Pyrimidinones. 3. N-Substituted 6-Phenylpyrimidinones and Pyrimidinediones with Diuretic/Hypotensive and Antiinflammatory Activity

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In an extensive analysis of the antiviral and interferon-induction structure–activity relationship of 6-arylpyrimidinones we found that modifications at positions 1–4 of the pyrimidine ring resulted in a loss of activity.[1-3] However, we uncovered interesting hypotensive and antiinflammatory activity with a series of N-substituted analogues, the results of which we report herein.

Over the past 30 years numerous pyrimidines have been prepared and their pharmacology evaluated[4] Various 2-amino-4-substituted-5-alkylpyrimidines were reported to have diuretic activity.[5-8] In addition, numerous 2-aminopyrimidines have exhibited bacteriostatic, fungicidal, and antiviral activity.[8-10] We therefore expanded the analogue scope of our antiviral 5-halo-6-phenylpyrimidines to search for cardioregulatory as well as antiinflammatory activity. These were exploited in a series of N-substituted analogues.

Chemistry. There are two obvious problems in attempting to synthesize N-substituted pyrimidines: regioselective introduction of the desired *N*-alkyl substituent and unequivocal determination of the position of the substituent. The latter problem was solved through the use of ^1H–^{13}C long-range gated decoupling NMR. This we found generally applicable to a variety of *N*-alkyl substituents. However, selectivity in introducing the N-substituent appeared to be a function of other substituents on the pyrimidine. Therefore, the results we described should not be generalized beyond the 6-aryl series.

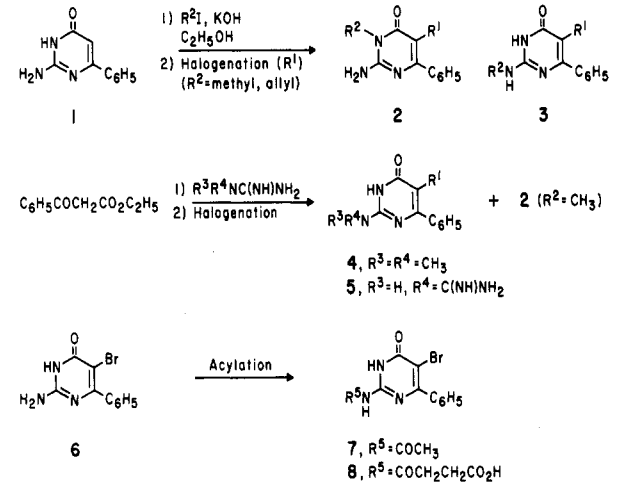
Methylation (CH_3I, KOH, EtOH) of 2-amino-6-phenyl-4-pyrimidinone yields the N_3-methyl product in 75% yield[8] (Scheme I). Halogenation readily affords the desired 5-halogen derivatives.[2] Condensation of ethyl benzoylacetate with methylguanidine affords a mixture of N_2-methyl and N_3-methyl products with the latter greatly predominating. This is in contrast to the report of N_1- and N_2-methyl products in the condensation with ethyl acetoacetate.[8]

The N_2-methyl is readily recognized in the ^1H NMR spectrum, since the methyl appears as a doublet which collapses to a singlet either with deuterium exchange or N–*H* irradiation. The position of the C_2, C_4, C_5, and C_6 can be readily assigned in the ^{13}C NMR spectrum by ^1H–^{13}C coupling (gated and off-resonance decoupling; see Experimental Section).[12,13] The C_5 exhibits 1,2-coupling (doublet); the C_4 exhibits a small 1,3-coupling; the C_6 exhibits multiple 1,3-coupling; and the C_2 exhibits none. Therefore, the N_3-methyl is readily assigned based on 1,4-coupling with both C_2 and C_4, and of course, the N_2-methyl exhibits a multiplet for C_2 only. No N_1-methyl or alkyl analogues were isolated in these approaches.

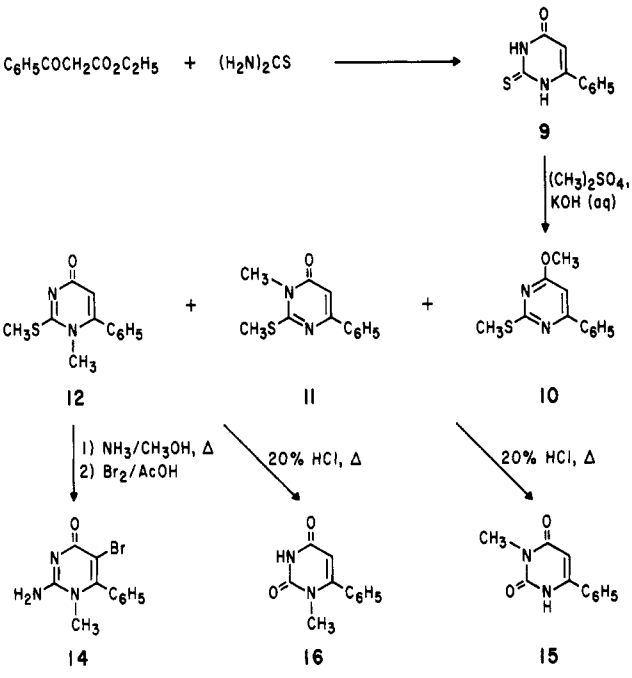
Additional N_2-substituted analogues were prepared via direct condensation, such as the N_2-dimethyl and the N_2-amidino, from dimethylguanidine and biguanide, respectively. Acetylation and succinylation of pyrimidinone **6** yielded the 2-acetyl and 2-succinyl derivatives, **7** and **8**.

