and 3.7 M HCl–dioxane (0.92 mL, 3.4 mmol) added to the stirred solution. The precipitated gum was dissolved in hot *i*-PrOH–EtOH (30 mL each) and the solution diluted with *i*-Pr_2O (40 mL). On cooling the title compound crystallized and was filtered off and dried. Yield 0.26 g (49%). Table III.

***S*-[2-(4-Nitrophenyl)ethyl]cysteamine Hydrochloride (13).** 4-Nitrophenethyl bromide (2.3 g, 10 mmol) was added to a stirred solution of cysteamine hydrochloride (1.14 g, 10 mmol) and NEt_3 (2.02 g, 20 mmol) in EtOH (10 mL). The mixture was stirred at ambient temperature for 2 days and then evaporated. The residue was dissolved in 2 M HCl (30 mL) and washed with Et_2O (25 mL) and EtOAc (3 × 25 mL). The aqueous phase was basified to pH 14 with 10 M NaOH and extracted with CH_2Cl_2 (2 × 25 mL). TLC indicated that the required product was present in the EtOAc and CH_2Cl_2 extracts. The EtOAc extract was washed with 1 M NaOH (3 × 10 mL) and brine (25 mL) and then combined with the CH_2Cl_2 extract. Drying and concentration gave ca. 1.5 g of a yellow oil. The oil was dissolved in dioxane (45 mL) and the stirred solution was treated with 4 M HCl–dioxane (10 mL). The precipitated salt was collected by filtration and recrystallized twice from MeCN (50 mL and 30 mL). Yield 0.91 g (34.6%). Table III.

***N*-(*tert*-Butyloxycarbonyl)-L-tyrosyl-D-methionine (25).** A solution of Boc-L-Tyr-OH (7.8 g, 27.8 mmol) and HOBt (7.50 g, 55.5 mmol) in DMF (25 mL) was cooled to −15 °C and treated with DCCI (5.72 g, 27.8 mmol). The reaction mixture was stirred at −15 °C for 10 min and then H-D-Met-OMe (4.52 g, 27.8 mmol) was added and the mixture was stirred at 4 °C overnight. The DCU was removed by filtration and the DMF evaporated in vacuo. The crude product was dissolved in EtOAc (450 mL) and washed with 10% aqueous citric acid (3 × 50 mL), half-saturated NaCl (50 mL), 10% aqueous $NaHCO_3$ (3 × 50 mL), and half-saturated NaCl (2 × 50 mL). The solution was dried and evaporated to leave a solid, which was crystallized from EtOAc–petroleum ether. Yield 9.94 g (84%); R_f (B) 0.93, R_f (C) 0.85, contaminated with DCU. The product was dissolved in MeOH (100 mL) and water (30 mL) and saponified at pH 12 by addition of 1 M NaOH with a pH stat. When the hydrolysis was complete, the MeOH was removed by evaporation in vacuo and insoluble material (DCU) was removed by filtration. The filtrate was cooled in ice and the pH adjusted to 2 by careful addition of 1 M HCl. The crude product was extracted into EtOAc (2 × 250 mL), and the combined extracts were washed with water (50 mL), dried, and concentrated to give an oily foam, which solidified on trituration with Et_2O. The product was filtered off, washed with Et_2O, and dried. Yield 7.77 g (81%), mp 76 °C dec; R_f (B) 0.78, R_f (C) 0.65. Anal. ($C_{19}H_{28}N_2O_6S$) C, H, N.

Pharmacological Methods. A. Isolated Guinea Pig Ileum. Segments of the terminal portion of the ileum of guinea pigs (300–350 g) were suspended in a 20-mL organ bath under 1 g tension and bathed in Krebs bicarbonate solution gassed with 95% O_2 and 5% CO_2. Contractions were induced by coaxial stimulation of the ileum with pulses at 0.1 Hz, 0.5-ms duration, and at supramaximal voltage and recorded by means of isometric transducers. Dose–response curves were constructed allowing 15-min washout between doses.

B. Writhing Assays. (a) Groups of five to six female Charles River mice of the CD1 strain were injected intraperitoneally with phenyl-*p*-benzoquinone (PBQ) at 2.5 mg kg^{-1} in a dose volume of 10 mL kg^{-1}. The irritant induced a syndrome (writhing) characterized by a series of abdominal constrictions and/or hind-limb extensions, which were counted for a 2.5-min period commencing 10 min after PBQ injection. Vehicle or drugs were administered either subcutaneously or orally via a blunt-ended intragastric needle, in a dose volume of 10 mL kg^{-1}, 30 min prior to PBQ. Antinociceptive activity was assessed in terms of an ED_{50} and determined by linear regression. The ED_{50} was defined as that dose of drug which induced a 50% reduction in the number of writhes obtained compared to vehicle administration alone.

(b) Groups of five to six male Tuck mice of the TFW strain were injected intraperitoneally with acetic acid (0.6%) in a dose volume of 25 mL kg^{-1}. Writhes were counted for a 5-min period commencing 15 min after acetic acid injection. *N*-Methylnalorphine (11.5 mg kg^{-1}) was administered intraperitoneally 20 min prior to the analogues, which were administered subcutaneously, both antagonist and test compounds being given in a dose volume of 10 mL kg^{-1}. Antinociceptive activity was determined as above. Dose ratios were determined as the shift of the parallel regression lines in the absence and presence of *N*-methylnalorphine.

C. Hot-Plate Assays. Groups of five to six male Hacking and Churchill mice of the CFLP strain were used. Each mouse was placed on a copper surface maintained at 55 °C and observed for signs of discomfort such as licking/shaking of the paw or jumping. A cut off time of 30-s exposure was used to prevent tissue damage. Drugs were administered subcutaneously in a dose volume of 10 mL kg^{-1}. ED_{50}s were defined as that dose of drug which increased the latency of response 2-fold compared to vehicle and were determined by parallel-line probit analysis.

Antiinflammatory Activity of a Series of Substituted 2,3-Dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-diones

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A series of substituted analogues based on the novel 2,3-dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione ring system have been synthesized and shown to exhibit antiinflammatory activity in the adjuvant-induced arthritis rat model (AAR). The activity exhibited by the pyrimidopurinediones in this model of chronic inflammation is comparable to that of their previously studied 2-oxo congeners, the 6-hydroxypyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-triones, the best of which show potency levels approximately equal to that of naproxen. On the basis of its potency in the AAR assay, 9-benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(3-methyl-2-butenyl)pyrimido-[2,1-*f*]purine-4,8(1*H*,9*H*)-dione was selected for further evaluation and found to exhibit cyclooxygenase inhibitory activity in the in vitro rat neutrophil model. With respect to side-effect liability, this prenylated derivative has been shown to be devoid of gastric ulcer inducing potential, as well as the ocular toxicity observed previously with the 2-oxo series.

In the course of investigating a series of 6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-triones, which are atypical nonsteroidal antiinflammatory agents,[1] the reduction of a member of the series, containing an ester side

