Articles

Substituted Xanthines, Pteridinediones, and Related Compounds as Potential Antiinflammatory Agents. Synthesis and Biological Evaluation of Inhibitors of Tumor Necrosis Factor α

Howard B. Cottam,* Hsiencheng Shih, Lida R. Tehrani, D. Bruce Wasson, and Dennis A. Carson

Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, University of California, San Diego, La Jolla, California 92093-0663

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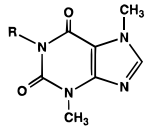
A series of analogues of pentoxifylline metabolites were prepared in the purine, pteridine, [1,2,5]-thiadiazolo[3,4-*d*]pyrimidine, and quinazoline ring systems and evaluated for their ability to inhibit the production of tumor necrosis factor-α (TNFα) in human peripheral blood monocytes stimulated with bacterial lipopolysaccharide (LPS). The more active compounds were also tested for inhibition of cyclic AMP phosphodiesterase type IV (PDE IV) from human neutrophils in order to help determine their mechanism of action. Selected compounds which showed good activity in the in vitro TNFα assay were evaluated in an in vivo LPS-induced leukopenia model in mice. The most potent compounds in the TNFα assay, **6**, **31**, and **58**, inhibited TNFα production at an IC_{50} of approximately 5 μM for each. Compound **58** was a very poor inhibitor of PDE IV but was the most active at preventing the leukopenia induced by TNFα in mice, providing more than 60% protection at 50 mg/kg. Thus, compounds such as **58**, which are good inhibitors of TNFα production but are devoid of PDE IV inhibitory properties, may have potential as new antiinflammatory agents.

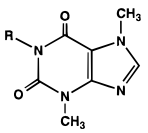
Introduction

Cytokines have been recognized as essential components in acute and chronic inflammatory processes. Tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1) are polypeptides which promote inflammation and are mainly produced by cells of the monocyte/macrophage lineage. Moreover, TNFα is a principal mediator of chronic inflammation. While the cytokine response to cell injury is a regulated process and is usually beneficial to the host, there are a number of important human diseases which are characterized by cytokine overproduction with resultant inflammation and fibrosis. These may include immunological diseases such as rheumatoid arthritis, multiple sclerosis, asthma, psoriasis, and inflammatory bowel disease.

The hemorheological agent pentoxifylline (**1**) is an alkylated xanthine which is clinically useful for the treatment of peripheral vascular and cerebrovascular disease and a number of other conditions involving defective regional microcirculation.[1] It has been shown to inhibit the synthesis of TNFα,[2] and this effect has been attributed to inhibition of cyclic AMP phosphodiesterase (PDE).[3] However, some experimental evidence does not support this explanation. For example, an extensive study of the inhibitory profile of pentoxifylline and related compounds toward several isoforms of PDE[4] showed that this agent only weakly inhibited PDE. Moreover, agents which elevate intracellular cyclic AMP levels inhibit epidermal growth factor mitogenesis, whereas pentoxifylline does not.[5,6] In addition, it is rapidly metabolized,[1] and therefore one or more metabolites of pentoxifylline may well be the active agent(s), albeit weak, of TNFα inhibition. As a starting point, the metabolite of most interest to us was the N-1 carboxypropyl derivative known as metabolite 5[1] (**2**) because of a report indicating that this metabolite was the most effective at reducing oxygen radical production by polymorphonuclear leukocytes (PMN).[7] These reactive oxygen species have been reported to facilitate the in vitro and in vivo release of TNFα from mouse peritoneal macrophages exposed to lipopolysaccharide (LPS).[8]



1
Pentoxifylline
R = (5-oxohexyl)



2
R = (3-carboxypropyl)

Phosphodiesterase type IV (PDE IV) is the predominant PDE isoenzyme in monocytes[9] and in neutrophils.[10] Two potent inhibitors of this isoform are the catechol ethers rolipram and Ro 20-1724[9] [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone] which inhibit the production of TNFα in LPS stimulated monocytes[11] and the respiratory burst in neutrophils.[12] While the inhibition of PDE IV may well be a major mechanism by which these drugs inhibit TNF production, their use as antiinflammatory agents has been limited by undesirable side effects, possibly the result of PDE inhibition at the higher doses.

With this in mind, we desired to design a series of analogues of pentoxifylline metabolites which might

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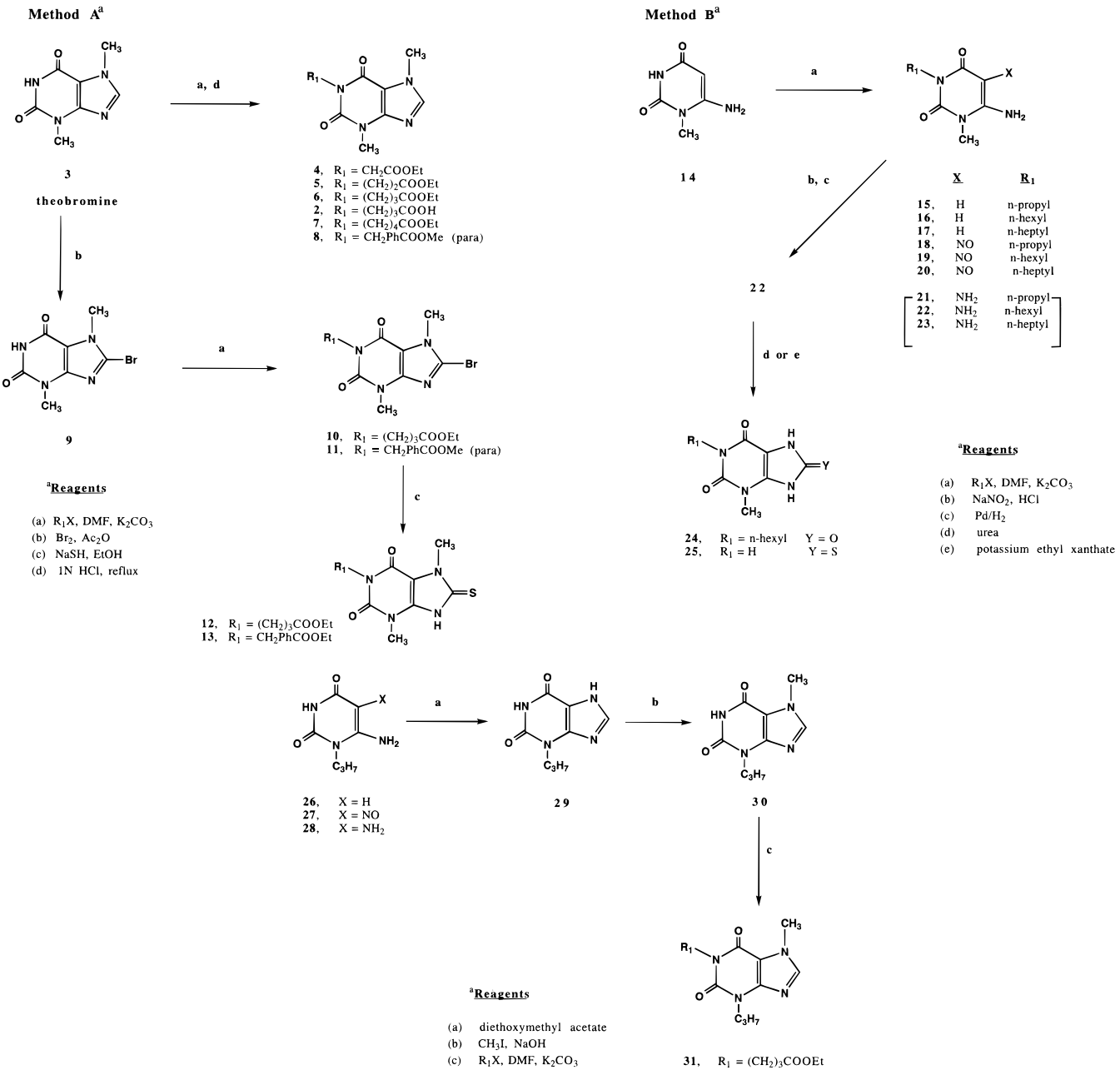
Scheme 1



