoxanthines and guanines have been synthesized and evaluated as potential purine nucleoside phosphorylase inhibitors. In this series, 9-(5-phosphono-5-endo)guanine was considered as the most potent with a \( K_i = 170 \) nM. 8-Amino-9-(carboxyethyl)guanine (9b), containing a carboxylate ion at the terminal end, which is considered as an isoster of phosphono or phosphate, is a multisubstrate analogue. However, compound 9b demonstrated a weak PNP inhibitory activity with a \( K_i = 10.0 \) \( \mu M \).

Modifications at C-5' of the ribose moiety in a purine nucleoside is considered to be a promising approach to develop PNP inhibitors. It is generally agreed that a halogen atom at the C-5' position provides a better affinity for the nucleoside to PNP. Similarly, the PNP inhibitory activity of acyclovir was increased 10-fold by replacing the 2-hydroxy group with an iodo atom to give 9-[2-iodoethoxyethyl]guanine (16). However, a similar replacement of the 2-hydroxy group of 8-aminocyclovir with an iodo atom did not enhance the affinity to PNP. We found that compound 19, having a phenylthio group at the C-5' position, with a \( K_i = 0.45 \) \( \mu M \) was 40-fold better than that of 8-aminoguanosine and possessed potency equal to 8-amino-5'-deoxy-5'-chloroguanosine. However, compound 23, with the same substituent at the C-5' position of formycin B, showed poor inhibitory activity with a \( K_i = 26 \) \( \mu M \) which is weaker than that of 19. Although compound 23 is not as potent as 5'-deoxy-5'-iodoformycin (\( K_i = 7.0 \) \( \mu M \)), it is still better than the parent compound formycin B (\( K_i = 100 \) \( \mu M \)). The results obtained from these studies coincide with reports that halogens or a thiomethyl group at the C-5' position of specific PNP substrates resulted in unchanged or improved affinities but greatly decreased substrate activity. The increased affinity of 8-amino-5'-deoxy-5'- (phenylthio)guanosine to PNP was probably due to an interaction of the 5'-

### Table I. Kinetic Constants for Human PNP

<table>
<thead>
<tr>
<th>Compounds</th>
<th>( K_i (\mu M) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-amino-9-benzylguanine (1)</td>
<td>0.2</td>
</tr>
<tr>
<td>8-amino-9-benzylguanine (9a)</td>
<td>12.4</td>
</tr>
<tr>
<td>8-amino-9-(carboxyethyl)guanine (9b)</td>
<td>10.0</td>
</tr>
<tr>
<td>8-amino-9-(carboxyethyl)guanine (9c)</td>
<td>a</td>
</tr>
<tr>
<td>8-amino-9-(carboxyethyl)guanine (9d)</td>
<td>180</td>
</tr>
<tr>
<td>8-amino-9-(carboxyethyl)guanine (9e)</td>
<td>13.4</td>
</tr>
<tr>
<td>9-(2-iodoethoxy)methylguanine (16)</td>
<td>15.0</td>
</tr>
<tr>
<td>8-amino-9-(2-iodoethoxy)methylguanine (17)</td>
<td>23.0</td>
</tr>
<tr>
<td>8-amino-5'-deoxy-5'- (phenylthio)guanosine (19)</td>
<td>0.45</td>
</tr>
<tr>
<td>5'- (fluorosulfonyl)benzoylguanosine (21)</td>
<td>93.0</td>
</tr>
<tr>
<td>8-amino-5'- [p-(fluorosulfonyl)benzoyl]guanosine (20)</td>
<td>37.0</td>
</tr>
<tr>
<td>9-[p-(fluorosulfonyl)benzoyl]guanosine (20)</td>
<td>7.0</td>
</tr>
<tr>
<td>5'- [p-(fluorosulfonyl)benzoyl]formycin B (24)</td>
<td>&gt;300</td>
</tr>
<tr>
<td>5'- [p-(fluorosulfonyl)benzoyl]formycin B (23)</td>
<td>26.0</td>
</tr>
</tbody>
</table>

- Insoluble
- No inhibition at 1 mM
- 95% inhibition at 1 mM
that two hydrophobic binding sites might exist in the
from the neighboring subunit). Accordingly, we propose
the other area is in the neighboring subunit and most likely
plays a minor role.

phenylthio group with the phenylalanine residue and is
indicative of a hydrophobic region adjacent to the active
site of PNP.

