Mesoionic Xanthine Analogues: Antagonists of Adenosine Receptors

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A variety of mesoionic xanthines including mesoionic thiazolo[3,2-α]pyrimidines, benzothiazolopyrimidines, and 1,3,4-thiadiazolo[3,2-α]pyrimidines were antagonists of A_1-adenosine receptors (inhibition of binding of [^{3}H]-cyclohexyladenosine) and A_2-adenosine receptors (inhibition of 2-chloroadenosine-elicited accumulations of cyclic AMP) in brain tissue. Most of the compounds were less potent than theophylline and none were remarkably selective for A_1- or A_2-adenosine receptors. However, members of the thiadiazolopyrimidine class of mesoionics exhibited very low or no activity as antagonists of A_2-adenosine receptors while exhibiting activity only 2–4-fold lower than that of theophylline at A_1-adenosine receptors. Unlike the case for theophylline, the presence of a phenyl substituent in the five-membered ring did not enhance the potency of a mesoionic thiadiazolopyrimidine. The nature of the substituents on the mesoionic ring did not appear to have marked effects on potency unlike the marked effect of the nature of 1,3-substituents on activity of nonmesoionic xanthines. The benzothiazolo[3,2-α]pyrimidines were the most potent antagonists, being nearly as potent as theophylline at A_1-adenosine receptors and somewhat more potent than theophylline at A_2-adenosine receptors.

Theophylline and other alkylxanthines were considered for decades to owe their pharmacological effects primarily to inhibition of cyclic nucleotide phosphodiesterases and a resultant elevation of cyclic AMP and/or cyclic GMP levels. It is now realized that theophylline and certain other xanthines are more potent as antagonists of adenosine receptors than they are as inhibitors of phosphodiesterases.[1] One class of adenosine receptors (A_1) is inhibitory to adenylate cyclase; blockade of this receptor is probably involved in the lipolytic[2] and central stimulation[3] activity of theophylline and other xanthines. The other class of adenosine receptors (A_2) is stimulatory to adenylate cyclase. Blockade of this receptor by xanthines could, under certain conditions, increase heart rate[4] and blood pressure,[4b] reduce coronary blood flow,[5] and reduce hormone production.[6,7] Antagonism of adenosine receptors might also be involved in the diuretic effects produced by xanthines.[8] At present, the xanthines are the only major class of adenosine receptor antagonists. In a search for more potent and/or selective antagonists, a series of mesoionic xanthine analogues was investigated. Such mesoionic analogues do, like the xanthines, inhibit phosphodiesterases.[9-12] In the current study, inhibition of binding of [^{3}H]cyclohexyladenosine to rat cerebral cortical membranes[13,14] was used to assess the potency of mesoionic analogues at an A_1-adenosine receptor. Inhibition of 2-chloroadenosine-elicited accumulations of cyclic AMP in guinea pig cerebral cortical slices[15] was used to assess potency of mesoionic analogues as antagonists of an A_2-adenosine receptor. A potent phosphodiesterase inhibitor (rolipram)[16] was included in the latter protocol to eliminate any possible effects of phosphodiesterase inhibition by the mesoionic analogues.

Chemistry. The synthesis of most of the mesoionic xanthines analogues has been previously reported;[9-12] for the most part, they were prepared by condensation of the appropriately substituted alkylamino heterocycle with bis(2,4,6-trichlorophenyl) malonate or bis(2,4,6-trichlorophenyl) alkylmalonate. The condensation is described for the preparation of the novel derivative **2b**. The thiadiazolopyrimidine derivative **3a** was prepared by condensation of 2-(ethylamino)-1,3,4-thiadiazole with carbon suboxide. The synthesis of **3d** required 2-(ethylamino)-5-phenyl-1,3,4-thiadiazole (**8**); 4-ethyl-3-thiosemicarbazide was allowed to react with trimethyl orthobenzoate in the presence of an acid catalyst to afford a white crystalline material (mp 173–175 °C). Although the spectral data obtained of this product were consistent with what might be anticipated for **8**, Chandra et al.[17] had previously reported that treatment of benzal thiosemicarbazone with ferric chloride affords **8** with a melting point of 238–240 °C. Ortho ester cyclizations of alkylthiosemicarbazides can yield mixtures of thiadiazoles and mercaptotriazoles,[18] and although our product was distinctly different from 3-phenyl-5-mercapto-1,2,4-triazole (mp 141–142 °C),[19] the

