data. The effect of the inhibitor on K_m and V_{max} was verified by analyzing the data using the nonlinear least-squares HYPMIC program of R. B. Barlow, Elsvevier-Biosoft, Cambridge, UK.

Acknowledgment. The financial support of the Alabama Affiliate of the American Diabetes Association and of an Auburn University Grant-in-Aid and Pfizer Central Research, Groton, CT, for providing us with sorbinil are gratefully acknowledged.

Antiinflammatory Activity of Substituted 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones. Atypical Nonsteroidal Antiinflammatory Agents

David J. Blythin,* James J. Kaminski,* Martin S. Domalski, James Spitler, Daniel M. Solomon, David J. Conn, Shing-Chun Wong, Laura Lehman Verbiar, Loretta A. Bober, Peter J. S. Chiu, Arthur S. Watnick, Marvin I. Siegel, James M. Hilbert, and Andrew T. McPhail†

Pharmaceutical Research Division, Schering-Plough Corporation, Bloomfield, New Jersey, 07003, and Department of Chemistry, Duke University, Durham, North Carolina 27706. *Received August 12, 1985*

A novel class of antiinflammatory drugs, which are substituted derivatives of the fused tricyclic system 6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (see, for instance, Table III), is described. Synthetic procedures and structure determination with the assistance of X-ray crystallography are discussed. Semiempirical molecular orbital calculations are used to investigate the relative stability of the possible isomers and tautomers of the title compounds. A biological profile of the class, and of several of the more potent analogues, in several antiinflammatory models, including the adjuvant-induced arthritis and the collagen II models, is defined. Several members of the class are shown to possess extremely low ulcerogenic effects in spite of exhibiting cyclooxygenase inhibition. A preliminary bioavailability study of two of the lead structures is presented. The compounds **6–72** appear to constitute a class of drugs that shows interesting potential antiarthritic activity and also exhibits an activity profile different from that of the standard classical NSAI drugs, as determined by a comparison of the profile of this class of drug with that of several standard agents. Certain findings from toxicological studies have precluded the further development of compounds within this group, although related structural types are being investigated.

A large amount of work has been performed, in many laboratories, over the past several years, to develop new types of antiinflammatory agents, mainly for use in patients with arthritis of varying degrees of severity.[1] Although numerous products have been introduced over the years, almost all contain a carboxylic acid function[1a] or equivalent[1b]. These products are subject to the serious, limiting side effects of gastrointestinal irritation and ulceration, probably due to their commonly shared ability to inhibit the cyclooxygenase enzyme in the GI tract.[2] In addition, they do not affect the long-term prognosis of the disease state. In our continuing attempts to discover new and useful drugs for the treatment of inflammatory diseases,[3] we have examined a series of tricyclic pyrimido-[2,1-*f*]purines (Table III) which has been found to possess an interesting profile of antiinflammatory activity, different in several respects from that of the known, standard agents, and, in some ways, exhibiting the profile of a disease-modifying drug.

The compounds described lack significant analgesic effects, acute toxicity, and GI side effects. After a single oral dose, significant plasma levels have been observed for two of the most interesting compounds. Finally, single-crystal X-ray analyses,[4] performed on three analogues (**32**, **67**, **76**), confirm unequivocally the direction of cyclization and demonstrate that an intramolecular O–H···O hydrogen-bonded interaction is a consistent feature of the 6-hydroxypyrimido[2,1-*f*]purinetrione system.

Chemistry

The basic ring system was entered by a modification of the classical pyrimidine-forming reaction between a 1,1-diamino derivative (in this case a substituted 8-aminopurine, Table I) and a malonate ester (Table II).[5] However, the typical conditions used for this type of reaction, i.e. NaOEt in refluxing EtOH, failed to produce the desired tricyclic products. A useful modification was found by using a catalytic quantity of NaOMe or NaOEt and employing the malonate as solvent at approximately 190–210 °C, as shown in Scheme I, method A. Use of a Dean–Stark separator to remove the volatile products often improved the yield.

An alternate procedure, using the same starting materials, utilized NaH in DMF to cause condensation and cyclization, as shown in Scheme I, method C.

Alkylation of the 7-unsubstituted derivative **6** occurred exclusively at carbon-7 and is the subject of a separate report[6] (Scheme I, method B).

† Duke University.

(1) (a) See, for instance, a review on Naproxen: Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1979**, *18*, 241. A review on Fenoprofen: Brogden, R. N.; Pinder, R. M.; Speight, T. M.; Avery, G. S. *Drugs* **1977**, *13*, 241. (b) Lombardino, J. G.; Wiseman, E. H.; McLamore, W. M. *J. Med. Chem.* **1971**, *14*, 1171. (c) Castaner, J.; Arrigoni-Martelli, E. *Drugs of the Future* **1977**, *2*, 124. (d) Wiseman, E. H.; Chang, Y.-H.; Lombardino, J. G. *Arzneim.-Forsch.* **1976**, *26*, 1300.

(2) (a) This tentative connection between the gastrointestinal side effects of the classical NSAI drugs is based on the fact that (a) many of these drugs inhibit cyclooxygenase[2b] and (b) administration of protective prostaglandins, e.g. PGE_2 and PGI_2, reduces the gastrointestinal side effects of many NSAI drugs.[2c,d] (b) Vane, J. *Nature (London), New Biol.* **1971**, *231*, 232. (c) Whittle, B. J. R. *Eur. J. Pharmacol.* **1976**, *40*, 233. (d) Rainsford, K. D. In *Prostaglandins and Inflammation*, Suppl. No. 6 (AAS 6) to *Agents Actions* **1979**, 193.

(3) Wong, S. C.; Sasso, S.; Jones, H.; Kaminski, J. J. *J. Med. Chem.* **1984**, *27*, 20.

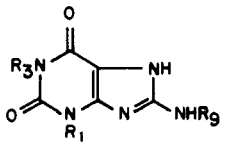
(4) The X-ray crystal data are available as supplementary material. Essential crystal data for compounds **32**, **67**, and **76** are also listed in the Experimental Section.

(5) See, for example: Brown, D. J. *The Pyrimidines*; Wiley: New York, London, 1962; p 31.

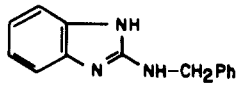
(6) Solomon, D. M.; Conn, D. J.; Wong, S. C.; Kaminski, J. J. *Heterocycles*, in press.

0022-2623/86/1829-1099$01.50/0

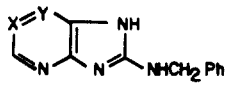
Table I. 8-Substituted Aminotheophyllines 1 and Heterocycles 2–4



1



2

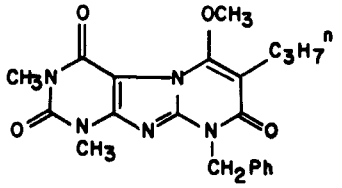


3. X = N; Y = CH
4. X = CH; Y = N

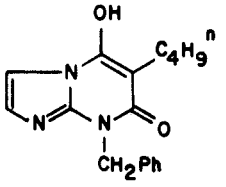
compd	R_1	R_3	R_9	mp, °C	recryst solv[a]	yield, %	formula	anal.
1a	CH_3	CH_3	$PhCH_2$	230–230.5		87	$C_{14}H_{15}N_5O_2$	C, H, N
1b	CH_3	CH_3	2-$FPhCH_2$	225–227		88	$C_{14}H_{14}FN_5O_2$	C, H, N, F
1c	CH_3	CH_3	3-$FPhCH_2$	230–240 dec		97	$C_{14}H_{14}FN_5O_2$	C, H, N, F
1d	CH_3	CH_3	4-$FPhCH_2$	257–260	DMF	68	$C_{14}H_{14}FN_5O_2$	C, H, N, F
1e	CH_3	CH_3	4-$ClPhCH_2$	225–226	DMF	66	$C_{14}H_{14}ClN_5O_2$	C, H, N, Cl
1f	CH_3	CH_3	2,4-$Cl_2C_6H_3CH_2$	260–263		74	$C_{14}H_{13}Cl_2N_5O_2$	C, H, N, Cl
1g	CH_3	CH_3	3,4-$Cl_2C_6H_3CH_2$	275–278		83	$C_{14}H_{13}Cl_2N_5O_2$	C, H, N, Cl
1h	CH_3	CH_3	4-CH_3OPhCH_2	248–250		91	$C_{15}H_{17}N_5O_3$	C, H, N
1i	CH_3	CH_3	4-CH_3SPhCH_2	238–240		90	$C_{15}H_{17}N_5SO_2$	
1j	CH_3	CH_3	4-EtO_2CPhCH_2	228–230		65	$C_{17}H_{19}N_5O_4$	
1k	CH_3	CH_3	2-$PyCH_2$	>275		87	$C_{13}H_{14}N_6O_2$	C, H, N
1l	CH_3	CH_3	2-thienyl-CH_2	257–259	MeOH	55	$C_{12}H_{13}N_5O_2S$	C, H, N, S
1m	CH_3	CH_3	Ph	309–312[b]		79	$C_{13}H_{13}N_5O_2$	C, H, N
1n	CH_3	CH_3	4-FPh	*c*		85	$C_{13}H_{12}N_5O_2F$	
1o	CH_3	CH_3	$PhCH_2CH_2$	248–250		93	$C_{15}H_{17}N_5O_2$	C, H, N
1p	CH_3	CH_3	$PhCHCH_3$	235–238		82	$C_{15}H_{17}N_5O_2$	C, H, N
1q	CH_3	CH_3	*n*-C_6H_{13}	236–238		72	$C_{13}H_{21}N_5O_2$	C, H, N
1r	CH_3	CH_3	c-$C_6H_{11}CH_2$	246–248		94	$C_{14}H_{21}N_5O_2$	C, H, N
1s	*n*-C_4H_9	*n*-C_4H_9	$PhCH_2$	163–164		65	$C_{20}H_{27}N_5O_2$	C, H, N
1t[c]	*n*-C_4H_9	*n*-C_4H_9	4-$FPhCH_2$	150–152		69	$C_{20}H_{26}FN_5O_2$	C*, H, N, F*
1u	*n*-C_4H_9	*n*-C_4H_9	4-CH_3OPhCH_2	164–165.5	MeOH	68	$C_{21}H_{28}N_5O_3$	C, H, N
1v	*n*-C_4H_9	*n*-C_4H_9	2-thienyl-CH_2	158–160	MeOH	58	$C_{18}H_{25}N_5O_2S$	C, H, N, S
1w	*n*-C_4H_9	*n*-C_4H_9	$PhCH_2CH_2$	154–156	MeOH	72	$C_{21}H_{28}N_5O_2$	C, H, N
2				155–160	Et_2O	88	$C_{14}H_{13}N_3$	C, H, N
3				287–289 dec		40	$C_{12}H_{11}N_5$	C, H, N
4				339–341 dec		22	$C_{12}H_{11}N_5$	C, H, N

[a] Isolated and used without further purification unless indicated otherwise. [b] Senga, K.; Ichiba, M.; Kanazawa, H.; Nishigaki, S.; Higuchi, M.; Yoneda, F. *Synthesis* 1977, 264. [c] Very insoluble. Used without purification. [d] For 1t; Anal. ($C_{20}H_{26}FN_5O_2$) H, N; C: calcd, 62.00; found, 61.50; F: calcd, 4.90; found, 4.19.

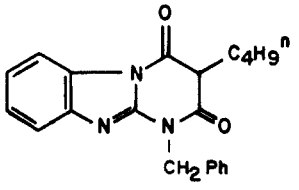
Compounds in which the purine moiety was replaced by other heterocyclic systems (74–77) were prepared from



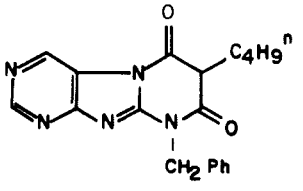
73



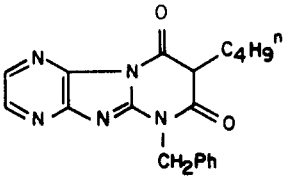
74



75



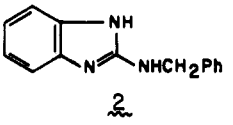
76



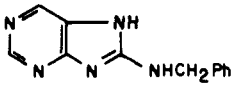
77

intermediates 2–4 and 2-[(phenylmethyl)amino]imidazole[7] by using modifications of standard procedures.

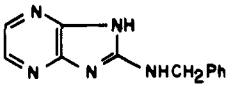
Preparation of the desired amino-substituted purines (1a–w, Table I) or the heterocyclic analogues 2–4 was



2



3



4

accomplished by thermal displacement of halo-substituted intermediates. Those intermediates not commercially available were prepared by a standard sequence of reactions starting from the correctly substituted 1,3-dialkylurea such as 1,3-di-*n*-butylurea and progressing through the pyrimidine intermediates to the desired purines. Since these are reactions based on well-established purine methodology,[8] they will not be described.

Certain of the malonate esters, which were not available commercially, were synthesized according to published procedures (5g;[9] 5h;[10] 5i[11]) or according to procedure (5j).