possess enhanced ability to inhibit the production of TNFα but which would be devoid of significant PDE IV inhibitory activity, thereby avoiding possible side effects attributable to PDE inhibition. These analogues might thus have potential as antiinflammatory agents in conditions where TNFα is known to be an important contributor. Accordingly, the candidate compounds were prepared and tested for their ability to inhibit the production of TNFα in human peripheral blood monocytes following exposure to LPS. In addition, the inhibitory activity of the more active compounds toward PDE IV from human neutrophils was determined relative to Ro 20-1724, which was commercially available and served as a positive control. Finally, a few selected compounds were tested in the LPS-induced leukopenia model in mice to assess whether results in this TNF-dependent system would correlate with in vitro observations.

Chemistry

The first series of analogues were purine derivatives of metabolite 5 (**2**). These were prepared by one of three general methods depicted in Scheme 1. In method A, theobromine (**3**) served as a convenient starting material and only required the appropriate alkylating conditions to assure N-1 versus O-6 alkylation. Alkyl halides were used in our studies since alkylation of oxopurines with these agents gives exclusively N-alkylation.[13] Thus, treatment of theobromine with the appropriate alkyl halide in DMF at 75 °C in the presence of potassium carbonate provided the corresponding N-1 alkylated products in good yield. Several 8-substituted derivatives were prepared by a variation of this method in which theobromine was first brominated to give 8-bromotheobromine (**9**)[10] and then alkylated as described above to yield **10** and **11**. The 8-bromo sub-

stituent was also displaced by NaSH to yield the corresponding 8-thioxo derivatives, **12** and **13**.

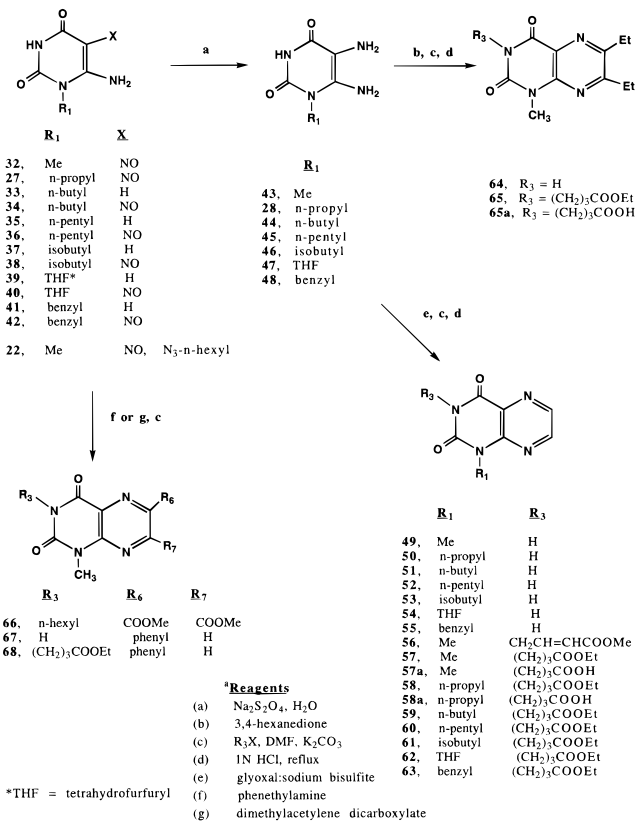
Method B (Scheme 1), an example of the Traube purine synthesis,[13] was used to prepare a few 1,3,8-trisubstituted xanthines bearing no alkyl group at the N-7. In this procedure, the N-1 substituted pyrimidine was alkylated at N-3. Formation of the purine ring was then completed in three steps: nitrosation, reduction of the nitroso to the amine by catalytic hydrogenation, and then ring closure using urea or potassium ethyl xanthate to provide the 8-oxo and 8-thioxo dervatives (**24** and **25**), respectively. Method C, a variation of method A, was used for the preparation of the N-3 propylpurines. Although 3-*n*-propylxanthine (**29**)[15] is commercially available, we chose to synthesize this intermediate since the precursors were not available and were required for the preparation of the other ring systems. Here, the requisite starting material was the commercially available *n*-propylurea which was condensed with ethyl cyanoacetate in the presence of sodium ethoxide to yield the 6-amino-1-propylpyrimidinedione[16] (**26**) in moderate yield. Ring closure to the purine **29** was accomplished in three steps as in method A, except that diethoxymethyl acetate was used as the one-carbon source in the ring closure step. Sequential alkylations were then performed using alkyl halides at N-7 followed by N-1 to provide the desired final compound ethyl 4-(2,3,6,7-tetrahydro-2,6-dioxo-7-methyl-3-*n*-propyl-1*H*-purin-1-yl)butanoate (**31**).

