

case of lung washings) or by the presence of urinary hydroxyproline which increases during periods of active collagen synthesis. The immunoassay procedures developed for the hydroxylase will probably not be useful here because they detect both inhibited and uninhibited enzyme. The amount of propeptide fragments formed and detected by the immunoassay for these would probably remain unchanged by a prolyl 4-hydroxylase inhibitor because the de novo pathway would not be suppressed but rather the products of the pathway, by failing to reach maturity, would be degraded immediately. The primary obstacle faced in the development of prolyl hydroxylase inhibitors is the high affinity of the enzyme for its endogenous peptidyl substrate. Successful inhibitors will need to gain access to the intracellular enzyme in concentrations sufficient to prevent enzyme-substrate interactions and, because of the kinetics of the system, will probably require very low inhibition constants to be active in vivo.

(4) Inhibitors of the procollagen peptidases would suppress collagen fibril formation and, therefore, prevent the accumulation of degradation-resistant forms of collagen in scar tissue. The specificity of antifibrotic agents directed toward these enzymes will become more apparent as the specific proteases are characterized. An inhibitor of a procollagen amino-terminal protease based on the amino acid sequence around the cleavage site in the  $\text{pro}\alpha 1$  chain of type I collagen has been described.<sup>28</sup> However, the procollagen carboxy-terminal peptidase, which would be the primary objective of an antifibrotic development program, has not been characterized. Thus, inhibitors of the procollagen peptidases would have to be considered at this time as possible second-generation antifibrotic agents.

(5) Selective activators of mammalian collagenase may be ultimately useful in resolving preformed scar tissue. The four types of activity described above might serve as

the basis for a drug to prevent the progression of a fibrotic lesion. The reversal of an existing fibrosis may, in fact, occur as a consequence of suppressed collagen accumulation since turnover of collagenous protein is continuous. Any reversal that occurs will clearly require the participation of an active collagenase. It would follow that the remodeling process could be accelerated by the selective activation of mammalian collagenases in the fibrosed organ. However, the regulation of mammalian collagenase is not understood sufficiently at present to identify the feasibility of this approach.

Inhibitors of collagen synthesis, including those with defined specificity for a prefibrotic or fibrotic lesion, would produce predictable toxicity if collagen synthesis were inhibited systemically. All known agents which influence connective tissue formation are teratogens. In addition, collagen domains (helical protein structures containing Pro-Hyp-Gly sequences) are found in a number of proteins. The impact of a collagen synthesis inhibitor on the function of these proteins cannot be assessed with accuracy until a useful inhibitor is available for systemic use. The proteins which may be influenced by inhibiting collagen synthesis or, more likely, by processing include the first component of complement (Clq), which contains six triple helical domains per molecule,<sup>32</sup> and acetylcholinesterase, where a collagenous tail has been implicated in the anchorage of this protein to the cell membrane matrix.<sup>33</sup> In view of the life-threatening nature of progressive fibrosis in organs like lung, liver, and kidney, the risks accepted with the use of an effective antifibrotic therapy would be justified if the dose-response curve of the drug provided reason to believe that the impact on these and other collagen-dependent systems could be minimized.

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## Mesoionic Xanthine Analogues: Phosphodiesterase Inhibitory and Hypotensive Activity

Richard A. Glennon,\* Michael E. Rogers, J. Doyle Smith, M. K. El-Said,

Department of Pharmaceutical Chemistry

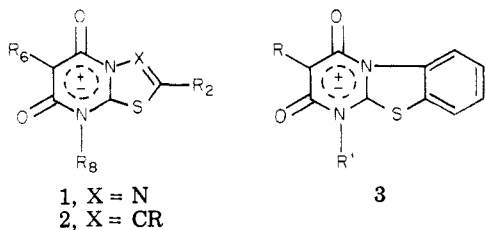
and John L. Egle

Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

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Several mesoionic thiazolo[3,2-*a*]pyrimidines and mesoionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidines were evaluated as inhibitors of cyclic-AMP phosphodiesterase. While small alkyl substituents at the 6 position have no significant effect on activity, phenyl and benzyl substituents enhance activity. Mesoionic structures such as 1 ( $R_2 = \text{H}$ ;  $R_3 = \text{Et}$ ) possess 20 to 40 times the activity of theophylline when the  $R_6$  substituent is phenyl or 4-chlorobenzyl. Methyl and ethyl substitution at the 2 position essentially abolishes activity. Although plagued by solubility problems, several of the mesoionic derivatives were found to display weak hypotensive effects in vivo.