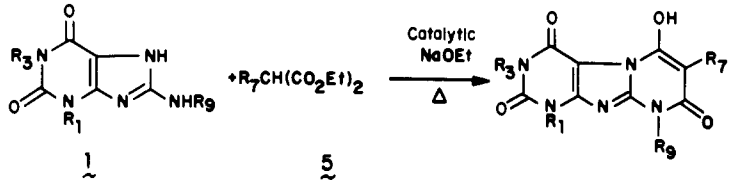
(7) Compernolle, F.; Castagnoli, N. *J. Heterocycl. Chem.* **1982**, *19*, 1403.

(8) (a) Traube, W. *Ber.* **1900**, *33*, 3035. (b) Speer, J. H.; Raymond, A. L. *J. Am. Chem. Soc.* **1953**, *75*, 114.

Scheme I

METHOD A.

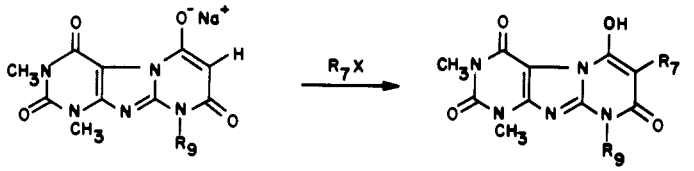
DE NOVO SYNTHESIS OF THE SUBSTITUTED 6-HYDROXYPYRIMIDO [2,1-f]-PURINE-2,4,8,(1H,3H,9H)-TRIONE RING SYSTEM



1 5

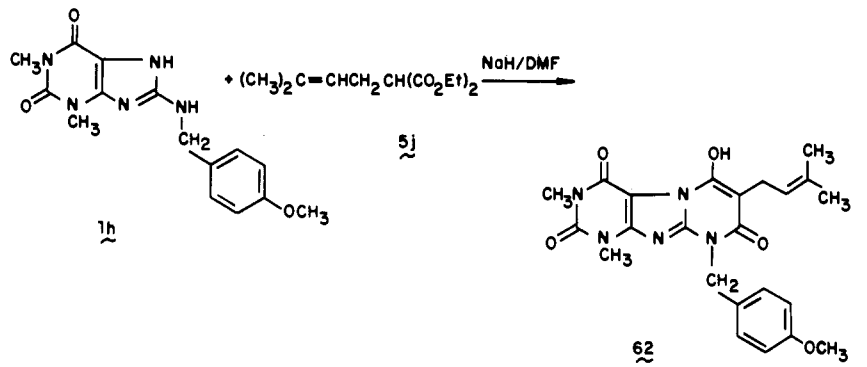
METHOD B.

ALKYLATION OF 9-SUBSTITUTED-1,3,-DIMETHYL-6-HYDROXYPYRIMIDOPURINE [2,1-f] PURINE-2,4,8 (1H,3H,9H)-TRIONE



METHOD C.

SYNTHESIS OF 1,3-DIMETHYL-6-HYDROXY-7-(3-METHYL-2-BUTENYL)-9-(4-METHOXYBENZYL) PYRIMIDO [2,1,-f] PURINE-2,4,8, (1H,3H,9H)-TRIONE (62)



1h 5j 62

The direction of cyclization of the malonate residue **5** to the aminopurine **1** was proposed on the basis of standard spectral data (proton NMR, UV, IR) and established unequivocally by single-crystal X-ray analyses of the representative 1,3-dimethyl (**32**) and 1,3-di-*n*-butyl (**67**) derivatives. Atomic positional and thermal parameters are in Tables S-I–S-VI (supplementary material). Interatomic distances, bond angles, and torsion angles are in Tables S-VII–S-X, while Tables S-XI and S-XII contain displacements of atoms from selected least-squares planes. Views of the structures and solid-state conformations, provided in Figure 1a and b, not only show that cyclization consistently occurred in the predicted direction but also reveal that the favored tautomer is the 6-OH-8-oxo form, which has a strong intramolecular O–H···O hydrogen bond between the C-6 hydroxy and C-4 carbonyl groups. Ring atoms of the pyrimido[2,1-*f*]purine skeleton in the two crystallographically independent molecules of **67** are approximately coplanar, but their directly bonded carbon and oxygen atoms are displaced from the least-squares plane through N-1–C-10a by varying amounts to accommodate intra- as well as intermolecular nonbonded interactions. In **37**, however, in addition to the substituent displacements, atoms C-6, C-7, and C-8 are displaced by significant amounts (Δ 0.104–0.190 Å) to the same side of the least-squares plane through N-1–N-5, C-9a–C-10a, and thus the tricyclic framework is slightly bowed.

In the case of heterocyclic analogue **76**, prepared for comparison, cyclization could also have taken place at the alternate nitrogen atom of the imidazole ring of **3**, but only one product was isolated. A single-crystal X-ray analysis of the monohydrate verified that ring formation occurred in the same direction as with aminopurine **1**. A view of the solid-state conformation is in Figure 2. Atomic positional and thermal parameters are in Tables S-XIII–S-XV (supplementary material). Interatomic distances and bond angles are in Table S-XVI. Torsion angles and displacements from least-squares planes are in Tables S-XVII and S-XVIII, respectively. This analogue, which lacks the potential for stabilization of a 6-OH function by intra-

(9) Efimovsky, O. *J. Rech. C.N.R. Sci.* **1959**, *No. 47*, 147; *Chem. Abstr.* **1962**, *56*, 4742*i*.
(10) Hayashi, K. *Chem. Pharm. Bull.* **1960**, *8* (3), 177.
(11) Edwards, D.; Hamer, D.; Stewart, W. H. *J. Pharm. Pharmacol.* **1964**, *16* (9), 618.

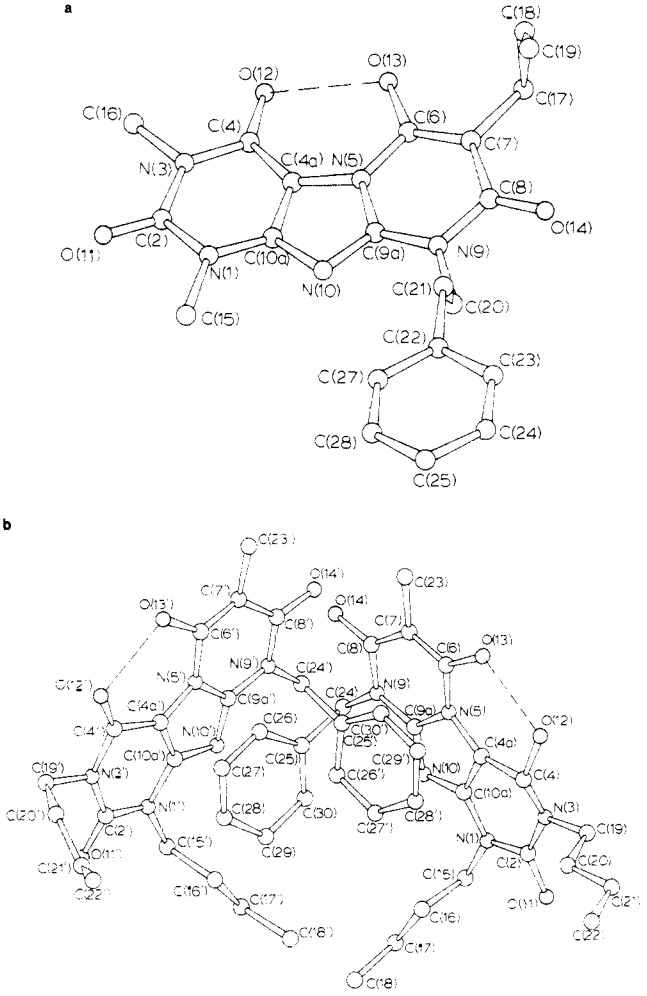


Figure 1. (a) Structure and solid-state conformation of **32**. The broken line denotes an intramolecular O–H···O hydrogen bond. (b) Structure and solid-state conformation of the two crystallographically independent molecules defining the asymmetric crystal unit for **67**. The broken lines denote intramolecular O–H···O hydrogen bonds.

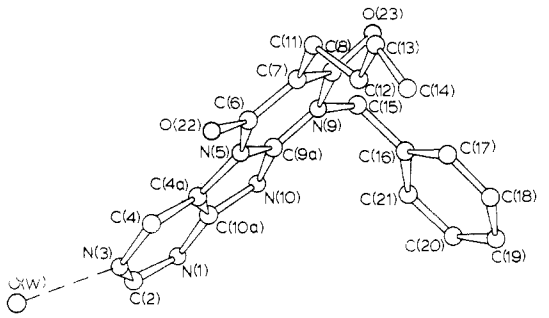


Figure 2. Structure and solid-state conformation of **76** in crystals of the monohydrate. The broken line denotes an N–H···O hydrogen bond.

molecular O–H···O hydrogen bonding, exists in zwitterionic form in the solid state with the directly bonded carbon and oxygen substituent atoms again displaced by significant amounts (Δ 0.059–0.108 Å) from the least-squares plane through the approximately planar C-1–C-10a skeleton.

Subsequent assignment of structures was by comparison of the proton NMR, UV, and IR spectra with the compounds of defined structure. The observed spectra were in accord with the assigned structures and were unremarkable.

Consistent with the experimental observations defining the direction of ring closure, semiempirical molecular orbital calculations predict the same result based on the differences in gas-phase stability between the two possible reaction products. Since the reaction was conducted under equilibrium conditions (thermodynamic control), the difference in the stabilities of the potential reaction products determines the course of the reaction. Using MNDO/2, the gas-phase heat of formation of the product formed by cyclization to N-7, **A** in Figure 3, is 18.8 kcal/mol lower than the product formed by cyclization to N-9 **B**. Much of the difference in energy may be assigned to the strong steric interaction between the hydroxyl and *N*-methyl groups in isomer **B**. This theoretical prediction is in agreement with the experimental observations.

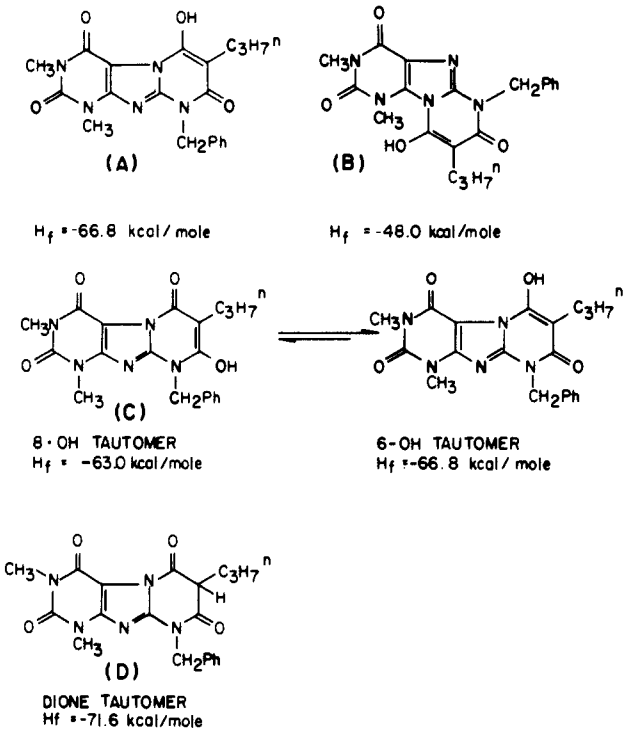


Figure 3. Comparative heats of formation for selected Substituted 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-triones.

The gas-phase stabilities of the possible tautomers of the reaction product were next compared. Using gas-phase heats of formation calculated using MNDO/2, the 6-OH-8-oxo tautomer, **A**, was predicted to be more stable than the 8-OH-6-oxo tautomer, **C**, by 3.8 kcal/mol. It is important to note that the MNDO/2 procedure employed does not take into account any additional stabilization that may arise from hydrogen bonding, which is known to exist in the solid state for the 6-OH-8-oxo tautomer **A**.

Comparison of the gas-phase heat of formation of the 6,8-dione tautomer **D** with that of **A** suggests that **D** is more stable than **A** by 4.8 kcal/mol. Since a hydrogen bond provides approximately 3–5 kcal/mol in stability,[12] the two possible tautomers may be of almost identical energy, at least in the gas phase. Experimentally, however, only the 6-OH-8-oxo tautomer **A** is observed both in the solid state and in Me_2SO solution, based on X-ray and ^1H NMR respectively. It cannot be stressed emphatically enough that the gas-phase calculations only provide a rational basis for prediction of the ideal state. The effects of solute–solute and solute–solvent interactions (perturbations of the ideal state) can provide the energy necessary to deviate from predicted results. In this case, these in-

(12) See, for instance: Eliel, E. L. *Stereochemistry of Carbon Compounds*; McGraw-Hill: New York, 1962; pp 131–132.

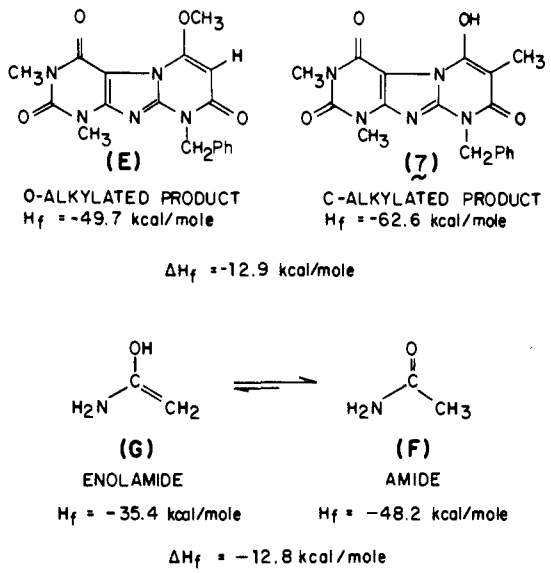


Figure 4. Comparative heats of formation of Alkylated Pyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-triones.

teractions must stabilize isomer **A** relative to isomer **D**.