The active site of purine nucleoside phosphorylase has
been recently characterized using X-ray diffraction data.\(^{18b}\)
This study revealed that guanine, hypoxanthine, and
certain analogues donate a hydrogen bond at N-1 and
accept a hydrogen bond at O-6. Asn-243 and Lys-244 are
near O-6 while Glu-201 is near N-1. Furthermore, the amino
group at C-2 of guanine is in position to form an additional
hydrogen bond with Glu-201. According to this description
of the purine binding site of PNP, we assumed that the
8-aminoguanine moiety and the pyrazolopyrimidine moiety
of formycin B could enter the active site of PNP with the
ribose moiety being outside of the binding site. On
the basis of this assumption, Figure 1 shows energy-
minimized conformations of 19 and 23 (superimposed),
generated using the ALCHEMY II molecular modeling
program.\(^{18b}\)

These 8-aminoguanine derivatives and formycin B analogues provide the same arrangement of hydrogen bond donors and receptors in the active site of
PNP while the ribose and the 5'-phenylthio group of 19
and 23 are distorted in different directions. However, the
5'-phenylthio moiety of 19 is anti to the 8-aminoguanine
while the 5'-phenylthio group of 23 is syn to the 8-position.
The latter conformation is very similar to that reported
for 5'-iodoformycin B binding to the active site of PNP
(in the X-ray diffraction data). The introduction of a
phenylthio group at the 5' position leads to a 40-fold
improvement in the 8-aminoguanosine series, but only a
4-fold improvement in the formycin B series, and it is
probably due to the phenylthio groups selecting a different
hydrophobic site. It should be noted that one side of the
"ribose pocket" has been characterized as hydrophobic
and includes at least 4 aromatic residues (Phe-200, Tyr-
88, and His-257 from one subunit, along with Phe-159
from the neighboring subunit). Accordingly, we propose
that two hydrophobic binding sites might exist in the
pocket as illustrated in Figure 2. The hydrophobic area
A is in or near the active site and plays a major role while
the other area is in the neighboring subunit and most likely
plays a minor role.

At the outset, we presumed that the 8-aminoguanine
nucleus might be responsible for the recognition and
binding of 8-aminoguanosine to the active site of PNP.
This prompted us to make the assumption that perhaps
there is another pocket for the C-5' position of the ribose
which would allow us to use a chemically reactive functional
group such as p-(fluorosulfonyl)benzoyl group on the C-5'
position of the ribose for irreversible binding to PNP.
However, nucleosides containing this sulfonyl fluoride
moiety have been previously synthesized and evaluated
as affinity labeling agents for adenosine nucleotide sites
in glutamate dehydrogenase,\(^{34}\) pyruvate kinase,\(^{35}\) RNA
polymerase,\(^{36}\) phosphofructokinase,\(^{37,38}\) and the
mitochondrial F1-ATPase.\(^{39}\)

We expected this sulfonyl fluoride moiety to be capable of functioning as an electrophilic functional group in covalent reactions with several classes
of amino acids such as lysine, tyrosine, histidine, and serine
in the active site of PNP.\(^{40}\) Thus, the synthesis of
8-aminoguanosine with several chemically reactive functional
groups attached to the C-5' position became one of our
primary goals in the design of PNP inhibitors, e.g., 9-[2-(
fluorosulfonyl)benzoyl]ethoxymethyl]guanine (18),
8-amino-5'-[p-(fluorosulfonyl)benzoyl]guanosine (20), 5'-
[p-(fluorosulfonyl)benzoyl]guanosine (21), and 5'-[p-(
fluorosulfonyl)benzoyl]formycin B (24) were synthesized
and evaluated. As can be seen from Table I, none of these
compounds showed irreversible inhibitory activity to PNP.
In fact, the inhibitory activities of these compounds are
even weaker than those of the parent compounds. This
might be because there is no nucleophilic residue at the
active site of PNP and/or the sulfonyl fluoride moiety is
too large to fit into the active site of PNP. However, the
enzymatic inhibitory activity of compound 20 is still better
than that of 21, indicative of the importance of having an
amino group at the 8-position.