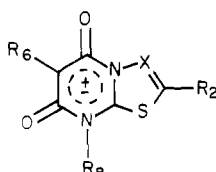
We have previously reported that derivatives of mesoionic 1,3,4-thiadiazolopyrimidines, 1, and mesoionic



thiazolopyrimidines, 2, possess theophylline-like activity as inhibitors of adenosine cyclic 3',5'-monophosphate (cyclic-AMP) phosphodiesterase (PDE).<sup>1</sup> Although the observed activity is rather low, this was the first demonstration that mesoionic xanthine analogues possess such activity. It appears that lengthening and branching of alkyl chains at the 8 position ( $R_8$ ) may enhance potency

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Table I. Properties of Mesoionic Xanthine Analogues



no.	R <sub>6</sub>	R <sub>8</sub>	R <sub>2</sub>	X	formula	mp, °C	% yield	recrystn solv	anal.
1d	<i>n</i> -Pr	Et	H	N	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	197-199	42	<i>i</i> -PrOH	C, H, N
1e	<i>i</i> -Pr	Et	H	N	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	176-177	31	EtOAc	C, H, N
1f	<i>s</i> -Bu	Et	H	N	C <sub>11</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub> S	187-188	50	EtOAc	C, H, N
1g	<i>i</i> -Bu	Et	H	N	C <sub>11</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub> S	180-182	30	EtOAc	C, H, N
1h	amyl	Et	H	N	C <sub>12</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub> S	166-168	19	EtOAc	C, H, N
1j	<i>p</i> -OMe-Bzl	Et	H	N	C <sub>15</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub> S·0.25MeOH	212-213	84	MeOH	C, H, N
1k	<i>p</i> -Cl-Bzl	Et	H	N	C <sub>14</sub> H <sub>12</sub> ClN <sub>3</sub> O <sub>2</sub> S	130-131	66	MeOH	C, H, N
1m	Ph	Et	H	N	C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub> S	140-142	82	EtOH	C, H, N
1n	Me	Et	Me	N	C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub> S	243-244	54	EtOAc	C, H, N
1o	Et	Et	Me	N	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	203-204	72	Me <sub>2</sub> CO	C, H, N
1p	Me	Et	Et	N	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	151-152	37	EtOAc	C, H, N
1q	Et	Et	Et	N	C <sub>11</sub> H <sub>15</sub> N <sub>3</sub> O <sub>2</sub> S	137-138	41	EtOAc	C, H, N
2b	Et	Et	H	CCH <sub>3</sub>	C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S·0.25H <sub>2</sub> O	145-147	66	95% EtOH	C, H, N
2f	Et	CPM <sup>b</sup>	H	C-Ph	C <sub>18</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S	188-190	25	<i>i</i> -PrOH	C, H, N

<sup>a</sup> Series 1 compounds melt with decomposition. <sup>b</sup> CPM = cyclopropylmethyl.

somewhat; however, little else is known about the structure-activity relationships (SAR) of these compounds.<sup>1</sup> Compounds 1 and 2 are isosteric with xanthine, and it is well established that substitution at the 1, 3, 7, and 8 positions of xanthine (i.e., the R<sub>6</sub>, R<sub>8</sub>, X, and R<sub>2</sub> positions, respectively, of 1 and 2) have a profound influence on PDE inhibition.<sup>2-6</sup> These various studies may have employed different enzyme forms and different enzyme sources; nevertheless, certain trends emerge (particularly when a theophylline standard is used for reference). Because of the structural relationship which exists between the mesoionic structures 1 and 2 and xanthine, it is conceivable that substitution at R<sub>6</sub>, R<sub>2</sub>, and R (where R<sub>8</sub> is held constant as an ethyl group, for the most part) might also influence the activity of the mesoionic compounds. Several such derivatives were prepared and evaluated using bovine heart PDE.