An unexpected result was obtained when the alkylation of the 7-unsubstituted derivative was examined. Since the X-ray and ^1H NMR data showed that the 6-OH-8-oxo tautomer was the most stable, it was anticipated that alkylation of **6** could at least produce a mixture of the 6-*O*-alkyl ether, **E**, and the 7-methyl isomer **7**, as shown in Figure 4. However, when the alkylation was carried out with methyl iodide, the exclusive product was the 7-methyl compound **7** and not the ether **E**. MNDO/2 calculation of the heats of formation of the two possible isomers **E** and **7** (Figure 4) confirm that the C-alkylated product, **7**, is more stable than the O-alkylated product by 12.9 kcal/mol. This energy difference correlates with the calculated difference in energy between the amide **F** and enol amide **G** tautomers (Figure 4) and suggests that this difference in energy may be reflected in the transition states leading to products and affects the course of reaction. Further discussion of this interesting alkylation has been published elsewhere.[6]

Biological Results and Discussion

The primary model used to detect antiinflammatory activity in this series of compounds was the reverse passive arthus reaction (RPAR)–synovitis assay,[13] which is described in the Experimental Section. A compound showing significant activity in this acute inflammatory reaction was screened subsequently in the chronic adjuvant arthritic rat (AAR) assay using prophylactic and/or therapeutic treatment regimens. In addition, certain of the more potent derivatives were screened for inhibition of the 5-lipoxygenase and cyclooxygenase enzymes; and several of the most interesting compounds were examined in the collagen II arthritis assay in rats[14] (Tables IV–VI, respectively).

Results from the acute screen indicate some generalizations in SAR. Although only a limited number of 1,3-dibutyl derivatives were synthesized (**66–72**), their level of activity appeared to be considerably below that of the corresponding 1,3-dimethyl derivatives, e.g. **8**, **10**, **17**, and **18**. Further modifications were carried out with the 1,3-dimethyl derivatives only.

Those derivatives in which R_9 was a substituted phenyl group (**34–39**) showed generally low levels of activity in the primary screen. A *p*-fluoro substituent appeared to improve activity (**38**, **39**), and the latter compound showed slight activity in the AAR screen. A 2-pyridylmethyl substituent (**27**) or an α-methylbenzyl group (**28**) at position 9 led to products with marginal activity in the primary screen, and such substitution patterns were not further pursued. Also leading to rather low levels of activity were the 2-phenylethyl substituent (**31–33**) and the 2-thienylmethyl group (**26**), which is considered isosteric with a benzyl group.[15] In light of the fact that a benzyl group appears to give the highest overall activity in this series (vide infra), this was a somewhat surprising result. Comparable levels of activity were observed with the 9-*n*-hexyl (**29**) and 9-cyclohexylmethyl (**30**) analogues. However, they did not seem to offer any advantages over the benzyl analogues and were not pursued.

Variation of R_9 yielded the best activity when a benzyl, or substituted benzyl, group was present, although activity was also greatly influenced by the R_7 substituent. Of the simple R_7 substituents it appeared that optimum activity was observed with a short straight alkyl chain of 1–4 carbon atoms, e.g. **8**, **10**, and **17**. The 7-H analogue **6** as well as the simple, branched alkyl analogues **9** (R_7 = isopropyl) and **11** (R_7 = isobutyl) all showed low levels of activity and were not explored further. A similar observation was made for the 7-benzyl compounds **12** and **19**. Initial SAR work was undertaken to optimize the substitution of the benzyl group, utilizing simple alkyl substituents at R_7. A 4-fluoro substituent retained or improved activity (**7** vs. **16**; **8** vs. **17**), whereas the 3- and 2-fluoro analogues both showed a reduction of activity (**13**, **14**). A number of chloro-substituted derivatives were made (**20–24**), but none of them exhibited superior activity to the benzyl or 4-fluorobenzyl analogues, and they were not pursued. A 4-methoxy substituent (**25**) resulted in a compound of low activity. In summary, a preliminary SAR generalization is that R_9 is preferably an unsubstituted benzyl or a 4-fluorobenzyl group, and the R_7 substituent could be a short straight alkyl group.

All of these compounds suffer from very low water solubility in the neutral state, whereas the sodium salts are quite water soluble. Introduction of hydrophilic groups into the R_7 side chain was investigated to improve the biological profile of the molecule. None of these hydrophilic analogues (**40–53**; Table IV) exhibited sufficient activity in the primary screen to be further examined.

Thus, it seemed that there should be a lipophilic grouping at C-7 in order to retain or enhance biological activity. To test this hypothesis, a series of unsaturated side chains was investigated (**54–65**). Simple allyl and propargyl substitution (**54**, **59**, **61**) yielded compounds with low levels of activity whereas introduction of a 3-methyl-2-butenyl (prenyl) side chain produced compounds that exhibited significantly greater potency than the alkyl analogues (e.g., **8** and **17**). A 4-fluoro substituent on the pendant benzyl group at C-9 did not significantly influence the activity in this series (**57** vs. **60**).

Finally, the purine nucleus was replaced by several other nitrogen-containing heterocyclic ring systems (**74–77**).

(13) Casmer, C.; Jones, H.; Watnick, A. S., unpublished results. To establish the validity of this procedure in detecting compounds with true antiinflammatory activity, and eliminating false positives, the following compounds were tested (po) and found inactive: theophylline (50 and 100 mg/kg); cyproheptadine (100 mg/kg); azatadine (100 mg/kg); propanolol (20 mg/kg).

(14) Bober, L. A.; Tivey, L. C.; DaFonseca, M.; Smith, S. R.; Watnick, A. S. *Immunopharmacology* **1985**, *9*, 97.

(15) See, for instance: Korolkovas, A.; Burckhalter, J. H. *Essentials of Medicinal Chemistry*; Wiley: New York, London, 1976; p 23.

Table II. 2-Substituted Diethyl Malonates 5

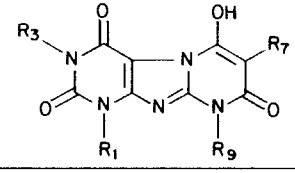
$R_7CH(CO_2Et)_2$

5

compd	R_7	bp, °C (mmHg)	ref	compd	R_7	bp, °C (mmHg)	ref
5a	H		*a*	**5f**	i-C_4H_9		*c*
5b	CH_3		*a*	**5g**	$EtOCH_2CH_2$	*d*	9
5c	n-C_3H_7		*a*	**5h**	$EtSCH_2CH_2$	*d*	10
5d	i-C_3H_7		*b*	**5i**	$(CH_3)_2NCH_2CH_2$	*d*	11
5e	n-C_4H_9		*a*	**5j**	$(CH_3)_2C{=}CHCH_2$	143–147 (17)	*e*

[a] Obtained from Aldrich Chemical Co. [b] Obtained from Fluka Chemical Co. [c] Obtained from Pfaltz and Bauer Chemical Co. [d] Isolated and used without further purification. [e] Experimental procedure described.

Table III. Substituted 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones

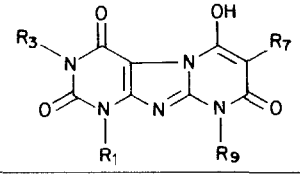


compd	R_1	R_3	R_9	R_7	method of synth	salt[a]	mp, °C	recryst[b] solvent	yield, %	formula	anal.	ref
6	CH_3	CH_3	$PhCH_2$	H	A	Na	>295		77	$C_{17}H_{14}N_5O_4Na{\cdot}H_2O$	C, H, N, Na	6
7	CH_3	CH_3	$PhCH_2$	CH_3	A		207–209	DMF	54	$C_{18}H_{17}N_5O_4$	C, H, N	
8	CH_3	CH_3	$PhCH_2$	n-C_3H_7	A	Na	275–285		76	$C_{20}H_{20}N_5O_4Na{\cdot}H_2O$	C, H, N, Na	
9	CH_3	CH_3	$PhCH_2$	i-C_3H_7	A		167–168.5		30	$C_{20}H_{21}N_5O_4$	C, H, N	
10	CH_3	CH_3	$PhCH_2$	n-C_4H_9	A	Na	266–280		58	$C_{21}H_{22}N_5O_4Na{\cdot}H_2O$	C, H, N, Na	
11	CH_3	CH_3	$PhCH_2$	i-C_4H_9	A		168–169		41	$C_{21}H_{23}N_5O_4$	C, H, N	
12	CH_3	CH_3	$PhCH_2$	$PhCH_2$	B		190–195	EtOAc	26	$C_{24}H_{21}N_5O_4$	C, H, N	6
13	CH_3	CH_3	2-$FPhCH_2$	CH_3	A		224–226	$CHCl_3/Et_2O$	52	$C_{18}H_{16}FN_5O_4$	C, H, N, F	
14	CH_3	CH_3	3-$FPhCH_2$	CH_3	A		218.5–219.5		58	$C_{18}H_{16}N_5O_4{\cdot}{}^1/_4H_2O$	C, H, N, F	
15	CH_3	CH_3	4-$FPhCH_2$	H	A	Na	260–280		68	$C_{17}H_{13}FN_5O_4Na{\cdot}H_2O$	C, H, N, F, Na	
16	CH_3	CH_3	4-$FPhCH_2$	CH_3	A		242–243.5	DMF	31	$C_{18}H_{16}FN_5O_4{\cdot}{}^1/_4H_2O$	C, H, N, F	
17	CH_3	CH_3	4-$FPhCH_2$	n-C_3H_7	A	Na	280–300		55	$C_{20}H_{19}FN_5O_4Na{\cdot}H_2O$	C, H, N, F, Na	
18	CH_3	CH_3	4-$FPhCH_2$	n-C_4H_9	A	Na	265–290		62	$C_{21}H_{21}FN_5O_4Na{\cdot}H_2O$	C, H, N, F, Na	
19	CH_3	CH_3	4-$FPhCH_2$	$PhCH_2$	A		247–248.5		44	$C_{24}H_{20}FN_5O_4$	C, H, N, F	
20	CH_3	CH_3	4-$ClPhCH_2$	CH_3	A		236–238		82	$C_{18}H_{16}ClN_5O_4$	*d*	
21	CH_3	CH_3	4-$ClPhCH_2$	n-C_3H_7	A		192–194	DMF	54	$C_{20}H_{20}ClN_5O_4$	C, H, N, Cl	
22	CH_3	CH_3	4-$ClPhCH_2$	n-C_4H_9	A		178–180.5	DMF	29	$C_{21}H_{22}ClN_5O_4$	C, H, N	
23	CH_3	CH_3	2,4-Cl_2-$C_6H_3CH_2$	n-C_4H_9	A		220–222	$CHCl_3$/EtOAc	82	$C_{21}H_{21}Cl_2N_5O_4$	C, H, N, Cl	
24	CH_3	CH_3	3,4-Cl_2-$C_6H_3CH_2$	n-C_4H_9	A		214–216	$CHCl_3$/EtOAc	76	$C_{21}H_{21}Cl_2N_5O_4$	C, H, N, Cl	
25	CH_3	CH_3	4-CH_3O-$PhCH_2$	n-C_3H_7	A		207–208	$CHCl_3/Et_2O$	40	$C_{21}H_{23}N_5O_5$	C, H, N	
26	CH_3	CH_3	2-thienyl-CH_2	n-C_3H_7	A		225–226	$CHCl_3/Et_2O$	72	$C_{18}H_{19}N_5O_4S$	C, H, N, S	