In order to investigate the effects of purine ring system variations on TNFα inhibition, a series of compounds was prepared in two systems related to purines, namely, the pteridine and the 8-thiapurine or thiadiazolopyrimidine. A few compounds in both systems were initially synthesized by a variation of method B described above. This method, however, was later found not to be as generally convenient as method C since alkylations of the pyrimidine intermediates gave a mixture of two major products, the *O*- and *N*-alkyls, while alkylation carried out after ring closure to the bicyclic systems (including purines) resulted in the desired *N*-alkyls as the exclusive products.

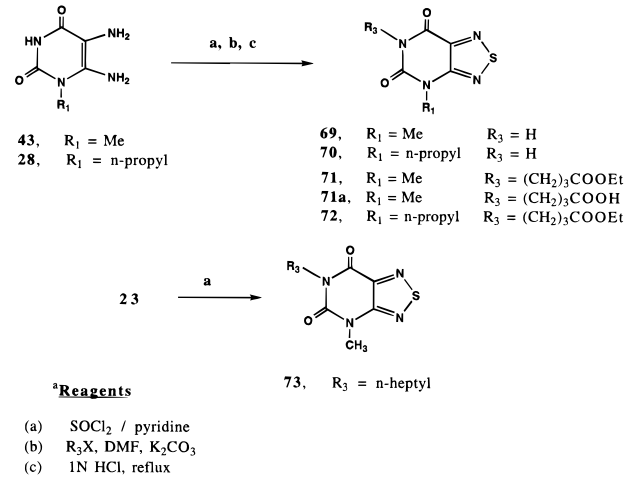
The synthesis of compounds in both ring systems was facilitated by the ability to use common intermediates, the *o*-diaminopyrimidines, as versatile precursors, one of which, **28,** was used in method C for the purines. Thus, several diaminopyrimidine precursors were prepared beginning with the corresponding monosubstituted ureas by the method described in method C, including the methyl, *n*-butyl, *n*-pentyl, isobutyl, tetrahydrofurfuryl, and the benzyl (**43**−**48**, respectively, Scheme 2). Interestingly, attempts to condense alicyclic ureas, such as cyclopentyl- and cyclohexylureas with ethyl cyanoacetate, under these same conditions failed to yield the corresponding pyrimidines. Steric hindrance is likely the reason for such failure. For the pteridine system, ring closure of the *o*-diamines with a two-carbon source such as glyoxal provided good yields of the N-1 substituted pteridines, **49**−**55**. Finally, alkylation at N-3 under the same conditions as described in method A for theobromine using ethyl 4-bromobutyrate resulted in the desired disubstituted pteridinediones **56**−**63**. In one case, the ester moiety of the *n*-propyl-substituted derivative **58** was hydrolyzed by heating in dilute hydrochloric acid to obtain the butanoic acid **58a**. In addition to these pteridines, use of the diketone, 3,4-hexanedione, in the ring closure provided the 6,7-diethylpteridine **65**, a more lipophilic derivative. Another 6,7-disubstituted pteridine was prepared by a type of Timmis reaction[17] wherein the *C*-nitrosopyrimidine intermediate **22** was condensed with dimethylacetylene dicarboxylate to form the dimethyl 1,3-dialkylpteridine-6,7-dicarboxylate **66**. In addition, treatment of **27** with phenethyl amine in a Timmis reaction followed by alkylation provided the 6-phenyl dialkylpteridine **68**.

Compounds in the other related ring system (Scheme 3), [1,2,5]thiadiazolo[3,4-*d*]pyrimidine, were prepared by brief treatment of the *o*-diamine intermediates with thionyl chloride in the presence of pyridine. Alkylation of these intermediates then resulted in the methyl- and *n*-propyl-substituted products **71** and **72,** respectively.

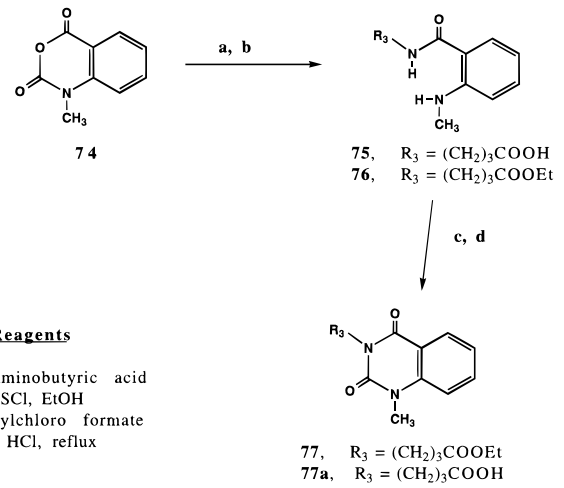
Scheme 2[a]



Scheme 3[a]



Scheme 4[a]



Acid hydrolysis of **71** gave the free acid **71a**. One derivative in this ring system having a straight chain (*n*-heptyl) at N-3 was synthesized from the preformed alkylated pyrimidine **23** by ring closure under the same conditions to provide the dialkylated compound **73**.

Finally, quinazoline derivatives were prepared bearing N-1 and N-3 substituents as "dideazapteridine" analogues (Scheme 4). The starting material for this ring system was *N*-methylisatoic anhydride (**74**), which on treatment with 4-aminobutyric acid yielded the substituted benzamide **75**. Esterification of the carboxylic acid function of **75** with dry ethanol in the presence of chlorotrimethylsilane provided the ethyl ester **76**. Reaction of **76** with ethyl chloroformate resulted in ring closure to ethyl 4-(1-methyl-2,4-dioxoquinazol-3-yl)butanoate (**77**). The corresponding free acid **77a** was prepared by treatment with 1 N HCl as described for the other ester/acid combinations.

Results and Discussion

Table 1 presents a compilation of the new and known compounds which were prepared in each ring system and shows the results of the TNFα inhibition assay performed by an ELISA method. It is evident from the IC_{50} values for TNFα production that a simple pattern of structure−activity has emerged.