**Chemistry.** The mesoionic compounds (Table I) were simply prepared by the thermal condensation of an (alkylamino)-1,3,4-thiadiazole or (alkylamino)thiazole with the appropriate bis(2,4,6-trichlorophenyl) malonate, 4, as previously reported.<sup>1</sup> The trichlorophenyl malonates were prepared by hydrolysis of the corresponding diethyl malonates, followed by reesterification using POCl<sub>3</sub> and trichlorophenol.

## Results and Discussion

The mesoionic compounds were tested in an assay previously described by Klee<sup>7</sup> using bovine heart PDE and

1 μM cAMP; the results are reported in Table II as I<sub>50</sub> values. A number of compounds were only poorly soluble in the buffer solution; ethanol (2.5% final concentration) was used to solubilize some of the test compounds prior to final dilution with buffer. Compounds 1a-m, 2c,d,f, and 3 were dissolved in this manner; compound 1j was even insoluble under these conditions. The presence of ethanol diminishes enzyme activity somewhat; as a consequence, theophylline control was run both in the presence and absence of ethanol and Table II lists the potencies of the test compound relative to their respective theophylline control. Another standard inhibitor, 1-methyl-3-isobutylxanthine (IBMX), was included for comparative purposes.

In the 1 series, replacement of an R<sub>6</sub> H, Me, or Et group with *n*-Pr, *i*-Pr or *s*-Bu results in a two- to fourfold increase in potency. Increasing the hydrophobicity in the R<sub>6</sub> region (i.e., R = amyl, phenyl, benzyl) further enhances activity, with the *p*-chlorobenzyl substituted compound, 1k, being the best of those studied. On the other hand, methyl and ethyl substitution at the 2 position (1n-q) essentially abolishes activity. In the thiazolopyrimidine (2) series, substitution at the 3 position by a methyl or phenyl group does not adversely effect activity, and, in fact, actually enhances activity, comparing 2a with 2b, 2e with 2f, and 2c with 2d (although in this latter case the R<sub>6</sub> group was also changed from H to Me). Based on the activity of the phenyl-substituted compound, the benz-fused analogue of 2a, (i.e., 3) was prepared and evaluated. Ring fusion was found to result in nearly a tenfold increase in activity, comparing 2a with 3.

Theophylline produces a hypotensive effect in animals; regardless of how theophylline produces this effect, it appeared reasonable to compare the hypotensive effects of several mesoionic derivatives with those of theophylline by monitoring blood pressure before and after intravenous injection into the femoral vein of anesthetized rats. The data are presented in Table III. The results of this preliminary study reveal that several of the mesoionic xanthine derivatives (1b, 1c, and 2a) can produce a weak hypotensive response, with 1b being almost comparable in activity with theophylline. Methylation at R<sub>2</sub> reduces activity; compounds 1n and 1o (the R<sub>2</sub> methyl derivatives of 1b and 1c) are much less active than their non-

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Table II. Inhibition of Cyclic-AMP Phosphodiesterase

compd	R <sub>2</sub>	R <sub>6</sub>	R <sub>8</sub>	X	I <sub>50</sub> , <sup>a,b</sup> μM	rel potency <sup>c</sup>
1a	H	H	Et	N	774	0.5
1b	H	Me	Et	N	1160	0.4
1c	H	Et	Et	N	550	0.7
1d	H	<i>n</i> -Pr	Et	N	252 (±70)	1.6
1e	H	<i>i</i> -Pr	Et	N	254 (±28)	1.6
1f	H	<i>s</i> -Bu	Et	N	233 (±27)	1.8
1g	H	<i>i</i> -Bu	Et	N	154 (±49)	2.7
1h	H	amyl	Et	N	156 (±19)	2.6
1i	H	Bzl	Et	N	52	7.9
1j	H	<i>p</i> -MeO-Bzl	Et	N	insoluble	
1k	H	<i>p</i> -Cl-Bzl	Et	N	9.6 (±01)	42.9
1m	H	Ph	Et	N	24.0 (±02)	17.2
1n	Me	Me	Et	N	2453 (±330)	0.1
1o	Me	Et	Et	N	1689 (±86)	0.2
1p	Et	Me	Et	N	2411 (±51)	0.1
1q	Et	Et	Et	N	1980	0.1
2a	H	Et	Et	CH	858	0.3
2b	H	Et	Et	CCH <sub>3</sub>	412 (±11)	0.7
2c	H	H	3-Cl-Bzl	CH	308 (±10)	1.3
2d <sup>d</sup>	H	Me	3-Cl-Bzl	C-Ph	152 (±30)	2.7
2e	H	Et	CPM <sup>e</sup>	CH	396 (±24)	0.7
2f	H	Et	CPM <sup>e</sup>	C-Ph	287 (±32)	1.5
3		Et	Et		153 (±27)	2.7
theophylline						1.00 <sup>c</sup>
IBMX					9.9 (±03)	28.9