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Table I. Effects of Xanthines and Various Mesoionic Xanthine Analogues on A_1- and A_2-Adenosine Receptors

no.	R	R′	R″	R‴	IC_{50},[a] μM: A_1 receptor	IC_{50},[a] μM: A_2 receptor
			Xanthines			
1a	CH_3	CH_3	H	H	28 ± 6	42 ± 6
1b	C_2H_5	C_2H_5	H	H	6.4 ● 0.4	12 ± 2
1c	n-C_3H_7	n-C_3H_7	H	H	1.5 ± 6	8.1 ± 2.4
1d	$CH_2C_6H_5$	$CH_2C_6H_5$	H	H	7 ± 2	35 ± 5
1e	CH_3	CH_3	CH_3	H	110 ± 22	150 ± 15
1f	CH_3	CH_3	H	C_6H_5	0.8 ± 0.3	1.65 ± 0.12
			Thiazolopyrimidines			
2a	C_2H_5	C_2H_5	H	H	120	150
2b	n-C_3H_7	n-C_3H_7	H	H	54 ± 6	250
2c	C_2H_5	$CH_2C_3H_5$	H	H	100	120
2d	C_2H_5	$CH_2C_6H_5$	H	H	160	150
2e	$CH_2C_6H_5$	$CH_2C_3H_5$	H	H	160	200
2f	C_2H_5	$CH_2C_3H_5$	C_6H_5	H	120	200
2g	C_2H_5	b	H	H	95	250
			Thiadiazolopyrimidines			
3a	H	C_2H_5		H	110 ± 10	≫250 (0%)
3b	CH_3	C_2H_5		H	60 ± 30	≫250 (0%)
3c	C_2H_5	C_2H_5		H	72 ± 20	≫250 (15%)
3d	C_2H_5	C_2H_5		C_6H_5	80 ± 40	250
3e	$CH_2C_6H_5$	C_2H_5		H	58 ± 2	≫200 (10%)
3f	$CH_2C_6H_4$(4-Cl)	C_2H_5		H	45 ● 7	>250 (35%)
3g	CH_3	n-C_5H_{11}		H	120 ± 30	≫250 (5%)
3h	C_2H_5	C_2H_5		CH_3	500	≫250 (0%)
			Benzothiazolopyrimidines			
4a	C_2H_5	$CH_2C_3H_5$	H		37 ± 3	18 ± 5
4b	C_2H_5	$CH_2C_3H_5$	OC_2H_5		20 ± 5	54
4c	n-C_3H_7	i-C_4H_9	H		30	20
4d	C_2H_5	i-C_4H_9	H		41 ± 4	15 ± 5
			Triazolopyrimidine			
5	n-C_3H_7	CH_3			≫250 (20%)	≫250 (5%)
			Imidazothiazine			
6	C_2H_5				290 ± 20	≫250 (25%)
			Isoquinopyrimidine			
7	C_2H_5	C_2H_5			27	30

[a] IC_{50} values for A_1 receptors were obtained from antagonism of binding of 1 nM [^{3}H]cyclohexyladenosine to rat cerebral cortical membranes. IC_{50} values for A_2-adenosine receptors were obtained from antagonism of accumulations of cyclic AMP elicited by 15 μM 2-chloroadenosine in guinea pig cerebral cortical slices. Values are from single determination or are means ● SEM for two to three determinations. Percentages in parentheses indicate the percent inhibition at the highest concentration tested. [b] R′ = 3,5-dimethoxybenzyl.

identity of **8** was verified by another synthetic route. 2-Amino-5-phenyl-1,3,4-thiadiazole was prepared by a literature procedure,[20] and acylated with acetic anhydride[21] and the resultant amide reduced with $LiAlH_4$ to afford **8** with a melting point of 173–174 °C. Compound **8** was cyclized to **3d** by condensation with bis(2,4,6-trichlorophenyl) ethylmalonate.