N-1 Substituent (Purine Numbering). First, in the purine series, compounds having N-1 chain lengths of from two to seven carbons were prepared, and four carbons was found to be optimum. Thus, the optimum side chain at N-1 is a butanoic ester. The free carboxylic acids were not as effective as their corresponding esters at inhibiting TNFα production in these in vitro assays. This pattern was observed not only for the purines but also for the other ring systems as well. It is conceivable that the esters provide a more cell-permeable form of the active compound whereas the free acids, being ionized at physiological pH, may cross cell membranes with greater difficulty. Compounds with variable chain lengths at N-1 were not prepared in the other ring systems on the assumption that subtle changes in the ring atoms would not alter the optimum chain length determined in the purine system.

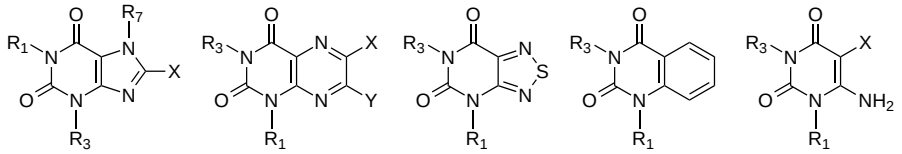
N-3 Substituent. The variations at N-3 included a few examples of aliphatic, alicyclic, and aralkyl groups. Following the observation that the purine compounds had, in general, much greater PDE IV inhibitory activity than the corresponding compounds in the other series (see discussion below), most of the work with variation at this position was performed in the pteridine series (Scheme 2). In this series, there appears to be slightly greater activity where N-3 is *n*-propyl compared to methyl or *n*-butyl. Thus, **58** was reproducibly about twice as potent as **57** and 2−3 times more potent than **59** in the TNFα assay. This activity difference between the methyl and *n*-propyl groups at N-3, however, was not so evident in the purine series, as compounds **6** and **31** had essentially the same activity. Interestingly, the tetrahydrofurfuryl derivative retained good activity while the aralkyl (benzyl) derivative was relatively inactive.

Ring System Variations. The most active compounds in the purine series were identified as **6** and **31** with a reproducible IC_{50} of less than 10 μM for TNFα inhibition. Substitution at the 8-position with bromo, oxo, or thioxo groups generally resulted in decreased activity, although the 7-methylated 8-oxo derivative was not prepared. A change from a purine to a pteridine ring, keeping all other groups constant, did not cause a dramatic loss of inhibitory activity. However, substitution of groups on the pteridine ring such as 6,7-diethyl (**65**) or 6-phenyl (**68**) caused a substantial decrease in activity. A change from purine to the thiadiazolopyrimidine system resulted in a significant decrease in activity. Thus, **6** was about 3−5 times more potent than **71**. It was anticipated that the pteridines and the thiadiazolopyrimidines would be quite similar in activity since the sulfur atom in a ring occupies about the same space as two ring carbons, and these two rings would therefore be nearly spatially equivalent. Finally, a change from the purine or pteridine to the quinazoline ring surprisingly resulted in a dramatic decrease in inhibitory activity (compare **6** and **77**). This suggests that the ring nitrogens, other than the pyrimidine nitrogens, may play a role in the binding to the target receptor responsible for inhibition of TNFα. Evaluation of additional "deaza" analogues of the purines or pteridines would be required to confirm this observation.

PDE IV Inhibition. PDE IV was prepared from human neutrophils and a comparison was made of the PDE IV inhibitory activity of several of the more active compounds identified in the TNF assay. The results, shown in Figure 1, indicate that there is little correlation between PDE inhibitory activity and that of TNFα in this assay. Moreover, there appears to be little or no PDE IV inhibitory activity among pteridines active in the TNFα assay. For example, **58**, the pteridine most active in the TNFα assay, was a very poor inhibitor of PDE IV even at millimolar concentrations. Interestingly, Ro 20-1724, a potent PDE IV inhibitor, was about 10-fold less active than **58** at inhibiting the production of TNFα while rolipram was found to be about 2-fold less potent than **58** in this assay. In general, the pteridines were studied more extensively than the purines as a group because of the general lack of PDE IV inhibitory activity associated with the pteridines, and thus lower probability of PDE side effects within this class.

In Vivo Leukopenia Model. The administration of TNFα or of stimuli which produce TNFα in mice results in a transient leukopenia (primarily a neutropenia) in the peripheral circulation. In this model,[21] mice were