Since we were unable to prepare any N_1-derivatives via either direct alkylation of the pyrimidinone or condensation of the appropriate guanidine, we explored an alternative route. Reasoning that the temporary removal of the N_2-amino group might allow N_1-alkylation to occur, we prepared the 2-(methylthio)-6-phenyl-4-pyrimidinone

Scheme I



Scheme II



(Scheme II). Methylation with excess dimethyl sulfate and 2 equiv of base afforded **10**, **11**, and **12** in approxi-

† Deceased.

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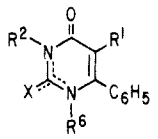
Table I



entry	R^1	R^2	R^6	X	analyses/ref	diuretic[a]	blood pressure change[b] 4 h	blood pressure change[b] 24 h	antiinflammatory,[c] % inhibition RPAR (mg/kg) 25	50	100	200
2	Br	CH_3		NH_2	$C_{11}H_{10}BrN_3O$: C, H, N, Br	5.52	−12	−7	36	64	74	−
3	Br	H		$NHCH_3$	$C_{11}H_{10}BrN_3O$: C, H, N, Br	−	NT		42	78	82	92
4	Br	H		$N(CH_3)_2$	$C_{12}H_{12}BrN_3O$: C, H, N	−	+1	+2				
5	Br	H		$NHC(NH)NH_2$	$C_{11}H_{10}BrN_5O$: C, H, N, Br	−	−2	−1				
6	Br	H		NH_2	ABPP/1	7.41	+0	−22	13	31	44	
7	Br	H		$NHCOCH_3$	$C_{12}H_{10}BrN_3O_2$: C, H, N, Br	−	+4	+1				
8	Br	H		$NHCOCH_2CH_2CO_2H$	$C_{14}H_{12}BrN_3O_4$: C, H, N, Br[d]	NT	NT				−	
14	Br		CH_3	NH_2	$C_{11}H_{10}BrN_3O$: C, H, N[g]	NT	NT				−	
17	I	H		NH_2	AIPP/1	−	−7	−12	−	3	8	17
18	I	CH_3		NH_2	$C_{11}H_{10}IN_3O$: C, H, N	4.46	−6	−13	18	30	79	−
19	I	H		$N(CH_3)_2$	$C_{12}H_{12}IN_3O$: C, H, N, I	−	+1	+2			−	
20	I	H		$NHCOCH_3$	$C_{12}H_{10}IN_3O_2$: C, H, N, I	−	+3	+9			−	
21	I	H		$NHC(NH)NH_2$	$C_{11}H_{10}IN_5O$: C, H, N, I, C[e]	−	−2	+3			−	
22	I	H		$NHCH_3$	$C_{11}H_{10}IN_3O$: C, H, N, I, N[f]	−	NT				−	
23	I	CH_2CHCH_2		NH_2	$C_{13}H_{12}IN_3O$: C, H, N	5.09	−6	−10			−	
24	Br		OH	NH_2	ref 17	−	+6	−1		−	−	
1	H	H		NH_2	ref 1	−					−	
15	H	CH_3	H	O	$C_{11}H_{10}N_2O_2$: C, H, N	−	0	+6		−	−	
25	Br	CH_3	H	O	$C_{11}H_9BrN_2O_2$: C, H, N, Br, Br[h]	−	−1	+6		−	−	
26	I	CH_3	H	O	$C_{11}H_9IN_2O_2$: C, H, N, I, I[i]	−	+1	+4		−	−	
16	H	H	CH_3	O	ref 11		+4	+6	27	47	79	95
27	Br	H	CH_3	O	$C_{11}H_9BrN_2O_2$: C, H, N, Br, Br[j]	−	+1	+1	22	43	57	67
28	I	H	CH_3	O	$C_{11}H_9IN_2O_2$: C, H, N, I	−	+15	−1	33	44	73	99
29	Cl	H	CH_3	O	$C_{11}H_9ClN_2O_2$: C, H, N, Cl	3.49	+2	+2		−		
36	Br	CH_3	CH_3	O	$C_{12}H_{11}BrN_2O_2$: C, H, N, Br	−	NT		−	36	59	−
31	Br	H	H	O	ref 11	3.53	+6	+5	−	46	58	−
32	I	H	H	O	ref 11	5.43	+7	+11	−	41	48	−
33			hydrochlorothiazide			7.18	0	+2				
34			furosemide			31.25	+1	−3				
35			guanethidine				−16	9				

[a] Numerical values indicate that the test substance was considered active (i.e., T/C for stage 1·T/C for stage 2 > 3.34) with the greater the value the greater the activity; − indicates inactive and NT is not tested; given orally at 40 mg/kg. [b] Refers to the average mmHg change in mean arterial blood pressure observed at 4 and 24 h after oral administration of 50 mg/kg. [c] Compounds were administered po 1 h before antigen sensitization to 24-h fasted rats. Dexamethasone served as the positive control exhibiting 50% inhibition at 5 mg/kg. [d] Equiv w = 389. [e] Calcd, 37.19; found, 37.79. [f] Calcd, 12.84; found, 13.30. [g] 1.0 HBr. [h] Calcd, 28.42; found, 26.87. [i] Calcd, 38.68; found, 40.6. [j] Calcd, 28.42; found, 27.07.

mately 10%, 70%, and 3% yields, respectively. The O-alkylated derivative **10** is readily identified by its ^1H NMR (OCH_3 at 4.01 ppm). Differentiation of **11** and **12** was accomplished by ^1H–^{13}C long-range coupling where **11** exhibited collapse to a multiplet with gated decoupling of the readily identified C_4. In the ^1H NMR the N–CH_3 of **11** is further downfield (3.55 ppm) than in **12** (3.23 ppm) as expected due to the deshielding of the adjacent carbonyl in **11**. In addition, **11** was hydrolyzed to the known N_3-methyl-6-phenyluracil and **12** to N_1-methyl-6-phenyluracil.[11]

Although the yield was poor, sufficient **12** was prepared and heated in a sealed tube with ammonia-saturated methanol to yield the 2-aminopyrimidinone. Bromination afforded **14** in an overall 1% yield from **9**. That no rearrangement occurred during the ammonia treatment was evident by the nonidentity of **14** with the previously prepared **2** and **3**.