Results and Discussion

Xanthines. Theophylline (**1a**) is nearly equipotent as an antagonist at A_1- and A_2-adenosine receptors[14] (Table I). Addition of a methyl group at the 7-position (R″) yields caffeine (**1e**) and reduces potency about 3-fold at both A_1- and A_2-adenosine receptors. A phenyl group at the 8-position (R‴) of theophylline (i.e., **1f**) greatly increases potency at A_1- and A_2-receptors[13,15,22-24] (Table I). The nature of substituents at the 1- and 3-positions (R, R′) of xanthines has significant effects on potency and selectivity at A_1- and A_2- adenosine receptors. The 1,3-diethyl derivative **1b** is 5-fold more potent than theophylline at both A_1 and A_2 receptors (Table I). The 1,3-dipropyl analogue of theophylline (i.e., **1c**) is 20-fold more potent at A_1 receptors but only 5-fold more potent at A_2 receptors than theophylline. The 1,3-dibenzyl analogue **1d** is 4-fold more potent at A_1 receptors than theophylline while being equipotent with theophylline at A_2 receptors. Further investigation of structure–activity relations for xanthine antagonists is in progress.

Mesoionic Analogues. Most of the mesoionic analogues are less potent than theophylline as adenosine antagonists. Whether or not this reflects the effect of the mesoionic ring or the effect of alterations in the five-membered ring on receptor affinity is unknown. There have been only limited studies on effects of replacement of N_7 and N_9 nitrogen atoms of xanthines with sulfur or carbon on potencies at adenosine receptors. 8-Azatheophylline, for example, was virtually inactive as an antagonist at A_2-adenosine receptors of fibroblasts, while 1-ethyl-3-propyl-7-thiaxanthine was 10-fold less potent than 1,3-diethylxanthine.[22] 9-Oxa-8-phenyltheophylline was at

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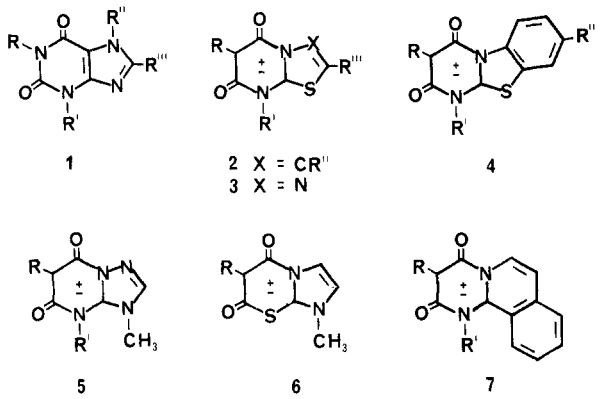

1 2 X = CR'' 3 X = N 4 5 6 7

least 500 times less active than 8-phenyltheophylline. One direct comparison of the effect of structural modification of the five-membered ring on activity can be made for a xanthine and a mesoionic xanthine analogue; in the case of the mesoionic triazolopyrimidine **5**, a methyl substituent is present at what would be the xanthine 9-position. Compound **5** is virtually inactive at adenosine receptors (Table I) as are the corresponding 9-methylxanthines isocaffeine and 1,9-dimethylxanthine.[3,22] The weakly active mesoionic imidazothiazine **6** also possesses an alkyl group at the xanthine 9-position.