Table 1. Physicochemical and TNFα Inhibition Data



I II III IV V

compd	class	R_1	R_3	R_7	X	Y	mp (°C)	formula	analyses[a]	TNFα IC_{50}[b]
rolipram										12
Ro 20-1724										50
1	I	5-oxohexyl	Me	Me	H		102−103	ref 18		85
2	I	$(CH_2)_3COOH$	Me	Me	H		208−210	ref 19		>200
4	I	CH_2COOEt	Me	Me	H		162−165	$C_{11}H_{14}N_4O_4$	C,H,N	200
5	I	$(CH_2)_2COOEt$	Me	Me	H		76−78	$C_{12}H_{16}N_4O_4$	C,H,N	10
6	I	$(CH_2)_3COOEt$	Me	Me	H		73−75	$C_{13}H_{18}N_4O_4$	C,H,N	6
7	I	$(CH_2)_4COOEt$	Me	Me	H		84−85	$C_{14}H_{20}N_4O_4$	C,H,N	11
8	I	$CH_2PhCOOMe$		Me	H		158−160	$C_{16}H_{16}N_4O_4$	C,H,N	>200
10	I	$(CH_2)_3COOEt$	Me	Me	Br		101−102	$C_{13}H_{17}BrN_4O_4$	C,H,N	60
11	I	$CH_2PhCOOMe$	Me	Me	Br		218−219	$C_{16}H_{15}BrN_4O_4$	C,H,N	>200
12	I	$(CH_2)_3COOEt$	Me	Me	SH		202−206	$C_{13}H_{18}N_4O_4S$	C,H,N	100
13	I	$CH_2PhCOOEt$	Me	Me	SH		>293 dec	$C_{17}H_{18}N_4O_4S$	C,H,N	>200
20	V	Me	*n*-heptyl		NO		>218 dec	$C_{12}H_{20}N_4O_3$	C,H,N	ND[c]
24	I	*n*-hexyl	Me	H	OH		>295 dec	$C_{12}H_{18}N_4O_3$	C,H,N	50
25	I	H	Me	H	SH		>320	ref 20		>200
31	I	$(CH_2)_3COOEt$	*n*-propyl	Me	H		oil	$C_{15}H_{22}N_4O_4$	C,H,N	5
43	V	Me	H		NH_2		>270 dec	$C_5H_8N_4O_2$	C,H,N	ND
49	II	Me	H		H	H	287−289	$C_7H_6N_4O_2$	C,H,N	ND
50	II	*n*-propyl	H		H	H	187−189	$C_9H_{10}N_4O_2$	C,H,N	ND
51	II	*n*-butyl	H		H	H	177−179	$C_{10}H_{12}N_4O_2$	C,H,N	ND
52	II	*n*-pentyl	H		H	H	>140 dec	$C_{11}H_{14}N_4O_2$	C,H,N	ND
53	II	isobutyl	H		H	H	195−197	$C_{10}H_{12}N_4O_2$	C,H,N	ND
54	II	THF	H		H	H	184−187	$C_{10}H_{10}N_4O_3$	C,H,N	ND
55	II	benzyl	H		H	H	230−232	$C_{13}H_{10}N_4O_2$	C,H,N	ND
56	II	Me	$CH_2CH{=}CHCOOMe$		H	H	118−119	$C_{12}H_{12}N_4O_4$	C,H,N	25
57	II	Me	$(CH_2)_3COOEt$		H	H	90−92	$C_{13}H_{16}N_4O_4$	C,H,N	12
57a	II	Me	$(CH_2)_3COOH$		H	H	199−202	$C_{11}H_{12}N_4O_4$	C,H,N	>200
58	II	*n*-propyl	$(CH_2)_3COOEt$		H	H	53−55	$C_{15}H_{20}N_4O_4$	C,H,N	5
58a	II	*n*-propyl	$(CH_2)_3COOH$		H	H	97−99	$C_{13}H_{16}N_4O_4$	C,H,N	>200
59	II	*n*-butyl	$(CH_2)_3COOEt$		H	H	47−48	$C_{16}H_{22}N_4O_4$	C,H,N	15
60	II	*n*-pentyl	$(CH_2)_3COOEt$		H	H	58−60	$C_{17}H_{24}N_4O_4$	C,H,N	16
61	II	isobutyl	$(CH_2)_3COOEt$		H	H	75−77	$C_{16}H_{22}N_4O_4$	C,H,N	20
62	II	THF	$(CH_2)_3COOEt$		H	H	56−58	$C_{16}H_{20}N_4O_5$	C,H,N	19
63	II	benzyl	$(CH_2)_3COOEt$		H	H	109−110	$C_{19}H_{20}N_4O_4$	C,H,N	175
64	II	Me	H		Et	Et	218−222	$C_{11}H_{14}N_4O_2$	C,H,N	ND
65	II	Me	$(CH_2)_3COOEt$		Et	Et	66−68	$C_{17}H_{24}N_4O_4$	C,H,N[d]	130
65a	II	Me	$(CH_2)_3COOH$		Et	Et	161−162	$C_{15}H_{20}N_4O_4$	C,H,N	ND
66	II	Me	*n*-hexyl		COOMe	COOMe	92−95	$C_{17}H_{22}N_4O_6$	C,H,N	ND
67	II	Me	H		Phenyl	H	>307 dec	$C_{13}H_{10}N_4O_2$	C,H,N[e]	ND
68	II	Me	$(CH_2)_3COOEt$		Phenyl	H	99−100	$C_{19}H_{20}N_4O_4$	C,H,N	>200
69	III	Me	H				210−213	$C_5H_4N_4O_2S$	C,H,N	ND
70	III	*n*-propyl	H				142−144	$C_7H_8N_4O_2S$	C,H,N	ND
71	III	Me	$(CH_2)_3COOEt$				45−47	$C_{11}H_{14}N_4O_4S$	C,H,N	25
71a	III	Me	$(CH_2)_3COOH$				121−122	$C_9H_{10}N_4O_4S$	C,H,N	>200
72	III	*n*-propyl	$(CH_2)_3COOEt$				oil	$C_{13}H_{18}N_4O_4S$	C,H,N	18
73	III	Me	*n*-heptyl				73−75	$C_{12}H_{18}N_4O_2S$	C,H,N	75
76	IV	Me	$(CH_2)_3COOEt$				oil	$C_{14}H_{20}N_2O_3$	C,H,N[f]	ND
77	IV	Me	$(CH_2)_3COOEt$				oil	$C_{15}H_{18}N_2O_4$	C,H,N[g]	55
77a	IV	Me	$(CH_2)_3COOH$				163−165	$C_{13}H_{14}N_2O_4$	C,H,N	>200

[a] All compounds analyzed for C, H, N; results were within ±0.4% of theoretical values. [b] Concentration of compound in μM which inhibited the production of TNFα by 50% of control. [c] ND = not determined. [d] C: calcd, 58.61; found, 59.22. [e] H: calcd, 4.13; found, 3.70. [f] N: calcd, 10.25; found 9.77. [g] C: calcd, 59.62; found, 60.61.

given LPS by intraperitoneal injection to induce the production of TNFα. The LPS was given in the presence or absence of preadministered drug and then blood samples from the animals were collected 2 h later and analyzed for neutrophil content. Figure 2 compares the effects of pentoxifylline (**1**), **6**, and **58** in this model. The pteridine **58** displayed excellent ability to prevent TNFα-induced leukopenia in a dose-dependent manner. At the highest dose, 50 mg/kg, this compound inhibited leukopenia by over 60%. The mechanism of action of this compound is probably independent of PDE IV inhibition since **58** shows very weak inhibition of this enzyme even at higher doses (1 mmol). Thus, compound **58** and derivatives of it may avoid the side effects associated with PDE inhibition and therefore may be promising candidates for further study as potential antiinflammatory agents.

Experimental Section

Chemistry. Melting points were obtained on a Mel-Temp II capillary melting point apparatus and are uncorrected.