Lastly, the corresponding N_3-methyl-5-halopyrimidinediones (uracil) were prepared for biological comparisons. These were prepared via conventional fusion procedures to afford the uracil followed by halogenation as outlined previously.[1]

Biology. 6-Phenylpyrimidinones **2**, **5**, **7**, and **8** were evaluated for antiviral activity against SFV challenge in mice and found inactive. In addition the N-alkylated-6-halogeno-6-phenyluracils were uniformly inactive in this virus model.

Table I depicts the cardiovascular/renal and antiinflammatory activities of both the 2-aminopyrimidinone and the pyrimidinedione series. Compounds **2**, **6**, **18**, and **23** exhibit diuretic activity when administered orally to fasted rats. These same 2-aminopyrimidinones do exhibit hy-

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Table II. Effect of ABPP and AIPP on Adjuvant-Induced Polyarthritis

treatment[a]	dose, mg/kg, b.i.d.	body wt change, gm	spleen wt, g	arthritis (day 15) incidence	score	% I	arthritis (day 33) incidence	score	% I	PIU	% I
normal control		+57	1.05 ± 0.13	0/10	0					3.6 ± 1.5	
arthritic control		+5	1.93 ± 0.19	25/30	11.3 ± 0.5		13/14	11.4 ± 1.6		196.9 ± 27.1	
cortisol	3.4	−13	1.79 ± 0.37	9/10	7.5 ± 1.5	34	5/5	15.6 ± 0.4	0	152.6 ± 35.4	42
	8.6	+1	1.12 ± 0.06	4/10	2.4 ± 1.1	79	5/5	6.6 ± 1.7	42	123.4 ± 32.5	37
	17.8	−6	1.18 ± 0.20	0/10	0	100	5/5	4.2 ± 1.5	63	43.0 ± 17.7	78
ABPP	106	−8	2.92 ± 0.35	6/10	3.8 ± 1.6	66	4/5	9.4 ± 3.1	18	79.0 ± 28.0	60
	208	−12	2.89 ± 0.38	3/10	1.3 ± 0.5	88	2/4	1.5 ± 1.0	87	54.6 ± 8.8	72
	321	−16	3.11 ± 0.61	2/10	1.8 ± 1.6	84	1/4	0.2 ± 0.2	98	80.6 ± 10.6	59
AIPP	35	−12	1.73 ± 0.14	10/10	10.0 ± 1.5	0	5/5	11.2 ± 2.1	0	162.6 ± 9.4	17
	106	−11	1.68 ± 0.27	9/10	10.0 ± 1.6	0	5/5	13.4 ± 1.7	0	124.8 ± 12.3	37
	208	−14	1.75 ± 0.16	9/10	9.6 ± 1.6	0	5/5	11.4 ± 2.5	0	149.8 ± 22.9	24
	327	−14	1.95 ± 0.15	10/10	10.9 ± 1.2	0	5/5	10.8 ± 1.1	0	147.0 ± 5.6	25

[a] Compounds were administered po to 50% of the animals for 15 days. Treatment was stopped in 50% of the animals on day 15, and this part of the assay was terminated on day 33.

Table III. Schedule Dependence of the Effects of ABPP on Adjuvant-Produced Polyarthritis

days of treatment[a]	dose, mg/kg, b.i.d.	body wt change, g	spleen wt, g	arthritis (day 15) incidence	arthr score	% I	PIU	% I
normal control[b]		114	1.22 ± 0.02	0/10			3.8 ± 1.0	
arthritic control		+34	1.78 ± 0.12	20/20	15.7 ± 0.1		146.7 ± 12.9	
1, 3, 5, 7, 9, 11, 13, 15	279	+23	2.09 ± 0.6	6/10	2.8 ± 0.6	82	62.5 ± 8.7	57
1, 4, 7, 10, 13	263	+37	1.77 ± 0.12	6/10	3.7 ± 1.4	76	34.9 ± 7.4	76
1, 5, 9, 14	267	+34	1.73 ± 0.10	9/10	7.6 ± 1.6	51	59.8 ± 9.5	59
1, 6, 12	281	+37	1.59 ± 0.08	6/10	5.4 ± 1.6	66	69.6 ± 12.7	53
1–15	292	+21	2.16 ± 0.17	0/10	0	100	21.1 ± 4.0	86

[a] Compounds were administered po for the stipulated time periods. [b] Vehicle was administered to the normal and arthritic control groups from days 1–15.

potensive activity when given orally to normotensive rats at essentially equivalent doses. Since compound **17** exhibits hypotensive activity but no diuretic activity, it cannot be concluded that these two phenomena are interdependent with this series of compounds. A similar conclusion must be drawn from the pyrimidinediones where several compounds (**29**, **31**, and **32**) are diuretic but exhibit no hypotensive response. For comparison, diuretic assay values and hypotensive assay values obtained with hydrochlorothiazide (HCTZ) and furosemide (diuretic standards) and guanethidine (hypotensive standard) are also depicted in Table I. Further evaluation of the diuretic activity of pyrimidinones revealed that natriuresis and kaliuresis accompanies the diuresis associated with these agents (Table IV). Results from identical tests with the standards hydrochlorothiazide and furosemide are also shown in Table IV. The most active pyrimidinones were as efficacious as hydrochlorothiazide but less efficacious than furosemide. The Na^+–K^+ excretion profile with these pyrimidinones was nearly identical to that apparent with the thiazide diuretic.