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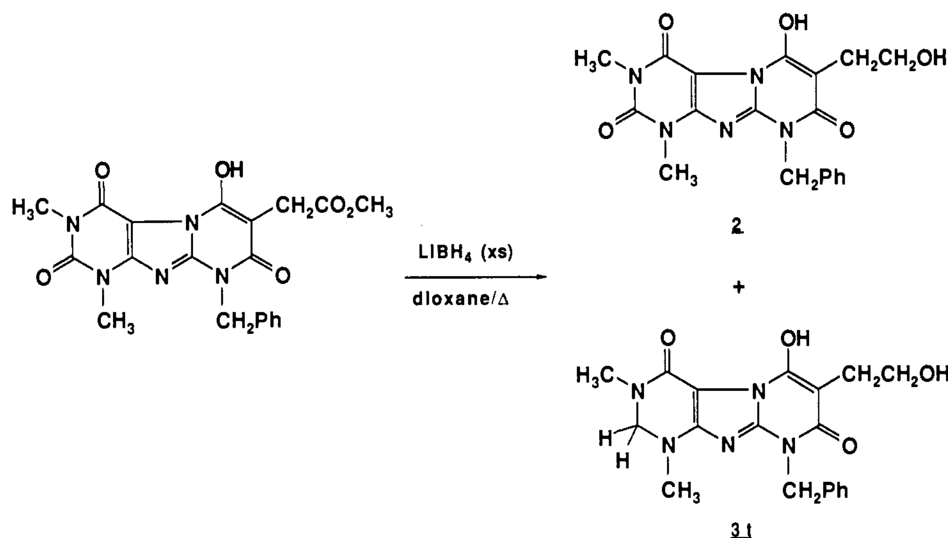
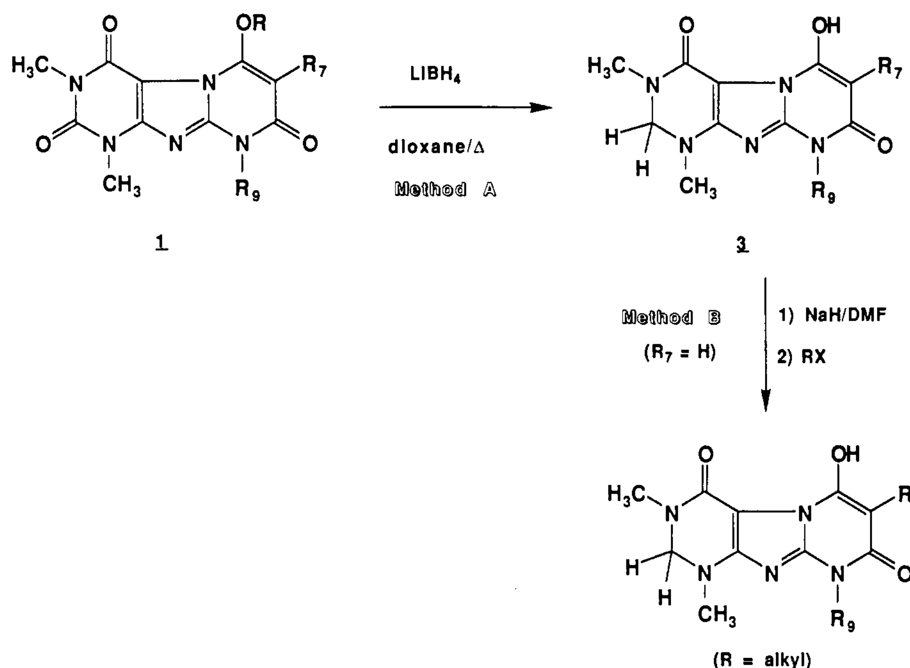
Scheme I



Scheme II



chain substituted on this nucleus, was carried out as illustrated in Scheme I with the intention of preparing alcohol **2**. The isolation and characterization of a side product (**3t**) of this reaction led us to the serendipitous discovery of a novel regiospecific reduction of the carbonyl group at position 2 and thus provided entry to the previously unknown 2,3-dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione ring system.[2] The accessibility of a novel heterocyclic ring system bearing a close structural relationship to, yet chemically distinct from, our original series provided the opportunity to explore the intriguing possibility that such a related system might retain the antiinflammatory activity of the "parent" compounds, but be free of the toxic liabilities that unfortunately accompanied the desirable properties of that original pyrimidopurine series. It was, of course, equally possible that the new system might exhibit a converse profile—that is, toxicity without antiinflammatory properties—or that it might be altogether lacking in noteworthy pharmacological activity. With the objective of distinguishing among these possibilities and defining the biological properties of this novel heterocyclic system, several substituted 2,3-dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-diones were prepared and their antiinflammatory activity was determined. The results of these studies and the structure–activity relationships that derive from them, supplemented by further pharmacological and toxicological evaluation of a key compound in the series, constitute the subject matter of this paper.

Chemistry

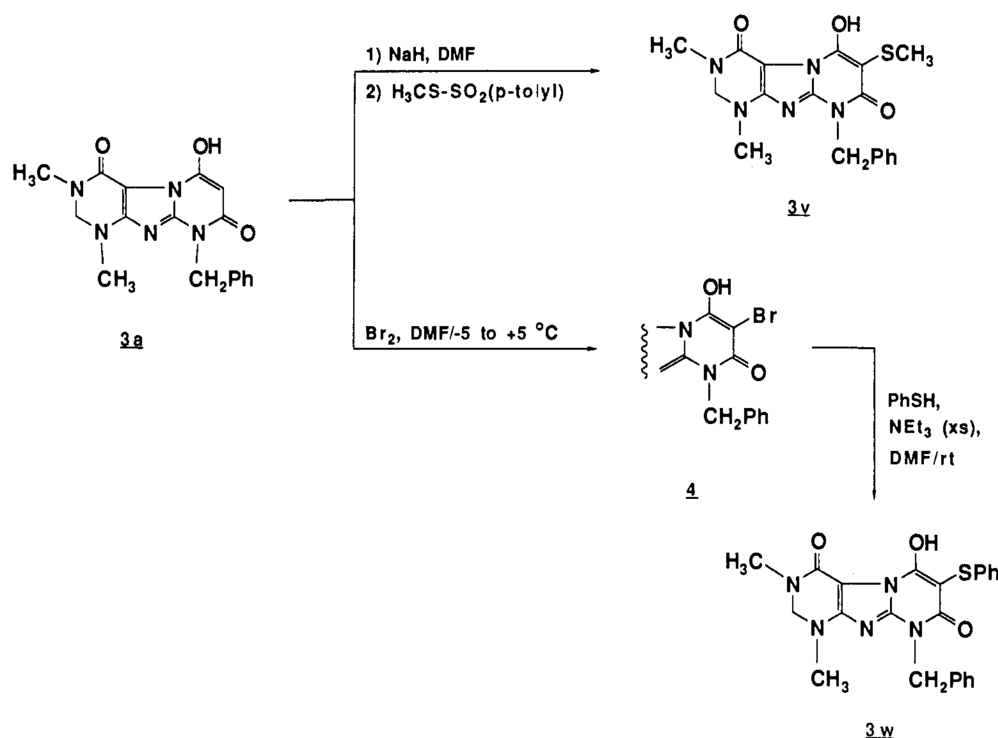
Synthesis of the tricyclic 6-hydroxypyrimido[2,1-*f*]purine-2,4,8(1*H*,3*H*,9*H*)-trione nucleus and its regiospecific alkylation at position 7 have been described previously.[1,3]

(1) Blythin, D. J.; Kaminski, J. J.; Domalski, M. S.; Spitler, J.; Solomon, D. M.; Conn, D. J.; Wong, S. C.; Verbiar, L. L.; Bober, L. A.; Chiu, P. J. S.; Watnick, A. S.; Siegel, M. I.; Hilbert, J. M.; McPhail, A. T. *J. Med. Chem.* **1986**, *29*, 1099.

(2) A full discussion of this reduction has been presented elsewhere: Conn, D. J.; Kaminski, J. J.; Solomon, D. M. *J. Org. Chem.* **1988**, *53*, 3265.

(3) Solomon, D. M.; Rizvi, R. K.; Kaminski, J. J. *Heterocycles* **1986**, *24*, 2179.

Scheme III



The key reaction that provides access to the title compounds is a novel regiospecific reductive process (Scheme II) that has recently been disclosed.[2] Treatment of the tricyclic trione 1 (or a silylated derivative thereof) with an excess of lithium borohydride in refluxing dioxane yields the corresponding 2,3-dihydro derivative 3 (Scheme II, method A). When the 7-position of 3 is unsubstituted (i.e., R_7 = H), 7-alkylated derivatives can be prepared by the methodology previously described[3] for the synthesis of the corresponding 2-oxo compounds 1 (Scheme II, method B). The option of introducing the 7-substituent before or after the reduction step is limited by the ability of the specific substituent to survive the reduction conditions. In general, it was found convenient to utilize the 7-unsubstituted-2,3-dihydro compound (**3a** or **3b**; see Table I) as a common intermediate, which was derivatized with the appropriate reagents to produce the desired target compounds.

In the specific case of the 7-prenyl analogue **3j**, the general conditions for the alkylation of **3a** (sodium hydride, DMF) were found to give poor yields (<10%). Therefore, several combinations of base, solvent, and reaction temperature were examined. Optimal yields, in the range of 31–39% depending upon reaction scale, were obtained with an "extractive alkylation" technique,[4] employing a two-phase methylene chloride–aqueous sodium hydroxide system in the presence of the phase transfer agent tetrabutylammonium hydrogen sulfate.

Hydrogenation of **3j** over 5% palladium-on-carbon yielded the 7-(3-methylbutyl) derivative **3n**.