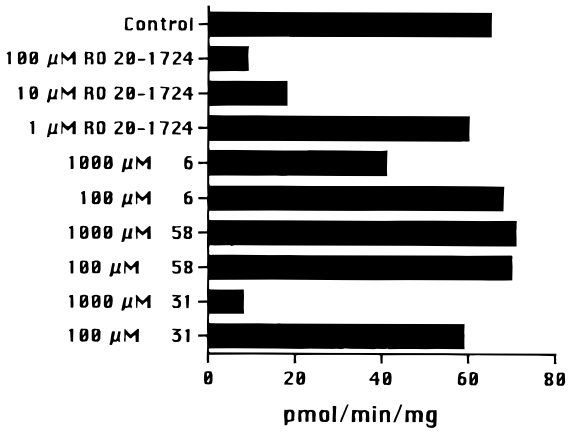


Figure 1. Inhibition of type IV phosphodiesterase from human neutrophils.

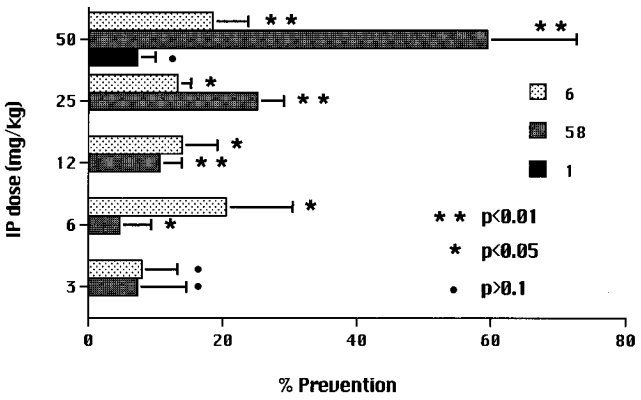


Figure 2. Prevention of LPS induced leukopenia in mice. Data shown is the average of three experiments.

Nuclear magnetic resonance spectra were obtained on a Varian Unity 500 at 499.8 MHz. The chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane as internal standard. Ultraviolet spectra were obtained using a Uvikon 930 (Kontron Instruments) spectrophotometer. Infrared spectra were obtained on a Nicolet model 205 spectrophotometer. Mass spectra were obtained using a Finnigan MAT spectrometer in the FAB (positive) mode. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ. In addition to elemental analysis, the identity of a few of the compounds was verified by exact mass spectral determinations. Thin-layer chromatography was performed on silica gel 60 F-254 plates (EM Reagents). E. Merck silica gel (230−400 mesh) was used for flash column chromatography.

Biological Materials. Cells. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized normal human blood by ficoll-histopaque density centrifugation. PBMC were plated in 96-well plates in RPMI 1640 supplemented with 20% autologous plasma and glutamine at a density of 5×10^5 cells/mL. In some experiments, monocytes were purified from PBMC by adherence to gelatin-coated flasks and used at a density of 5×10^4 cells/mL. Polymorphonuclear leukocytes (PMN) were purified by ficoll−histopaque density centrifugation followed by hypotonic lysis to remove red blood cell contamination.

Mice. Female ICR mice (6 to 8 weeks old) were from Harlan Sprague−Dawley (Indianapolis, IN).

Reagents. Lipopolysaccharides from *Escherichia coli* serotype 026:B6 (LPS), cyclic 3′,5′-adenosine monophosphate (cAMP), adenosine monophosphate (AMP), human tumor necrosis factor α (TNF α), and biotinylated rabbit anti human tumor necrosis factor-α were purchased from Sigma Chemical (St. Louis, MO). [2,8-^{3}H]cAMP (1.5 TBq/mmol) was purchased from Moravek Biochemicals (Brea, CA). Mouse anti human tumor necrosis factor α was purchased from Upstate Biotechnology Incorporated (Lake Placid, NY). Streptavidin, horseradish peroxidase (poly-HRP), was purchased from Accurate Chemical (Westbury, NY). Tetramethylbenzidine came from Kirkegaard and Perry (Gaithersburg, MD). *n*-Propylurea, isobutylurea, and *n*-butylurea were purchased from Lancaster Synthesis (Windham, NH). Other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). Rolipram and Ro 20-1724 were purchased from Biomol Research Labs, Inc. (Plymouth Meeting, PA).

3′,5′-cAMP Phosphodiesterase (PDE). PMN pellets were sonicated in 10 volumes of 20 mM Bis-Tris, 5 mM 2-mercaptoethanol, 2 mM EDTA, 2 mM benzamidine, 0.05 mM phenylmethanesulfonyl fluoride (PMSF), and 50 mM sodium acetate pH 6.5 and then centrifuged for 10 min at 16000*g*. The supernatant was desalted on G-25 sephadex. Phosphodiesterase activity was assayed radiochemically for 10 min at 30 °C. The 100 μL assay volume contained 50 mM Tris-HCl pH 7.5, 6 mM $MgCl_2$, 2.5 mM 2-mercaptoethanol, 50 μg/mL nucleotidase, 200 μg/mL bovine serum albumin, 10 μM [^{3}H]cAMP, the indicated concentration of test compound (Figure 1), and 30 μL of PMN extract. The assay was stopped by addition of 1 mL of a 30% slurry of AG 1 × 8 anion exchange resin (Biorad). A 500 μL sample of the supernatant was assayed by liquid scintillation spectrophotometry.

In Vitro TNFα. Compounds in RPMI-1640 (2X concentrations) were added in equal volume to the PBMC in 96-well microtiter plates, followed by a 60 min incubation. LPS was then added to the cultures at a final concentration of 10 μg/mL. Then, 18 h later, 100 μL of each well was collected and assayed for TNFα content by ELISA. Compounds found to possess good activity were evaluated in the same assay using purified PBMC monocytes.

TNFα ELISA. ELISA plates were coated for 4 h with 2 μg/mL mouse anti TNFα and then blocked overnight with 3% BSA. Then 50 μL of culture supernatant was transferred to the plate, incubated for 2 h at 37 °C, and then washed. The plate was then incubated for 1 h with 50 μL of 1:2000 rabbit anti TNFα and washed. Poly-HRP at 1:5000 was then added for 1 h and the plate developed.

In Vivo Leukopenia. Mice were injected intraperitoneally with the indicated amount of compound in 250 μL of saline, followed 60 min later with 50 μg/kg of LPS in saline. Two hours later, blood was collected into heparinized tubes by retro-orbital puncture. Nucleated cells were enumerated.