Both series of compounds exhibit antiinflammatory effects as measured in the reverse passive Arthus reaction (RPAR). The pyrimidinediones are the more potent and consistently active compounds exhibiting a dose-related (25-200 mg/kg) suppression of the RPAR.

The summary structure–activity relationship (SAR) data indicate that (1) for cardiovascular activity N_1- or N_2-substituents abolish activity whereas N_3-substituents are allowed but not required and (2) for antiinflammatory activity the N_1-substituent of the pyrimidinedione series is compatible but not necessary for activity whereas the N_3 is tolerated only where N_1 is methylated.

The antiarthritic activity of compounds **6** and **17** was further evaluated in adjuvant-induced polyarthritic rats. The data in Table II indicate that compound **6** (ABPP) caused a dose-related inhibition of this chronic arthritic process as reflected in a significant decrease in the incidence and severity of arthritis on day 15. Further, if treatment is terminated on day 15, the activity of ABPP is sustained as illustrated by the suppression of arthritis on day 33. Inhibition of the PIU values relative to controls also demonstrates the antiarthritic activity of this compound. In contrast, compound **17** (AIPP) was essentially inactive in this model of arthritis (Table II).

As an extension of the aforementioned studies, we further evaluated the effects of ABPP on polyarthritis in rats as a function of the dosing regimen with this compound. The results of these studies (Table III) indicate that compound **6** exerted maximum antiarthritic activity when administered daily from day 1 through day 15. However, greater than 80% suppression of the arthritic response was achieved when ABPP was administered on an alternate-day basis.

It has been reported that selective defects in T-cell function and immune interferon regulation may contribute to the etiology of rheumatoid arthritis.[19] In addition there are preliminary reports that interferon treatment of rheumatoid arthritis results in a partial amelioration of some of the symptoms.[20] Since (1) ABPP is better tolerated in the clinic than interferon,[21,22] (2) ABPP exhibits

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Table IV. Dose–Response Relationships for Effects of Selected Pyrimidinones on Urine Volume and Na^+ and K^+ Excretion in Conscious Rats[a]

entry	urinary excretion	dose, mg/kg					
		0.3	1.0	3.0	10	30	100
2	UV[b]	1.0	0.9	1.4	2.3	2.5	2.8
	Na^+	1.0	1.2	1.3	2.1	2.5	2.8
	K^+	1.1	1.0	1.3	1.7	2.2	2.2
6	UV	1.1	1.2	1.3	2.5	2.6	2.7
	Na^+	1.1	1.3	1.5	2.3	2.9	2.8
	K^+	1.1	1.0	1.1	1.3	1.5	1.3
18	UV	1.3	1.4	1.6	2.2	2.7	2.0
	Na^+	1.1	1.2	1.6	1.9	2.4	2.7
	K^+	1.1	1.1	1.2	1.4	2.1	1.6
29	UV	0.9	0.9	0.8	1.1	1.6	1.6
	Na^+	1.1	1.1	0.9	1.1	1.6	1.6
	K^+	0.9	1.2	1.1	1.3	1.7	1.9
31	UV	0.9	1.0	1.1	1.2	1.5	2.1
	Na^+	1.2	1.2	1.0	1.2	1.4	1.9
	K^+	1.0	1.0	1.0	0.9	1.2	1.8
HCTZ[c]	UV	1.9	2.3	2.2	2.4	2.6	2.7
	Na^+	1.7	2.0	2.1	2.4	2.4	2.5
	K^+	1.2	1.6	1.6	1.5	1.5	1.7
furosemide	UV	1.1	1.2	1.3	2.2	4.7	7.2
	Na^+	1.1	1.1	1.1	1.5	3.1	4.5
	K^+	1.0	1.0	1.3	1.6	2.2	3.2

[a] Values are urinary excretion in treated rats/urinary excretion in control rats. Underscoring indicates values significantly greater than 1.0 ($P < 0.05$); i.e., excretion in treated was significantly greater than in the corresponding control. Mean urine volume of all control rats was 4.2 ± 0.1 (SE) mL/5 h; control Na^+ excretion was 0.68 ± 0.02 mequiv/5 h; and K^+ excretion was 0.24 ± 0.01 mequiv/5 h. [b] Urine volume. [c] Hydrochlorothiazide.

antiarthritic activity as noted above, and (3) there appears to be a correlation with antiarthritic activity and interferon induction (note that AIPP is inactive and a poor interferon inducer), it appears justifiable to evaluate ABPP and other interferon inducers in patients with rheumatoid arthritis.

Experimental Section

Melting points were determined on a Thomas-Hoover unimelt and are uncorrected. ^1H NMR spectra were run on a Varian T60-A, FT-80 or EM-390. ^{13}C NMR spectra were recorded on a Varian CFT-20. All data are relative to internal Me_4Si. All solvents and reagents employed were reagent grade and used as received.

^1H–^{13}C coupling spectra (gated decoupling) were performed by employing a filter bandwidth of 4000, sweep width of 2304, acquisition time of 1.77 s, pulse width of 9, pulse delay of 3.00 s, and the decoupler turned off.

Halogenation procedures are as previously described.[2]

6-Phenyl-2,4-pyrimidinedione. To 24 g (0.4 mol) of urea was added 200 mL of ethyl benzoylacetate, and the reaction mixture was allowed to stir at 135 °C for 72 h. The mixture was cooled, allowed to stand at room temperature for 18 h, and filtered. The resulting solids were washed well with ether and dried at 60 °C to yield 16.0 g. The solids were triturated with water, cooled, and filtered to yield 14.0 g (18.6%) of 6-phenyl-2,4-pyrimidinedione: ^1H NMR (Me_2SO-d_6) 7.86–7.46 (m, 5 H, Ph), 5.81 (s, 1 H, vinyl).