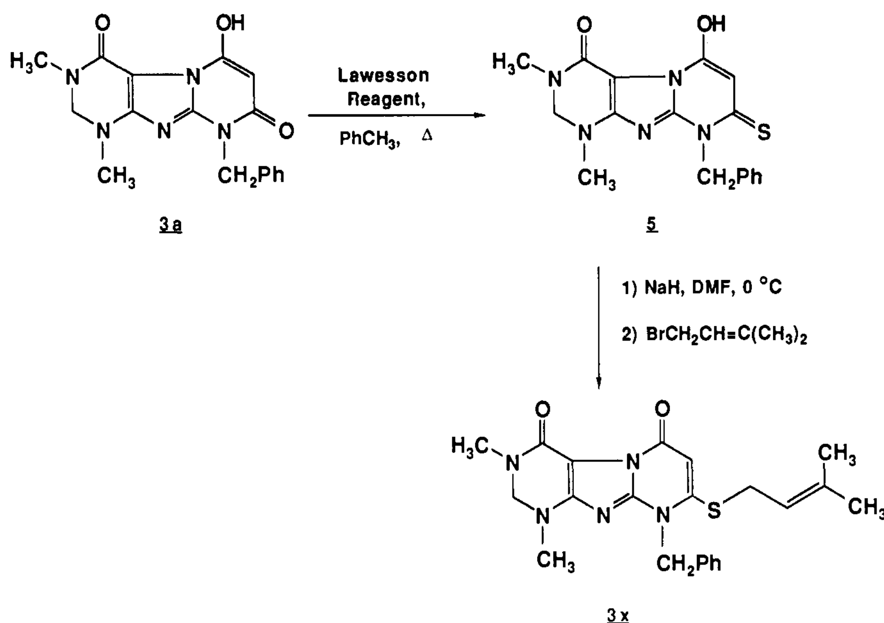
The two analogues (**3v** and **3w**) containing sulfur at the 7-position were prepared as shown in Scheme III. The 7-methylthio compound **3v** was obtained by the reaction of 7-unsubstituted intermediate **3a** with *S*-methyl-4-toluenethiosulfonate. The phenylthio derivative **3w** was synthesized by treatment of the intermediate bromide **4** with benzenethiol. Prenylthio analogue **3x** was prepared in a two-step sequence as shown in Scheme IV. Thionation of dione **3a** with Lawesson's reagent gave a single isolable monosulfurated product, identified as the 8-thione **5**. The position of thionation was established by comparison of the proton and carbon magnetic resonance spectra of **3a** and **5**. The observation of downfield shifts of both carbon (by 17 ppm) and hydrogen (by 1.20 ppm) at position 7, as well as the smaller, but significant, downfield shifts of the benzylic carbon (5.4 ppm) and protons (0.68 ppm) of **5** relative to **3a**, suggested that sulfuration had occurred at the 8-position. Sulfuration at position 4 would be expected to affect significantly at least the adjacent 3-*N*-methyl group; sulfuration at position 6 (or 4) would not account for the observed substantial effect on the benzylic position. In contrast to the regiospecific 7-carbon alkylation that has been consistently observed with the 6,8-dioxygenated compounds, the 8-thiono analogue **5**, upon treatment with sodium hydride in dimethylformamide, followed by the addition of prenyl bromide, yielded the 8-S-prenylated compound **3x**. The assignment of the position of prenylation in **3x** is based upon the following ^{1}H NMR spectral characteristics: (1) the detection of 7-H (1-proton singlet at δ 5.77) requires prenylation to have occurred on the 6-oxygen or 8-sulfur, and (2) the observed chemical shift (δ 3.53) for the prenyl methylene group is a reasonable value for the product of substitution on sulfur, but much too far upfield to accord with alkylation at the 6-oxygen.[5]

The 7-[3-(trifluoromethyl)-2-butenyl] analogue **3m** was synthesized by direct alkylation of 7-unsubstituted substrate **3a** with 1-chloro-3-(trifluoromethyl)-2-butene (**11**), which was in turn prepared from 1,1,1-trifluoroacetone, as shown in Scheme V. The Wittig process produced an isomeric mixture (89:11) of esters (**9**), the predominant of which had the ester and methyl functions cis to one another (as deduced from the ^{1}H NMR spectrum in which the methyl protons cis to ethoxycarbonyl appeared 0.26 ppm further downfield than the trans methyl protons).

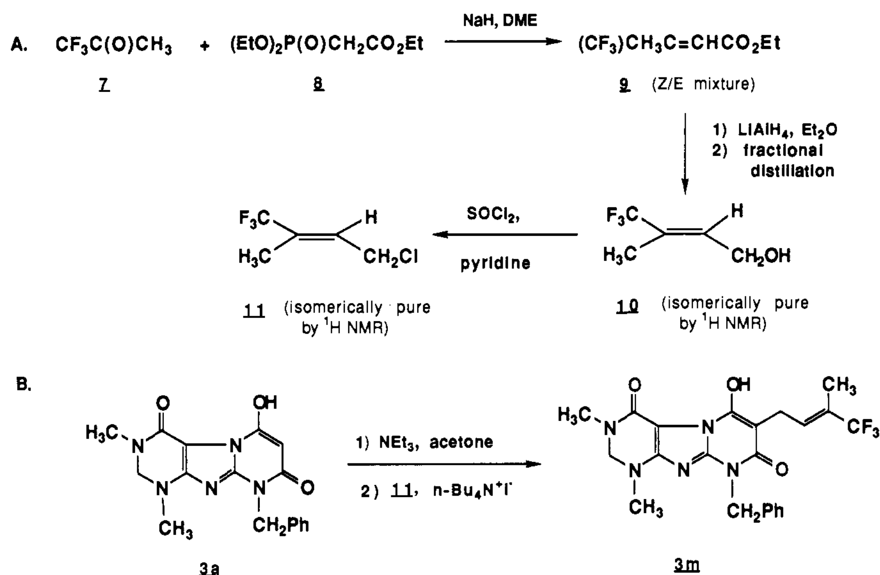
(4) Brandstrom, A.; Junggren, U. *Tetrahedron Lett.* **1972**, 473.

(5) For comparison, the 6-*O*-methyl group protons of 9-benzyl-1,3-dimethyl-6-methoxy-7-propylpyrimido[2,1-*f*]purine-2,4,8-(1*H*,3*H*,9*H*)-trione appear at δ 4.08. See ref 3.

Scheme IV



Scheme V



Distillation of the crude product resulting from a lithium aluminum hydride reduction of **9** gave an isomerically pure alcohol **10**, identified as having the methyl and hydroxymethyl groups cis to each other, both on the basis of the chemical shift of the methyl protons and from the amount of pure isomer obtained from the original mixture of esters (see the Experimental Section). Treatment of **10** with thionyl chloride and pyridine gave isomerically pure chloromethyl compound **11**, in which the cis relationship of the methyl and chloromethyl moieties follows from the derivation of **11** from pure cis alcohol **10**. Thus, target compound **3m** was also obtained as a single isomer with the structure shown in Scheme V.

The 7-formyl compound **3r** was prepared by means of a Vilsmeier–Haack reaction[6] on **3a**. The intermediate 7-(*N*,*N*-dimethylamino)methenyl compound **3q** was isolated and then hydrolyzed with aqueous base to obtain the sodium salt of **3r**.

The 7-(*N*-phenylcarbamoyl) derivative **3u** was synthesized by the acylation of 3a with phenyl isocyanate and isolated as a triethylammonium salt.

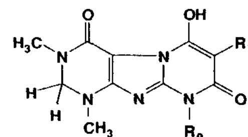
Biological Results and Discussion

At the inception of this study, the primary animal model used to detect potential antiinflammatory activity was the reverse passive Arthus reaction (RPAR)–synovitis assay.[1] However, in the course of examining the 2,3-dihydropyrimidopurine series, it became evident that the synovitis results showed no apparent correlation with data obtained in our secondary model, the adjuvant arthritic rat (AAR) assay.[7] Indeed, few compounds in this series exhibited significant activity in the synovitis assay. Thus, in several instances (Table II) compounds were examined in the AAR and found to be active despite an apparent lack of activity in the RPAR–synovitis test. Conversely, in at least one example (**3g**), moderate activity in the synovitis assay was not substantiated in the prophylactic AAR. A substantial

(6) Vilsmeier, A.; Haack, A. *Ber.* **1927**, 119.

(7) (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*, 155–71.