General Alkylation Procedure for Compounds 4−8, 10, and 11 and for 15−17, 31, 56−68, 71, 72, and 77 (Method A). Theobromine or 8-bromotheobromine (2 mmol) was combined with anhydrous K_2CO_3 (2.5 mmol) and dry DMF (15 mL), and the mixture was brought to 75 °C. The appropriate alkyl halide (2.5 mmol) was added, and the mixture was stirred at 75 °C for 2−18 h. The reaction mixture was cooled, poured into water (125 mL), and extracted with ethyl acetate (2 × 75 mL). The organic layer was dried over magnesium sulfate and evaporated to yield a colorless oil or white solid which was triturated with ethyl ether. The resulting solid, often analytically pure, may be purified further if desired by crystallization from a small amount of ethanol (yields 58−89%). Compounds **15**−**17**, **31**, **56**−**68**, **71**, **72**, and **77** were prepared by this same procedure only using the appropriate precursors in place of theobromine. ^{1}H NMR sprectra, elemental analyses, and exact mass data were consistent with the assigned structures.

General Procedure for Ester Hydrolysis to Free Carboxylic Acids for Compounds 6a, 57a, 58a, 65a, 71a, and 77a. The appropriate ester (0.1−2 mmol) was heated to boiling with 1 N HCl (1−5 mL, replenishing as necessary) for 1 h. The mixture was then allowed to cool, often producing microneedles of desired product, or evaporated to dryness to yield an off-white solid (80−90% yield). ^{1}H NMR sprectra were consistent with the assigned structures.

General Thiation Procedure for Compounds 12 and 13. The 8-bromoxanthine **10** or **11** (0.25 mmol) was suspended in anhydrous ethanol (10 mL) and heated to reflux. $NaSH{\cdot}H_2O_x$ (2.5 mmol) was added, and the mixture became clear green almost immediately. The mixture was stirred under reflux for 30 min, cooled, and evapoereated onto silica gel. Flash column chromatography using 5−7% MeOH in CH_2Cl_2 pro-

vided a 63% and 75% yield of **12** and **13**, respectively, as white solids. Note: Compound **13** was found by ^{1}H NMR to be the ethyl ester due to transesterification under the reaction conditions.

General Procedure for C-Nitrosation of Pyrimidines. Compounds 18−20, 27, 32, 34, 36, 38, 40, and 42. The pyrimidine (15 mmol) was suspended in 1 N HCl (30 mL), and an aqueous solution of sodium nitrite (20 mmol in 10 mL) was added dropwise with stirring over 10 min. The suspension went from off-white to purple almost immediately. Stirring was continued for 1 h, the pH was adjusted to 5 with ammonia water, and the purple solid product was collected to provide 75−90% yield after drying. The characteristic lack of the C-5 proton in the ^{1}H NMR was evident for each compound.

General Procedure for the Reduction of 5-Nitroso- to 5-Amino pyrimidines. Compounds 21−23, 28, and 43−48. The 5-nitrosopyrimidine (15 mmol) was suspended in water (50 mL) and heated to 80−90 °C. Sodium hydrosulfite (45 mmol) was added with stirring in portions over 5 min. The color quickly changed from purple to light green, and stirring was continued for an additional 10 min. The mixture was cooled in ice and filtered. The filtered solid was washed with cold water, EtOH, and Et_2O to provide the *o*-diamine in 70−88% yield as an off-white to pale green solid.

1-*n*-Hexyl-3-methyluric Acid (24). The nitrosopyrimidine **19** (270 mg, 1.06 mmol) was dissolved in ethanol (20 mL) with warming, and palladium on carbon (75 mg, 10%) was added under argon. Hydrogenation was performed at room temperature and 15 psi for 2 h, filtered to remove catalyst, and evaporated to dryness. The residue was combined with urea (600 mg, 10 mmol) and heated neat on the hot plate with stirring. The temperature reached 140 °C, which produced a clear melt and was maintained for about 10 min with additional urea added (1 g). Upon cooling, the melt solidified and was dissolved in 1 N NaOH (25 mL) and boiled with decolorizing carbon for 10 min, filtered, and acidified to pH 3−4 while hot. The resulting precipitate was collected after cooling, washed with water, and dried to yield 160 mg (57%) of **24** as an off-white solid: mp >290 °C dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.80 and 10.73 (2s, 2H, N-7 H, N-9 H), 3.78 (t, 2H, N-CH_2), 3.30 (s, 3H, N-CH_3, under H_2O signal), 1.48 (m, 2H, 2′CH_2), 1.24 (m, 6H, 3′, 4′, 5′ CH_2), 0.85 (t, 3H, CH_3). Anal. $C_{12}H_{18}N_4O_3$.

3-Methyl-8-thiouric Acid (25). The pyrimidinediamine **32** (100 mg, 0.63 mmol) was combined with potassium ethyl xanthate (810 mg, 5 mmol) and DMF (10 mL) and heated at 100 °C. The suspension became green almost immediately, and the reaction was complete after 30 min by TLC. After a total reaction time of 1 h, the mixture was cooled, filtered, washed with Et_2O, and dried to yield an off-white solid (310 mg) which presumably contained the unreacted potassium ethyl xanthate and the potassium salt of the desired product. The solid was suspended in water (5 mL) and heated to dissolve. Glacial acetic acid was added to pH 5, and a vigorous effervescence was noted. A white solid formed which was filtered warm, washed with water and then ethanol, and dried to yield 99 mg (79%) of the title compound: mp >320 °C; ^{1}H NMR (DMSO-d_6) δ 13.40, 12.92 and 11.80 (3br s, 3H, NHs), 3.28 (s, 3H, CH_3). Anal. $C_6H_6N_4O_2S$.

3-*n*-Propylxanthine (29). The pyrimidinediamine **28** (750 mg) was combined with diethoxymethyl acetate (7 mL) and heated at 80 °C for 2 h. The mixture was evaporated to dryness, water (5 mL) was added, and the mixture heated for 20 min to near boiling. The resulting solution was then allowed to evaporate slowly to yield off-white crystals: yield 680 mg (86%); mp 282−284 °C (lit.[15] mp 291−292 °C).