1,3-Dimethyl-5-bromo-6-phenyl-2,4-pyrimidinedione (36). To 2.67 g (10 mmol) of 5-bromo-6-phenyl-2,4-pyrimidinedione was added 50.0 mL of water and 1.0 g (41 mM) of NaOH. When all was in solution, 2.24 mL (≈2.97 g, ≈23.5 mmol) of dimethyl sulfate was added and the reaction mixture was heated at reflux for 18 h. The reaction mixture was cooled and poured into a separatory funnel containing 250 mL of CH_2Cl_2. The aqueous layer was washed twice with methylene chloride, and the combined organic layers were dried over Na_2SO_4 (anhydrous), filtered, and evaporated to dryness under vacuum to yield 2.40 g of an amorphous foam. The crude material was dissolved in 50 mL of acetone, 20 g of silica gel added, and the slurry evaporated. The powder was placed on top of 300 g of silica gel and the column eluted with 1:1 ethyl acetate/hexane (R_f 0.3 in 1:2 EtOAc/hexane) to yield 1.50 g (50.8%): ^1H NMR ($CDCl_3$) 7.50 (m, 3 H, Ph), 7.15 (m, 2 H, Ph), 3.45 (s, 3 H, $CONCH_3CO$), 3.10 (s, 3 H, NCH_3). Anal. ($C_{12}H_{11}BrN_2O_2$) C, H, N, Br.

3-Methyl-6-phenyl-2,4-pyrimidinedione (15). To 2.4 g (26.6 mmol) of *N*-methylurea was added 25.0 mL of ethyl benzoylacetate, and the mixture was allowed to stir at 135 °C for 18 h. After cooling to room temperature, the precipitate formed was filtered and washed well with ether to yield 1.70 g (31.6%). Recrystallization from acetone/ethanol/hexane gave 1.40 g (26%). This material was identical (spectroscopically and chromatographically) with the pyrimidinone obtained via hydrolysis at the 2-SCH_3 analogue.

Hydrolysis of (Methylthio)pyrimidinones (15, 16). To 12.7 g of 2-(methylthio)-3-methyl-6-phenyl-4-pyrimidinone (54.7 mmol) was added 150 mL of a 20% aqueous HCl solution. The reaction mixture was heated at reflux, with stirring, for 2 1/2 h. After cooling, the resulting solids were filtered, washed with water, and dried at 60 °C to yield 10.5 g. Recrystallization from ethanol/DMF yielded 7.90 g (71.5%): ^1H NMR (Me_2SO-d_6) 7.76 (m, 2 H, Ph), 7.56 (m, 3 H, Ph), 5.96 (s, 1 H, vinyl), 3.16 (s, 3 H, NCH_3). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

In similar fashion 1.0 mmol of 2-(methylthio)-1-methyl-6-phenyl-4(3*H*)-pyrimidinone was hydrolyzed to the N_1-methyl isomer in 65% yield: ^1H NMR (Me_2SO-d_6) 7.44 (s, 5 H), 5.40 (s, H), 2.96 (s, 3 H). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

The assignments are consistent with the ^1H NMR spectra wherein the enforced orthogonal phenyl group of the N_1 isomer appears as a singlet (2) and the N_3-methyl group is further downfield due to additional deshielding effects of both carbonyl groups.

2-Amino-1-methyl-6-phenyl-4-pyrimidinone. To 400 mg (1.72 mM) of 1-methyl-2-(methylthio)-6-phenyl-4-pyrimidinone was added, in a glass bomb, 12 mL of a saturated solution of NH_3/CH_3OH. The glass bomb was sealed and heated at 170 °C for 18 h. The reaction was cooled, the bomb opened, and the solution evaporated to a yellow oil under vacuum. The desired material had R_f 0.3 in 15% MeOH/$CHCl_3$ and was contaminated with 1-methyl-6-phenyl-2,4-pyrimidinedione as the major by-product. The crude material was dissolved in ethanol, 8 g of silica gel was added, and the slurry was evaporated to a powder under vaccuum. The powder was placed on top of 25 g of silica gel and eluted with 7% MeOH/$CHCl_3$, collecting 15.0-mL fractions until desired material was obtained. Evaporation of solvent to dryness gave 180 mg (52%). Recrystallization from methanol/ether gave 60 mg (17%): ^1H NMR (Me_2SO-d_6) 7.50 (s, 5 H, Ph), 5.5 (s, 1 H, vinyl), 3.3 (s, 3 H, NCH_3). MS: calcd, 201.0902; found, 201.0893.

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2-Thioxo-6-phenyl-4-pyrimidinone (9). To 106 g (1.4 mol) of thiourea was added 1 L of ethanol and 400 mL of 25% sodium methoxide/methanol and 180 g (0.93 mol) of ethyl benzoylacetate. The reaction solution was heated at reflux for 18 h and cooled to 5 °C. A precipitate formed that was filtered and washed with methanol, dissolved in water (1 L), and neutralized by the addition of concentrated HCl. The resulting solids were filtered, washed well with water followed by acetone, and dried at 60 °C overnight to yield 119.7 g: mp 263–5 °C.[18]

Methylation of 2-Thioxo-6-phenyl-2,4-pyrimidinedione (10–12). To 119.7 g (0.586 mol) of 2-thio-6-phenyl-2,4-pyrimidinedione was added 250 mL of water and 51.2 g (1.28 mol) of sodium hydroxide. The reaction mixture was stirred at ambient temperature until solution was complete. Dimethylsulfate (122 mL, ≈166.9 g) was added dropwise at 50–60 °C. When the addition was complete, the pH was slightly acidic and a precipitate formed. The solids were filtered and washed well with water and dried at 40 °C under vacuum. The aqueous solution was extracted twice with toluene and chloroform. The organic layers were combined, dried (Na_2SO_4 anhydrous), and evaporated to dryness to yield 0.9 g of 1-methyl-2-(methylthio)-6-phenyl-4-pyrimidinone: ^{1}H NMR (Me_2SO-d_6) 7.46 (s, 5 H, Ph), 5.70 (s, 1 H, vinyl), 3.23 (s, 3 H, NCH_3), 2.46 (s, 3 H, SCH_3). Anal. ($C_{12}H_{12}N_2OS$) C, H, N, S.