Table I. Substituted 2,3-Dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-diones



compd	R_7	R_9	method of synthesis[a]	salt[b]	mp, °C	chromatography solvent	recrystn solvent	isolated yield, %[c]	formula	anal.
3a	H	$PhCH_2$	A		176–182	EtOAc–MeOH–HOAc (99:1:0.1)	MeOH–EtOAc	43	$C_{17}H_{17}N_5O_3$	C,H,N
3b	H	4-$FPhCH_2$	A		214–232	CH_2Cl_2–acetone–HOAc (75:25:0.15)	MeCN	18	$C_{17}H_{16}N_5O_3F$	C,H,N,F
3c	CH_3	$PhCH_2$	B	Na	250–257 dec	$CHCl_3$–MeOH (96:4)	$CHCl_3$–MeOH	21	$C_{18}H_{18}N_5O_3Na{\cdot}0.5H_2O$	C,[d]H,N,Na
3d	C_3H_7-*n*	$PhCH_2$	A		177–178.5	EtOAc–hexane (75:25)	EtOAc	44	$C_{20}H_{23}N_5O_3$	C,H,N
3e	C_3H_7-*n*	4-$FPhCH_2$	A	Na	210 dec	$CHCl_3$–EtOH–NH_4OH (90:2:0.1)		16	$C_{20}H_{21}N_5O_3FNa{\cdot}0.5H_2O$	C,H,N,Na
3f	C_3H_7-*n*	2-thienyl-CH_2	A[e]		190–192	$CHCl_3$–MeOH (97:3)	$CHCl_3$–MeOH	*f*	$C_{18}H_{21}N_5O_3S$	C,H,N,S
3g	$CH_2CH{=}CH_2$	4-$FPhCH_2$	B		131.5–133 dec	EtOAc–hexane (3:1)		5	$C_{20}H_{20}N_5O_3F$	C,H,N
3h	$CH_2C{\equiv}CH$	$PhCH_2$	B		163–164	$CHCl_3$–MeOH (97:3)	$CHCl_3$–MeOH	13	$C_{20}H_{19}N_5O_3$	C,H,N
3i	(*E*)-$CH_2CH{=}CHCH_3$	$PhCH_2$	B		180–183	$CHCl_3$–MeOH (98:2)	$CHCl_3$–MeOH	30	$C_{21}H_{23}N_5O_3$	C,H,N
3j	$CH_2CH{=}C(CH_3)_2$	$PhCH_2$	B[e]		153–154.5	EtOAc–hexane (1:1)		9/39[f]	$C_{22}H_{25}N_5O_3{\cdot}0.33H_2O$	C,H,N
3k	$CH_2CH{=}C(CH_3)_2$	4-$FPhCH_2$	B		188–188.5	EtOAc–hexane (3:1)		20	$C_{22}H_{24}N_5O_3F$	C,H,N,F
3l	$CH_2CH{=}C(CH_3)_2$	4-CH_3OPhCH_2	*e*		157–159	$CHCl_3$–MeOH (98:2)	$CHCl_3$–MeOH	*f*	$C_{23}H_{27}N_5O_4$	C,H,N
3m	$CH_2CH{=}C(CH_3)CF_3$	$PhCH_2$	B[e]		110–112	$CHCl_3$–MeOH (97:3)	Et_2O–hexane	2[f]	$C_{22}H_{22}N_5O_3F$	C,H,N,F
3n	$(CH_2)_2CH(CH_3)_2$	$PhCH_2$	B[e]		141–142		MeOH–EtOAc	*f*	$C_{22}H_{27}N_5O_3{\cdot}0.33H_2O$	C,H,N
3o	2-cyclohexen-1-yl	$PhCH_2$	B		208–210	$CHCl_3$–MeOH (98:2)	$CHCl_3$–MeOH	26	$C_{23}H_{25}N_5O_3$	C,H,N
3p	$PhCH_2$	$PhCH_2$	B	Na	175–185	$CHCl_3$–MeOH (96:4)	$CHCl_3$–MeOH	27	$C_{24}H_{22}N_5O_3Na{\cdot}0.5H_2O$	C,H,N,Na
3q	$=CHN(CH_3)_2$	$PhCH_2$	B[e]		235 dec			95[f]	$C_{20}H_{22}N_6O_3{\cdot}H_2O$	C,H,N
3r	CHO	$PhCH_2$	*e*	Na	325–326 dec			*f*	$C_{18}H_{16}N_5O_4Na{\cdot}0.125H_2O$	C,H,N,Na
3s	CH_2CO_2Et	$PhCH_2$	B		148–150	$CHCl_3$–MeOH (98:2)	$CHCl_3$–MeOH	11	$C_{21}H_{23}N_5O_5$	C,H,N
3t	CH_2CH_2OH	$PhCH_2$	A		193–194 dec		MeOH	7	$C_{19}H_{21}N_5O_4$	C,H,N
3u	CONHPh	$PhCH_2$	B[e]	NEt_3	174.5 dec			87[f]	$C_{24}H_{22}N_6O_4{\cdot}N(C_2H_5)_3{\cdot}0.4H_2O$	C,H,N
3v	SCH_3	$PhCH_2$	B[e]		196–199		$CHCl_3$–MeOH	60[f]	$C_{18}H_{19}N_5O_3S$	C,H,N,S
3w	SPh	$PhCH_2$	B[e]		227–238 dec	CH_2Cl_2–acetone–HOAc (1:1:0.1)		*f*	$C_{23}H_{21}N_5O_3S$	C,H,N,S
3x	H [8-$SCH_2CH{=}C(CH_3)_2$]	$PhCH_2$	B[e]		144.5–151 dec	acetone (67–90% gradient)–CH_2Cl_2		*f*	$C_{22}H_{25}N_5O_2S$	C,H,N,S

[a] A, reduction of corresponding 2-oxo compound; B, derivatization of **3a** or **3b**. [b] Sodium salts were prepared by neutralization of the enolic acid with 1 equiv of sodium hydroxide, followed by lyophilization (except **3r**, which formed directly during preparation; see the Experimental Section). [c] Refers to the yield of the step indicated under method of synthesis. [d] Calcd: C, 56.24. Found: C, 55.82. [e] Details of the synthesis are presented in the Experimental Section. [f] See the Experimental Section for yield information.

Table II. In Vivo Antiinflammatory Activity of Substituted 2,3-Dihydro-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-diones

					adjuvant arthritic rat					
				RPAR–synovitis	prophylactic, % inhibition			therapeutic, % inhibition		
compd	R_7	R_9	salt	% inhibn (50 mg/kg, po)	dose, mg/kg	1° paw	2° paw[a]	dose, mg/kg	1° paw	2° paw[a]
3a	H	$PhCH_2$		0						
3b	H	4-$FPhCH_2$		9	12.5	22	21			
					25	36	24			
					50	37	46			
3c	CH_3	$PhCH_2$	Na	0	12.5	0	36			
					25	0	22			
					50	46	69			
3d	C_3H_7-*n*	$PhCH_2$	Na	45	12.5	16	38			
					25	24	65			
					50	38	70			
3e	C_3H_7-*n*	4-$FPhCH_2$	Na	0	25	0	56			
3f	C_3H_7-*n*	2-thienyl-CH_2			12.5	20	13			
					25	20	51			
3g	$CH_2CH{=}CH_2$	4-$FPhCH_2$		40	50	10	49			
3h	$CH_2C{\equiv}CH$	$PhCH_2$		6	25	35	21			
					50	36	13			
3i	(*E*)-$CH_2CH{=}CHCH_3$	$PhCH_2$		0	12.5	20				
					25	24	40			
					50	44	71			
3j	$CH_2CH{=}C(CH_3)_2$	$PhCH_2$		20[b]	3	17	47	3	20	28
					9	33	65	10	33	40
					27	54	92[c]	25	63	76[d]
3k	$CH_2CH{=}C(CH_3)_2$	4-$FPhCH_2$		18	25	24	49	6.25	25	42
								12.5	53	84
								25	47	95[e]
3l	$CH_2CH{=}C(CH_3)_2$	4-CH_3OPhCH_2		6.25	40	48				
					12.5	40	63			
					25	45	69[f]			
3m	$CH_2CH{=}C(CH_3)CF_3$	$PhCH_2$		25	6.25	33	60	3	37	52
					12.5	47	64	10	63	84
					25	45	74[g]	25	75	98[h]
3n	$(CH_2)_2CH(CH_3)_2$	$PhCH_2$			45	23	51			
3o	2-cyclohexen-1-yl	$PhCH_2$		16	12.5	9	6			
					25	12	15			
					50	13	13			
3p	$PhCH_2$	$PhCH_2$	Na	5	25	34	49			
					50	46	63			
3q	$=CHN(CH_3)_2$	$PhCH_2$		27						
3r	CHO	$PhCH_2$	Na	34	50	6	32			
3s	CH_2CO_2Et	$PhCH_2$		14	25	18	20			
3t	CH_2CH_2OH	$PhCH_2$		8						
3u	CONHPh	$PhCH_2$			50	−21[i]	23			
3v	SCH_3	$PhCH_2$		10	50	33	61			
3w	SPh	$PhCH_2$			50	15	23			
3x	H [8-$SCH_2CH{=}C(CH_3)_2$]	$PhCH_2$			25	−16[j]	8			