27	CH_3	CH_3	2-$PyCH_2$	n-C_3H_7	A		208–210	DMF	9	$C_{19}H_{20}N_6O_4$	C, H, N	
28	CH_3	CH_3	$PhCHCH_3$	n-C_3H_7	A		197–200	$CHCl_3/Et_2O$	55	$C_{21}H_{23}N_5O_4$	C, H, N	
29	CH_3	CH_3	n-C_6H_{13}	n-C_3H_7	A	Na	245–260	$CHCl_3$/EtOAc	52	$C_{19}H_{26}N_5O_4Na$	C, H, N, Na	
30	CH_3	CH_3	$C_6H_{11}CH_2$	n-C_4H_9	A		151–153	$CHCl_3$/hexanes	53	$C_{21}H_{29}N_5O_4$	C, H, N	
31	CH_3	CH_3	$PhCH_2CH_2$	CH_3	A		222–224	MeOH	25	$C_{19}H_{19}N_5O_4$	C, H, N	
32	CH_3	CH_3	$PhCH_2CH_2$	n-C_3H_7	A		168–170	MeOH	75	$C_{21}H_{23}N_5O_4$	C, H, N	
33	CH_3	CH_3	$PhCH_2CH_2$	i-C_3H_7	A		209–211	MeOH	31	$C_{21}H_{23}N_5O_4$	C, H, N	
34	CH_3	CH_3	Ph	CH_3	A		>260	CH_3CN/EtOH	41	$C_{17}H_{15}N_5O_4$	*e*	
35	CH_3	CH_3	Ph	n-C_3H_7	A		>260		62	$C_{19}H_{19}N_5O_4$	C, H, N	
36	CH_3	CH_3	Ph	n-C_4H_9	A		232–234.5	CH_3CN	49	$C_{20}H_{21}N_5O_4$	C, H, N	
37	CH_3	CH_3	Ph	i-C_4H_9	A		>250	DMF	10	$C_{20}H_{21}N_5O_4$	*f*	
38	CH_3	CH_3	4-FPh	n-C_3H_7	A		>260	CH_3CN	7	$C_{19}H_{18}FN_5O_4$	C, H, N	
39	CH_3	CH_3	4-FPh	n-C_4H_9	A	Na	290–315		62	$C_{20}H_{19}FN_5O_4Na\cdot H_2O$	C, H, N, F, Na	
40	CH_3	CH_3	$PhCH_2$	NH_2	Ex[c]		245 dec		27	$C_{17}H_{16}N_6O_4\cdot{}^1/_2H_2O$	C, H, N	
41	CH_3	CH_3	$PhCH_2$	$NHCOCH_3$	Ex[c]		277–279 dec	$CHCl_3/CH_3OH$/EtOAc	43	$C_{19}H_{18}N_6O_5$	C, H, N	
42	CH_3	CH_3	$PhCH_2$	$CH_2CH_2N(CH_3)_2$	A	HCl	195–197 dec	CH_3OH	12	$C_{21}H_{24}N_6O_4\cdot HCl$	C, H, N, Cl	
43	CH_3	CH_3	$PhCH_2$	CO_2Et	Ex[c]	Na	>240 dec	CH_3OH	77	$C_{20}H_{18}N_5O_6Na\cdot H_2O$	C, H, N, Na	
44	CH_3	CH_3	$PhCH_2$	$CH_2CO_2CH_3$	B	Na	227–230 dec		43	$C_{20}H_{19}N_5O_6Na\cdot 1^1/_4H_2O$	C, H, N, Na	
45	CH_3	CH_3	$PhCH_2$	CH_2CO_2H	Ex[c]		258–258.5 dec		99	$C_{19}H_{17}N_5O_6\cdot{}^3/_4H_2O$	C, H, N	
46	CH_3	CH_3	$PhCH_2$	CHO	Ex[c]	Na	>360 dec		86	$C_{18}H_{14}N_5O_5Na\cdot 1^1/_4H_2O$	C, H, N, Na	
47	CH_3	CH_3	$PhCH_2$	CH_2CN	B		>195	CH_3OH/CH_3CN	17	$C_{19}H_{16}N_5O_4$	C, H, N	
48	CH_3	CH_3	$PhCH_2$	CH_2CH_2OH	Ex[c]		183.5–184 dec		71	$C_{19}H_{19}N_5O_5$	C, H, N	
49	CH_3	CH_3	$PhCH_2$	$CH_2CH_2O_2CCH_3$	Ex[c]		68		40	$C_{21}H_{21}N_5O_6$	C, H, N	
50	CH_3	CH_3	$PhCH_2$	CH_2CH_2OEt	A	Na	>300		23	$C_{21}H_{22}N_5O_5Na\cdot{}^3/_4H_2O$	C, H, N, Na	
51	CH_3	CH_3	$PhCH_2$	CH_2CH_2SEt	A	Na	298–300 dec	CH_3OH/CH_3CN	39	$C_{21}H_{22}N_5O_4SNa\cdot 1^1/_4H_2O$	C, H, N, S, Na	
52	CH_3	CH_3	$PhCH_2$	CH_2CH_2SOEt	Ex[c]	Na	207–210 dec		12	$C_{21}H_{22}N_5O_5SNa\cdot 1^3/_4H_2O$	C, H, N, S, Na	
53	CH_3	CH_3	$PhCH_2$	$CH_2CH_2SO_2Et$	Ex[c]		192–193.5		20	$C_{21}H_{23}N_5O_6S$	C, H, N, S	
54	CH_3	CH_3	$PhCH_2$	$CH_2CH_2{=}CH_2$		Na						6
55	CH_3	CH_3	$PhCH_2$	*trans*-$CH_2CH{=}CHCH_3$		Na						6
56	CH_3	CH_3	$PhCH_2$	2-cyclohexen-1-yl		Na						6
57	CH_3	CH_3	$PhCH_2$	$CH_2CH{=}C(CH_3)_2$		Na						6
58	CH_3	CH_3	$PhCH_2$	2-cyclohexylideneethyl	B		150–152.5		16	$C_{25}H_{27}N_5O_4$	C, H, N	
59	CH_3	CH_3	$PhCH_2$	$CH_2C{\equiv}CH$		Na						6
60	CH_3	CH_3	4-$FPhCH_2$	$CH_2CH{=}C(CH_3)_2$	B	Na	240–260		47	$C_{22}H_{21}FN_5O_4Na\cdot H_2O$	C, H, N, F, Na	
61	CH_3	CH_3	4-$FPhCH_2$	$CH_2C{\equiv}CH$	B	Na	>300		38	$C_{20}H_{15}FN_5O_4Na$	C, H, N, Na	
62	CH_3	CH_3	4-CH_3O-$PhCH_2$	$CH_2CH{=}C(CH_3)_2$	C	Na	240–250		77	$C_{23}H_{24}N_5O_5Na\cdot H_2O$	C, H, N, Na	
63	CH_3	CH_3	4-CH_3S-$PhCH_2$	$CH_2CH{=}C(CH_3)_2$	C		172–174	$CHCl_3/CH_3OH$	45	$C_{23}H_{25}N_5O_4S$	C, H, N, S	
64	CH_3	CH_3	4-CH_3SO-$PhCH_2$	$CH_2CH{=}C(CH_3)_2$	Ex[c]		233–235	$CHCl_3/CH_3OH$	63	$C_{23}H_{25}N_5O_5S$	C, H, N, S	
65	CH_3	CH_3	4-EtO_2C-$PhCH_2$	$CH_2CH{=}C(CH_3)_2$	C		190–192	$CHCl_3/CH_3OH$	54	$C_{25}H_{27}N_5O_6$	C, H, N	
66	n-C_4H_9	n-C_4H_9	$PhCH_2$	n-C_3H_7	A		113–115	MeOH	68	$C_{26}H_{33}N_5O_4$	C, H, N	
67	n-C_4H_9	n-C_4H_9	$PhCH_2$	CH_3	A		137–138	i-PrOH	81	$C_{24}H_{29}N_5O_4$	C, H, N	
68	n-C_4H_9	n-C_4H_9	4-$FPhCH_2$	n-C_3H_7	A		129–130.5	MeOH	28	$C_{26}H_{32}FN_5O_4\cdot{}^1/_4H_2O$	C, H, N, F	
69	n-C_4H_9	n-C_4H_9	4-$FPhCH_2$	n-C_4H_9	A		136.5–138	MeOH	37	$C_{27}H_{34}FN_5O_4$	C, H, N, F	
70	n-C_4H_9	n-C_4H_9	4-CH_3O-$PhCH_2$	n-C_3H_7	A		136–138	MeOH	75	$C_{27}H_{35}N_5O_5$	C, H, N	
71	n-C_4H_9	n-C_4H_9	2-thienyl-CH_2	n-C_3H_7	A		115–117	MeOH	43	$C_{24}H_{31}N_5O_4S$	C, H, N, S	
72	n-C_4H_9	n-C_4H_9	$PhCH_2CH_2$	n-C_3H_7	A		117–118.5	MeOH	66	$C_{27}H_{35}N_5O_4$	C, H, N	

[a] Sodium salts are prepared by neutralization of the acid with 1 equiv of sodium hydroxide followed by lyophilization. [b] Isolated and used without further purification unless indicated otherwise. [c] Ex = experimental procedure described. [d] For **20**: Anal. ($C_{16}H_{16}ClN_5O_4$) H, N, Cl; C: calcd, 53.81; found, 53.25. [e] For **34**: Anal. ($C_{17}H_{15}N_5O_4$) H, N; C: calcd, 57.79; found, 57.38. [f] For **37**: Anal. ($C_{20}H_{21}N_5O_4$) C, H; N: calcd, 17.71; found, 17.22.

Table IV. Acute and Chronic in Vivo Antiinflammatory Activity of Substituted 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones

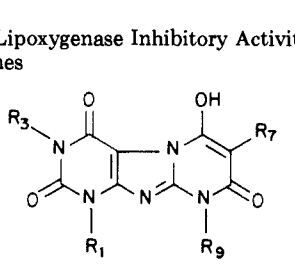


						acute RPAR synovitis % inhibn (50 mg/kg, po)	chronic adjuvant arthritic rat							
							prophylactic % inhibn				therapeutic % inhibn			
compd	R_1	R_3	R_9	R_7	salt		dose, mg/kg, po	1° paw	2° paw	rel potency[a]	dose, mg/kg, po	1° paw	2° paw	rel potency[a]
6	CH_3	CH_3	$PhCH_2$	H	Na	11								
7	CH_3	CH_3	$PhCH_2$	CH_3		8								
8	CH_3	CH_3	$PhCH_2$	n-C_3H_7	Na	33[p]	9	16	38		9	17	15	
							18	41	52	1.0[b]	18	35	52	1.0[c]
							36	59	78		36	64	77	
9	CH_3	CH_3	$PhCH_2$	i-C_3H_7		0								
10	CH_3	CH_3	$PhCH_2$	n-C_4H_9	Na	48	9	17	55					
							18	49	64	2.1[d]				
							36	52	79					
11	CH_3	CH_3	$PhCH_2$	i-C_4H_9		17								
12	CH_3	CH_3	$PhCH_2$	$PhCH_2$		16								
13	CH_3	CH_3	2-$FPhCH_2$	CH_3		0								
14	CH_3	CH_3	3-$FPhCH_2$	CH_3		0								
16	CH_3	CH_3	4-$FPhCH_2$	CH_3		36								
17	CH_3	CH_3	4-$FPhCH_2$	n-C_3H_7	Na	53	9	42	49		3.3	7	30	
							18	56	49	1.2[e]	10	27	38	0.9[f]
							36	60	67		30	47	56	
18	CH_3	CH_3	4-$FPhCH_2$	n-C_4H_9	Na	27	9	28	59					
							18	46	70	3.8[g]				
							36	74	74					
19	CH_3	CH_3	4-$FPhCH_2$	$PhCH_2$		10								
20	CH_3	CH_3	4-$ClPhCH_2$	CH_3		28								
21	CH_3	CH_3	4-$ClPhCH_2$	n-C_3H_7		53								
22	CH_3	CH_3	4-$ClPhCH_2$	n-C_4H_9		16								
23	CH_3	CH_3	2,4-$Cl_2C_6H_3CH_2$	n-C_4H_9		31	10	0	29					
24	CH_3	CH_3	3,4-$Cl_2C_6H_3CH_2$	n-C_4H_9		33	10	0	17					
25	CH_3	CH_3	4-CH_3OPhCH_2	n-C_3H_7		11								
26	CH_3	CH_3	2-thienyl-CH_2	n-C_3H_7	Na	9	10	20	9					
27	CH_3	CH_3	2-$PyCH_2$	n-C_3H_7		17								
28	CH_3	CH_3	$PhCHCH_3$	n-C_3H_7		0								
29	CH_3	CH_3	n-C_6H_{13}	n-C_3H_7	Na	37	10	28	43					
30	CH_3	CH_3	$C_6H_{11}CH_2$	n-C_4H_9	Na	25	10	11	34					
31	CH_3	CH_3	$PhCH_2CH_2$	CH_3		14								
32	CH_3	CH_3	$PhCH_2CH_2$	n-C_3H_7	Na	27	10	14	38					
33	CH_3	CH_3	$PhCH_2CH_2$	i-C_3H_7		17								
34	CH_3	CH_3	Ph	CH_3		14								
35	CH_3	CH_3	Ph	n-C_3H_7		11								
36	CH_3	CH_3	Ph	n-C_4H_9		11								
37	CH_3	CH_3	Ph	i-C_4H_9		16								
38	CH_3	CH_3	4-FPh	n-C_3H_7		27								
39	CH_3	CH_3	4-FPh	n-C_4H_9	Na	26	10	29	35					