A series of seven mesoionic thiazolopyrimidines, **2**, with various substituents were evaluated (Table I). Compounds **2a–2g** were considerably weaker than theophylline, and, in contrast to the marked effects of substituents on activity in the **1** series, substituent effects in series **2** were less pronounced. Nevertheless, dipropyl derivative **2b** was, as in the **1** series, more active than its diethyl derivative **2a** at A_1 sites. Compound **2b** also displays selectivity for A_1 sites.

Benz-fusion of the series **2** compounds affords mesoionic benzothiazolopyrimidines **4**. These compounds are as potent or are slightly more potent than theophylline (**1a**) as adenosine antagonists and, with the exception of the ethoxy-substituted derivative **4b**, exhibit some selectivity for A_2 sites. There was little effect of substituent variation on activity, although, admittedly, substituent selection was not very large.

Several of the mesoionic thiadiazolopyrimidines **3** were quite selective for A_1-adenosine receptors, showing nearly no activity at A_2-adenosine receptors at the highest concentration tested (250 μM). Remarkably, the thiadiazolopyrimidine **3a** was as active at A_1 receptors as was **3b**; in a corresponding nonmesoionic xanthine pair, 3-methylxanthine is about 120-fold less active than theophylline.[24] Increasing the size of the xanthine 3-position substituent from ethyl (**3b**) to *n*-pentyl (**3g**) had only a slight effect on activity. The presence of the 4-chlorobenzyl substituent of **3f** resulted in a relatively potent A_1-adenosine antagonist, which now has significant, albeit weak, activity as an A_2 antagonist. The presence of a methyl group in the five-membered ring of **3c** (i.e., **3h**) is not tolerated, **3h** being virtually inactive. This is remarkable, since in the case of theophylline, the presence of a methyl substituent in an equivalent position (i.e., 8-methyltheophylline) has little effect on the potency at either A_1 (unpublished results) or A_2 receptors.[22] An 8-phenyl group in theophylline (i.e., **1f**) increases potency by 35-fold, and again the lack of corresponding effect when the potency of **3c** is compared with that of **3d** is remarkable. Possibly, the sharper bond angle of the C–S–C portion of the thiadiazole ring of **3h** and **3d**, imparted by the presence of the sulfur atom, might locate the methyl or phenyl group in a somewhat different orientation than that in 8-methyltheophylline or **1f**, respectively.

The mesoionic thiazine analogue **6** was essentially devoid of activity, while the mesoionic isoquinopyrimidine **7** was equipotent with theophylline.

Nearly all of the mesoionic compounds discussed herein have been previously examined as inhibitors of cyclic-AMP phosphodiesterase (PDE).[9-12] In general, the benzothiazolopyrimidines and thiadiazolopyrimidines are more active than their thiazolopyrimidine counterparts, while the mesoionic triazolopyrimidine **5** and the methyl-substituted thiadiazolopyrimidine **3h** are inactive. There does not appear to exist, for these few compounds, a significant relationship between the data in Table I and the ability of these agents to inhibit cAMP PDE.[9-12]

The data indicate some analogies and some differences between the structure–activity requirements for mesoionics and the structure–activity requirements for xanthines as adenosine antagonists. Further comparisons are required, and at that time speculations as to the nature of interactions of the receptor surface with the planar heterocyclic ring of adenosine antagonists (xanthines, mesoionics, pteridines,[22] pyrazolopyridines,[25-27] pyrazolopyrimidines,[28] etc.) may be possible.

Experimental Section

Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Perkin-Elmer R-24 high-resolution spectrometer and chemical shifts are reported relative to Me_4Si as an internal standard. Infrared spectra were obtained on a Perkin-Elmer 257 spectrophotometer and mass spectra were determined on a Finnigan 4000 series GC/MS. Spectral data were consistent with the assigned structures. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, and determined values were within 0.4% of theoretical. Most of the compounds used in this study were those previously reported, or they were synthesized according to literature procedures.[9-12]