<sup>a</sup> All compounds with the exception of 1n-q, 2a,b,d, and IBMX were dissolved in 95% EtOH (2.5% final concentration) prior to dilution with buffer. <sup>b</sup> Substrate (cyclic AMP) concentration is 1 μM. Data for compounds 1b,a,c,i and 2a are included only for comparative purposes. <sup>c</sup> See text for explanation. I<sub>50</sub> for theophylline control without ethanol is 286 (±19) μM and with ethanol (2.5% final concentration) is 412 (±20). <sup>d</sup> Prepared according to literature procedure. <sup>e</sup> CPM = cyclopropylmethyl.

Table III. Decrease in Arterial Blood Pressure Produced by Several Mesoionic Xanthine Analogues<sup>a</sup>

compd	dose, mg/kg					
	2.5	5	10	15	30	60
1b	0.9 (7)	2.2 (7)	1.6 (7)	1.2 (7)	7.5 (7)	
1c	0.4 (8)	0.6 (9)	1.6 (7)	0.7 (8)		
1n	0.2 (2)	0.3 (3)	0.4 (2)	0.5 (4)		
1o	0	0.3 (6)	0.6 (5)	0.3 (6)	1.2 (6)	1.9 (6)
2g <sup>b</sup>	0.9 (6)	0.6 (6)	1.2 (6)	1.2 (6)	2.9 (6)	
2a	0.3 (5)	0.6 (7)	0.8 (7)	1.2 (6)		
theophylline	1.00 (19)	2.34 (26)		2.36 (19)		

<sup>a</sup> The decrease in mean arterial pressure produced by each dose of compound is reported relative to a control dose of theophylline (2.5 mg/kg) run in the same animal. Numbers in parentheses refer to the number of determinations. Theophylline was also run at doses of 5 and 15 mg/kg in each animal, and the mean values for all determinations, relative to the 2.5 mg/kg dose, are also reported. Actual drops in blood pressure for theophylline at 2.5, 5, and 15 mg/kg (minus control values) are 13.5 (±3.1), 31.6 (±6.1), and 31.9 (±4.7) mmHg, respectively. <sup>b</sup> Compound 2g: R<sub>6</sub> = H; R<sub>8</sub> = *n*-Bu; X = CH.

methylated counterparts. Additional studies with these compounds are planned but are hampered by problems with solubility.

As shown in Table II, substituents on the mesoionic nucleus can dramatically alter the effect on PDE inhibition, and although there may be some parallelisms between the xanthines and the mesoionic derivatives, there are also some obvious differences. Goodsell et al.,<sup>3</sup> for example, have found that 8-alkylation of theophylline enhances its potency as an inhibitor of PDE (using different preparations, others have found that 8-alkylation has little effect on, or enhances, the activity of xanthine analogues).<sup>2,6</sup> On the other hand, 8-alkylation of the mesoionic thiadiazolopyrimidines (1) abolishes activity. Furthermore, replacement of the 3-methyl group of theophylline with an isobutyl group (to give IBMX) enhances activity, while the isobutyl mesoionic derivatives do not show this enhancement of activity and are even less active than theophylline.<sup>1</sup>

The mesoionic xanthine analogues are isosteric with the xanthines and, like theophylline, display a moderate degree of activity in both the PDE and hypotensive assays. While none of the simple alkyl derivatives of 1 is significantly

more potent than theophylline, the R<sub>6</sub> benzyl and phenyl derivatives show some promise. Based on these results, synthesis and evaluation of substituted phenyl and benzyl derivatives appear warranted.

### Experimental Section

**Chemistry.** Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 257 spectrophotometer, and <sup>1</sup>H NMR spectra were determined on a Perkin-Elmer R-24 spectrometer using Me<sub>4</sub>Si as an internal standard. Mass spectra were obtained on a Finnigan 4015 GC/MS data system at 70 eV. The structures of all compounds are consistent with their IR, NMR, and mass spectral data. Microanalyses were performed by Atlantic Microlab, Atlanta, GA, and results are within 0.4% of the calculated values. Radioactivity was measured using a Beckman Ls 355 liquid scintillation counter.