In summation, the influence of an amino substituent at
the 8-position of guanosine was consistent with that of the
previous assumption that 8-aminoguanine plays an
important role in recognition and binding to the active site
of purine nucleoside phosphorylase. The area around the
9-position of 8-aminoguanine is most likely a pocket which
can accommodate a larger group and could provide a labeled
and chemically reactive functional group. The same
phenomena was also observed at the C-5' position of the
ribose moiety of 8-aminoguanosine. However, a chemically
reactive functional group at the C-5' position did not

\(\text{Figure 1. Superimposition of compound 19 and 23 in energy-minimized conformation state.}\)

\(\text{Figure 2. Proposed three binding sites for purine nucleoside phosphorylase inhibitors. Shaded part is purine binding site; A and B represent two hydrophobic areas.}\)
provide an irreversible inhibitor and would suggest that nucleophilic residues (if they are in that area) are not in the proper juxtaposition for a reaction to occur.

**Experimental Section**

Melting points were obtained on a Electrothermal apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 983 G spectrophotometer; 1H and 13C nuclear magnetic resonance spectra were recorded on a Bruker Model AM-300 spectrometer using Me2SO-d6 as the solvent and internal standard. UV spectra were recorded on a Shimadzu UV-210 A spectrophotometer. AICA hydrochloride and AICA-riboside were purchased from Sigma Chemical Co.

Elemental analysis was carried out in Cheng-Kung University, Tainan, Taiwan.

2-Amino-4-(n-hexylamino)pyrimidin-6-one (3a). A mixture of 2-amino-4-chloropyrimidin-6-one (2) (1.5 g, 10 mmol) and 6-aminohexanoic acid (1.31 g, 9.49 mmol) and n-hexylamine (15 mL, 0.11 mol) in anhydrous ethanol (100 mL) was heated at reflux in an oil bath for 6 h. The white solid was collected by filtration and recrystallized from a mixture of ethanol and water to give 1.78 g (60% from 3a): mp 221–222°C; 1H NMR (200 MHz, DMSO-d6) δ 1.26 (q, 2H, CH3); 1.5–1.8 (3m, 4H, CH2), 2.17 (t, 2H, CH2), 3.19 (q, 2H, CH2), 3.70 (s, 3H, CH3), 6.17 (t, 1H, NH, D2O exchangeable), 6.27 (s, 2H, NH2, D2O exchangeable), 9.37 (br s, 1H, NH, D2O exchangeable), 10.07 (s, 1H, NH, D2O exchangeable), 11.11 (s, 1H, NH, D2O exchangeable), 11.85 (s, 1H, NH, D2O exchangeable). Anal. (C13H18N4O) C, H, N.

A solid was formed by the addition of methanol (50 mL) to this oily residue. The solid was collected by filtration and washed with water (50 mL). The red solid was collected by filtration and washed with water (50 mL). The crude product was recrystallized from 1N sodium hydroxide solution and glacial acetic acid to afford 0.51 g (65% from 3a): mp 224–225°C; 1H NMR (100 MHz, DMSO-d6) δ 3.70 (s, 3H, CH3), 4.87 (t, 2H, CH2), 6.29 (s, 2H, NH2, D2O exchangeable), 6.95 (t, 1H, NH, D2O exchangeable), 7.36–8.14 (m, 7H, ArH), 9.34 (br s, 1H, NH, D2O exchangeable), 11.15 (s, 1H, NH, D2O exchangeable). Anal. (C13H18N4O) C, H, N.

A mixture of 2-amino-4-(naphthylmethyl)aminopyrimidin-6-one (3d). Compound 6d was prepared in 70% yield using a procedure similar to that which afforded 3a; mp 228–230°C; 1H NMR (100 MHz, DMSO-d6) δ 3.70 (s, 3H, CH3), 4.87 (t, 2H, CH2), 6.29 (s, 2H, NH2, D2O exchangeable), 6.95 (t, 1H, NH, D2O exchangeable), 7.36–8.14 (m, 7H, ArH), 9.34 (br s, 1H, NH, D2O exchangeable), 11.15 (s, 1H, NH, D2O exchangeable). Anal. (C13H18N4O) C, H, N.

2-Amino-4-(naphthylmethyl)amino)pyrimidin-6-one (3e). Compound 3e was prepared in 45% yield using a procedure similar to that which afforded 3a. An analytical sample was prepared by recrystallization from a mixture of ethanol and water: mp 226°C dec; 1H NMR (100 MHz, DMSO-d6) δ 0.78–1.75 (m, 11H, 2CH3, 4.40 (s, 1H, =CH), 6.07 (s, 2H, NH2, D2O exchangeable), 6.35 (s, 1H, NH, D2O exchangeable), 6.95 (s, 1H, NH, D2O exchangeable). Anal. (C13H16N4O) C, H, N.

2-Amino-4-(naphthylmethyl)amino)pyrimidin-6-one (3d). Compound 3d was prepared in 34% yield using a procedure similar to that which afforded 3a: mp 260°C dec; 1H NMR (100 MHz, DMSO-d6) δ 4.48 (s, 1H, =CH), 4.78 (d, 2H, CH2), 6.17 (s, 2H, NH2, D2O exchangeable), 6.93 (t, 1H, NH, D2O exchangeable), 7.42–8.10 (m, 7H, ArH), 9.64 (br s, 1H, NH, D2O exchangeable). Anal. (C13H16N4O) C, H, N.

2-Amino-4-(naphthylmethyl)amino)pyrimidin-6-one (3b). Sodium nitrite (4.0 g, 57.97 mmol) was added to the hot stirring mixture in small portions over a period of 1 hour until the color had changed to a light yellow color. After the mixture was cooled to room temperature, the solid was collected by filtration and mixed with 3 equiv of methylocarbonyl isothiocyanate in acetonitrile (50 mL). The mixture was heated at reflux in an oil bath for 8 h. The white solid was collected by filtration and recrystallized from a mixture of ethanol and water to give 1.78 g (60% from 3b): mp 221–222°C; 1H NMR (200 MHz, DMSO-d6) δ 1.26 (q, 2H, CH2), 1.5–1.8 (3m, 4H, CH2), 2.17 (t, 2H, CH2), 3.19 (q, 2H, CH2), 3.70 (s, 3H, CH3), 6.17 (t, 1H, NH, D2O exchangeable), 6.27 (s, 2H, NH2, D2O exchangeable), 9.37 (br s, 1H, NH, D2O exchangeable), 10.07 (s, 1H, NH, D2O exchangeable), 11.11 (s, 1H, NH, D2O exchangeable), 11.85 (s, 1H, NH, D2O exchangeable). Anal. (C13H18N4O) C, H, N.

A mixture of 2-amino-4-(naphthylmethyl)amino)pyrimidin-6-one (3e) and sodium dithionite was added to the hot stirring mixture in small portions over a period of 1 hour until the color

8-Amino-9-hexylguanine (9a). A mixture of 7a (1.35 g, 4.38 mmol) and anhydrous potassium carbonate (1.31 g, 9.49 mmol) in anhydrous methanol (50 mL) was heated at reflux in an oil bath. After 6 h, the solvent was removed by evaporation in vacuo (water pump) at 70°C. Water (100 mL) was added to the residue, and the mixture was then heated at reflux in an oil bath for 24 h. The mixture was cooled to room temperature and the pH was adjusted to 5 with glacial acetic acid. The solid was collected by filtration and washed with water (50 mL). The crude product was recrystallized from 1N sodium hydroxide solution and glacial acetic acid to afford 0.82 g (75% of 9a): mp 300°C; UV Lambda (nm) (MeOH–DMSO, v:v, 8:2) 259 (1.5), 293 (0.9); (pH 1) 289 (1.5), 320 (0.9); (pH 11) 255 (1.7); 1H NMR (270 MHz, DMSO-d6) δ 0.83 (t, 3H, CH3), 1.23 (s, 4H, 2CH2), 1.55 (m, 2H, CH2), 3.73 (t, 2H, CH2), 5.38 (s, 2H, NH2, D2O exchangeable), 6.17 (s, 2H, NH2, D2O exchangeable), 10.44 (s, 1H, NH, D2O exchangeable). Anal. (C13H18N4O) C, H, N.

8-Amino-9-(carboxypropyl)guanine (9b). Dicyclohexylcarbodiimide (1.1 g, 5.34 mmol) was added to a mixture of 6b (1.0 g, 2.93 mmol) and methylene chloride (15 mL). The mixture was evaporated to dryness in vacuo (water pump) at 70°C and then suspended in small portions over a period of 1 hour until the color changed to a light yellow color. After the mixture was cooled to room temperature, and the solid was collected by filtration and mixed with 3 equiv of methylocarbonyl isothiocyanate in acetonitrile (50 mL). The mixture was heated at reflux in an oil bath for 8 h. The white solid was collected by filtration and recrystallized from a mixture of ethanol and water to give 1.78 g (60% from 9b): mp 221–222°C; 1H NMR (200 MHz, DMSO-d6) δ 1.26 (q, 2H, CH2), 1.5–1.8 (3m, 4H, CH2), 2.17 (t, 2H, CH2), 3.19 (q, 2H, CH2), 3.70 (s, 3H, CH3), 6.17 (t, 1H, NH, D2O exchangeable), 6.27 (s, 2H, NH2, D2O exchangeable), 9.37 (br s, 1H, NH, D2O exchangeable), 10.07 (s, 1H, NH, D2O exchangeable), 11.11 (s, 1H, NH, D2O exchangeable), 11.85 (s, 1H, NH, D2O exchangeable). Anal. (C13H18N4O) C, H, N.
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(t, 2 H, CH₃), 3.74 (t, 2 H, CH₂), 5.99 (s, 2 H, NH₂, D₂O exchangeable), 6.30 (s, 2 H, NH₂, D₂O exchangeable), 10.78 (s, 1 H, NH, D₂O exchangeable). (t, 2 H, CH₂), 5.62 (s, 2 H, NH₂, D₂O exchangeable), 10.14 (s, 1 H, NH, D₂O exchangeable). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.

8-Amino-9-(cyclohexylmethyl)guanine (9c). Compound 9c was prepared in 71% yield using a procedure similar to that which afforded 9a: mp >300 °C; UV λ_max nm (ε x 10³) (0.1 N NaOH) 270 (1.2); H NMR (100 MHz, DMSO-d₆) δ 6.55 (s, 2 H, CH₂), 7.37 (t, 2 H, CH₂), 5.37 (s, 2 H, NH₂, D₂O exchangeable), 6.14 (s, 2 H, NH₂, D₂O exchangeable), 7.30–7.21 (m, 5 H), 3.58 (s, 2 H, CH₂), 5.79 (s, 2 H, D₂O exchangeable), 10.09 (s, 1 H, NH, D₂O exchangeable). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.

8-Amino-9-(naphthylmethyl)guanine (9d). Compound 9d was prepared in 67% yield using a procedure similar to that which afforded 9a: mp >300 °C; UV λ_max nm (ε x 10³) (0.1 N NaOH) 282 (1.08); H NMR (100 MHz, DMSO-d₆) δ 6.55 (s, 2 H, CH₂), 7.37 (t, 2 H, CH₂), 5.37 (s, 2 H, NH₂, D₂O exchangeable), 6.14 (s, 2 H, NH₂, D₂O exchangeable), 7.24–7.35 (m, 6 H), 5.4 Hz, D₂O exchangeable), 5.63 (d, 1 H, H-1', J = 5.4 Hz), 5.34 (d, 1 H, H-2' OH, J = 6.08 Hz), 5.58 (d, 1 H, H-1', J = 5.76 Hz), 5.98 (s, 2 H, NH₂, D₂O exchangeable), 6.39 (s, 2 H, NH₂, D₂O exchangeable), 7.14–7.35 (m, 5 H, ArH), 11.04 (s, 1 H, NH), UV λ_max nm (ε x 10³) (methanol) 254 (2.3), 292 (0.83); (pH 1) 251 (2.1), 291 (0.87); (pH 11) 254 (2.0). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.

8-Amino-5'-[5-(fluorosulfonyl)benzoyl]guanosine (21). Concentrated hydrochloric acid (0.7 mL) was added to a stirred suspension of 8-aminoguanosine (12, 1.0 g, 3.38 mmol) in anhydrous DMF (10 mL). The mixture was allowed to stir at room temperature for 1.5 h. (pH 11) 250 (1.3), 293 (0.7); (pH 1) 250 (1.1); (pH 11) 250 (1.1); (pH 1) 288 (1.2). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.

5'-[5-(Fluorosulfonyl)benzoyl]guanosine (21). Guanosine (1.0 g, 3.5 mmol) was coevaporated with anhydrous DMF (2 x 10 mL) at 60 °C in vacuo and then was dissolved in hexamethylphosphoramide (10 mL). The solution was immersed in an ice bath and to this solution was added p-(fluorosulfonyl)benzoyl chloride (1.5 g, 6.7 mmol) in small portions. The mixture was allowed to stir at room temperature for 4 h and was then extracted with petroleum ether (70 mL) and a mixture of ethyl acetate–ether (1:1) (100 mL). The resulting yellow oil was then dissolved in hexamethylphosphoramide (10 mL). Ether (100 mL) was added to this solution to afford a precipitate. The white solid was collected by filtration and subsequently dissolved in anhydrous DMF (10 mL). Anhydrous triethylamine (1 mL) was added to the resulting solution to afford a precipitate. To the suspension was added ether (100 mL) slowly with stirring. The yellow solid was collected by filtration and air-dried. The yellow solid was recrystallized from a mixture of ethanol and water (2:3) (150 mL). The resulting yellow oil was then dissolved in methanol (10 mL) and was added to this mixture to afford 9a: mp >300 °C; UV λ_max nm (ε x 10³) (0.1 N NaOH) 270 (1.2); H NMR (100 MHz, DMSO-d₆) δ 7.37 (t, 2 H, CH₂), 5.37 (s, 2 H, CH₂), 6.76 (m, 1 H, CH), 3.58 (s, 2 H, CH₂), 5.79 (s, 2 H, D₂O exchangeable), 10.09 (s, 1 H, NH, D₂O exchangeable). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.

8-Amino-5'-[5-(fluorosulfonyl)benzoyl]guanosine (21). Concentrated hydrochloric acid (0.7 mL) was added to a stirred suspension of 8-aminoguanosine (12, 1.0 g, 3.38 mmol) in anhydrous DMF (10 mL). The mixture was allowed to stir at room temperature for 1.5 h. (pH 11) 250 (1.3), 293 (0.7); (pH 1) 250 (1.1); (pH 11) 250 (1.1); (pH 1) 288 (1.2). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.

5'-[5-(Fluorosulfonyl)benzoyl]guanosine (21). Guanosine (1.0 g, 3.5 mmol) was coevaporated with anhydrous DMF (2 x 10 mL) at 60 °C in vacuo and then was dissolved in hexamethylphosphoramide (10 mL). The mixture was immersed in an ice bath and to this solution was added p-(fluorosulfonyl)benzoyl chloride (1.5 g, 6.7 mmol) in small portions. The mixture was allowed to stir at room temperature for 4 h and was then extracted with petroleum ether (50 mL). The upper layer was decanted and the lower layer became a yellow oil by the addition of ethyl acetate–ether (1:1) (100 mL). The resulting yellow oil was then dissolved in anhydrous DMF (10 mL). Ether (100 mL) was added to this solution to afford a precipitate. The white solid was collected by filtration and subsequently dissolved in anhydrous DMF (10 mL). Anhydrous triethylamine (1 mL) was added to the resulting solution to afford a precipitate. To the suspension was added ether (100 mL) slowly with stirring. The yellow solid was collected by filtration and air-dried. The yellow solid was recrystallized from a mixture of ethanol and water (2:3) (150 mL) with activated charcoal (1 g) to afford 8-amino-9-(5'-[5-(fluorosulfonyl)benzoyl]guanosine (21). (0.5 g, 37%): mp 240 °C dec; UV λ_max nm (ε x 10³) (0.1 N NaOH) 270 (1.2), 4.65–4.45 (m, 2 H, H-5'), 4.65 (q, 1 H, H-3'), 4.75 (q, 1 H, H-2'), 5.07 (d, 1 H, H-3' OH, J = 6.3 Hz, D₂O exchangeable), 5.43 (d, 1 H, H-2' OH, J = 5.4 Hz, D₂O exchangeable), 5.63 (d, 1 H, H-1', J = 4.13 Hz), 5.86 (s, 2 H, NH₂, D₂O exchangeable), 6.17 (s, 2 H, NH₂, D₂O exchangeable), 8.24 (s, 4 H, ArH), 10.50 (s, 1 H, NH, D₂O exchangeable); UV λ_max nm (ε x 10³) (methanol) 254 (2.3), 293 (0.83); (pH 1) 251 (2.1), 291 (0.87); (pH 11) 254 (2.0). Anal. (C₁₇H₁₄N₆O₂·H₂O) C, H, N.
(3.0 mL). The mixture was allowed to stir at room temperature for 3 h and then concentrated in vacuo at 60 °C to 10 mL. To the concentrated mixture was added ether (50 mL), with shaking, to afford a white solid. The solid was collected by filtration and washed with ether (100 mL) to afford 1.0 g of the crude product. The filtrate was evaporated to dryness and then to this oil residue was added water (20 mL), methanol (5 mL), and ether (100 mL) to obtain another 0.4 g of crude product. The total crude product (4.1 g) was recrystallized from water to furnish 5'-deoxy-5'-[phenylthio]formycin B (1.2 g, 69%); mp 207-209 °C; UV (H2O): 290 nm, (ε x 10^4) 255 (1.5), (pH 11) 252 (1.2), 580 (0.6), 292 (0.3); 1H-NMR (270 MHz, DMSO-d6) 6 5.96 (3 H, H-5'), 5.92 (q, 1 H, H-4'), 4.14 (q, 1 H, H-3'), 4.56 (q, 1 H, H-2'), 4.92 (d, 1 H, H-1', J = 5.5 Hz), 5.09 (d, 1 H, H-2' OH, J = 5.8 Hz, D2O exchangeable), 7.30 (m, 5 H, ArH), 7.89 (s, 1 H, D2O exchangeable), 14.07 (s, 1 H, NH, D2O exchangeable). Anal. (C50H42O2N6S2) C, H, N.

5'-[Fluorosulfonyl]benzoyl|formycin B (24). To a suspension of formycin B (0.5 g, 1.57 mmol) in HMPA (10 mL) was added p-[fluorosulfonyl]benzoyl chloride (0.5 g). The mixture was allowed to stir at room temperature for 12 h. The mixture was then poured into ice-water to furnish a white solid precipitate. The solid was collected by filtration and recrystallization from methanol to afford 5'-[fluorosulfonyl]benzoyl|formycin B (0.6 g, 71%); mp 244-246 °C; UV (H2O): 292 (0.3), 290 (0.6), 285 (1.5); 1H-NMR (270 MHz, DMSO-d6) 6 4.15 (t, 1 H, H-4'), 4.61-4.24 (m, 4 H, H-3', H-2' and H-3'), 5.04 (d, 1 H, H-1', J = 4.35 Hz), 5.15 (t, 2 H, H-2' OH, D2O exchangeable), 7.33 (m, 5 H, ArH), 7.89 (s, 1 H, D2O exchangeable), 12.25 (s, 1 H, NH, D2O exchangeable). Anal. (C42H36O6F2S2) C, H, N.

PNN Inhibitory Assay. Substrate, [8-14C]inosine, is used at a specific activity of 22.5 mCi/mmol. For Kd determinations, a fixed amount of diluted cell extract is incubated with variable amounts of radiolabeled substrate (12.5-100 mM) and inorganic phosphate (50 mM). Kd determinations were performed with variable radiolabeled inosine concentrations (12.5-100 mM), fixed inorganic phosphate concentrations (50 mM), and variable inhibitor concentrations (0.03 mM to 1.0 µM). All reactions are incubated for 30 min at 37 °C. Substrate and product (inosine and hypoxanthine, respectively) are separated by high voltage paper electrophoresis. The radiolabeled substrate of the reaction is visualized by UV light (290 nm), cut from the paper, and counted in a toluene-based scintillation fluid in a Packard Tri-carb liquid scintillation spectrometer. Enzyme-free blank reactions are used as controls for all reactions. Inhibition constants (Ki) for all determinations, with or without inhibitor, not more than 15% of the substrate is converted to product. Double reciprocal plots of the initial velocity values versus the substrate concentrations are linear.

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References
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