[a] The oral ED_{50} calculated is based on the percent inhibition observed in the noninjected (2°) paw. [b] The oral dose given was 100 mg/kg. [c] The oral ED_{50} for **3j** in the prophylactic adjuvant arthritic rat was 6.02 (2.6–14.6), $p = 0.05$. [d] The oral ED_{50} for **3j** in the therapeutic adjuvant arthritic rat was 15.0 (5–93), $p = 0.05$. [e] The oral ED_{50} for **3k** in the therapeutic adjuvant arthritic rat was 7.6 (3.1–13.5), $p = 0.05$. [f] The oral ED_{50} for **3l** in the prophylactic adjuvant arthritic rat was 14.0 (6.2–31.2), $p = 0.05$. [g] The oral ED_{50} for **3m** in the prophylactic adjuvant arthritic rat was 8.8 (4.2–17.9), $p = 0.05$. [h] The oral ED_{50} for **3m** in the therapeutic adjuvant arthritic rat was 4.0 (0.9–9.0), $p = 0.05$. [i] The paw weight was 121% of control. [j] The paw weight was 116% of control.

level of activity in the AAR screen was regarded as an essential element in the profile of a compound to qualify it as a candidate for further evaluation.

A limited number of analogues were prepared in the 2,3-dihydro series compared with the more extensively investigated parent (2-oxo) series.[1] Initial target selection was based on the known structure–activity relationships of the 2-oxo series with emphasis focused on modifications of the 7-substituent. The results in the 2-oxo series had suggested that a short alkyl chain (saturated or unsaturated) at the 7-position, coupled with a simple benzyl or 4-fluorobenzyl group at the 9-position, might be expected to produce compounds with the most desirable profiles. Although with the limited comparative data available it cannot be stated categorically that the two series exhibit precisely parallel structure–activity relationships, it is indeed a fact that the analogues having a 3-methyl-2-butenyl (prenyl) substituent at the 7-position and a benzyl or 4-fluorobenzyl moiety at the 9-position were among the most efficacious compounds in both series. Structure–activity data are collected in Table II.

Compound **3j**, having a prenyl group at the 7-position

and a benzyl group at position 9, exhibits oral ED_{50} values of 6 and 15 mg/kg, respectively, in the prophylactic and therapeutic variants of the AAR assay.[8]

Retaining the 7-prenyl group and substituting a *p*-fluorine in the 9-benzyl moiety results in compound **3k**, which exhibited potency in the therapeutic AAR assay comparable to that of **3j**. The introduction of a *p*-methoxy substituent in the benzyl group produced compound **3l**, which retained antiinflammatory activity in the prophylactic AAR with a level of potency statistically indistinguishable from that of **3j**.

A more lipophilic version of the prenyl function was prepared by substituting a trifluoromethyl moiety for one of the terminal methyls to obtain compound 3m, which exhibited potency in the prophylactic AAR assay equivalent to that of **3j** and was perhaps somewhat more potent than **3j** when administered in the therapeutic regimen. Saturation of the prenyl to a 3-methylbutyl (isopentyl) function gave **3n**, which exhibited a lower level of activity than did **3j** or the other aforementioned analogues.

Analogues with methyl (**3c**), propyl (**3d**), or benzyl (**3p**) substituents at the 7-position showed significant levels of activity in the prophylactic AAR, but their potency appeared to be substantially lower than that of **3j** and its most active congeners. With the 7-propyl group held constant, the 9-benzyl group was changed to 4-fluorobenzyl and to 2-thienylmethyl to obtain **3e** and **3f**, respectively. These structural modifications appeared to have an adverse effect on activity in the prophylactic AAR. Among the compounds having unsaturated hydrocarbon substituents (excluding prenyl) at position 7 (**3g**, **3h**, **3i**, **3o**; all 9-benzylated except **3g**), only **3i**, bearing a 2-butenyl moiety, showed significant activity in the prophylactic AAR.

Analogues containing relatively hydrophilic substituents at position -7—including formyl (**3r**), (ethoxycarbonyl)-methyl (**3s**), and phenylcarbamoyl (**3u**)—exhibited little activity in the chronic assay.

A 7-methylthio-substituted analogue (**3v**) showed a modest level of activity, while the phenylthio analogue (**3w**) was virtually inactive. Introduction of a prenylated sulfur function at position 8 produced **3x**, which was devoid of activity in the prophylactic AAR assay.

In summary, then, **3j** showed a level of potential antiinflammatory activity comparable to that exhibited by the most efficacious compounds in the original 2-oxo series. Other members of the 2,3-dihydro series—such as, **3k** and **3m**—showed similar levels of activity in the AAR assay, but none was superior to **3j**, which was selected for further biological evaluation.

Additional Biological Properties of 3j. Cyclooxygenase Inhibition. Compound **3j** exhibited in vitro cyclooxygenase inhibitory activity in the rat neutrophil assay with an estimated IC_{50} of approximately 6 μM, comparable to the level of inhibitory activity shown by the prenylated 2-oxo series compounds reported previously.[1] As observed with its 2-oxo analogues, **3j** was devoid of inhibitory activity against the rat neutrophil 5-lipoxygenase enzyme. The anticyclooxygenase activity of **3j** may contribute to its observed pharmacological action in the AAR assay.

(8) For comparison, the 2-oxo analogue of 3j had oral ED_{50} values of 2.5 (not statistically significant) and 4.4 (1.9–8.2) mg/kg in the prophylactic and therapeutic AAR regimens, respectively. These values may be further compared with the respective oral ED_{50} values of 6.13 (1.9–29.8) mg/kg (prophylactic) and 1.1 (0.6–2.0) mg/kg (therapeutic) obtained for the standard agent naproxen in the same assay.

PDE Inhibition.[9] Selected members of the parent 2-oxo series had been found to exhibit phosphodiesterase (PDE) inhibitory activity. Since PDE inhibition would not be expected to contribute to antiinflammatory efficacy, this activity was regarded as extraneous and undesirable. Indeed, the 2-oxo analogue of **3j** showed an IC_{50} of approximately 37 μM in the PDE inhibition assay, comparable to that of aminophylline (IC_{50} = 30 μM in the same assay). In contrast, **3j** exhibited significantly less potency against phosphodiesterase with an IC_{50} of approximately 200 μM.

GI Ulcerogenic Potential.[1] Key compounds in the 2-oxo series were examined for their GI ulcerogenic potential and were found to exhibit substantially greater therapeutic ratios than indomethacin. Compound **3j** also exhibited a favorable profile: there was no detectable induction of gastric ulceration in rats administered oral doses of **3j** of up to 210 mg/kg, or approximately 35 times its ED_{50} in the AAR.

Toxicological Assessment. Finally, investigation of the parent 2-oxo series had been halted when toxicity studies in rats revealed that representative members of the series induced ocular changes of unexplained origin. It was therefore deemed crucial to examine **3j** for any signs of such an effect. When rats were dosed orally over a 5-day period with 75–300 mg/kg of **3j** (up to 50 times its ED_{50} in the AAR), no compound-related gross or microscopic changes were observed.[10]

Conclusion

With respect to the questions raised in our introductory remarks, it has been demonstrated that the 2,3-dihydropyrimidopurines, as a class, do indeed retain the antiinflammatory activity exhibited by the 2-oxo parent series and that there are some observable parallels in the structure–activity relationships of the two series. The compound that has emerged as the most interesting example of this new class of pyrimidopurine derivatives is **3j**.