40	CH_3	CH_3	$PhCH_2$	NH_2		11								
41	CH_3	CH_3	$PhCH_2$	$NHCOCH_3$		20								
42	CH_3	CH_3	$PhCH_2$	$CH_2CH_2N(CH_3)_2$	HCl	0								
43	CH_3	CH_3	$PhCH_2$	CO_2Et	Na	0								
44	CH_3	CH_3	$PhCH_2$	$CH_2CO_2CH_3$	Na	14								
45	CH_3	CH_3	$PhCH_2$	CH_2CO_2H		11								
46	CH_3	CH_3	$PhCH_2$	CHO		14								
48	CH_3	CH_3	$PhCH_2$	CH_2CH_2OH		17								
49	CH_3	CH_3	$PhCH_2$	$CH_2CH_2O_2CCH_3$		1								
50	CH_3	CH_3	$PhCH_2$	CH_2CH_2OEt	Na	8								
51	CH_3	CH_3	$PhCH_2$	CH_2CH_2SEt	Na	0								
52	CH_3	CH_3	$PhCH_2$	CH_2CH_2SOEt	Na	0								
53	CH_3	CH_3	$PhCH_2$	$CH_2CH_2SO_2Et$		6								
54	CH_3	CH_3	$PhCH_2$	$CH_2CH{=}CH_2$	Na	29	10	16	16					
55	CH_3	CH_3	$PhCH_2$	*trans*-$CH_2CH{=}CHCH_3$	Na	12	10	17	24					
56	CH_3	CH_3	$PhCH_2$	2-cyclohexen-1-yl	Na	25	10	8	2					
57	CH_3	CH_3	$PhCH_2$	$CH_2CH{=}C(CH_3)_2$	Na	26	9	30	76		3.3	24	42	
							3	22	51	3.1[h]	10	54	70	4.3[i]
							1	0	37		30	63	83	
58	CH_3	CH_3	$PhCH_2$	2-cyclohexylideneethyl		7	36	6	39					
59	CH_3	CH_3	$PhCH_2$	$CH_2C{\equiv}CH$	Na	27	10	6	27					
60	CH_3	CH_3	4-$FPhCH_2$	$CH_2CH{=}C(CH_3)_2$	Na	29	9	22	35		3.3	21	43	
							18	39	69	1.1[j]	10	38	67	4.8[k]
							36	44	69		30	59	87	
61	CH_3	CH_3	4-$FPhCH_2$	$CH_2C{\equiv}CH$	Na	24	10	10	19					
62	CH_3	CH_3	4-CH_3OPhCH_2	$CH_2CH{=}C(CH_3)_2$		10								
63	CH_3	CH_3	4-CH_3SPhCH_2	$CH_2CH{=}C(CH_3)_2$							25	0	63	
64	CH_3	CH_3	4-$CH_3SOPhCH_2$	$CH_2CH{=}C(CH_3)_2$							25	13	47	
65	CH_3	CH_3	4-EtO_2CPhCH_2	$CH_2CH{=}C(CH_3)_2$							25	0	10	
66	*n*-C_4H_9	*n*-C_4H_9	$PhCH_2$	*n*-C_3H_7		12								
67	*n*-C_4H_9	*n*-C_4H_9	$PhCH_2$	CH_3		27								
68	*n*-C_4H_9	*n*-C_4H_9	4-$FPhCH_2$	*n*-C_3H_7		8								
69	*n*-C_4H_9	*n*-C_4H_9	4-$FPhCH_2$	*n*-C_4H_9		6								
70	*n*-C_4H_9	*n*-C_4H_9	4-CH_3OPhCH_2	*n*-C_3H_7		5								
71	*n*-C_4H_9	*n*-C_4H_9	2-thienyl-CH_2	*n*-C_3H_7		14								
72	*n*-C_4H_9	*n*-C_4H_9	$PhCH_2CH_2$	*n*-C_3H_7		0								
73						17								
74					Na	17								
75					Na	0								
76					Na	21	10	0	26					
77					Na	15	10	0	8					
piroxicam						*l*	2	66	84		5	68	80	
naproxen						*m*	1		14		0.3		27	
							5		44	*n*	1		50	*o*
							25		58		3		70	

[a] Relative potency is based on the ED_{50} of the noninjected (2°) paw relative to 8; relative potency of 8 = 1.0. [b] The oral ED_{50} for 8 determined prophylactically was 14.5 (9.6–20.9) mg/kg, $p = 0.05$. [c] The oral ED_{50} for 8 determined therapeutically was 18.8 (12.0–29.8) mg/kg, $p = 0.05$. [d] The oral ED_{50} determined for **10** was 6.6 (1.2–13.1) mg/kg, $p = 0.05$. [e] The oral ED_{50} for 17 determined prophylactically was 12.4 (0.4–42.7) mg/kg, $p = 0.05$. [f] The oral ED_{50} for 17 determined therapeutically was 20.8 (5.1–484) mg/kg, $p = 0.05$. [g] The oral ED_{50} determined for 18 was 3.9 (0.11–9.4) mg/kg, $p = 0.05$. [h] The oral ED_{50} for **57** determined prophylactically is approximately 2.5 mg/kg; the regression is not significant. [i] The oral ED_{50} for **57** determined therapeutically was 4.4 (1.9–8.2) mg/kg, $p = 0.05$. [j] The oral ED_{50} for **60** determined prophylactically was 13.4 (2.7–34.1) mg/kg, $p = 0.05$. [k] The oral ED_{50} for **60** determined therapeutically was 3.9 (1.3–8.0) mg/kg, $p = 0.05$. [l] The oral ED_{50} determined for piroxicam was 3.5 mg/kg (0.9–43.5) $p = 0.05$. [m] The oral ED_{50} determined for naproxen was 24.4 mg/kg (12.5–56.5) $p = 0.05$. [n] The oral ED_{50} determined for naproxen was 6.13 mg/kg (1.9–29.8) $p = 0.05$. [o] The oral ED_{50} determined for naproxen was 1.1 mg/kg (0.6–2.0) $p = 0.05$. [p] Compound 8 was equiactive in normal and adrenalectomized animals in this assay.

Table V. Comparative in Vitro Cyclooxygenase and Lipoxygenase Inhibitory Activities of Selected Substituted 6-Hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones



compd	R_1	R_3	R_9	R_7	salt	% inhibn at 50 μM: CO-ase	% inhibn at 50 μM: LO-ase 5-HETE	% inhibn at 50 μM: LO-ase LT-B	CO-ase IC_{50}, μM
8	CH_3	CH_3	$PhCH_2$	n-C_3H_7	Na	43	0	5	
17	CH_3	CH_3	4-$FPhCH_2$	n-C_3H_7	Na	36	0	8	
54	CH_3	CH_3	$PhCH_2$	$CH_2CH{=}CH_2$	Na	16	16	20	
55	CH_3	CH_3	$PhCH_2$	*trans*-$CH_2CH{=}CHCH_3$	Na	49	26	29	
56	CH_3	CH_3	$PhCH_2$	2-cyclohexen-1-yl	Na	17	25	38	
57	CH_3	CH_3	$PhCH_2$	$CH_2CH{=}C(CH_3)_2$	Na	92	17	22	2.0
58	CH_3	CH_3	$PhCH_2$	2-cyclohexylideneethyl		0	10	9	
59	CH_3	CH_3	$PhCH_2$	$CH_2C{\equiv}CH$	Na	9	11	7	
60	CH_3	CH_3	4-$FPhCH_2$	$CH_2CH{=}C(CH_3)_2$	Na	93	21	22	4.2
61	CH_3	CH_3	4-$FPhCH_2$	$CH_2C{\equiv}CH$	Na	3	8	5	
62	CH_3	CH_3	4-CH_3OPhCH_2	$CH_2CH{=}C(CH_3)_2$	Na	73	0	15	
piroxicam									0.1
naproxen									0.06
BW-755C							25[a]	38[a]	

[a] Values are for IC_{50} (μM).

Table VI. Effects of Antiinflammatory Drugs on the Development of Collagen II Arthritis in Rats

treatment[a]	% pos for arthritis[b]	% inhibn paw edema[c]: inj paw	% inhibn paw edema[c]: noninj paw	% pos for bone lesions[d]	immune resp to collagen II antigen[e]: DTH (×10^{-3} mm)	immune resp to collagen II antigen[e]: antibody titer (−log 2)
collagen (C)	71.4			61.5	60.8 ± 3.6	13.4 ± 0.7
C + D-penicillamine	31.3	36.0	42.6[f]	31.5	53.8 ± 6.4	10.7 ± 0.6[f]
C + piroxicam	73.3	0	0	60.0	52.6 ± 4.1	12.2 ± 0.9
C + auranofin	43.7	15.9	23.5	43.8	44.5 ± 3.8[f]	12.9 ± 0.9
C + **57**	46.7	0	10.1	40.0	58.2 ± 6.4	10.9 ± 0.7
C + **8**	26.7	9.6	37.5	26.7	46.4 ± 3.8[f]	9.2 ± 0.5[f]
C + **17**	31.3	38.2	54.9[f]	18.8	34.1 ± 4.1[f]	6.6 ± 0.3[f]
C + **60**	21.4	53.9[f]	80.6[f]	14.3	47.3 ± 7.0[f]	8.4 ± 0.5[f]

[a] 16 rats per group. [b] Based on animals positive for arthritis by either paw edema or the presence of bone lesions. [c] Percent inhibition = [Δ paw vol (collagen-tr) − Δ paw vol (collagen + drug)]/[Δ paw vol (collagen-tr) − Δ paw vol (ICFA control)] × 100. [d] Based on number of animals positive for periostitis, cartilage space changes, and surface bone erosions as determined by radiography. [e] Delayed-type hypersensitivity (DTH) measured by increase in ear thickness 24 h after antigen injection into pinna. Antibody titer was determined by passive hemagglutination. [f] $p \leq 0.05$, Student's t-test.

None of these congeners exhibited more than marginal activity in the primary screens and were not pursued further.

Even though none of the compounds studied in the primary assay was as active as either of the standards piroxicam and naproxen, a number of the more active analogues (**8**, **10**, **17**, **18**, **57**, **60**) were selected for further evaluation, including dose–response measurements in the AAR, with prophylactic and/or therapeutic regimens. Compound **8** was taken as the unit standard of potency for the AAR screening, and greatest potency was observed with the prenyl-substituted derivatives (**57**, **60**). Of particular interest was the activity of this group of compounds in the therapeutic AAR, in which the disease state is allowed to develop and progress for 7 days before the first drug dose is given. This assay is probably among the best of the available chronic models of rheumatoid arthritis that are in general use today.[16]

Comparisons of potency of the most interesting compounds in this class with those of two standard agents, piroxicam and naproxen, are shown in Table IV. The prenyl compounds **57** and **60** are slightly more potent than naproxen, while the 7-alkyl derivatives **8** and **17** are slightly less potent. None of the compounds approaches the potency of piroxicam in the standard screens.

The alkyl-substituted derivatives (**8**, **17**) show no activity against the rat neutrophil 5-lipoxygenase enzyme, as shown in Table V, nor do they show significant inhibition of the cyclooxygenase enzyme from the same source. Their IC_{50} values are greater than 50 μM against this latter enzyme, making it unlikely that their mode of action is by inhibition of either of these enzymes. On the contrary, the compounds substituted with a prenyl group (**57**, **60**) unexpectedly show strong inhibition of the cyclooxygenase enzyme, as shown in Table V. Their structure does not resemble that of the known cyclooxygenase inhibitors,[17] and the difference between the 7-alkyl-substituted compounds **8** and **17** and the prenyl compounds **57** and **60** is quite striking. It is possible that these latter compounds exert their biological activity, at least in part, by way of inhibition of cyclooxygenase.

(16) Billingham, M. E. J.; Davies, G. E. In *Anti-Inflammatory Drugs*; Vane, J. R., Ferreira, S. H., Eds.; Springer-Verlag: Berlin, Heidelberg, New York, 1979; pp 108–144.

(17) Some PG synthetase inhibitors are discussed by: Shen, T. Y. Reference 16, pp 305–347.

Table VII. Comparative Gastrointestinal Ulcerogenic Effects of Indomethacin and the Pyrimidopurines 17, 57, and 60

		gastric ulceration		intestinal ulceration	
compd	dose,[a] mg/kg	ulcer incidence no./gp	lesion length mm/rat (±SE)	ulcer incidence no./gp	lesion score (±SE)
indomethacin	10	7/8	5.0 ± 1.2	8/8	4.2 ± 0.2
17	10	0/8	0	0/8	0
	30	0/8	0	0/8	0
	100	1/8	0.2 ± 0.2	0/8	0[b]
	300	0/8	0	0/8	0[b]
57	10	0/8	0	0/8	0
	30	0/8	0	0/8	0
	100	3/8	1.0 ± 0.7	1/8	0.1 ± 0.1
	300	5/8	9.6 ± 6.6	not tested	
60	10	0/8	0	0/8	0
	30	1/8	0.1 ± 0.1	0/8	0
	100	1/8	0.1 ± 0.1	0/8	0
	300	1/8	3.0 ± 3.0	2/8	0.2 ± 0.2

[a] For gastric study: single oral dose. For intestinal study: three oral doses, (see the Experimental Section). [b] Some deaths were seen on multiple dosing. They were not related to ulceration, however.

The four most interesting compounds from the series (8, 17, 57, 60) were examined in the collagen II arthritis screen, in rats.[14] A brief description of the methodology is given in the Experimental Section. In this assay, arthritis is caused in a test rat by injection of bovine type II collagen. Arthritis is assayed by measurement of paw edema, by radiographic evidence of bone erosion, and by immunological parameters (Table VI). The standard antiarthritic drug piroxicam appears to be completely inactive in this screen (Table VI), whereas levamisole[14] and D-penicillamine, drugs classified as disease-modifying agents, show definite activity in reducing both paw edema and radiographically detected bone erosion and other damage when administered concomitantly with antigen. Another such drug, auranofin, was not active in this study. However, it is not to be expected that all disease-modifying agents work by the same mechanism or will show activity at a specific dose level.