The dried solids were chromatographed on 1.5 kg of silica gel and eluted with 1:2 EtOAc/hexane until R_f 0.9 (1:1, EtOAc/hexane) appeared. Evaporation of the material yielded 10.3 g of 2-(methylthio)-4-methoxy-6-phenylpyrimidine. This pyrimidine was recrystallized from *n*-hexane to yield 8.5 g of pure material: ^{1}H NMR ($CDCl_3$) 8.15–7.98 (m, 2 H, Ph), 7.55–7.4 (m, 3 H, Ph), 7.8 (s, 1 H, vinyl), 4.01 (s, 3 H, OCH_3), 1.65 (s, 3 H, SCH_3). Anal. ($C_{12}H_{12}N_2OS$) C, H, N, S.

At this point the eluent was switched to 1:1 EtOAC/hexane and solvent collected until R_f 0.5 (2:1 EtOAc/hexane) appeared. Evaporation of solvents left 71 g of 3-methyl-2-(methylthio)-6-phenyl-4-pyrimidinone. Recrystallization gave 58.5 g of pure compound: ^{1}H NMR ($CDCl_3$) 8.05–7.85 (m, 2 H, Ph), 7.50–7.35 (m, 3 H, Ph), 6.65 (s, 1 H, vinyl), 3.55 (s, 3 H, NCH_3), 2.70 (s, 3 H, SCH_3). Anal. ($C_{12}H_{12}N_2OS$) C, H, N, S.

The third spot (R_f 0.3 in 1:1 EtOAc/hexane), which was found to be starting material, was discarded and the eluent switched to 2.5% MeOH/$CHCl_3$ and the elution continued until spot 4 was 1.90 g of 1-methyl-2-(methylthio)-6-phenyl-4-pyrimidinone, which was recrystallized from water to yield 750 mg.

(3,4-Dihydro-4-oxo-6-phenyl-2-pyrimidinyl)guanidine (5). To 5.0 g (50 mmol) of biguanide in 300 mL of absolute ethanol was added 30 mL of toluene and heated to reflux to azeotrope off 100 mL of solvent. The suspension was cooled, and 9.6 g (50 mmol) of ethyl benzoylacetate was added. This was heated to reflux for 6 h. Then 20 mL of water was added and continued to heat at reflux for an additional 30 min. Upon cooling to room temperature CO_2 chips were added to neutralization. The precipitate was filtered and washed with water, followed by acetone and ether, and dried at 60 °C in vacuo to yield 3.6 g (31.5%) of product: ^{1}H NMR (Me_2SO-d_6/Me_4Si) 11.9–11.6 (b, 1 H, NH), 8.58–8.08 (b, 4 H, NH), 8.0–7.25 (m, 2 H, Ph), 7.63–7.38 (m, 3 H, Ph), 6.28 (s, 1 H, vinyl). Anal. ($C_{11}H_{11}N_5O$) C, H, N.

2-(Acetylamino)-5-iodo-6-phenyl-4(3*H*)-pyrimidinone (20). To 2.0 g (6.38 mmol) of 2-amino-5-iodo-6-phenyl-4(3*H*)-pyrimidinone was added 70.0 mL of acetic anhydride and heated at reflux for 2 h. The reaction mixture was cooled and evaporated to a volume of 2 mL under vacuum. Addition of 20 mL of ethanol induced a precipitate, which was filtered, washed with ethanol, acetone, and ether, and dried at 60 °C to yield 1.60 g (71%) of product: ^{1}H NMR (Me_2SO-d_6/TMS) 12.16–11.66 (b, 1 H, NH), 7.75–7.33 (m, 5 H, Ph), 3.58–3.16 (b, 1 H, NH), 2.13 (s, 3 H, CH_3C). Anal. ($C_{12}H_{10}IN_3O$) C, H, N, I.

2-(Acetylamino)-5-bromo-6-phenyl-4(3*H*)-pyrimidinone (7). By employing the same procedure as for compound **20**, 1.90 g (83%) of product was isolated: ^{1}H NMR (Me_2SO-d_6/Me_4Si) 12.1–11.8 (b, 1 H, NH), 7.8–7.36 (m, 5 H, Ph), 3.5–3.08 (b, 1 H, NH), 2.16 (s, 3 H, CH_3C). Anal. ($C_{12}H_{10}BrN_3O_2$) C, H, N, Br.

2-Succinimido-5-bromo-6-phenyl-4(3*H*)-pyrimidinone (8). To 2.8 g (10.5 mmol) of 2-amino-5-bromo-6-phenyl-4(3*H*)-pyrimidinone was added 70 mL of dry pyridine and 4.5 g (45 mmol) of succinic anhydride. After heating at reflux for 1 h, the solution was cooled and evaporated to dryness under vacuum. The residue was azeotroped twice with water and once with ethanol, then triturated twice with boiling water and filtered, while hot. Repetition of this procedure gave a uniform solid, which was recrystallized from 100 mL of hot ethanol to yield 400 mg (9.6%) of product. Anal. ($C_{14}H_{12}BrN_3O_4$) C, H, N, Br.