In common with its 2-oxo progenitors, **3j** is a structurally novel nonsteroidal antiinflammatory agent exhibiting substantial activity in the AAR model of inflammation, despite a lack of significant activity in the acute synovitis model. The compound further shares the cyclooxygenase inhibitory properties exhibited by key members of the parent series. Compound **3j** shows only relatively weak inhibition of phosphodiesterase. Finally, and most importantly, **3j** is devoid of the toxic effects that precluded clinical development of the 2-oxo series. However, since a novel mechanism of action has not been established for **3j**, further progression of this compound has not been pursued.

Experimental Section

Melting points were determined on a Thomas-Hoover (melting points below 240 °C; corrected) or Electrothermal (melting points above 240 °C; uncorrected) capillary melting point apparatus. ^1H NMR spectra were recorded on a Varian CFT-20 (79.5 MHz), EM-390 (90 MHz), or XL-400 (400 MHz) spectrometer and are expressed as ppm (δ) from Me_4Si internal standard. Microanalyses were performed by the Physical and Analytical Research Department of the Schering-Plough Pharmaceutical Research Division, and carbon, hydrogen, nitrogen, and sulfur results were within ±0.4% of theory except as noted in the text. Flash chromatography[11] was performed on silica gel supplied by E.

(9) The protocol used to obtain these data is described in the following reference: Ahn, H. S.; Eardley, D.; Watkins, R.; Prioli, N. *Biochem. Pharmacol.* **1986**, *35*, 1113.

(10) The details of these studies have not been published.

(11) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Merck (No. 9385) or J.T. Baker (No. 7024). Unless otherwise indicated, all reagents and chemicals were obtained commercially and were used without pretreatment or further purification.

Reduction of the 1,3-Dimethyl-6-hydroxypyrimido[2,1-f]purine-2,4,8(1H,3H,9H)-trione System. Full experimental details for the reduction of the title system have been disclosed in a separate paper.[2] Preparation of the following compounds is explicitly detailed in that reference: **3a**, **3b**, **3d**, **3e**, and **3t**. All other 2,3-dihydropyrimidopurinediones in this paper, except compounds **3f** and **3l**, are derived from either **3a** or **3b** either by direct alkylation with commercially available reagents (**3c**, **3g**, **3h**, **3i**, **3k**, **3o**, **3p**, **3s**; see following paragraph) or by more elaborate derivatization, which is described in detail below. Relevant experimental data and physical constants are contained in Table I.

General Procedure for the Alkylation of 7-Unsubstituted 2,3-Dihydro-6-hydroxypyrimido[2,1-f]purine-4,8(1H,9H)-diones. The general method utilized for the alkylation of the 7-unsubstituted 2,3-dihydropyrimido[2,1-f]purines **3a** and **3b** was the same procedure previously described for the alkylation of their 2-oxo analogues.[3] Full experimental details are given below for those syntheses that entailed significant variations on the general alkylation theme or the preparation of novel intermediates.

2,3-Dihydro-1,3-dimethyl-6-hydroxy-7-propyl-9-(2-thienylmethyl)pyrimido[2,1-f]purine-4,8(1H,9H)-dione (3f). 1,3-Dimethyl-6-hydroxy-7-propyl-9-(2-thienylmethyl)pyrimido-[2,1-f]purine-2,4,8(1H,3H,9H)-trione[1] was reduced with lithium borohydride in dioxane according to the general procedure[2] to obtain **3f** in 7% yield as an off-white powder, mp 190–192 °C (from chloroform–methanol). Anal. ($C_{18}H_{21}N_5O_3S$) C, H, N, S.

9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(3-methyl-2-butenyl)pyrimido[2,1-f]purine-4,8(1H,9H)-dione (3j). Extractive Alkylation Method. Compound **3j** was prepared in low yield (9%; see Table I) by means of the general alkylation procedure referenced above. An improved yield was obtained when the "extractive alkylation" technique of Brandstrom and Junggren[4] was employed as described herein. A solution of 1.41 g (35.3 mmol) of sodium hydroxide and 6.0 g (17.7 mmol) of tetrabutylammonium hydrogen sulfate in 20 mL of water was added to a rapidly stirred, precooled (5 °C) solution of 5.0 g (14.7 mmol) of **3a** and 5.2 g (34.9 mmol) of 1-bromo-3-methyl-2-butene in 40 mL of methylene chloride. The mixture was stirred, and the temperature was allowed to rise to room temperature over a period of 3 h. The layers were separated, and the aqueous phase was extracted with two 5-mL portions of methylene chloride. The combined extracts were washed successively with 5 mL each of 3 N HCl and water and then dried over anhydrous magnesium sulfate. Drying agent was filtered, and the filtrate was concentrated under reduced pressure. The concentrate was taken up in hexane–ethyl acetate (2:1) and placed on a pad of silica gel (Baker 7024) on a medium-porosity sintered glass funnel. The pad was eluted with hexane–ethyl acetate (1:1) and the filtrate stripped under vacuum to obtain 2.33 g (39%) of the title compound with mp 145–146.5 °C. Trituration of this material in ether–hexane (1:1) gave a 92% recovery of **3j** with mp 147–148 °C. Anal. ($C_{22}H_{25}N_5O_3$) C, H, N. (The data in Table I pertain to a $^1/_3$hydrate obtained by means of the general alkylation procedure.)

2,3-Dihydro-1,3-dimethyl-9-[(4-methoxyphenyl)methyl]-6-hydroxy-7-(3-methyl-2-butenyl)pyrimido[2,1-f]purine-4,8(1H,9H)-dione (3l). (a) 8-[[(4-Methoxyphenyl)methyl]-amino]theophylline and diethyl malonate were reacted by means of the general method previously described[1] to obtain 1,3-dimethyl-9-[(4-methoxyphenyl)methyl]-6-hydroxypyrimido[2,1-f]purine-2,4,8(1H,3H,9H)-trione in 77% yield, mp 226–228 °C (from chloroform–methanol). Anal. ($C_{18}H_{17}N_5O_5$) C, H, N.

(b) Reduction of the above trione with lithium borohydride in dioxane via the general procedure[2] gave 2,3-dihydro-1,3-dimethyl-9-[(4-methoxyphenyl)methyl]-6-hydroxypyrimido[2,1-f]purine-4,8(1H,9H)-dione in 28% yield, mp 180–182 °C. Anal. ($C_{18}H_{19}N_5O_4$) C, H, N.

(c) Alkylation of 1,3-dimethyl-9-[(4-methoxyphenyl)-methyl]-6-hydroxypyrimido[2,1-f]purine-4,8(1H,9H)-dione with 1-bromo-3-methyl-2-butene was effected by means of the general procedure[3] to obtain title compound **3l** in 27% yield, mp 157–159 °C (from chloroform–methanol). Anal. ($C_{23}H_{27}N_5O_4$) C, H, N.

9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-[3-(trifluoromethyl)-2-butenyl]pyrimido[2,1-f]purine-4,8-(1H,9H)-dione (3m) (Scheme V). (a) (Z/E)-Ethyl 3-(Trifluoromethyl)-2-butenoate (9). The reaction was performed under nitrogen. The reaction flask was charged with 22.5 g (0.52 mol) of sodium hydride (55% dispersion; prewashed with three 200-mL portions of petroleum ether) and 800 mL of dry tetrahydrofuran, followed by 112 g (0.5 mol) of diethyl [(ethoxycarbonyl)methyl]phosphonate. The resultant mixture, after stirring for 1 h at room temperature, was cooled to −10 °C, and 65 g (0.58 mol) of 1,1,1-trifluoroacetone was added. The cooling bath was removed, and the reaction mixture was stirred at room temperature. After 1 h the mixture was transferred to a separatory funnel and was diluted with 500 mL of ether and then washed with two 250-mL portions of water. The organic layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the filtrate was distilled at atmospheric pressure. A cut boiling at approximately 35–63 °C and weighing 21.7 g (59%) was collected and found by ^1H NMR analysis (400 MHz; $CDCl_3$) to consist of a mixture of the geometric isomers of the title compound: the Z isomer, defined as that in which the methyl and ethoxycarbonyl groups are cis to one another, was identified by the chemical shift (δ 2.24) of the methyl group, as compared with the E isomer in which the methyl group appeared significantly further upfield (δ 1.98). The Z:E ratio was 89:11. This isomeric mixture was used without further treatment in reduction step b.