**Bis(2,4,6-trichlorophenyl) *n*-Pentylmalonate (4a).** A mixture of diethyl *n*-pentylmalonate (25 g, 0.11 mol), KOH (25 g, 0.45 mol), and water (25 mL) was stirred at 90 °C for 3 h. The mixture was acidified to pH 2 by the addition of 50% H<sub>2</sub>SO<sub>4</sub> and then filtered. The filtrate was extracted with ether (3 × 50 mL); the ether portion was extracted with aqueous Na<sub>2</sub>CO<sub>3</sub> (3%) and the carbonate solution was readjusted to pH 2 by the addition of 50% H<sub>2</sub>SO<sub>4</sub>. The acid solution was extracted with Et<sub>2</sub>O (3 ×

50 mL), and the ether portion was dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to dryness to give 10 g (53%) of crude *n*-pentylmalonic acid (mp 77–79 °C), which was used without further purification. A mixture of this acid (8.7 g, 0.05 mol), 2,4,6-trichlorophenol (15.8 g, 0.08 mol), and  $\text{POCl}_3$  (16.1 g, 0.105 mol) was heated on an oil bath (80 °C) for 7 h. The crude reaction mixture was cooled to room temperature and poured onto ice (250 g). The aqueous portion was decanted and the residual mass was dissolved in  $\text{Et}_2\text{O}$  (150 mL). The  $\text{Et}_2\text{O}$  solution was washed with aqueous  $\text{NaHCO}_3$  (5%, 3 × 30 mL), water (3 × 30 mL), and dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation of the solvent under reduced pressure gave a brown oily residue which crystallized upon standing. Recrystallization from hexane afforded 18 g (84%) of **4a** as white crystals, mp 75–77 °C. Anal. ( $\text{C}_{20}\text{H}_{16}\text{Cl}_6\text{O}_4$ ) C, H.

**Bis(2,4,6-trichlorophenyl) Isobutylmalonate (4b)**. Compound **4b** was prepared in 16% yield from the appropriate diethyl malonate in the same manner as **4a**, mp 87–88 °C after recrystallization from hexane. Anal. ( $\text{C}_{19}\text{H}_{14}\text{Cl}_6\text{O}_4$ ) C, H.

**Bis(2,4,6-trichlorophenyl) sec-Butylmalonate (4c)**. Compound **4c** was prepared in 21% yield from the appropriate diethyl malonate in the same manner as **4a**, mp 79–80 °C after recrystallization from hexane. Anal. ( $\text{C}_{19}\text{H}_{14}\text{Cl}_6\text{O}_4$ ) C, H.

**Bis(2,4,6-trichlorophenyl) 4-Methoxybenzylmalonate (4d)**. Compound **4d** was prepared in 43% yield from the appropriate substituted diethyl malonate in the same manner as **4a**, mp 130–131 °C after recrystallization from  $\text{EtOAc}$ . Anal. ( $\text{C}_{23}\text{H}_{14}\text{Cl}_6\text{O}_5$ ) C, H.

**Bis(2,4,6-trichlorophenyl) 4-Chlorobenzylmalonate (4e)**. Compound **4e** was prepared in 63% yield from the appropriately substituted diethyl malonate in the same manner employed for **4a**. Recrystallization from hexane afforded white crystals, mp 117–119 °C. Anal. ( $\text{C}_{22}\text{H}_{11}\text{Cl}_7\text{O}_4$ ) C, H.

The unsubstituted, methyl, ethyl, and isopropyl,<sup>10</sup> as well as the propyl,<sup>12</sup> esters have been previously reported.

**N-(4-Phenyl-2-thiazolyl)cyclopropylcarboxamide (5)**. A solution of cyclopropylcarboxylic acid chloride (5.23 g, 50 mmol) in THF (25 mL) was added dropwise to a stirred solution of 2-amino-4-phenylthiazole (8.81 g, 50 mmol) and  $\text{NET}_3$  (5.57 g, 55 mmol) in THF (125 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 4 h and was filtered. The filtrate was dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to dryness to yield the crude product. Recrystallization from 95%  $\text{EtOH}$  gave 9.25 g (76%) of **5** as dark gold crystals, mp 204–207 °C. Anal. ( $\text{C}_{13}\text{H}_{12}\text{N}_2\text{OS}$ ) C, H, N.