Preparation of 2-Amino-3-methyl-6-phenyl-4(3*H*)-pyrimidinone and 2-(Methylamino)-6-phenyl-4(3*H*)-pyrimidinone (2, 3). Following the procedure employed for compound **5**, 75 mmol of methylguanidine sulfate was heated with 18.8 g of ethyl benzoylacetate and 36 mL of 25% sodium methoxide/methanol in 1.1 L of ethanol for 28 h to afford 21.7 g of solids. Recrystallization twice from 100 mL of hot 95% ethanol yieled 4.2 g of a solid, mp 238–239 °C, identical with that obtained from the methylation of 2-amino-6-phenyl-4(3*H*)-pyrimidinone and assigned N_3-methyl isomer (**2**, vide infra).

To the solution from the crystallization was added 50 g of silica gel, and the mixture was evaoporated to a powder under vacuum. The powder was placed on 800 g of silica gel (dry), and the column was eluted with 3% methanol/ethyl acetate. The crude solid had two spots on TLC (R_f 0.7 and 0.65, 5% MeOH/EtOAc) with the N_3-methyl isomer having a R_f 0.7. The fractions containing pure R_f 0.65 were combined and evaporated to dryness to yield 40 mg of the N_2-methyl isomer (**3**). ^{1}H NMR (Me_2SO-d_6/Me_4Si) 8.16–7.91 (m, 2 H, Ph), 7.58–7.36 (m, 3 H, Ph), 2.93–2.86 (d, J = 4.5 Hz, 3 H, NCH_3). Anal. ($C_{11}H_{11}N_3O$) C, H, N.

Methylation of 2-Amino-6-phenyl-4(3*H*)-pyrimidinone (2). To 7.0 g (36.4 mmol) of **1** was added 350 mL of absolute ethanol and 3.3 g (57 mmol) of KOH. When all the solid had dissolved, 18.2 g of potassium hydroxide and 7.98 mL (≈0.129 mol) of methyl iodide were added and the reaction mixture was heated at reflux for 5 h. At this point an additional 0.5 g of potassium hydroxide was added and heating was continued for an additional 3 h. The mixture was cooled and evaporated to dryness under vacuum. To the residue was added 150 mL of H_2O, and, at 50 °C, 1 N HCl(aq) was added to dissolve the solid. The solution was cooled to 20 °C and aqueous $NaHCO_3$ solution added until no further precipitation occurred. The solids were filtered, washed well with water, and dried at 50 °C in vacuo to yield 5.4 g (74%) of product. Recrystallization of an analytical sample from methanol/ether gave crystals: mp 240–240.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.88–8.05 (m, 2 H, Ph), 7.38–7.58 (m, 3 H, Ph), 7.05–7.31 (b, 2 H, NH_2), 6.23 (s, 1 H, vinyl), 3.33 (s, 3 H, NCH_3). Anal. ($C_{11}H_{11}N_3O$) C, H, N.

2-(Dimethylamino)-6-phenyl-4(3*H*)-pyrimidinone (4). Following the procedure employed for compound **5**, 0.1 mol of 1-(dimethylamino)guanidine hydrochloride was heated with 14.8 g of lithium carbonate, 6.8 g of sodium ethoxide, and 19.2 g of ethyl benzoylacetate to afford 16.5 g of product (77% yield): ^{1}H NMR (Me_2SO-d_6/Me_4Si) 8.13–7.91 (m, 2 H, Ph), 7.58–7.33 (m, 3 H, Ph), 6.11 (s, 1 H, vinyl), 3.10 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{12}H_{13}N_3O$) C, H, N.

Reversed Passive Arthus Reaction. Male Wistar rats (180–200 g, Charles River, Wilmington, MA) were sensitized with an intravenous injection of 1 mg of bovine serum albumin in 0.2 mL of saline (n = 5–10 rats/group). Sixty minutes following antigen administration rats were challenged with antibody via a subplantar injection of 200 μg of anti-bovine serum albumin in 0.2 mL of saline in the left hindpaw. The degree of hindpaw swelling (inflammation) was determined by mercury displacement plethysmography. Changes in paw volume over time were determined at 2 h postantibody challenge.

Developing Arthritis Assay. Adjuvant-Induced Polyarthritis. Male rats (200–225 g, Charles River, Wilmington, MA) received a subcutaneous injection of 0.1 mL of complete Freund's adjuvant (4 mg/mL *Mycobacterium butyricum* in mineral oil) in the tail (n = 10 rats/group) on day 0. An arthritic score, used to assess drug effects on the arthritic process, was determined by assigning a rating of 0–4 to each paw (the maximum severity of arthritis would be 16 if each paw received a rating of 4) on the last day of the assay. The incidence of arthritis was also determined at this time.

Plasma Inflammation Units (PIU). PIU values were determined as described previously.[14]

Preparation and Administration of Compounds. Test compounds and reference standards were suspended in water containing 1–2 drops of Tween-80 (pyrogen-free, Accurate

Chemicals, Hicksville, NY) and shaken overnight with large glass beads. All compounds were administered orally, in milligrams per kilogram of body weight, according to the respective dosing regimens described in the table legends. Normal rats and the respective control groups received water plus Tween-80.

Diuretic Assays. A primary assay for diuretic activity was conducted in male rats weighing approximately 160 g. The rats were deprived of food 24 h and water $1^1/_2$ h before test time. During testing both food and water were withheld. Testing was initiated by simultaneous hydration and oral administration of test agent. This was accomplished by gavage with 25 mL/kg of normal saline (0.9%) containing (carboxymethyl)cellulose (0.5%) and test substance. Rats were placed in metabolism cages and urine collected over the ensuing 5 h. In all instances, the test dose was 40 mg/kg. Criteria for declaring test substances active or inactive were established from a two-stage test as described by Roseberry and Gehan.[15] For each stage, the ratio (denoted T/C) of urine volume in treated animals to urine volume in control rats was determined. When the product of T/C for stage 1 and stage 2 was 3.34 or greater, the test substance was declared active. Secondary testing was carried out on selected pyrimidinones. These agents were subjected to dose–response analyses using a wide range of doses (0.3–100 mg/kg) and tested for effects on urinary Na^+ and K^+ excretion. Urinary Na^+ and K^+ were measured by flame photometry. In all other respects, i.e., tests animals, route of administration, hydration, and collection periods, secondary testing was identical to primary testing.