General Procedure for Ring Closure of Pyrimidinediamines to Pteridines (49−55). The *o*-diamine (2 mmol) was suspended in water (20 mL) and heated to above 70 °C before a solution of glyoxal−sodium bisulfite addition product (10 mmol in 25 mL water) was added with stirring. The pale green suspension slowly became light amber and clear. After the suspension was heated for 5 min, TLC indicated reaction was complete. The mixture was cooled, extracted with ethyl acetate (5 × 40 mL), dried over $MgSO_4$, and evaporated to provide the desired product in 70 and 85% yield. ^{1}H NMR showed the appearance of two aromatic signals at about 8.74 and 8.55 as doublets (J = 2.5 Hz) for all compounds.

6,7-Diethyl-1-methylpteridine-2,4-dione (64). Compound **43** (200 mg, 1.27 mmol) was suspended in acetonitrile (5 mL), and 3,4-hexanedione (185 μL, 1.52 mmol) was added. The mixture was heated at 70 °C for 15 min with minimal product formation due to insolubility of **43**. Therefore DMF (3 mL) and water (3 mL) were added, and the temperature was raised to 100 °C. After 90 min total reaction time the mixture was cooled and poured into water (100 mL) and extracted with ethyl acetate (3 × 75 mL). The organic layer was dried over $MgSO_4$ and evaporated to provide the colorless crystalline product: yield 240 mg (81%); mp 218−222 °C; ^{1}H NMR (DMSO-d_6) δ 11.78 (br s, 1H, NH), 3.46 (s, 3H, NCH_3), 2.95 and 2.93 (2q, 4H, $2CH_2$ of ethyls), 1.28 and 1.23 (2t, 6H, $2CH_3$ of ethyls). Anal. $C_{11}H_{14}N_4O_2$.

Dimethyl 3-*n*-Hexyl-1-methyl-2,4-dioxopteridine-6,7-dicarboxylate (66). Compound **22** (300 mg, 1.18 mmol) was combined with dimethyl acetylenedicarboxylate (430 mg, 0.37 mL, 3 mmol) in dry DMF (5 mL), and the mixture was heated at 180 °C (bath temperature) for 3 h. The mixture was cooled, added to water (75 mL), extracted with EtOAc (2 × 75 mL), dried ($MgSO_4$), and evaporated to yield a thick oil. The crude product was purified by silica gel flash chromatography using 2% EtOAc in CH_2Cl_2. Crystallization of the pure product fraction from EtOH/H_2O gave **66** in 46% yield (205 mg): mp 92−95 °C; ^{1}H NMR ($CDCl_3$) δ 4.12 (t, 2H, NCH_2), 4.01 and 4.05 (2s, 6H, 2 COOMe), 3.72 (s, 3H, NCH_3), 1.70, 1.56, 1.31, and 0.86 (3m, 11H, 2′-6′ of hexyl group). Anal. $C_{17}H_{22}N_4O_6$.

1-Methyl-6-phenylpteridine-2,4-dione (67). The nitrosopyrimidine **32** (220 mg, 1.28 mmol) was mixed thoroughly with phenethylamine hydrochloride (1.5 g, 9.5 mmol) and heated in an open beaker on the hot plate. After a few minutes at about 160 °C the purple reaction mixture fused to a brown paste. TLC indicated many products, so sulfolane (1 mL) was added and heating was continued for 15 min. The reaction mixture was heated in water (10 mL), then diluted to 50 mL in water, and extracted with EtOAc (2 × 50 mL), and the organic layer was dried over $MgSO_4$, and then concentrated. The residue was flash chromatographed on silica gel using 4% MeOH in CH_2Cl_2: yield 75 mg (23%) of **67** as a pale yellow-orange solid; mp >307 °C dec; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.95 (br s, 1H, NH), 9.37 (s, 1H, C-7 H), 8.17 (m, 2H, 2′,6′ phenyl), 7.55 (m, 3H, 3′,4′,5′ phenyl), 3.51 (s, 3H, NCH_3). Anal. $C_{13}H_{10}N_4O_2$.

General Method for Ring Closure of Pyrimidines to [1,2,5]Thiadiazolo[3,4-*d*]pyrimidines (Compounds 69, 70, and 73). The *o*-diamine **23, 28,** or **43** (2.3 mmol) was suspended in dry acetonitrile (5 mL), and dry pyridine (1.5 mL) was added. Thionyl chloride (1 mL, 13.7 mmol) was added quickly, and the mixture, which became clear and darkened, was heated at 60 °C for 10 min. The mixture was then cooled and poured into 1 N HCl (40 mL) with stirring. The resulting yellow solution was extracted with ethyl acetate (3 × 40 mL), dried over $MgSO_4$, and evaporated to yield a pale yellow solid which was triturated with ether (yield 65−74%).

Ethyl 4-[[2-(Methylamino)benzoyl]amino]butanoate (76). A mixture of *N*-methylisatoic anhydride (3.5 g, 19.8 mmol) was combined with 4-aminobutyric acid (2.5 g, 24.3 mmol) in dry DMF (50 mL) and heated at 100 °C for 2 h. TLC indicated the reaction to be complete, and the DMF was removed in vacuo. The residue was used directly for esterification which was accomplished by dissolving the residue in 100% ethanol (50 mL) and adding chlorotrimethylsilane (2.5 mL, 20 mmol). The mixture was heated at 65 °C for 6 h and then evaporated to yield a brown syrup. Crude yield 87% from isatoic anhydride. A small sample was purified for characterization and biological testing by preparative TLC using 7% MeOH in CH_2Cl_2. The remainder of the material was used directly for preparation of compound **77**. Anal. $C_{14}H_{20}N_2O_3$.

Ethyl 1-Methyl-1,4-dihydro-2,4-dioxo-3(2*H*)-quinazolinebutanoate (77). The residue from **76** was combined with ethyl chloroformate (10 mL) and heated at 90 °C for 1 h. The mixture was cooled and poured into saturated aqueous sodium bicarbonate (50 mL) with stirring and after 10 min extracted

with ethyl acetate (2 × 75 mL). The organic layer was dried over $MgSO_4$ and evaporated to yield a brown syrup. The crude product was flash chromatographed on silica using 3% MeOH in CH_2Cl_2 to yield **77** as a thick oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.27−7.42 (2m, 4H, C-5,6,7,8), 4.04 (t, 2H, CH_2 of ethyl), 3.88 (m, 2H, NCH_2), 3.11 (s, 3H, NCH_3), 2.33 (t, 2H, 2′ CH_2), 1.71 (m, 2H, 3′ CH_2). Anal. $C_{15}H_{18}N_2O_4$.

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