**2-[(Cyclopropylmethyl)amino]-4-phenylthiazole (6)**. A solution of **5** (3.66 g, 15 mmol) in dry THF (75 mL) was added dropwise to a stirred suspension of  $\text{LiAlH}_4$  (1.14 g, 30 mmol) in THF (50 mL) at 0 °C. After the mixture was heated at reflux

for 4 h,  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  was added in small portions to decompose any unreacted  $\text{LiAlH}_4$ . The mixture was filtered (Celite), and the filtrate was dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to dryness to yield a dark yellow solid. Recrystallization from absolute  $\text{EtOH}$  gave 2.73 g (73%) of **6** as yellow needles, mp 81–83 °C. Anal. ( $\text{C}_{18}\text{H}_{14}\text{N}_2\text{S}$ ) C, H, N.

**Anhydro-3-phenyl-6-ethyl-7-oxo-8-(cyclopropylmethyl)-5-hydroxythiazolo[3,2-a]pyrimidinium Hydroxide (2f)**. An intimate mixture of **6** (1.15 g, 5 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate<sup>10</sup> was heated neat, under a stream of  $\text{N}_2$ , at 160 °C until a clear melt resulted (2 min). The cooled melt was crystallized by trituration with anhydrous  $\text{Et}_2\text{O}$  (20 mL), and the product was collected by filtration. Recrystallization from *i*-PrOH gave 0.39 g (25%) of **2f** as beige needles: mp 189–191 °C; IR ( $\text{CHCl}_3$ ) 1690, 1635  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.6 (d, 4 H, cyclopropyl  $\text{CH}_2$ ), 1.1 (t, 3 H,  $\text{CH}_3$ ), 1.2 (m, 1 H, cyclopropyl CH), 2.5 (q, 2 H,  $\text{CH}_2\text{CH}_2$ ), 4.2 (d, 2 H,  $\text{CH}_2$ ), 6.8 (s, 1 H), 7.4 (s, 5 H, aromatic protons); mass spectrum, *m/e* (relative intensity) 327 (18), 311 (100), 257 (93). Anal. ( $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ ) C, H, N.

Compounds **1d–m** (Table I) were prepared in the same manner as that employed for the synthesis of **2f**, using 2-(ethylamino)-1,3,4-thiadiazole and the appropriate bis(2,4,6-trichlorophenyl) malonate, while the synthesis of **1n** and **1o**, **1p** and **1q**, and **2b** employed 2-(ethylamino)-5-alkyl-1,3,4-thiadiazole<sup>11</sup> or 2-(ethylamino)-4-methylthiazole,<sup>1</sup> respectively.

**PDE Assay.** The assay of Klee<sup>7</sup> was used, employing bovine heart phosphodiesterase (Sigma Chemical Co.) and 1  $\mu\text{M}$  [ $^3\text{H}$ ]adenosine cyclic monophosphate as we have previously described in more detail.<sup>1</sup>  $I_{50}$  values were determined by plotting uninhibited velocity/inhibited velocity ( $V_0/V$ ) vs. the inhibitor concentration. The  $I_{50}$  is the inhibitor concentration where  $V_0/V = 2$ . At least five different inhibitor concentrations, giving 25–75% inhibition, were used for each inhibitor. Each  $I_{50}$  value is an average of two experiments run consecutively and the data in Table II represent duplicate or triplicate determinations of these averaged runs.

**Pharmacology.** Male Sprague–Dawley rats (250–385 g) were anesthetized with sodium pentobarbital (50 mg/kg) administered by intraperitoneal injection. Cannulation of the right femoral artery (PE 50 tubing) allowed arterial blood pressure to be measured by means of a Stratham P-23 DC pressure transducer and a Grass Model 7 polygraph. The left femoral vein was cannulated (PE 50) to allow intravenous administration of test compounds. Each drug injection was flushed with 0.3 mL of saline and was immediately preceded by a control injection of an equivalent volume of saline. Injections were made at 5-min intervals or after return of blood pressure to preinjection levels, whichever occurred first. Results are expressed as the maximal effect observed.

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