Hypotensive Assay—Blood Pressure in Rats. The arterial blood pressures of restrained female Sprague–Dawley rats were measured directly from indwelling aortic cannulas exteriorized at the nape of the neck.[16] The rats were restrained in a towel when observations were made with a Statham transducer (P23G) and Grass polygraph. Mean arterial pressure was obtained by electrical integration of the phasic pressure. Observations were made before, as well as 4 and 24 h after, oral treatment. Compounds were suspended in a (carboxymethyl)cellulose vehicle to provide a dose of 50 mg/kg in a volume of 10 mL/kg. Mean arterial blood pressure values of two animals were averaged at each of the three observation periods. An average change of at least 5 mmHg was required posttreatment to attain statistical significance ($P < 0.05$).

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Registry No. 1, 56741-94-7; 2 (R^1 = H, R^2 = CH_3), 92519-10-3; 2 (R^1 = Br, R^2 = CH_3), 92519-08-9; 2 (R^1 = I, R^2 = H), 72943-43-2; 2 (R^1 = I, R^2 = CH_3), 92519-09-0; 2 (R^1 = I, R^2 = CH_2CHCH_2), 102649-71-8; 3 (R^1 = H, R^2 = CH_3), 100008-30-8; 3 (R^1 = Br, R^2 = CH_3), 102649-63-8; 3 (R^1 = I, R^2 = $COCH_3$), 102649-68-3; 3 (R^1 = I, R^2 = CH_3), 102649-70-7; 4 (R^1 = I), 102649-67-2; 4 (R^1 = H), 31937-04-9; 4 (R^1 = Br), 102649-64-9; 5 (R^1 = H), 102649-58-1; 5 (R^1 = Br), 102649-65-0; 5 (R^1 = I), 102649-69-4; 6, 56741-95-8; 7, 74856-68-1; 8, 102649-59-2; 9, 36822-11-4; 9 (dione), 102649-77-4; 10, 102649-60-5; 11, 74303-68-7; 12, 102649-61-6; 14, 102649-66-1; 15, 61736-36-5; 15 (5-bromo), 102649-72-9; 15 (5-iodo), 102649-73-0; 16, 42542-57-4; 16 (5-bromo), 102649-74-1; 16 (5-iodo), 102649-75-2; 16 (5-chloro), 102649-76-3; H_2NCONH_2, 57-13-6; $C_6H_5COCH_2CO_2CH_2CH_3$, 94-02-0; $H_3CNHCONH_2$, 598-50-5; H_2NCSNH_2, 62-56-6; $H_3CNHC(NH)NH_2 \cdot {}^1/_2H_2SO_4$, 598-12-9; $(CH_3)_2NC(NH)NH_2 \cdot HCl$, 1186-46-5; 6-phenyl-2,4-pyrimidinedione, 13345-09-0; 1,3-dimethyl-5-bromo-6-phenyl-2,4-pyrimidinedione, 98854-09-2; 5-bromo-6-phenyl-2,4-pyrimidinedione, 16290-56-5; 2-amino-1-methyl-6-phenyl-4-pyrimidinone, 102649-62-7; biguanide, 56-03-1; 1-hydroxy-2-amino-5-bromo-6-phenyl-4-pyrimidinone, 78222-48-7.

Topical Nonsteroidal Antipsoriatic Agents. 1. 1,2,3,4-Tetraoxygenated Naphthalene Derivatives[1]

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On the basis of previous observations that both 2,3-dihydro-2,2,3,3-tetrahydroxy-1,4-naphthoquinone (oxoline, **1**) and 6-chloroisonaphthazarin (**2**) had demonstrated antipsoriatic activity in vivo, a series of structural derivatives of **2** were prepared and examined in the Scholtz–Dumas topical psoriasis bioassay. Of these six (**5**, **6**, **9a**, **10**, **11a**, **11b**), the most effective compound was found to be 6-chloro-1,4-diacetoxy-2,3-dimethoxynaphthalene (RS-43179, lonapalene, **11a**). An extensive series of 1,2,3,4-tetraoxygenated naphthalenes (**16–74**) incorporating variations of the ester, ether, and aryl substituents were prepared as analogues of **11a** to examine the structural requirements for activity and were screened in vivo as inhibitors of arachidonic acid induced mouse ear edema, a topical bioassay capable of detecting 5-lipoxygenase inhibitors. Net lipophilicity, hydrolytic stability, and ring substitution play significant roles in determining the observed in vivo activity. Lonapalene (**11a**) is currently in clinical development as a topically applied nonsteroidal antipsoriatic agent.

Psoriasis is a chronic, relapsing hyperproliferative and inflammatory disease of the skin characterized by symmetrically distributed silvery red, scaling plaques found on the scalp, the extremities, back, buttocks, and especially the knees and elbows. In severe cases, most of the body surface may be involved. An estimated 6–8 million persons in the United States have psoriasis, contributing to a 2–6% incidence worldwide. While the exact etiology of the disease remains elusive, there now exist a number of treatments which provide temporary relief,[2] including coal tar and UV irradiation (the Goeckermann regimen), psoralen and UV-A irradiation (PUVA), oral retinoids, corticosteroids, and antimetabolites. Probably the most widespread treatment used, however, is topical anthralin, an irritating and mutagenic compound that leaves a brown

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