Anhydro-6,8-di-*n*-propyl-5-hydroxy-7-oxothiazolo[3,2-*a*]pyrimidinium Hydroxide (2b). 2-(*n*-Propylamino)thiazole (0.1 g, 0.7 mmol) and bis(2,4,6-trichlorophenyl) *n*-propylmalonate (0.35 g, 0.7 mmol) were heated, neat, at 160 °C, under a stream of N_2, until a clear melt resulted (ca. 3 min). When cool, the resultant yellow oil was triturated with anhydrous Et_2O (20 mL) and the crude solid product was collected by filtration. Recrystallization from EtOAc afforded 0.08 g (45%) of **2b** as white crystals, mp 128–130 °C. Anal. ($C_{12}H_{16}N_2O_2S$) C, H, N.

Anhydro-8-ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium Hydroxide (3a). Dibromomalonyl dichloride[29] (3.0 g, 10 mmol) in anhydrous Et_2O (30 mL) was added dropwise to zinc shavings (2.0 g), under a slow stream of N_2, at such a rate that the stirred reaction mixture boiled gently. The carbon suboxide that was formed was bubbled into a solution of 2-(ethylamino)-1,3,4-thiadiazole (0.05 g, 0.38 mmol) in anhydrous Et_2O (5 mL) to yield an almost instantaneous precipitate of crude **3a**. The solid was collected by filtration and recrystallized from MeCN to afford 0.07 g (92%) of **3** as white crystals, mp 208–210 °C (lit.[30] 208–209 °C).

2-(Ethylamino)-5-phenyl-1,3,4-thiadiazole (8). Method A. Concentrated HCl (0.05 mL) was added to a solution of 4-ethyl-3-thiosemicarbazide (1.19 g, 10 mmol) and trimethyl orthobenzoate (3.6 g, 20 mmol) in 95% EtOH (15 mL). The reaction mixture was stirred at room temperature for 1.5 h, heated at reflux for 1.5 h, and allowed to cool. The solvent was evaporated under

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reduced pressure to near dryness to yield a crude white product; this product was collected by filtration, washed well with petroleum ether, and recrystallized from 95% EtOH to afford 0.65 g (30%) of 8: mp 173–175 °C; IR (KBr) 3200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9–7.5 (m, 5, arom H), 7.6 (br s, 1, NH), 3.5 (q, 2, CH_2), 1.3 (t, 3, CH_3).

Method B. A solution of 2-acetamido-5-phenyl-1,3,4-thiadiazole[21] (0.2 g, 1 mmol) in THF (15 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (0.08 g, 2 mmol) in THF (15 mL) at 0 °C. The reaction mixture was heated at reflux for 3 h and cooled to 0 °C and the excess $LiAlH_4$ destroyed by the successive dropwise addition of H_2O (1 mL), 15% aqueous NaOH (1.5 mL), and H_2O (3 mL). The mixture was filtered, the filtrate was dried ($MgSO_4$) and evaporated to dryness to afford 0.09 g (40%) of 8, mp 173–174 °C after recrystallization from 95% EtOH. Anal. ($C_{10}H_{11}N_3S$) C, H, N.

Anhydro-2-phenyl-6,8-diethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium Hydroxide (3d). Bis(2,4,6-trichlorophenyl)ethylmalonate (0.39 g, 0.8 mmol) and 8 (0.2 g, 0.8 mmol) were heated, neat, at 160 °C until a clear melt resulted (ca. 5 min). The cooled product was triturated with anhydrous Et_2O (20 mL) and collected by filtration. Recrystallization from *i*-PrOH yielded 0.17 g (98%) of **3d** as pale yellow crystals: mp 233–235 °C; IR (KBr) 1685, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.0–7.6 (m, 5, arom H), 4.25 (q, 2, CH_2), 2.6 (q, 2, NCH_2), 1.6 (t, 3 CH_3), 1.1 (t, 3, NCH_2CH_3). Anal. ($C_{15}H_{15}N_3SO_2$) C, H, N.