(b) 3-(Trifluoromethyl)-2-buten-1-ol (10). To a stirred, ice-cooled suspension of 11.0 g (0.289 mol) of lithium aluminum hydride in 1 L of ether, maintained under a nitrogen atmosphere, was added 69.0 g (0.379 mol) of **9**. The reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 2 h, before being recooled to 0 °C and decomposed by the cautious sequential addition of 11 mL of water, 11 mL of 15% aqueous sodium hydroxide, and 33 mL of water. Solids were removed by filtration, and ether was evaporated from the filtrate at reduced pressure. The crude residual product was distilled at atmospheric pressure and the title compound obtained as a 39.0-g (73%) cut, boiling at 140–145 °C. The ^1H NMR spectrum ($CDCl_3$) showed the presence of only a single isomer (methyl doublet at δ 1.85, methine multiplet at δ 6.26) which, based upon the isomeric composition of starting material **9**, must have the methyl and hydroxymethyl groups cis to one another.

(c) 1-Chloro-3-(trifluoromethyl)-2-butene (11). To a solution of 14.0 g (0.10 mol) of **10** in 8.0 g (0.10 mol) of pyridine was added dropwise with vigorous stirring during about 5 min 13.1 g (0.11 mol) of thionyl chloride. The reaction mixture was stirred for 15 min at room temperature, refluxed for 30 min, and then allowed to cool to room temperature again, before being poured into 200 mL of ether. The ether solution was washed successively with three 150-mL portions of ice–water, 150 mL of aqueous sodium bicarbonate, and 150 mL of water and then dried over anhydrous magnesium sulfate. Drying agent was removed by filtration, and the filtrate was distilled at atmospheric pressure. After removal of a fraction containing mostly solvent, 8.3 g (52%) of the title compound was obtained as a cut boiling at 105–107 °C. The ^1H NMR ($CDCl_3$) confirmed the isomeric purity of the product (methyl doublet at δ 1.88, methine multiplet at δ 6.28).

(d) 9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-[3-(trifluoromethyl)-2-butenyl]pyrimido[2,1-f]purine-4,8-(1H,9H)-dione (3m). To a suspension of 10.2 g (30 mmol) of **3a** in 400 mL of acetone was added 3.2 g (32 mmol) of triethylamine. The mixture was stirred at room temperature under a nitrogen atmosphere for 15 min before addition of 0.2 g (0.54 mmol) of tetrabutylammonium iodide and 7.9 g (50 mmol) of **11**. The resultant solution was refluxed under nitrogen for 4 h. Solvent was removed under reduced pressure, and the residue was partitioned between chloroform and dilute aqueous hydrochloric acid. Layers were separated, and the aqueous phase was extracted with two 100-mL volumes of chloroform. The combined extracts were dried over anhydrous magnesium sulfate, drying agent removed by filtration, and solvent stripped from the filtrate under reduced pressure. The crude product was treated with methanol and filtered to recover 2.1 g (21%) of starting material **3a**. Column chromatography of the filtrate on silica gel with chloroform–methanol (97:3) gave the title compound. Recrys-

tallization from ether–hexane gave 3.0 g (22%) of **3m** in analytically pure form with mp 110–112 °C. (A separately prepared batch of analytically indistinguishable **3m** had mp 118–120 °C.) Anal. ($C_{22}H_{22}N_5O_3F_3$) C, H, N, F.

9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(3-methylbutyl)pyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione (3n). On a Parr hydrogenation apparatus, a mixture of 3.41 g (8.37 mmol) of **3j** and 0.5 g of 5% palladium on activated carbon catalyst in 200 mL of methanol and 70 mL of methylene chloride was hydrogenated at 50 psi and room temperature for 13.5 h. The reaction mixture was filtered through a pad of Celite. The pad was washed with methanol, and combined filtrate and washes were stripped of solvent on a rotary evaporator. The residual solid was crystallized from methanol–ethyl acetate to obtain 1.79 g (51%) of **3n**, mp 141–142 °C. Anal. ($C_{22}H_{27}N_5O_3 \cdot 0.33H_2O$) C, H, N.

9-Benzyl-2,3-dihydro-1,3-dimethyl-7-[(*N*,*N*-dimethylamino)methenyl]pyrimido[2,1-*f*]purine-4,6,8(1*H*,7*H*,9*H*)-trione (3q). To 75 mL of *N*,*N*-dimethylformamide was added 2.47 g (16.2 mmol) of phosphorus oxychloride. The resultant solution was allowed to stir at room temperature for approximately 5 min during which time an orange color gradually developed. To this stirred orange solution 5.0 g (14.7 mmol) of **3a** was added (exotherm) as a well-ground powder, and the yellow solution that formed was allowed to stir at room temperature under an argon atmosphere. TLC [Merck silica gel plates; chloroform–methanol–acetic acid (95:5:0.2)] indicated that the reaction was complete within 2.5 h. Solvent was removed under vacuum, and the residual oil was partitioned between methylene chloride and 10% aqueous sodium bicarbonate. Considerable frothing was observed at this stage. The methylene chloride layer was washed successively with aqueous sodium bicarbonate, water, and brine and was then dried by filtration through anhydrous magnesium sulfate. Methylene chloride was removed under vacuum, and the residual solid was triturated with 250 mL of ether. Filtration yielded 5.50 g (95%) of title compound **3q** as an analytically pure monohydrated yellow powder, mp 235 °C dec. Anal. ($C_{20}H_{22}N_6O_3 \cdot H_2O$) C, H, N.

9-Benzyl-2,3-dihydro-1,3-dimethyl-7-formyl-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione, Sodium Salt (3r). A suspension of 4.0 g (10.1 mmol) of **3q** in 200 mL (20 mmol) of 0.1 N aqueous sodium hydroxide was stirred in a bath maintained at 100 °C for 75 min. The reaction mixture was allowed to cool to room temperature before being filtered through a sintered glass funnel. The solid thus isolated was washed successively with water (150 mL in two portions), ether–isopropyl alcohol (75:25 (v/v); two 100-mL volumes), and ether (100 mL) and was then dried at 45 °C under vacuum. Title compound **3r** was thus obtained as a yellow powder (2.35 g; 60%) containing $^1/_8$ mol of water, mp 325–326 °C dec. Anal. ($C_{18}H_{16}N_5O_4Na \cdot {}^1/_8H_2O$) C, H, N, Na.

9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(*N*-phenylcarbamoyl)pyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione, Triethylamine Salt (3u). To a suspension of 3.39 g (10 mmol) of **3a** in 7 mL of dry methylene chloride was added portionwise via syringe 1.19 g (10 mmol) of phenyl isocyanate, followed by 1.16 g (11.5 mmol) of triethylamine. An exotherm occurred, and all solids dissolved, forming a light orange solution, which was allowed to stir at room temperature. Within 75 min a light tan suspension had formed, which was allowed to stir at room temperature for another 15 h. The reaction mixture was diluted with 20 mL of ether and stirred briefly, and the solid was isolated by filtration. The collected solid was triturated in 75 mL of fresh ether, filtered, and dried under vacuum to obtain 4.93 g (87%) of **3u** as an analytically pure off-white powder, mp 174.5 °C dec. Anal. ($C_{24}H_{22}N_6O_4 \cdot C_6H_{15}N \cdot 0.4H_2O$) C, H, N.

9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(methylthio)pyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione (3v) (Scheme III). To a suspension of 0.36 g (9.00 mmol) of 60% sodium hydride (prewashed with petroleum ether) in 19 mL of *N*,*N*-dimethylformamide was added portionwise 2.76 g (8.14 mmol) of **3a**. The resultant mixture was stirred at room temperature for 1 h before dropwise addition of a solution of 1.8 g (8.91 mmol) of methyl thiotosylate in 8 mL of DMF to obtain a yellow solution, which was allowed to stir at room temperature for 65 h. The reaction mixture was poured into a large volume of water and was extracted with several volumes of methylene chloride. The combined extracts were washed successively with water and brine and were then dried over anhydrous magnesium sulfate. Drying agent was removed by filtration, and the filtrate was stripped of solvent at reduced pressure. The residual oil was diluted with ether, stirred at room temperature for 1 h, and filtered to obtain a yellow solid, which was crystallized from chloroform–methanol. The product thus isolated was triturated in 40 mL of methanol at room temperature for 5.5 h. Filtration yielded 1.88 g (60%) of the title compound as an analytically pure light yellow powder, mp 196–199 °C. Anal. ($C_{18}H_{19}N_5O_3S$) C, H, N, S.