Of the test drugs examined (8, 17, 57, 60), three compounds (8, 17, 60) were effective in reducing the incidence of disease significantly (Table VI). Compounds 17 and 60 inhibited the amount of paw edema significantly, especially in the noninjected paw, which is taken as a measure of systemic disease. Additionally, all three active compounds caused a decreased incidence of bone lesions and a reduction in both the cellular and humoral immune responses to collagen.

Examination of GI Ulcerogenic Potential

Most of the standard antiinflammatory drugs available at this time exhibit significant ulcerogenic potential that can be demonstrated in animal models using indomethacin as a positive control (Table VII). At small multiples of its effective dose in the AAR assay it causes 85–100% of the rats to develop gastric and/or intestinal ulcers. In contrast, compounds 17 and 60 show virtually no ulcerogenic effect even at 300 mg/kg po, and 57 shows only slight effects at 100 mg/kg po, levels that are at least 10-fold higher than their ED_{50} values in the AAR. Measurement of lesion size showed that, in almost all cases, the induced lesions were much smaller than those seen with indomethacin. Thus, members of the present series appear to manifest considerably greater therapeutic ratios than indomethacin. In particular, compound 60, a potent cyclooxygenase inhibitor, shows essentially no gastrointestinal ulcerogenic potential.

Bioavailability Studies with 17 and 57

An experiment was performed to gain some insight into the absorption and distribution of this class of drug. The compounds chosen for study were administered, as their sodium salts, both po and iv to rats. Plasma levels after a single dose were measured by HPLC methods at suitable time intervals. As shown in Table VIII, both compounds were well absorbed orally and had long half-lives. In addition, their bioavailability by the oral route was high, being 50% for 17 and about 90% for 57, as determined by area under curve measurements. Absorption of both drugs from the gastrointestinal tract was relatively slow. The maximum plasma levels were not attained until 2 or 3 h after dosing. The elimination half-life of 57 was about 15 h, while that of 17 was at least 27 h, as estimated from the iv plasma level data. Thus, a single oral or iv dose of 20 mg/kg of 57 or 36 mg/kg of 17 provided very significant plasma levels of drug substance for extended periods of time.

Table VIII. Summary of Preliminary Plasma Level Data[a]

	compd 17, 36 mg/kg		compd 57, 20 mg/kg	
time, h	iv, μg/mL	po, μg/mL	iv, μg/mL	po, μg/mL
0.25	140.0 ± 1.9	20.0 ± 7.3	95.1 ± 26.4	2.4 ± 0.6
0.5	106.7 ± 34.7	45.1 ± 32.6	78.6 ± 6.1	10.8 ± 3.0
1.0	106.9 ± 8.0	23.1 ± 10.3	74.7 ± 1.2	17.3 ± 1.9
2.0	128.0 ± 4.5	66.1 ± 38.8	53.8 ± 6.9	21.9 ± 18.4
4.0	72.4 ± 31.5	41.8 ± 9.7		39.0 ± 16.4
6.0	78.3 ± 30.9	21.0 ± 2.5	35.1 ± 4.4	37.0 ± 30.5
12.0	88.3 ± 17.4	35.9 ± 15.7	10.6 ± 3.2	26.7 ± 6.4

[a] Mean (n = 3) plasma levels (±SD) of 17 and 57 following iv and po administration.

Conclusion

The 6-hydroxypyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-triones represent a novel class of nonsteroidal antiinflammatory drugs with an unusual range of activity. While exhibiting only moderate antiinflammatory activity in the primary synovitis assay, several members (8, 17, 57, and 60) exhibit potency in the chronic models of inflammation. Two members of the series, 57 and 60, are structurally novel cyclooxygenase inhibitors that do not resemble the classical carboxylic acid derivatives and lack ulcerogenic activity at effective antiinflammatory doses. Most importantly, several members (8, 17, 60) exhibit activity in a model of inflammation that is sensitive to drugs defined as disease-modifying agents.[14] Further progression of these compounds has been precluded as a result of unexplained ocular toxicity. However, analogues of this series are currently under active investigation.

Experimental Section

Melting points were determined with a Thomas-Hoover or an Electrothermal melting point apparatus and are uncorrected.

NMR spectra were obtained on a Varian CFT-20 or a Varian XL-400 spectrometer and are consistent with the assigned structures. Microanalyses were performed by the Physical and Analytical Chemistry Research Department of Schering-Plough Research Division, and carbon, hydrogen, and nitrogen results were within ±0.4% of theory except where noted in the tables. Reagents, solvents, and other chemicals obtained commercially were used without pretreatment or further purification, unless otherwise indicated.

Method A. 9-(Phenylmethyl)-1,3-dimethyl-6-hydroxy-7-*n*-propylpyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (8). To a suspension of 8-[(phenylmethyl)amino]theophylline[18] (**2g**) in diethyl *n*-propylmalonate (15 mL) was added NaOMe (100 mg). The mixture was heated, in an atmosphere of nitrogen, at 200–210 °C until TLC (silica gel, $CHCl_3$ (90)/MeOH (10)) showed that no starting material remained (ca. 8–9 h). The mixture was allowed to cool to ambient temperature and was diluted with EtOH (100 mL). After stirring, the product was filtered off, washed with fresh EtOH and then Et_2O, and dried in vacuo at 60 °C to yield the product: 1.85 g (67%); mp 208–210 °C; UV (MeOH) λ_{max} (log ϵ) 261 nm (4.55), ca. 289 (sh) (3.97), (MeOH/HO^-) 261 (4.66), 290 (3.97), 300 (3.95); approximate pK_a, 5.6. Anal. ($C_{20}H_{21}N_5O_4$) C, H, N.

The pK_a of an analogue **16**, was measured as approximately 4.9. Thus, this group of compounds shows acidity comparable to that of other weakly acidic antiinflammatory agents.

Numerous analogues were synthesized by this method or by utilizing minor modifications. They are listed in Table III under method of synthesis.

Method B. This method, the alkylation of the sodium salt of **6** or its analogues, has been described elsewhere.[6]

Method C. 1,3-Dimethyl-9-[(4-methoxyphenyl)methyl]-6-hydroxy-7-(3-methyl-2-butenyl)pyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (62). To a suspension of 5.8 g (0.13 mol) of sodium hydride (50% in mineral oil) in 400 mL of DMF was added 31.5 g (0.10 mol) of 4-[[(methoxyphenyl)methyl]amino]-theophylline dissolved in 100 mL of DMF. The mixture was stirred at room temperature for 0.5 h, and diethyl (3-methyl-2-butenyl)malonate (41.0 g, 0.18 mol) was added. The resulting solution was refluxed for 18 h. Upon cooling to ambient temperature, the reaction mixture was added to ice–water (1 L) and acidified to pH 1 with concentrated HCl. The solid that formed was filtered off and dissolved in 400 mL of hot $CHCl_3$. The solution was washed with water (2 × 100 mL) and dried ($MgSO_4$). After filtration and evaporation under reduced pressure an oil was obtained. Addition of methanol caused crystallization, and the product was recrystallized from $CHCl_3$/MeOH to yield 35.9 g (80%) of **62**, mp 192–195 °C.

By this method, or minor modifications, several other products were obtained, as indicated in Table III.

Preparation of the Sodium Salt of 62. A mixture of 0.6 g (0.013 mol) of sodium hydride in mineral oil (50%) and 4.5 g (0.01 mol) of 1,3-dimethyl-9-(4-methoxyphenyl)-6-hydroxy-7-(3-methyl-2-butenyl)pyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione (**62**) in 400 mL of dimethoxyethane was stirred at ambient temperature for 0.25 h. The solution was filtered to remove any insoluble material, and the dimethoxyethane was removed under reduced pressure to give a solid. This solid was washed thoroughly with ether and dried at 60 °C in vacuo to give 3.6 g (0.0077 mol, 77%) of **62**; sodium salt, mp 240–250 °C.

By this process, or with minor modifications, several other sodium salts were prepared, as listed in Table III.

1,3-Di-*n*-butyl-8-[(2-thienylmethyl)amino]-1*H*,3*H*,7*H*-purine-2,6-dione (1v). A mixture of 8-bromo-1,3-di-*n*-butyl-1*H*,3*H*,7*H*-purine-2,6-dione[19] (10.0 g) and 2-thienylmethylamine (45 mL) was heated, under N_2, at reflux for 4 h. After cooling, the product was poured into 200 mL of H_2O to produce an oil that solidified after some time. The solid was filtered off, washed with water, dried, and recrystallized from MeOH (charcoal) to yield 1v: 6.76 g (58%); mp 158–160 °C. Anal. ($C_{18}H_{25}N_5O_2S$) C, H, N, S.

Many of the other substituted aminopurine derivatives in Table I were prepared either by this method or by obvious and straightforward modifications.

Diethyl (3-Methyl-2-butenyl)malonate (5j). To a stirred suspension of 22 g (0.55 mol) of NaH (60% in oil, prewashed with petroleum ether) in 500 mL of DMF, under N_2, was added 80 g (0.5 mol) of diethyl malonate. Another 100 mL of DMF was added, and the mixture was stirred at room temperature for 30 min. It was then cooled in ice–water, and 77.5 g (0.52 mol) of 1-bromo-3-methyl-2-butene was added over 1.5 h. Another 100 mL of DMF was added, and the mixture was stirred at 0 °C for 1.5 h and then at room temperature for 16 h. The suspension was poured into ice (1 L) and was extracted (4 × 325 mL) with ether. The extracts were washed with 200 mL of water and of brine and were dried ($MgSO_4$). After filtration, the solvent was removed under reduced pressure. Distillation of the residual yellow oil yielded 79.1 g (69%) of the title compound (**5j**) as a clear, colorless oil, bp 145–147 °C (16–17 mmHg). Anal. ($C_{12}H_{20}O_4$) C, H.

7-Amino-9-benzyl-1,3-dimethyl-6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (40). (a) 9-Benzyl-1,3-dimethyl-6-hydroxy-7-nitrosopyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione. To an ice-cold suspension of **6** (2.0 g, 0.006 mol), in 25 mL of AcOH and 6 mL of H_2O, was added portionwise and with stirring 0.469 g (0.007 mol) of sodium nitrite. After 5 min the mixture was allowed to warm to room temperature and was stirred for 75 min. Water (35 mL) was added, and the reaction mixture was filtered, washed with water, partially air-dried, and then triturated with ether. Filtration and drying in vacuo at 50 °C yielded 1.75 g (81%) of the title compound as a yellow powder, mp 250–252.5 °C dec. Anal. ($C_{17}H_{14}N_6O_5$) C, H, N.

(b) A mixture of 3.0 g (0.008 mol) of the product from (a) and 1.49 g (0.008 mol) of *p*-TSA·H_2O in 240 mL of dry DMF was warmed and agitated until all solids dissolved. To the solution was added 300 mg of 10% Pd/C, and the mixture was hydrogenated at 50 psi for 2 h at room temperature. The product, containing a voluminous precipitate, was transferred to a 2-L RB flask and was refluxed (Ar atmosphere) with 360 mL each of MeOH and $CHCl_3$ until most of the organic solids had dissolved. Catalyst was removed by filtration, and solvent was removed at 45 °C under reduced pressure. The residual reddish purple solid was treated with 350 mL of hot MeOH, refluxed (under Ar) for a few minutes, then cooled to room temperature and filtered. The resulting solid was subjected to a second trituration with 125 mL of hot MeOH. Cooling and filtration, followed by washing with 3:2 Et_2O/MeOH and then with Et_2O, yielded 1.78 g (61%) of the free base monohydrate form of the title compound as a pale purple powder, mp >246 °C dec. Anal. ($C_{17}H_{16}N_6O_4·H_2O$) C, H, N.

7-Acetamido-9-benzyl-1,3-dimethyl-6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione (41). A mixture of 5.0 g (0.013 mol) of the 7-nitroso derivative from (a) above and 500 mg of 10% Pd/C in 200 mL of acetic anhydride and 300 mL of DMF was hydrogenated at 45 psi for 3.5 h at room temperature. After filtration, solvent was removed rapidly under reduced pressure (T < 40 °C). The residue was treated successively with $CHCl_3$ and Et_2O/CH_2Cl_2 and evaporated in vacuo after each treatment to obtain a purple slurry, which was triturated with ca. 125 mL of 1:1 Et_2O/CH_2Cl_2. Filtration and washing with the same solvent mixture gave 3.1 g (58%) of yellow powder, which was precipitated from solution in hot $CHCl_3$/MeOH (90:10) by addition of hot EtOAc to yield the title compound with a 76% recovery (44% overall yield) as a white fluffy solid, mp 277–279 °C dec. Anal. ($C_{19}H_{18}N_6O_5$) C, H, N.