Biochemical Assay. Inhibition of binding of 1 nM [^3H]-cyclohexyladenosine (New England Nuclear Corp.) to A_1-adenosine receptors in rat cerebral cortical membranes was assayed as described.[14] The K_D for [^3H]cyclohexyladenosine was about 1 nM. Inhibition of binding by a range of concentrations of each compound was assessed in triplicate in one to three separate experiments. Inhibition of 2-chloroadenosine-stimulated accumulation of cAMP in [^3H]adenine-labeled guinea pig cerebral cortical slices was determined essentially as described.[14] In addition, 10 μg/mL of adenosine deaminase was present in final incubations to eliminate contributions from endogenous adenosine and 30 μM rolipram [4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone, ZK 62711, Schering AG, West Berlin] was present in final incubations to inhibit phosphodiesterases. The EC_{50} of 2-chloroadenosine was approximately 7 μM. Inhibition of the response to 15 μM 2-chloroadenosine by a range of concentrations of each compound was determined in triplicate in one to two separate experiments. K_i values can be calculated from the observed IC_{50} values (Table I) by using the equation: $K_i = IC_{50}/1 + [\text{adenosine analogue}]/K_D$ or EC_{50} of the adenosine analogue.

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Registry No. **1a**, 58-55-9; **1b**, 5169-95-9; **1c**, 31542-62-8; **1d**, 31542-68-4; **1e**, 58-08-2; **1f**, 961-45-5; **2a**, 91265-82-6; **2b**, 91265-80-4; **2c**, 91265-83-7; **2d**, 91265-84-8; **2e**, 91280-59-0; **2f**, 91265-85-9; **2g**, 91265-86-0; **3a**, 39456-06-9; **3b**, 91265-87-1; **3c**, 53528-96-4; **3d**, 91265-81-5; **3e**, 53528-87-3; **3f**, 91265-88-2; **3g**, 53528-93-1; **3h**, 91265-89-3; **4a**, 91265-90-6; **4b**, 91265-91-7; **4c**, 91265-92-8; **4d**, 91265-93-9; **5**, 91265-76-8; **6**, 91265-77-9; **7**, 91265-78-0; **8**, 91265-79-1; 2-(*n*-propylamino)thiazole, 78508-32-4; bis(2,4,6-trichlorophenyl) *n*-propylmalonate, 77427-41-9; carbon suboxide, 12795-06-1; 2-(ethylamino)-1,3,4-thiadiazole, 13275-68-8; 4-ethyl-3-thiosemicarbizide, 13431-34-0; trimethyl orthobenzoate, 707-07-3; 2-acetamido-5-phenyl-1,3,4-thiadiazole, 28898-88-6; bis(2,4,6-trichlorophenyl) ethylmalonate, 15781-72-3.

Synthesis of a Tricyclic Aphidicolin Analogue That Inhibits DNA Synthesis in Vitro

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We have hypothesized that the biological activity of the antiviral antitumor diterpene aphidicolin requires a specific stereochemical relationship between two rigidly held hydroxyl groups on the α face of the molecule. The complex tetracyclic carbon skeleton is not necessary but appears to serve only as a framework on which to hold the hydroxyls. In support of this theory, we have prepared a simple tricyclic triol analogue (**7**) whose activity approaches that of the natural product in inhibiting in vitro DNA synthesis.

Aphidicolin (**1**), a diterpenoid tetrol produced by the mold *Cephalosporium aphidicola* Petch,[2] has provoked wide interest in recent years owing to its striking biological activity; more than 100 publications have appeared in the last 5 years reporting its use in biochemical studies. For example, aphidicolin shows marked activity against herpes virus, both in vitro and in the rabbit eye.[3,4] In addition, aphidicolin possesses considerable antitumor activity in the C6 mouse colon and B16 mouse melanosarcoma screens[5] and has been shown to inhibit the growth of leukemic T- and B-lymphocytes.[6] These biological properties, together with the unusual structure of aphidicolin, have also occasioned much activity among synthetic organic chemists. Six different total syntheses of the natural product have been recorded,[7–12] but there has

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