9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(phenylthio)pyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione (3w) (Scheme III). (a) 9-Benzyl-7-bromo-2,3-dihydro-1,3-dimethyl-6-hydroxypyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione (4). To a stirred suspension of 20 g (58.9 mmol) of **3a** in 200 mL of DMF, maintained at 0 °C, was added dropwise, over 2 h, a solution of 10.85 g (67.9 mmol) of bromine in 50 mL of DMF. When addition was complete, cooling of the reaction mixture was discontinued and stirring maintained at room temperature for 3.5 h. The reaction mixture was poured into a large excess of water, and the resultant suspension was stirred for 0.5 h at room temperature. The mixture was then filtered, and the solid thus isolated was washed with ether and dried under vacuum to obtain 21.9 g (89%) of **4** as a yellow solid, mp 189–91 °C. This crude product was used without further purification in the following reaction step.

(b) 9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-7-(phenylthio)pyrimido[2,1-*f*]purine-4,8(1*H*,9*H*)-dione (3w). To a solution of 8.0 g (19.1 mmol) of **4** and 2.77 g (27.5 mmol) of triethylamine in 90 mL of DMF, maintained at 0–5 °C, was added a solution of 2.82 g (25.7 mmol) of thiophenol in 15 mL of DMF. The cooling bath was removed, and the reaction mixture was allowed to stir at room temperature for 15 h before the solvent was evaporated under vacuum. The residual yellow oil was dissolved in chloroform, and the resultant solution was washed successively with water and brine and then dried over anhydrous sodium sulfate. The drying agent was removed by filtration, and the filtrate was stripped of solvent under reduced pressure to obtain a yellow solid. This crude product was subjected to flash chromatography, eluting first with methylene chloride–acetone (2:1) and finally with methylene chloride–acetone–acetic acid (1:1:0.1) to obtain 2.2 g (26%) of **3w** as a light brown solid, mp 227–238 °C dec. Anal. ($C_{23}H_{21}N_5O_3S$) C, H, N, S.

9-Benzyl-2,3-dihydro-1,3-dimethyl-8-[(3-methyl-2-butenyl)thio]pyrimido[2,1-*f*]purine-4,6(1*H*,9*H*)-dione (3x) (Scheme IV). (a) 9-Benzyl-2,3-dihydro-1,3-dimethyl-6-hydroxy-4-oxo-8-thioxopyrimido[2,1-*f*]purine (5). To a suspension of 6.12 g (18.0 mmol) of **3a** in 150 mL of toluene was added in one portion as a solid 2.79 g (6.90 mmol) of 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent). The resultant mixture was placed in an oil bath and stirred under an argon atmosphere for 10 h at 100 °C, at which time another 0.60 g (1.49 mmol) of Lawesson's reagent was added and stirring at 100 °C continued for another 8 h. After cooling to room temperature, the yellow suspension was filtered. The yellow powder thus isolated (4.71 g, 74%) was washed thoroughly with ether and was then flash chromatographed on silica gel, eluting with chloroform–methanol–acetic acid (990:10:1), to obtain 1.65 g (26%) of **5** as a brown-gray powder, mp 195.5–199 °C dec. Anal. ($C_{17}H_{17}N_5O_2S$) C, H, N, S.

(b) 9-Benzyl-2,3-dihydro-1,3-dimethyl-8-[(3-methyl-2-butenyl)thio]pyrimido[2,1-*f*]purine-4,6(1*H*,9*H*)-dione (3x). To an ice-cooled suspension of 1.60 g (4.51 mmol) of **5** in 33 mL of *N*,*N*-dimethylformamide was added 216 mg (5.41 mmol) of sodium hydride (60% dispersion in mineral oil). The mixture was stirred in an ice–water bath for 20 min before introduction via syringe of 1.01 g (6.76 mmol) of 1-bromo-3-methyl-2-butene as a neat oil. The resultant solution was stirred in an ice–water bath for 190 min and was then poured into a mixture of 125 mL of chloroform and 350 mL of brine acidified with 5.7 mL (5.7 mmol) of 1.0 N hydrochloric acid. The layers were separated, and the aqueous phase was extracted with three portions of chloroform. The combined extracts were washed twice with water, once with a 1:1 mixture of brine and water, and finally once with brine before drying by filtration through anhydrous magnesium sulfate. The filtrate was stripped of solvent under reduced pressure, and the residue was triturated in ether and filtered to obtain 1.54 g (81%) of a yellow powder. This crude product was flash chromatographed on silica gel, eluting with acetone–methylene chloride

(in a stepped gradient of 2:1, followed by 9:1) to obtain 948 mg (50%) of the title compound as a pale yellow powder, mp 144.5–151 °C. Anal. ($C_{22}H_{25}N_5O_2S$) C, H, N, S.

RPAR-Synovitis Technique.[1] Lewis rats were dosed orally with drug or placebo 1 h prior to iv administration 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, "Δ synovial weight", were considered to represent the inflammatory response for each animal. Differences in "Δ synovial weight" between lesion controls and drug-treated rats (five animals per treatment group) are presented as the mean percent inhibition. For compounds producing greater than 20% inhibition, the reported value is statistically different from control by an analysis of variance.

Adjuvant-Induced Arthritis in Rats (AAR).[7] **(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 methylcellulose. Injection of 0.1 mL of the adjuvant was made into the left hind paw. The left and right hand 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 35 days. Treatment of controls and measurement of "Δ paw volume" were conducted as described in (a) above.

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Dynamics and Thermodynamics of the Counterion Effect in a 7*H*-Pyridocarbazole Dimer (Ditercalinium). Hypothesis of a Nonbisintercalative Binding Mode to Calf Thymus DNA at High Drug/Base Ratio

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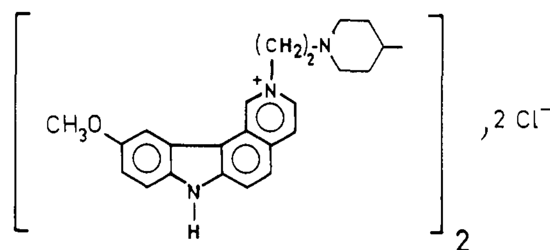
Ditercalinium, a 7*H*-pyridocarbazole dimer designed to bisintercalate into DNA, forms tight ion pairs in water with inorganic and organic anions. The thermodynamics and kinetics of the acetate–ditercalinium pairing has been investigated by means of T-jump spectroscopy. The formation of the pair has a constant estimated to 1000 M^{-1} and proceeds via a fast two-step mechanism with a relaxation time of 12 μs (acetate pH 5) to 50 μs (cacodylate, pH 7.5) involving an intermediate solvent-separated ion pair. A strong association of ditercalinium to cardiolipid has been observed and is expected to be involved in the respiratory chain inhibition induced by ditercalinium (unpublished results). Direct estimates of the binding constants of the drug to calf thymus DNA were obtained by means of UV titrations at high drug/base ratio (>0.17). The maximum number of binding sites per base both at pH 5 and pH 7.5 was found to be 0.22, a value consistent with monointercalation as expected from the prediction of Shafer's model for the interaction of bifunctional ligands to DNA. This work also supports the hypothesis that significant ionic binding may account for the ditercalinium/DNA interaction at high base/drug ratios (0.2).

Intercalation into DNA of planar aromatic molecules such as phenantridines, acridines, ellipticines, etc. is thought to be the primary act (if not the significant) that accounts for the cytotoxicity and eventual antitumor properties of these compounds. To this respect and in order to potentiate the affinity and specificity of their monofunctional counterparts, numerous bifunctional derivatives consisting of two intercalative moieties linked together by an aliphatic or alicyclic chain have been synthesized and tested against various experimental tumors.[1] One of this compounds, a 7*H*-pyridocarbazole dimer known under the generic name of ditercalinium (2,2′-([4,4′-bipiperidine]-1,1′-diyldi-2,1-ethanediyl)bis[10-methoxy-7*H*-pyrido[4,3-*c*]carbazole]),[2,3] specially designed to be a bisintercalator, showed promising antitumor activity (NSC366241) and had been introduced in phase 1 clinical trial until it proved to be highly hepatotoxic (possibly as a strong inhibitor of the electron-transport chain in the mitochondrion inner membrane[4,5] and was hence withdrawn from human test.



The numerous experimental data gained on DNA/ditercalinium interactions through various methods (ethidium binding competition, viscosimetry, NMR spectroscopy, electron microscopy) were interpreted in terms of the monointercalation of ditercalinium into DNA at physiological pH ($K_a = 10^7$ M^{-1}, pH 7.4) and of its bisin-

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