9-Benzyl-1,3-dimethyl-7-(ethoxycarbonyl)-6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione, Sodium Salt (43). To a stirred suspension of 8.0 g (0.028 mol) of 8-(benzylamino)theophylline in 80 mL of DMF was added, portionwise, over 7 min, 2.2 g (0.055 mol) of NaH (60% dispersion, used without washing). The temperature of the reaction mixture reached 38 °C. The mixture was warmed at 50 °C for 35 min, during which time a clear yellow solution was formed. Heating was stopped and a solution of 12.96 g (0.056 mol) of triethyl methanetricarboxylate was added dropwise during several minutes. When

(18) Cacace, F.; Masironi, R. *Ann. Chim. (Rome)* **1957**, *47*, 362.
(19) (a) Biltz, H.; Strufe, K. *Ann.* **1914**, *404*, 131. (b) Lespagnol, A.; Gaumeton, A. *Bull. Soc. Chim. Fr.* **1961**, 253.
(20) (a) Harrison, D.; Ralph, J. T.; Smith, A. C. B. *J. Chem. Soc.* **1963**, 2930. (b) Haerter, H. P.; Stauss, U.; Schindler, O. *Helv. Chim. Acta* **1971**, *54*, 2114.

addition was complete, the reaction mixture was heated at 150 °C (under N_2) for 20 h. Solvent was removed at 60 °C under reduced pressure, and the residue was triturated for several hours with Et_2O and then with MeOH. The mixture was filtered, and the solid was washed with EtOAc and dried in vacuo at 65 °C to yield 9.75 g (77%) of the title compound as a white solid, mp >240 °C dec. Anal. ($C_{20}H_{18}N_5O_6Na\cdot H_2O$) C, H, N, Na.

9-Benzyl-1,3-dimethyl-7-formyl-6-hydroxypyrimido[2,1-f]purine-2,4,8(1*H*,3*H*,9*H*)-trione (46). (a) To a well-stirred solution of 2.22 g (0.015 mol) of $POCl_3$ in 75 mL of dry DMF was added portionwise 5.0 g (0.014 mol) of **6**. The resultant thick yellow suspension was stirred (under Ar) for 6 h, another 0.21 g (0.0014 mol) of $POCl_3$ was added, and stirring at room temperature was resumed for an additional 16 h. The suspension was diluted with 100 mL of Et_2O and the mixture filtered. The solid was washed with Et_2O and was then triturated with 125 mL of fresh Et_2O. Filtration and further washing with Et_2O gave 5.3 g (91%) of 9-benzyl-1,3-dimethyl-7-[(*N*,*N*-dimethylamino)methenyl]-6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione as a pale yellow powder, mp 293–299 °C dec, which was used without further purification.

(b) A suspension of 4.0 g (0.0098 mol) of the derivative from (a), in 200 mL of 0.1 N NaOH was stirred at 95–100 °C for 75 min and then at room temperature for 23 h. Filtration and washing with water and then Et_2O gave 3.52 g (89%) of the Na salt of the title compound as an off-white powder, mp >360 °C dec. Anal. ($C_{18}H_{14}N_5O_3Na\cdot{}^1/_3H_2O$) C, H, N, Na.

9-Benzyl-1,3-dimethyl-6-hydroxy-7-(2-hydroxyethyl)pyrimido[2,1-f]purine-2,4,8(1*H*,3*H*,9*H*)-trione (48). To a stirred suspension of 5.46 g (0.013 mol) of 44 (free base) in 240 mL of dry dioxane was added portionwise (*caution*) 1.35 g (0.062 mol) of $LiBH_4$ (under N_2). The mixture was stirred for 20 min at room temperature and then for 16 h at 60 °C (under N_2). After cooling to room temperature, the well-stirred reaction mixture was cautiously acidified (*foaming*) to pH 2 by the dropwise addition of 3 N HCl under a stream of N_2. $CHCl_3$ (200 mL) was added, the mixture was stirred, and the layers were separated. The aqueous phase was extracted (3×) with $CHCl_3$. The combined extracts were dried (Na_2SO_4) and evaporated, and the residue was stirred with hexane. Filtration gave 3.63 g (71%) of the title compound as a white solid, mp 183.5–184 °C dec. Anal. ($C_{19}H_{19}N_5O_5$) C, H, N.

9-Benzyl-1,3-dimethyl-7-[2-(ethylsulfonyl)ethyl]-6-hydroxypyrimido[2,1-f]purine-2,4,8(1*H*,3*H*,9*H*)-trione (53) and 9-Benzyl-1,3-dimethyl-7-[2-(ethylsulfoxy)ethyl]-6-hydroxypyrimido[2,1-f]purine-2,4,8(1*H*,3*H*,9*H*)-trione (52). A mixture of 4.55 g (0.01 mol) of **51** (free acid) and purified *m*-chloroperbenzoic acid in 50 mL of $CHCl_3$ was refluxed for 6 h and then allowed to stand overnight at room temperature. The precipitate was removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was subjected to flash chromatography on silica gel. Elution with CH_3CN gave the title sulfone (**53**), which was recrystallized from methanol to yield 1.0 g (20%) of a pale pink solid, mp 192–193.5 °C. Anal. ($C_{21}H_{23}N_5O_5S$) C, H, N, S.

8-(Phenylmethyl)-6-*n*-butyl-5-hydroxyimidazo[1,2-*a*]pyrimidin-7-one (74). Sodium metal (1.4 g, 0.063 mol) was dissolved in 120 mL of absolute EtOH, under N_2. To this solution was added dropwise, with stirring, diethyl *n*-butylmalonate (6.75 g, 0.031 mol), dissolved in 30 mL of absolute EtOH followed by 2.5 g (0.012 mol) of 2-[(phenylmethyl)amino]imidazole hydrochloride. The resulting mixture was refluxed overnight. Upon cooling to ambient temperature, the volatiles were removed under reduced pressure. The residue was dissolved in water, and the aqueous solution was adjusted to pH 4.5 by the addition of 2 N HCl. The acidic solution was extracted with EtOAc (3 × 150 mL). The EtOAc extracts were combined and dried (Na_2SO_4). Following filtration, the EtOAc was removed under reduced pressure to give an oil. Trituration of the oil with ether gave a solid. The solid was isolated by filtration and dried. Recrystallization from Et_2O/MeOH gave 3.1 g (0.01 mol, 83%) of **74**, mp 105–106 °C. Anal. ($C_{17}H_{19}N_3O_2$) C, H, N. Neutralization of **74** using 1 equiv of NaOH, followed by lyophilization, gave the sodium salt of **74**, which was used without further purification.

1-(Phenylmethyl)-3-*n*-propylpyrimido[1,2-*a*]benzimidazole-2,4(1*H*,3*H*)-dione (75). A mixture of 108 g (0.81 mol) of 2-hydroxybenzimidazole and 350 mL of $POCl_3$ was heated under reflux for 4.5 h. Upon cooling to ambient temperature, the solution was added cautiously to a mixture of ice and water (3 L) with vigorous stirring. The acidic, aqueous solution was basified to pH 10 by the addition of concentrated NH_4OH. The solid that formed was isolated by filtration, washed thoroughly with water, and dried. Recrystallization from EtOAc/Et_2O gave 58 g (0.38 mol, 47%) of 2-chlorobenzimidazole, mp 202–206 °C.[20] Anal. ($C_7H_5ClN_2$) C, H, N, Cl.

2-Chlorobenzimidazole (15 g, 0.1 mol) and 37.5 g (0.35 mol) of phenylmethylamine were heated at 195–200 °C for 5 h under an argon atmosphere. The reaction mixture solidified and was dissolved in 300 mL of hot MeOH. EtOAc (500 mL) was added to the MeOH solution, and the precipitated phenylmethylamine hydrochloride was isolated by filtration. The EtOAc filtrate was washed with water (2 × 200 mL) and dried ($MgSO_4$). Following filtration, the volatiles were removed under reduced pressure, leaving an oil. Crystallization from Et_2O gave, after drying, 19.7 g (0.088 mol, 88%) of 2-[(phenylmethyl)amino]benzimidazole (**2**)[21], mp 155–160 °C. Anal. ($C_{14}H_{13}N_3$) C, H, N.

Using method A, condensation of 2-[(phenylmethyl)amino]-benzimidazole (**2**) with diethyl *n*-propylmalonate gave 8.0 g (0.024 mol, 80%) of **75**, mp 184–186 °C. Neutralization of **75** using 1 equiv of NaOH, followed by lyophilization, gave the sodium salt of **75**, mp 250–280 °C. Anal. ($C_{20}H_{18}N_3O_2Na\cdot H_2O$) C, H, N, Na.

9-(Phenylmethyl)-7-*n*-butyl-6-hydroxypyrimido[2,1-f]-purin-8(9*H*)-one, Sodium Salt (76). (a) **8-[(Phenylmethyl)amino]purine (3).** A mixture of 13.5 g (81.3 mmol) of 8-methylthiopurine[22] and 87 g (813 mmol) of phenylmethylamine was placed in an oil bath preheated to 150 °C. The solution was allowed to heat with stirring at this temperature (under Ar) for 34 h. The opaque red solution was diluted with 500 mL of Et_2O, and the resultant mixture was triturated with a mechanical stirrer. Filtration gave a 75% yield of crude product as a red powder that was recrystallized from MeOH (1100 mL) and $CHCl_3$ (500 mL) (charcoal). After filtration the solution was concentrated under reduced pressure to approximately 200 mL, and the precipitate was filtered and washed with Et_2O/MeOH (9:1) to obtain 5.97 g (32%) of **3** as an analytically pure pale pink powder that begins to decompose at 287 °C. Anal. ($C_{12}H_{11}N_5$) C, H, N.

(b) **9-(Phenylmethyl)-7-*n*-butyl-6-hydroxypyrimido[2,1-f]purin-8(9*H*)-one, Sodium Salt (76).** To a stirred suspension of 6.85 g (30.4 mmol) of **3** in 170 mL of DMF (under Ar) was added 1.4 g (35 mmol) of NaH (60% dispersion). The mixture was stirred at room temperature for 5 min and then placed in an oil bath at 50 °C for 20 min. To the warm reaction mixture was added 26.3 g (122 mmol) of diethyl *n*-butylmalonate. The resultant solution was placed in a bath heated to 100 °C, and the temperature was raised to 150 °C over about 30 min. It was stirred at 150 °C for 16.5 h and, after cooling, was filtered. The filtrate was evaporated under reduced pressure, and the residue was triturated in Et_2O (500 mL)/CH_2Cl_2 (50 mL). Filtration gave a brown powder that was purified by two recrystallizations from CH_3CN/Et_2O to yield 8.36 g (74%) of **76** as a light tan powder, mp 283 °C. Anal. ($C_{19}H_{18}N_5O_2Na\cdot 0.2H_2O$) C, H, N, Na.

6-(Phenylmethyl)-8-*n*-butyl-9-hydroxypyrazino[2′,3′:4,5]imidazo[1,2-*a*]pyrimidin-7(6*H*)-one, Sodium Salt (77). (a) **2-[(Phenylmethyl)amino]-1*H*-imidazo[4,5-*b*]pyrazine (4).** A mixture of 3.0 g (22.1 mmol) of 1*H*-imidazo[4,5-*b*]pyrazin-2-ol,[23] 18 mL of hexamethyldisilazane, and 90 mg of $(NH_4)_2SO_4$ was refluxed under Ar for 18 h. Excess reagent was distilled under reduced pressure from the light orange solution, leaving a residual oil to which were added 7.09 g (66.3 mmol) of phenylmethylamine and 597 mg (2.2 mmol) of $HgCl_2$. The mixture was placed in an oil bath at 175 °C and heated for 22 h. After cooling, 65 mL of MeOH was added and the mixture was refluxed, with stirring, for 1 h. The hot mixture was filtered, and the collected solids were washed with hot MeOH and then with Et_2O. The solid isolated was an off-white powder, containing a small quantity of heavier, dark gray solid and coarse yellow powder,

(21) Omar, A. M. M. E. *Synthesis* **1974**, 41. The compound was isolated as the picrate, mp 246–247 °C, by this author.
(22) Albert, A.; Brown, D. J. *J. Chem. Soc.* **1954**, 2060.
(23) Tong, Y. C. *J. Heterocycl. Chem.* **1981**, *18*, 751.

from which it was separated by decantation from Et_2O suspension and filtration to yield 3.57 g (72%) of 4, decomposing at 340 °C. Anal. ($C_{12}H_{11}N_5$) C, H, N.

(b) 6-(Phenylmethyl)-8-*n*-butyl-9-hydroxypyrazino-[2',3':4,5]imidazo[1,2-*a*]pyrimidin-7(6*H*)-one, Sodium Salt (77). To a stirred suspension of 4.47 g (19.8 mmol) of 4 in 110 mL of DMF (under Ar) was added 911 mg (22.8 mmol) of NaH (60% dispersion). The mixture was stirred at room temperature for 2 min, and then it was placed in an oil bath at 65 °C for 5 min. To the warm mixture was added 17.2 g (79.6 mmol) of diethyl *n*-butylmalonate. The mixture was heated to 150 °C and was stirred for 17 h. After cooling, the product was filtered, and the filtrate was evaporated under reduced pressure. The tacky residual solid was triturated in Et_2O (250 mL)/CH_2Cl_2 (25 mL). Filtration and washing with ether gave a pale yellow powder, which was crystallized from 475 mL of EtOH to yield **77** as yellow crystals that decomposed above 250 °C. Anal. ($C_{19}H_{18}N_5O_2Na$) C, H, N, Na.

RPAR Synovitis Technique.[13] Lewis rats were dosed orally with drug or placebo 1 h prior to iv administration of 2.28 mg of bovine serum albumin (BSA) in 0.2 mL of pyrogen-free saline, followed by the intraarticular injection of 0.54 mg of rabbit anti-BSA antibody in 0.03 mL of pyrogen-free saline in one knee, and saline alone in the contralateral joint, all under light anesthesia. After 3 h the rat was again dosed orally with drug or placebo. The drug dose used in calculations was the total administered both before and after lesion induction.

