# Inhibitors of Human Purine Nucleoside Phosphorylase. Synthesis, Purine Nucleoside Phosphorylase Inhibition, and T-Cell Cytotoxicity of 2,5-Diaminothiazolo[5,4-*d*]pyrimidin-7(6*H*)-one and 2,5-Diaminothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one. Two Thio Isosteres of 8-Aminoguanine

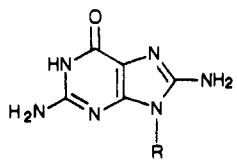
Jagadish C. Sircar,*† Mark J. Suto,† Mary E. Scott,‡ Mi K. Dong,‡ and Richard B. Gilbertsen‡

*Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105.* *Received March 3, 1986*

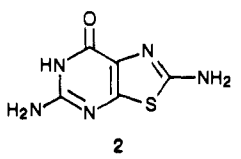
8-Aminoguanine is a potent inhibitor of purine nucleoside phosphorylase (PNP) and also a substrate of PNP. Two thioisosteres of 8-aminoguanine, 2,5-diaminothiazolo[5,4-*d*]pyrimidin-7(6*H*)-one (**2**) and 2,4-diaminothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (**3**), which cannot be substrates of PNP, were synthesized and evaluated for their inhibitory activity against PNP. They were found to be weak inhibitors of PNP and to be noncytotoxic for MOLT-4 T-cells in culture.

Purine nucleoside phosphorylase (PNP) is a purine metabolizing enzyme that catalyzes the phosphorolysis of nucleosides such as (deoxy)inosine or (deoxy)guanosine to their respective bases and the corresponding 1-(deoxy)-ribose phosphate. The reaction is a revesible one, i.e. PNP enzyme also catalyzes the formation of the nucleosides from the bases and 1-(deoxy)ribose phosphate. PNP deficiency has been identified as a genetic defect associated with severe T-cell immune deficiency.[1-4] T-cells are the central element in the regulation of the immune system. Imbalance in T-cell function may be a major factor contributing to the development of autoimmune diseases. Proliferating T-cells are associated with certain autoimmune diseases including rheumatoid arthritis and with transplant rejection and T-cell leukemia. Thus, PNP inhibitors have the potential to be useful as T-cell selective immunosuppressive agents for the treatment of autoimmune diseases, for the prevention of transplant rejection, and also for the treatment of malignant lymphoproliferative diseases. PNP inhibitors may also be useful in the treatment of insulin-dependent diabetes.

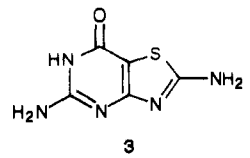
8-Aminoguanine (**1a**) and 8-aminoguanosine[5] (**1b**) have been identified as competitive inhibitors of human PNP that markedly potentiate the toxicity of 2′-deoxyguanosine for, and the accumulation of dGTP in, T lymphoblasts.[6-8]



**1a**, R = H
**b**, R = ribose



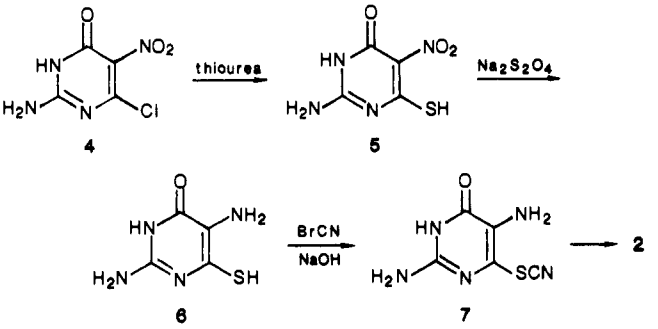
**2**



**3**

Accumulation of dGTP in T-cells causes cell death and blocks lymphocytes clonal expansion. 8-Aminoguanosine has been shown to be a substrate for PNP and is readily phosphorylzed to 8-aminoguanine.[6] Since 8-aminoguanosine is a nucleoside, it is not unusual that it acts as a substrate of PNP. Thus, a nonnucleoside PNP inhibitor that could not partake in the reactions associated with the enzyme would be desirable. We have synthesized two thio isosteres of 8-aminoguanine, **2** and **3**, which by virtue of their chemical structure cannot act as substrates of PNP. This report describes the ability of these thio isosteres to inhibit PNP and also their effects on the growth of human T- and B-lymphoblastoid cell lines.

**Scheme I**




**Chemistry.** Initial attempts to synthesize **2** from 2-amino-6-chloro-5-nitro-4-pyrimidinol[9] with thiourea in EtOH followed by reduction with sodium dithionite gave a mixture of products from which **2** could not be purified. Similarly, reaction of **4** with potassium thiocyanate in DMF followed by reduction with sodium dithionite failed to give the desired product **2**. So, **4** was converted to 2,5-diamino-6-mercapto-4(3*H*)-pyrimidinone (**6**).[10] The sodium salt of **6** was reacted with cyanogen bromide to give the desired product **2** in about 15% yield (Scheme I).

† Department of Chemistry.
‡ Department of Pharmacology.

(1) Gilblet, E. R.; Ammann, A. J.; Wara, D. W.; Sandman, R.; Diamond, L. K. *Lancet* **1975**, *1*, 1010.
(2) Cohen, A.; Doyle, D.; Martin, D. W., Jr.; Ammann, A. J. *N. Engl. J. Med.* **1976**, *295*, 1449.
(3) deBree, P. K.; Wadman, S. K. *Clin. Chem. Acta* **1977**, *174*, 271.
(4) Stoop, J. W.; Zegers, B. J. M.; Hendrick, G. F. M.; van Heukelom, L. H. C.; Staal, G. E. H.; de Bree, P. K.; Wadman, S. K.; Ballieux, R. E. *N. Engl. J. Med.* **1977**, *296*, 651.
(5) Holmes, R. E.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 1772.
(6) Stoeckler, J. D.; Cambor, C.; Kuhns, V.; Chu, S.; Parks, R. E., Jr. *Biochem. Pharm.* **1982**, *31*, 163.
(7) Kazmers, I. S.; Mitchell, B. S.; Dadonna, P. E.; Wotring, L. L.; Townsend, L. B.; Kelley, W. N. *Science (Washington, D.C.)* **1981**, *214*, 1137.
(8) Sidi, Y.; Mitchell, B. S. *J. Clin. Invest.* **1984**, *74*, 1640.
(9) Stuart, A.; Wood, H. C. S. *J. Chem. Soc.* **1963**, *4*, 186.
(10) Nair, M. G.; Boyce, L. H.; Berry, M. A. *J. Org. Chem.* **1981**, *46*, 3354.

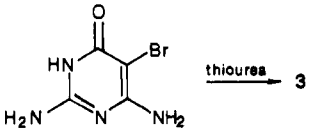
0022-2623/86/1829-1804$01.50/0 

Table I. Purine Nucleoside Phosphorylase (PNP) Inhibition and T-Lymphoblastoid Cell Line Toxicity

| compd | PNP inhibn: $IC_{50}$,[b] μM | $IC_{50}$,[a] μM: Molt-4 (T-cell) Wo/GdR | Molt-4 (T-cell) W/GdR | MGL-8 (B-cell) Wo/GdR | MGL-8 (B-cell) W/GdR |
|---|---|---|---|---|---|
| **1a** | 1.55 | ~400 | 2.35 | >500 | >500 |
| **1b** | 1.33 | ~500 | 3.25 | >500 | >500 |
| **2** | 142.5 | >50[c] | >50 | >50 | >50 |
| **3** | ~330 | >50 | >50 | >50 | >50 |

[a] Concentration required to inhibit cell growth by 50% of the control as assayed by [$^3$H]thymidine uptake. Assays were conducted in the presence or absence of 10 μM 2'-deoxyguanosine (GdR). [b] Concentration required to inhibit 50% of the control reaction. Results are from at least two determinations at multiple concentrations. Formation of radiolabeled hypoxanthine from [$^{14}$C]inosine was determined by TLC and a scintillation counter. [c] Could not be tested at higher concentrations due to limited solubility in culture medium.

The second thio isostere **3** was synthesized from 2,6-diamino-5-bromo-4(3*H*)-pyrimidinone (**8**) by reaction with thiourea in ethanol following the literature procedure.[11]




**8**

## Results and Discussion

8-Aminoguanine (**1a**) and 8-aminoguanosine (**1b**) are very potent human PNP inhibitors.[6] 8-Aminoguanosine is also a substrate for PNP and is converted to **1a** by PNP. Thus, a thio isostere of 8-aminoguanine could retain the potency but not the substrate activity. Both **2** and **3** were evaluated for their PNP activity by a radiochemical assay and also for their effects on the growth (thymidine uptake) of T-lymphoblastoid cell lines (Table I). Both compounds were found to be very weak inhbitors of PNP and were not T-cell cytotoxic under the experimental conditions used. The inactivity of compounds **1a,b** for MGL-8 is consistent with published results on the lack of cytotoxicity of PNP inhibitors, even in the presence of exogenous 2'-deoxyguanosine, for B-lymphoblasts.[7] Thus, the ribose portion and/or the second nitrogen seem to be important for binding to the enzyme. Since thio isosteres were only weakly active PNP inhibitors, the corresponding oxygen isosteres were not synthesized.

## Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. The analyses were run on E. Merck precoated silica gel 60F-254 plates of 0.25-mm thickness. Compounds of interest were detected either by ultraviolet lamp (Mineralight, 254 nm) or by staining with iodine. Ultraviolet and infrared spectra were obtained, respectively, with a Cary 118 spectrophotometer and a Nicolet SX60 instrument. The former were determined as solution in methanol and the latter as KBr plates. Mass spectra were taken on a Finnigan 4523 mass spectrometer with an electron impact of 70 eV. Combustion analyses were performed on a Perkin-Elmer 24 elemental analyzer.

**2,5-Diaminothiazolo[5,4-*d*]pyrimidin-7(6*H*)-one (2).** 2,5-Diamino-6-mercapto-4(3*H*)-pyrimidinone[10] (**6**; 1.0 g, 6.32 mmol) was dissolved in 1 N sodium hydroxide solution 7 mL) and then concentrated to dryness. The dry sodium salt was suspended in THF (25 mL), and cyanogen bromide (0.8 g, 7.6 mmol) was added. The mixture was stirred at room temperature for 24 h and concentrated, and the solid residue was diluted with water and filtered. The crude product **2** was recrystallized twice from a 10% $H_2SO_4$–water mixture to give white crystals: 0.25 g; mp >300 °C dec; IR (KBr) 1690, 1640 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ 266 nm (log ε 4.07), 316 (log ε 3.89); mass spectrum, *m/e* 183. Anal. ($C_5H_5N_5OS\cdot0.75H_2SO_4$) C, H, N, S.

**2,5-Diaminothiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (3).**[11] 2,6-Diamino-5-bromo-4(3*H*)-pyrimidinone (**8**; 5.13 g, 0.025 mol) was added to a solution of thiourea (3.81 g, 0.05 mol) in absolute ethanol (250 mL), and the reaction mixture was heated to reflux under nitrogen for 2.5 h when a solid separated out. The crude product was filtered off, dissolved in 0.2 N NaOH solution, and filtered again. The filtrate was acidified with glacial acetic acid and the solid collected by filtration and dried in vacuo: yield 2.7 g; mp >300 °C (lit.[11] mp >300 °C); IR (KBr) 1655, 1605, 1590 cm$^{-1}$; UV (0.1 N NaOH) $\lambda_{max}$ 261 and 350 nm. Anal. ($C_5H_5N_5OS\cdot H_2O$) C, H, N.

**Radiochemical Assay of Purine Nucleoside Phosphorylase Inhibitory Activity.** This assay procedure was adapted from the one described by Fox et al.[12]

**Materials and Methods.** [8-$^{14}$C]Inosine (58 mCi/mmol) was obtained from Amersham/Searle, and the thin-layer cellulose chromatography sheets with fluorescent indicator (No. 6065) were from Eastman Kodak (No. 13254).

**Human PNP (EC 2.4.2.1) Isolation.** One unit of human whole blood, obtained from a normal donor, was allowed to clot. Serum was removed and the clot frozen at −20 °C (volume 250 mL). The volume of the thawed clot was brought to 500 mL with 0.5 mM Tris containing 0.1 mM EDTA. After standing 2 h at ambient temperature, the material was dialyzed vs. 4 L of the same buffer. After 4 h of dialysis at ambient temperature, the material was filtered through several layers of cheesecloth and stored at −20 °C in 200-μL aliquots. Just prior to use, the material was thawed and diluted with 0.05 M potassium phosphate buffer, pH 7.0.

**Assay for PNP Activity.** Human PNP activity was measured by the conversion of [8-$^{14}$C]inosine to [8-$^{14}$C]hypoxanthine. The assay mixtures were composed of (in order of addition) 60 μL of 0.05 M potassium phosphate buffer (KPB), pH 7.0 (or 60 μL of KPB containing inhibitor); 20 μL of diluted PNP; and 20 μL of [8-$^{14}$C]inosine (30 μM). The assays were run at 37 °C and were started by the addition of inosine to 2-min-preincubated tubes containing inhibitors at varying concentrations in a final volume of 100 μL. The reaction was terminated after incubation by the addition of 20 μL of 8 M formic acid, and the tubes were then maintained on ice.

The product, hypoxanthine, was separated from inosine by chromatography of 20 μL of the reaction mixture, with 5 μL of marker solution (hypoxanthine–inosine, 0.5 mg and 1 mg/mL of $H_2O$, respectively) on cellulose TLC sheets. The sheets were developed to 10 cm above the origin with 1.6 M lithium chloride.

The spots identified under UV light as inosine or hypoxanthine were cut out and counted in 6 mL of scintillation counting solution.

Total cpm per sample were approximately 13 000. Counts due to hypoxanthine contamination of the substrate were less than 0.2%, and random counts between the origin and front were less than 2% of the total counts in the sample. Zero-time incubation of substrate with enzyme yielded background counts of <1.0% of total reaction counts (inosine + hypoxanthine). Percent conversion of substrate to product was calculated by the following equation:

$$\frac{\text{hypoxanthine cpm} - \text{bkgd cpm}}{\text{inosine cpm} + \text{hypoxanthine cpm}} \times 100 = \%\ \text{conversn}$$

$$1 - \frac{\%\ \text{conversn w inhib}}{\%\ \text{conversn w/o inhib}} \times 100 = \%\ \text{inhibn}$$

Duplicate samples and duplicate assays vary less than 10%.

**Assay of Toxicity in the Human Lymphoblast Tissue Culture System.** Compounds were tested for the ability to potentiate the cytotoxicity of 2'-deoxyguanosine for human T-lymphoblasts in culture.[13-15] This assay is based on the finding

(11) Horiuchi, M. *Chem. Pharm. Bull. (Tokyo)* **1959**, *7*, 383.
(12) Fox, I. H.; Andres, C. M.; Gelfand, E. W.; Biggar, D. *Science (Washington, D.C.)* **1977**, *197*, 1084.
(13) Mitchell, B. S.; Mejias, E.; Daddona, P. E.; Kelley, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5011.
(14) Gelfand, E. W.; Lee, J. J.; Dosch, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1998.

that, in the presence of an inhibitor of PNP, exogenously added 2′-deoxyguanosine is highly cytotoxic for T-lymphoblasts, presumably because it is phosphorylated to dGTP, a potent inhibitor of ribonucleotide reductase.[16,17]

Human MOLT-4 T-lymphoblasts (H.E.M. Research, Inc.) and MGL-8 B lymphoblasts (a gift of Dr. B. S. Mitchell, University of Michigan) were maintained in RPMI 1640 containing 10% heat-inactivated fetal calf serum, 1% L-glutamine (2 mM/mL of medium), and 1% penicillin–streptomycin solution. For the cell line assay, RPMI 1640 plus 10% heat-inactivated horse serum was used. Lymphoblasts (100 μL at $5 \times 10^6$ cells/mL) were added to the wells of 96-well U-bottom plates (Costar), and 100 μL of test compound or medium and 50 μL 2′-deoxyguanosine (Sigma) or medium were then added. Cultures were incubated in a humidified atmosphere of 5% $CO_2$ in air at 37 °C for 48 h and labeled with 20 μL of [$^3$H]thymidine (New England Nuclear) for the final 16 h of culture. Incorporation of radioactivity was determined by liquid scintillography. Inhibitory activity of compounds was quantitated in counts/minute and expressed as a percent of the counts incorporated by control cultures. $IC_{50}$ values (the concentration of test compound required to inhibit by 50% the incorporation of [$^3$H]thymidine by control cultures when assayed in the presence of 10 μM 2′-deoxyguanosine) were calculated from log-transformed linear regression analysis.

**Acknowledgment.** We thank Joel R. Wolfe for technical assistance and Dr. F. A. Mackellar and his associates for spectral data and microanalyses.

**Registry No.** **1a**, 28128-41-8; **2**, 103322-70-9; **3**, 22288-77-3; **6**, 37489-38-6; **8**, 6312-72-7; PNP, 9030-21-1; thiourea, 62-56-6.

(15) Carson, D. A.; Kaye, J.; Matsumoto, S.; Seegmiller, J. E.; Thompson, L. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2430.
(16) Moore, E. C.; Hurlbert, R. B. *J. Biol. Chem.* **1966**, *241*, 4802.
(17) Thelander, L.; Reichard, P. *Ann. Rev. Biochem.* **1979**, *48*, 133.

# *Book Reviews*

**Nonsteroidal Antiinflammatory Drugs. Volume 5 of Chemistry and Pharmacology of Drugs.** Edited by J. G. Lombardino. Wiley, New York, Chichester, Brisbane, Toronto, Singapore. 1985. xvii + 442 pp. 16 × 23.5 cm. $99.50

This comprehensive work considers in four chapters (1) "Normal Articular Cartilage and the Alterations in Osteoarthritis" by H. J. Mankin of the Massachusetts General Hospital (Boston), (2) "Inflammation Mechanisms and Mediators" by W. Dawson of the Lilly Research Centre, Ltd. (Surrey), and D. A. Willoughby of St. Bartholomew's Hospital (London), (3) "Laboratory Models for Testing Nonsteroidal Antiinflammatory Drugs" by I. G. Otterness and M. L. Bliven of Pfizer Inc. (Groton), and (4) "Medicinal Chemistry of Acidic Nonsteroidal Antiinflammatory Drugs" by J. G. Lombardino of Pfizer Inc. (Groton). The volume is extensively referenced through 1983 and into 1984 and has a good index. All chapters are interesting, and the four adequately summarize the multidisciplinary aspects of acidic nonsteroidal antiinflammatory drugs and the inflammation process at the animal, organ, cellular, and molecular levels.

Volume 5 is recommended reading for medicinal chemists and others interested in the topic. Educators will find the book an excellent source of material for both graduate and undergraduate courses. In addition to biochemical mechanisms of inflammation and the modes of action and pharmacological properties of numerous clinically important drugs, synthetic schemes are presented for various prototypes. To limit the review size, only acidic antiinflammatory drugs having approved generic names and some clinical data are included. Generally, drugs withdrawn from clinical trials are excluded as are specialized salts, formulations, or obvious prodrugs. Also noteworthy reading for medicinal chemists are discussions on proteoglycans and metaboism of articular cartilage (Chapter 1), biochemical mechanisms and chemical mediators of inflammation (Chapter 2), and operant mechanisms of animal models and the importance of such mechanisms to human disease (Chapter 3).

*The Ohio State University* **Donald T. Witiak**
*Columbus, Ohio 43210*

**Radiation-Protective Drugs and Their Reaction Mechanisms.** By J. C. Livesey, D. J. Reed, and L. F. Adamson. Noyes Publications, Park Ridge, NJ. 1985. xii + 146 pp. 16 × 24 cm. ISBN 0-8155-1051-9. $32.00

The concept that biological systems can be protected from lethal doses of ionizing radiation by chemicals first became established in 1948, from the work of Dale in England and Latarjet in France. In the nearly 40 years that have elapsed since, a variety of substances have shown some effect in preventing radiation damage to living cells, and a number of postulations have been put forth to explain their action. Considering the considerable amount of research on this subject, relatively few comprehensive treatments have been published, although reviews on various aspects of the subject have been adequate. The work by Livesey et al., which provides an up-to-date review of the radiation-protective mechanisms that have been postulated, is therefore most welcome. The coverage of chemical substances that provide protection is limited to a small selection of the more active agents, however.

The book begins with a concise account of the effects of radiation on biological systems. This considers the effects of ionizing radiation at the atomic and molecular levels as well as at the functional and systemic levels. Modes of energy transfer, critical radiation targets, and effects on cell survival are discussed briefly but adequately. Effects of nonionizing and ultraviolet radiation on biological systems are also included.

The second chapter is concerned with endogenous factors that influence radioresistance. A relatively extensive account of the oxygen effect on radiation damage is included, along with the mechanisms proposed. The endogenous radioprotective substances, including glutathione, other thiols, vitamins, and antioxidants, are treated in regard to the free-radical reactions involved. An explanation of the competition model for oxygen and glutathione is included.

Chemical radioprotection from exogenous substances is taken up in the third chapter and provides a thoroughly current treatment. The principal theories of radioprotection that are discussed were all elaborated more than 20 years ago, but much more information on the biochemical bases of these mechanisms is now available. Plausible explanations for the protective activity of specific types of agents can now be made, although no one mechanism has been generally accepted. Repair of radiation damage to macromolecules, including DNA, now a subject of much investigation, still requires further research to delineate the role of thiols and other protectors.

Therapeutic measures for treatment of radiation damage is taken up in the fourth chapter. This includes a discussion of the effects of interferon, tissue extracts, endotoxins, hormones, and exogenous thiols and enzymes. A very brief chapter five proposes recommendations by the authors for future research. There are two appendices: one is entitled "Chemical Radioprotection—Research in Progress" and lists a number of research projects supported by government agencies in the U.S. No dates for these projects are given. Appendix B includes "Some Compounds Showing Radio-Protective Activity" and includes a very brief discussion of structure–activity relationships and a list of protective compounds with relevant test data. The list is quite brief