About 17 h after lesion induction, the animal was killed and both knee joints were exposed. The subpatellar areolar tissue, with attendant synovium, was excised and weighed. Differences between the weight of antibody- and saline-injected knees were considered to represent the inflammatory response for each animal (Δ synovial weight). Differences in Δ synovial weight between lesion controls and drug-treated rats were evaluated for statistical significance by an analysis of variance.

Adjuvant-Induced Arthritis in Rats (AAR).[24] **(a) Prophylactic Regimen.** Heat-killed *Mycobacterium tuberculosis* (from the Ministry of Agriculture, Fisheries and Food Central Veterinary Laboratory, Weybridge, Surrey, England) was prepared by grinding to a fine powder. It was then weighed, mixed with paraffin oil (6 mg/mL), and homogenized.

The animals were dosed with drug 1 h prior to challenge with adjuvant and then for 21 consecutive days. Control animals were given methyl cellulose. Injection of 0.1 mL of the adjuvant was made into the left hind paw. The left and right hind paw volumes were measured immediately on a plethysmograph. Final measurements were taken on both paws on day 21 of the assay. Data were reported as Δ paw volume.

(b) Therapeutic Regimen. The same procedure was followed except that the lesion was allowed to develop for 7 days before the first drug treatment. Treatment was continued for 21 days, as in a, above.

Collagen II Arthritis Method.[14] Type II collagen antigen was prepared from bovine articular cartilage according to the method of Trentham et al.[25] It was dissolved in 0.01 M AcOH for 24 h at 4 °C before use. After dialysis against saline, equal volumes of collagen and incomplete Freunds adjuvant (ICFA; Difco, Detroit, MI) were mixed until a stable emulsion was formed. The emulsion was injected intradermally into the left hind footpad of outbred Wistar female rats in a total volume of 0.1 mL. Drugs were added to the mixture prior to emulsifying, in an amount such that each animal received 5 mg of drug contained in the emulsion. Controls were treated similarly.

Animals were examined weekly for 7 weeks for the development of paw inflammation. An animal was considered arthritic with a Δ paw volume of ≥8.0 for the injected paw or ≥4.0 for the noninjected paw. The incidence of disease is based on the above criteria while the percent inhibition of paw edema is calculated from the mean Δ paw volume of the entire group.

Bone lesions were determined on anesthetized animals positioned so that the foot was taken in the lateral projection, on a standard X-ray unit. Bone damage was scored on a system adapted from Clark et al.[26]

Delayed-type hypersensitivity responses to collagen II were determined at day 21. The method using ear thickness was adapted from Trentham et al.[27] Measurement of the antibody response utilized the passive hemagglutination method of Andriopoulos et al.[28]

Rat Neutrophil Cyclooxygenase/Lipoxygenase Assay.[29] **Cell Source.** Male Wistar–Lewis rats were injected iv with 5 mg of BSA in 0.2 mL of pyrogen-free saline followed by an intrapleural injection of 500 μg of the IgG fraction of rabbit anti-BSA in 0.2 mL of pyrogen-free saline, all under light anesthesia. After 4 h the pleural cavity exudate, consisting of 85–95% neutrophils, was removed. Neutrophils were isolated from the pleural exudates by centrifugation at 4 °C for 10 min at 200*g*. The cell pellet was resuspended in 17 mM Tris-HCl buffer, pH 7.2, containing 0.75% NH_4Cl, followed by centrifugation at 4 °C for 5 min at 200*g*. The pelleted neutrophils were rewashed in 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, by centrifugation at 4 °C for 5 min at 200*g*. The cell pellet was resuspended in 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl and 1 mM $CaCl_2$, for a final cell count of approximately 5×10^6 intact neutrophils/0.1 mL of suspension.

Assay. Arachidonic acid metabolism via cyclooxygenase and 5-lipoxygenase was determined by preincubating the above cell suspension with the test compound for 4 min at room temperature and then adding 0.1 mL of the preincubated cells to assay tubes. The final assay mixture (0.1 mL) contained 40 μM [1-^{14}C]arachidonic acid, 10 μM calcium ionophore A_{23187}, and 0.1% BRIJ 56 detergent. After 1 min at 37 °C, assays were terminated by the addition of 2.4 mL of a $CHCl_3$/MeOH (1:1, v/v) mixture and 0.9 mL of 0.1% HCOOH. The suspension was vortexed, immediately cooled on ice, and centrifuged and the organic layer withdrawn. The extract was evaporated and resuspended in 0.1 mL of $CHCl_3$/MeOH (1:2, v/v) for spotting on silica gel TLC plates (without gypsum). Development was with petroleum ether/Et_2O/AcOH (40:60:1, v/v/v). Products were located by autoradiography and the appropriate regions scraped and counted in a liquid scintillation counter. Metabolites were identified by cochromatography with authentic standards on TLC plates. Results are reported as percent inhibition of cyclooxygenase and lipoxygenase metabolite formation.

Gastrointestinal Ulceration Studies. (1) Gastric Ulceration. Rats were fasted for 24 h (with water ad libitum). Test drugs were suspended in methyl cellulose vehicle and administered orally by gavage in a volume of 0.5 mL/100 g of body weight. The animals were sacrificed after 4 h, and the stomachs were examined for lesions under a dissecting microscope.

(2) Intestinal Ulceration. Test drugs were administered to normal, fed rats by gavage for three consecutive days. The rats were sacrificed 24 h after the last dosing and examined for intestinal ulcers.

Bioavailability Studies. Male Lewis rats were dosed iv or po with the compound suspended in 0.4% (w/v) MC. While under anesthesia, blood was collected from the axillary vein at various time intervals. Plasma was immediately separated and samples (0.25 mL) were extracted with 5 mL of EtOAc. The organic layer was separated and evaporated under nitrogen. The residue was reconstituted in the HPLC mobile phase and analyzed by HPLC (Whatman ODS-3 RAC reversed-phase column; CH_3CN/MeOH/H_2O/AcOH (50:30:20:1, v/v/v/v)). Compounds were quantitated by the external standard method. Oral bioavailability

(24) (a) Newbould, B. B. *Br. J. Pharmacol.* **1963**, *21*, 127. (b) Rosenthale, M.; Nagra, C. *Proc. Soc. Exp. Biol. Med.* **1967**, *125*, 149. (c) Watnick, A. S. *Monogr. Physiol. Soc., Phila.* **1975**, *1*, 155–171.

(25) Trentham, D. E.; Townes, A. S.; Kong, A. H. *J. Exp. Med.* **1977**, *146*, 857.

(26) Clark, R. L.; Cuttins, J. T.; Anderle, S. K.; Cromartie, W. J.; Schwab, J. H. *Arth. Rheum.* **1979**, *22*, 25.

(27) Trentham, D. E.; McCune, W. J.; Susman, P.; David, J. R. *J. Clin. Invest.* **1980**, *66*, 1109.

(28) Andriopoulos, N. A.; Nestecky, J.; Miller, E. J.; Bradley, E. L. *Arth. Rheum.* **1976**, *19*, 613.

(29) Myers, R. F.; Siegel, M. I. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 586.

(F) was determined from the ratio

$$F = \text{AUC (po dose)}/\text{AUC (iv dose)}$$

where AUC is the area under the curve of the plasma level vs. time graph when the iv and po doses are identical.

Crystal Data. $C_{21}H_{23}N_5O_4$ (**32**): mol wt 409.45; monoclinic; a = 9.525 (1) Å, b = 9.978 (1) Å, c = 21.736 (2) Å; β = 102.12 (1)°; V = 1986.3 Å^3; Z = 4; D_{calcd} = 1.369 g cm^{-3}; μ(Cu Kα radiation, λ = 1.5418 Å) = 7.6 cm^{-1}; space group $P2_1/c$ (C_{2h}^5) uniquely from systematic absences $0k0$ when $k \neq 2n$, $h0l$ when $l \neq 2n$; sample dimensions 0.10 × 0.18 × 0.60 mm.

$C_{24}H_{29}N_5O_4$ (**67**): mol wt 451.53; triclinic; a = 18.790 (9) Å, b = 13.611 (7) Å, c = 9.277 (5) Å; α = 97.69 (1)°, β = 89.59 (1)°, γ = 92.64 (1)°; V = 2348.8 Å^3; Z = 4; D_{calcd} = 1.277 g cm^{-3}; μ(Cu Kα radiation) = 6.9 cm^{-1}; space group $P1$ (C_1^1) or $P\bar{1}$ (C_i^1) from Laue symmetry, shown to be the latter by structure solution and refinement; sample dimensions 0.20 × 0.30 × 0.14 mm.

$C_{19}H_{19}N_5O_2 \cdot H_2O$ (**76**): mol wt 367.41; monoclinic; a = 15.161 (9) Å, b = 11.633 (7) Å, c = 10.626 (7) Å; β = 102.79 (1)°; V = 1827.6 Å^3; Z = 4; D_{calcd} = 1.355 g cm^{-3}; μ(Cu Kα radiation) = 7.3 cm^{-1}; space group $P2_1/c$ (C_{2h}^5) uniquely as for **32**; sample dimensions 0.22 × 0.22 × 0.02 mm.

Crystallographic Measurements. Preliminary unit cell parameters and space group information were obtained from oscillation, Weissenberg, and precession photographs. Intensity data[30] for **32** were recorded on an Enraf-Nonius CAD-4 diffractomer (Cu Kα radiation, incident beam graphite monochromator; ω–2θ scans, θ_{max} = 67°) while those for **67** and **76** were measured on an Enraf-Nonius CAD-3 unit (Ni-filtered Cu Kα radiation; ω–2θ scans, θ_{max} = 67°). From totals of 3270 (**32**), 8119 (**67**), and 3273 (**76**) independent measurements, only those 2468, 3509, and 992 reflections, respectively, with $I > 3.0\sigma(I)$ were retained for the structure analyses and corrected for the usual Lorentz and polarization effects. Refined unit cell parameters for each crystal were derived by least-squares treatment of the diffractometer setting angles for 25 (**32**), 40 (**67**), and 40 (**76**) high-order reflections widely separated in reciprocal space.

Structure Analyses. All three crystal structures were solved by direct methods.[31] Approximate coordinates for the majority of non-hydrogen atoms in each molecule were obtained from E maps, and subsequent F_0 Fourier syntheses, phased by these partial structures, yielded positions for the remaining non-hydrogen atoms. Hydrogen atom coordinates were evaluated on the basis of expected molecular geometries in combination with the results of difference Fourier syntheses evaluated at late stages in the analyses. Full-matrix least-squares adjustment of non-hydrogen atom positional and anisotropic thermal parameters, with hydrogen atoms included at their calculated positions in the later iterations, converged to R[32] values of 0.054 (**32**), 0.083 (**67**), and 0.075 (**76**). Final atomic positional and thermal parameters are in Tables S-I–S-VI and S-XIII–S-XV (supplementary material). Lists of observed and calculated structure amplitudes are available from the authors.

Neutral-atom scattering factors used in the structure factor calculations for **67** and **76** were those for C, N, and O from ref 33 and for H from ref 34; for **32** all values were taken from ref 35. In the least-squares iterations, $\sum w\Delta^2$ ($\Delta = ||F_o| - |F_c||$) was minimized with weights; w, assigned according to the scheme $w^{1/2} = 1$ for $|F_o| < K$ and $w^{1/2} = K/|F_o|$ for $|F_o| > K$ [K = 16.8 for **32**, K = 22.0 for **67**, and K = 23.0 for **76**] to ensure no systematic dependence of $(w\Delta^2)$ when analyzed in ranges of $|F_o|$.

Acknowledgment. We thank J. Schwerdt for the preparation of compounds **10**, **18**, and **22**. The skilled technical assistance of J. Anthes, J. Berkenkopf, C. Casmer, M. DaFonseca, H. Gilchrest, H. Jones, Dr. T. Kung, M. Monahan, R. Myers, Dr. I. Rivkin, R. K. Rizvi, L. Tivey, and S. Williams is gratefully acknowledged. We appreciate the effort of Florence V. Cline, Computer Information Science, for her contribution in the computational aspects of this work. We are deeply indebted to Drs. A. K. Ganguly and T. L. Popper for many helpful discussions throughout the course of this work.

Supplementary Material Available: Tables of fractional atomic coordinates, thermal parameters, bond lengths, bond angles, torsion angles, displacements of atoms from least-squares planes, and observed and calculated structure factors for **32**, **67**, and **76** (88 pages). Ordering information is given on any current masthead page.

(30) Miller, R. W.; McPhail, A. T. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1527.
(31) Main, P.; Lessinger, L.; Woolfson, M. M.; Germain, G.; Declerq, J.-P. *MULTAN76, A System of Computer Programs for the Automated Solution of Crystal Structures*; Universities of York and Louvain: York, England, and Louvain, Belgium, 1976.
(32) $R = \sum||F_o| - |F_c||/\sum|F_o|$.
(33) Cromer, D. T.; Waber, J. T. *Acta Crystallogr.* **1965**, *18*, 104.
(34) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175.
(35) *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV.