1,2,5-Thiadiazole Analogues of Aceclidine as Potent m_1 Muscarinic Agonists

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The acetyl group of the muscarinic agonist aceclidine **4** was replaced by various 1,2,5-thiadiazoles to provide a new series of potent m_1 muscarinic agonists **17** and **18**. Optimal m_1 muscarinic agonist potency was achieved when the 1,2,5-thiadiazole substituent was either a butyloxy, **17d**, or butylthio, **18d**, group. Although 1,2,5-oxadiazole **37** and pyrazine **39** are iso-π-electronic with 1,2,5-thiadiazole **17d**, both analogues were substantially less active than **17d**. Compounds with high muscarinic affinity and/or m_1 muscarinic agonist efficacy were also obtained when the 3-oxyquinuclidine moiety of **17d** or **18c** was replaced by ethanolamines, hydroxypyrrolidines, hydroxyazetidine, hydroxyisotropanes, or hydroxyazanorbornanes. The structure−activity data support the participation of the oxygen or sulfur atom in the substituent on the 1,2,5-thiadiazole in the activation of the m_1 receptor. Several of these new 1,2,5-thiadiazoles have m_1 agonist efficacy, potency, and selectivity comparable to those of xanomeline **2** in the muscarinic tests investigated.

Introduction

The cholinergic deficits observed in Alzheimer's disease have generated several different strategies for treating the symptoms of this disease through enhancement of cholinergic neurotransmission.[1−7] Among these approaches, the development of subtype-selective m_1 receptor agonists for the treatment of Alzheimer's disease has been the focus of many research efforts.[8−14] Analogues of arecoline **1** and related compounds in which the ester group of arecoline has been replaced by a hydrolytically stable heterocycle have been thoroughly investigated as potentially m_1-subtype-selective muscarinic agonists.[10,13,15−18] Xanomeline **2** is a noteworthy product of these strategies in which the (hexyloxy)-1,2,5-thiadiazole group that replaces the ester group of arecoline confers surprising m_1 functional selectivity.[10−12]

Cursory comparison of **1** and **2** led to the observation that, at least superficially, the 1,2,5-thiadiazole could be mimicking the carbonyl group of arecoline at the receptor level while the hexyloxy group of **2** and the methoxy group of **1** could serve the same electronic and spatial functions for receptor binding and activation (Figure 1). This hypothesis was supported by our earlier computational work on **2** that clearly showed regions of high electrostatic potential over the nitrogens of the 1,2,5-thiadiazole ring that could serve as hydrogen-binding sites in the same manner as the high electrostatic potential regions about the ester carbonyl group.[13] In some sense, the natural muscarinic agonist acetylcholine **3** can be simply related to **1** by transposition of carbonyl and oxygen groups, i.e., the "reverse ester" tactic of medicinal chemistry (Figure 1). In addition, the nonquaternary, rigid analogue of **3**, aceclidine[19] **4** is a potent muscarinic agonist and the reverse esters of **4** are also potent muscarinic agonists.[20] Taken together, these observations led to the hypothesis that replacement of the ester carbonyl group of **3** or **4** by a 1,2,5-thiadiazole group could provide muscarinic agonists that would show functional m_1 subtype selectivity.

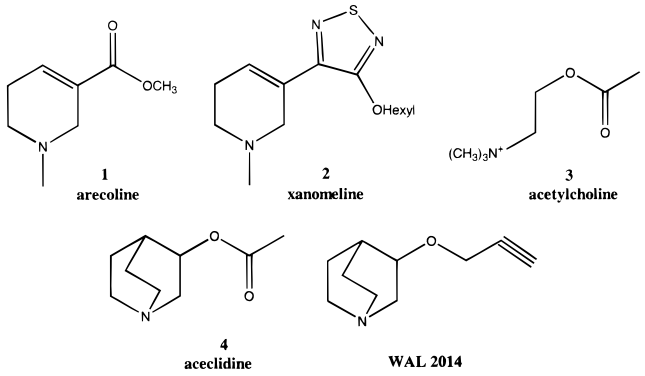

Figure 1. Is 1,2,5-thiadiazole an ester carbonyl mimic?

A goal of our present study was to synthesize 1,2,5-thiadiazole analogues of **3** and **4** and to test these analogues for biological activity in assays previously used to evaluate **2** and **4** as muscarinic agonists. The structure−activity relationship among 1,2,5-thiadiazole substituents on the resulting analogues was explored, and the ethylamine and quinuclidine moieties of these initial analogues were replaced with other azacycles to try to obtain compounds with improved m_1 efficacy, potency, and selectivity. The iso-π-electronic pyrazine and 1,2,5-oxadiazole heterocycles that have successfully replaced the 1,2,5-thiadiazole in other muscarinically active series were also investigated as potential mimics of the ester carbonyl group.[10,13,14] The observations obtained from this structure−activity relationship study led us to revise our previously proposed model of the m_1 pharmacophore.

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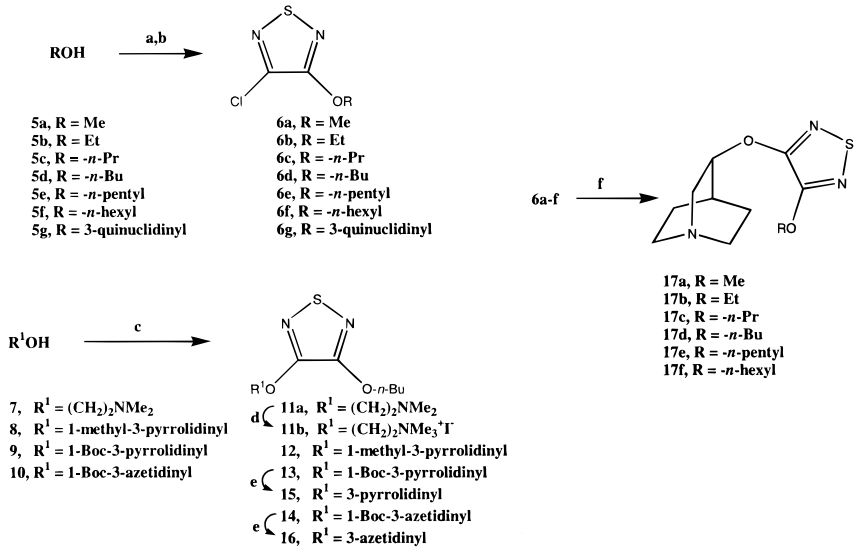
Scheme 1. Preparation of Dialkoxy-1,2,5-thiadiazoles[a]



[a] Reagents: (a) cyanogen, TEA; (b) S_2Cl_2; (c) KO-*t*-Bu, **6d**; (d) MeI; (e) HCl; (f) KO-*t*-Bu, **5g**.

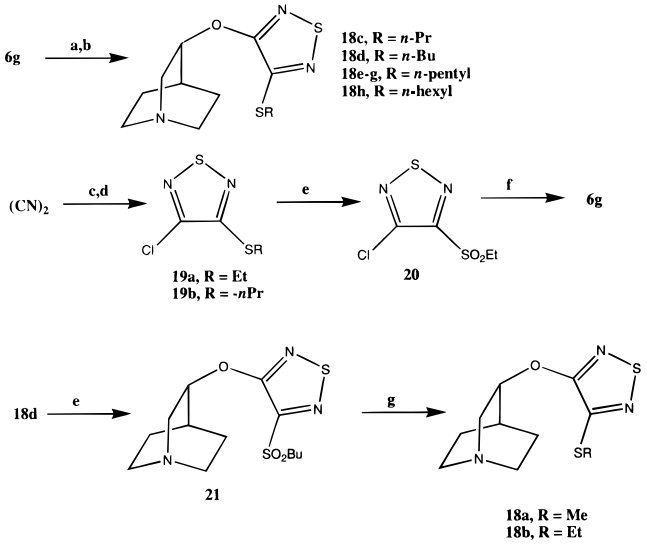
Chemistry

Although chlorine substituents on 1,2,5-thiadiazoles have been shown to participate in aromatic nucleophilic substitution reactions,[21,22] our initial attempt to prepare the parent thiadiazole analogue of **3** by treating chloro-1,2,5-thiadiazole[21] with the potassium salt of 2-dimethylamino alcohol in THF only decomposed the thiadiazole. In our experience, unsubstituted 1,2,5-thiadiazoles are often more sensitive to decomposition by base than are substituted thiadiazoles. Because alkoxythiadiazole derivatives in the **2** series had been shown to have high affinities for muscarinic receptors[10] and substituents on the 1,2,5-thiadiazole ring were anticipated to stabilize the heterocyle toward base, the alkoxy-1,2,5-thiadiazole analogues of **3** became the alternative target in our study. Treating alcohols **5a**−**g** with cyanogen in the presence of triethylamine and oxidizing the intermediate cyano imidates with sulfur monochloride gave the respective thiadiazoles **6a,b**[21] and **6c**−**g** in good to moderate yield (Scheme 1). (Butyloxy)-1,2,5-thiadiazole derivatives **11a** and **12**−**14** were obtained by treating **6d** with the appropriate potassium salt of **7**−**10** in THF (Scheme 1). The quaternary salt **11b** was obtained by treating **11a** with iodomethane. The N-protected thiadiazoles **13** and **14** were not fully characterized before being converted to the respective free amines **15** and **16** with acid. The homologous series of thiadiazoles **17a**−**f** were obtained by treating the potassium salt of **5g** with **6a**−**f,** respectively.

We had previously shown that chloro-1,2,5-thiadiazoles can be converted to (alkylthio)-1,2,5-thiadiazoles by treatment with sodium sulfide followed by alkylation of the intermediate mercapto-1,2,5-thiadiazole with alkyl halides.[10] A similar sulfurization and alkylation route using **6g** provided **18c**−**h** (Scheme 2). While this reaction sequence relatively efficiently introduced alkylthio substituents onto 1,2,5-thiadiazoles, the unpleasant isolation procedures used in the synthesis of **6g** and the modest yields led us to develop alternative convergent approaches to these alkylthio analogues.

Cyanogen reacted with either ethanethiol or propanethiol in the presence of diethylamine to form thiol

Scheme 2. Preparation of 3-Alkoxy-4-(alkylthio)-thiadiazoles[a]

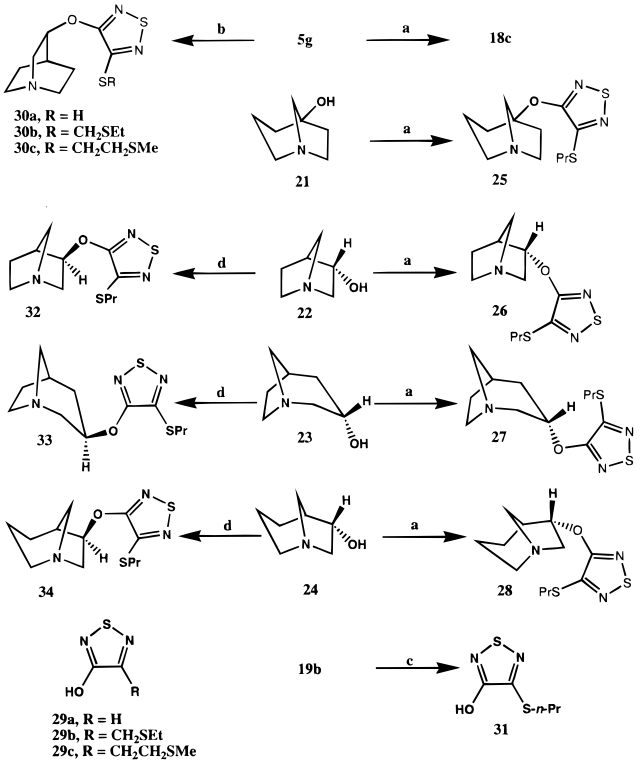


[a] Reagents: (a) Na_2S, DMF; (b) RBr; (c) DEA, RSH; (d) S_2Cl_2; (e) $KHSO_5$; (f) Li-5g; (g) NaSMe or KSEt.

imidates that were directly oxidized with sulfur monochloride to provide **19a** and **19b**, respectively, in good yield. Attempted substitution of the chloro group of **19b** using the lithium salt of 3-quinuclidinol failed to produce **18c**, suggesting that the chloro group was not an active enough leaving group. Sulfonyl groups have been found to be very effective leaving groups in aromatic nucleophilic displacements on other heterocycles, so the ethylthio group of **19a** was oxidized to the corresponding sulfone **20** using $KHSO_5$.[10,23] When **20** was treated with the lithium salt of 3-quinuclidinol, good yields of **6g** were obtained although small amounts of the corresponding ethylsulfonyl compound were also detected by NMR (Scheme 2).

The ease with which **6g** was produced from **20** suggested another alternative approach to the (alkylthio)thiadiazoles **18** that took advantage of the facile sulfonyl group displacement reaction. When **18d** was treated with $KHSO_5$ under acidic conditions, excellent

Scheme 3. Synthesis of Azabicyclic 1,2,5-Thiadiazoles[a]



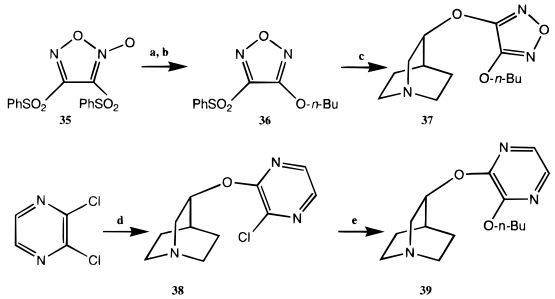
[a] Reagents: (a) KO-*t*-Bu, **19b**; **29a**−**c**, DEAD, $P(Ph)_3$; (c) NaOH, DMSO; (d) **31**, DEAD, $P(Ph)_3$.

yields of sulfone **21** were obtained if care was taken to destroy excess oxidant before neutralization of the reaction mixture in the isolation process. When **21** was treated with either the sodium or potassium salts of alkyl thiols, the sulfonyl group was easily displaced even at ambient temperature, e.g. **18a,b**. The ease of displacement of the sulfonyl group from the thiadiazole by alkylthio anions is in marked contrast to our experience with alkyl thiol anion displacements on related chloro-1,2,5-thiadiazoles where reduction of the chlorothiadiazole to the parent thiadiazole was observed rather than displacement of the chloro substituent.[10]

The development of the efficient conversion of one alkyl thiol substituent into a different alkyl thiol substituent by an intermediate sulfone led to a more thorough investigation of the direct production of **18c** from **5g** and **19b** because **18c** could serve as a key intermediate in the synthesis of additional examples of alkyl thiol **18**. The lithium salt of **5g** reacted with **19b** to give **18c** in very poor yield, but the potassium salt of **5g** as well as the potassium salts of other azacyclic alcohols **21**, **22**, **23**, and **24** did give the desired (propylthio)-1,2,5-thiadiazoles, e.g. **18c**, **25**−**28** (Scheme 3), in reasonable yield. The assignment of the endo stereochemistry for **26**, **27**, and **28** was based on the known stereochemistry of the starting azabicyclic alcohol precursor **22,**[24] **23,**[25] and **24**.[26] Very small amounts of the exo isomers were also obtained in these reactions, probably due to the presence of small amounts of exo alcohols being produced in the hydrogenation of the ketone precursors to the alcohols. Subsequently, much more efficient syntheses of these exo thiadiazole isomers were developed.

The preparation of the unsubstituted quinuclidinol **30a** also required the development of some new 1,2,5-thiadiazole chemistry (Scheme 3). Hydroxy-1,2,5-thiadiazoles are well-known to be very acidic[21] and, although not previously reported, are expected to be good substrates in the Mitsunobu reaction.[27] Addition of **5g** to a solution of **29a**, triphenylphosphine, and diethyl diazodicarboxylate gave **30a** in moderate yield. No N-alkylation product was isolated from the reaction in contrast to what is often seen when hydroxy-1,2,5-thiadiazoles are alkylated.[28] In a similar manner, **30b** and **30c** were prepared from 3-hydroxy-1,2,5-thiadiazoles **29b** and **29c**,[29] respectively.

Scheme 4. Preparation of (Quinuclidinoxy)pyrazines and -1,2,5-Oxadiazole[a]



[a] Reagents: (a) NaO-*n*-Bu; (b) $P(OMe)_3$; (c) Li-**5g**; (d) Li-3-*O*-quinuclidinyl; (e) NaO-*n*-Bu.

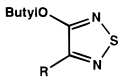
The *endo*-azabicyclic alcohols used to prepare **22**, **23**, and **24** were readily available by reduction of the corresponding ketones, but the regioisomeric exo alcohols that could be used to produce **32**−**34** by the same procedures were more difficult to obtain in adequate quantities. However, the Mitsunobu reaction generally proceeds by an S_N2 mechanism, leading to inversion at the center of attack and suggesting that **32**−**34** could be obtained from the hydroxy-1,2,5-thiadiazole **31** and the endo azabicyclic alcohols **22**−**24**. Heating **19b** with sodium hydroxide in DMSO produced the hydroxy-1,2,5-thiadiazole **31** in good yield (Scheme 3). As expected, application of the Mitsunobu reaction to **31** and the endo alcohols **22**−**24** led to the efficient preparation of **32**, **33**, and **34**, respectively (Scheme 3). The respective NMRs of **32**−**34** were identical with those obtained on small amounts of isomeric material obtained during the respective syntheses of **26**−**28** and were clearly different from the respective NMRs of **26**−**28**, permitting us to assign the expected exo stereochemistry for **32**−**34**.

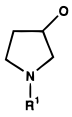
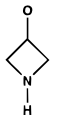
The iso-π-electronic 1,2,5-oxadiazole **37** and pyrazine **39** analogues of **17d** were prepared as shown in Scheme 4. The 3,4-bis(phenylsulfonyl)furoxan **35**[30] was treated with sodium butyl oxide followed by reduction with trimethyl phosphite[31] to give 3-(butyloxy)-4-(phenylsulfonyl)-1,2,5-oxadiazole **36.** Subsequently, **36** was converted to **37** with the lithium salt of **5g**. The lithium salt of **5g** also reacted with 3,4-dichloropyrazine to give **38**, which, in turn, was converted to (butyloxy)pyrazine **39** with sodium butyl oxide.

Biological Evaluation

Competitive radioligand binding assays using [^{3}H]-oxotremorine-M (Oxo-M) or [^{3}H]pirenzepine (Pz) in rat hippocampus membranes, where m_1 receptors predominate, were used to determine the affinities of compounds for muscarinic receptors. The affinities of the compounds for the receptors labeled by Pz were considered

Table 1. Effects of Small Azacycles on the Pharmacology of Dialkoxy-1,2,5-thiadiazoles



R	no.	receptor binding to rat brain membranes IC_{50} nM (Range)[a] [^{3}H]OxoM	[^{3}H]Pz	PI hydrolysis in A9 L-m_1 cells % max ± SEM	EC_{50}, nM[b] ± SEM	salivation in mice 10 mg/kg, i.p. Score
	11a	67.5 (51-84)	68.5 (48-89)	6.5 ± 2.8	n.d.	0
	11b	36 (23-49)	41 (33-49)	17.9 ± 2.4	n.d.	0
	12 (R^1 = Me)	42 (37-47)	44 (25-63)	13.9 ± 13.5	n.d.	0
	15 (R^1 = H)	158 (123-176)	361 (352-370)	32.7 ± 4.2	4867 ± 293	0
	16	105 (92-118)	351 (312-390)	52.6 ± 8.8	6202 ± 271	0
xanomeline		2 ± 0.5[c]	5 ± 1[c]	55 ± 2	200 ± 80	0
aceclidine		120 ± 27[c]	2427 ± 409[c]	60.5 ± 5.3	4466 ± 107	2

[a] All values are the geometric means of results from two to three separate experiments. [b] n.d. means not determined. [c] ±SEM.

to be the compounds' affinities for m_1 receptors[32] while affinities for the receptors labeled by Oxo-M were considered to be the compounds' affinities for the "agonist conformational state"[33] of the muscarinic receptor sites.

Stimulation of phosphoinositol (PI) hydrolysis in the A9 L cell line transfected with the m_1 receptor[34] is mediated through m_1 receptors.[6,7] The ability of each compound to stimulate PI hydrolysis in the A9 L−m_1 cell line was determined up to a concentration of 100 μM and the efficacy of the compound expressed as a percentage of that produced by 100 μM carbachol. The EC_{50} for PI hydrolysis was determined for each compound producing at least a 25% increase in hydrolysis. The EC_{50}'s for less efficacious compounds were not determined because of the substantial error incurred in the determinations.

The production of salivation in mice was considered to be a measure of a compound's ability to stimulate m_3 receptors.[6,7] Mice were injected with compound ip and visually scored for the appearance of salivation using a scale of 0−2.[11]

Results and Discussion

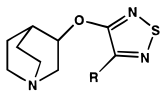
The rigid acetylcholine analogue aceclidine was chosen as the comparator for the compounds in this study because aceclidine is a tertiary amine capable of crossing the blood−brain barrier and it is much more metabolically stable than acetylcholine.[19] The (butyloxy)-1,2,5-thiadiazole analogues of acetylcholine (**11a,b**, Table 1) have higher affinity for muscarinic receptors than aceclidine, but they do not strongly stimulate PI hydrolysis in A9 L−m_1 cells. The more rigid acetylcholine analogue **12**, while improving upon the affinity of **11a**, also did not strongly stimulate PI hydrolysis. However, sterically less demanding desmethyl analogue **15**, while losing some muscarinic affinity relative to **12**, produces about half the PI stimulation seen with aceclidine. Azetidine **16** is even more efficacious than the larger and less rigid pyrrolidine **15** and almost as efficacious as aceclidine and xanomeline. The increase in m_1 efficacy with increasing rigidity, smaller ring size, and desmethylation suggests that activation of the m_1 receptor is very sterically demanding, a conclusion that is also supported by structure−activity studies in a series of 1,2,4-thiadiazole analogues of **1**.[17]

The increased conformational rigidity afforded by the quinuclidine ring in **17d** (Table 2) clearly leads to enhanced affinity for muscarinic receptors compared to monoazacyclic analogues **12**, **15**, and **16**. Despite this increased affinity, the m_1 efficacy of **17d** only approaches that of desmethylpyrrolidine **15** and is much less than that of **16.** The greater m_1 efficacy of smaller **16** compared to **17d** is again an indication of the high steric demands for m_1 receptor activation and is clearly in contrast to the demands for high affinity receptor binding. However, the need for conformationally rigid molecules that maintain important pharmacophoric groups in a proper alignment to achieve potent muscarinic receptor activation has been demonstrated for several series of heterocyclic arecoline analogues.[20,35] These precedents, the high affinity of **17d** for muscarinic receptors, and the high potency of **17d** at m_1 receptors caused us to focus our efforts on the quinuclidine series **17** rather than the monocyclic amine series.

Both **37** and **39**, the iso-π-electronic derivatives of **17d**, have much less affinity for muscarinic receptors than **17d**, and neither is efficacious in the A9 L−m_1 cells (Table 3). The relative order of the affinity of these three compounds for muscarinic Oxo-M receptors, **17d** > **39** > **37**, is the same as that previously observed in a series of tetrahydropyridyl muscarinic agonists[21] where the oxadiazoles diminished affinity was attributed to the relatively lower magnitude of electrostatic potential over the potential hydrogen-bonding sites proximal to the nitrogens of the heterocycles. The lower affinity of the pyrazine analogue in that study was attributed to increased deleterious steric interactions resulting from the larger size of the pyrazine ring relative to the 1,2,5-thiadiazole ring. The relative order of affinity in the present study is consistent with those previous explanations and suggests that the two series share a similar binding motif.

The substituent on the 1,2,5-thiadiazole in the **17** series has a marked effect on both muscarinic affinity and m_1 efficacy in A9 L−m_1 cells (Table 2), and the structure−activity relationships for muscarinic affinity resemble those seen with the 1,2,5-thiadiazole−tetrahydropyridines (TZTP) of which **2** is a member where Oxo-M affinity maximized with the butyloxy analogue.[10] A major difference between the TZTP series and the present quinuclidine **17** series can be seen with the unsubstituted thiadiazole **30a**. Derivative **30a** has relatively poor affinity and efficacy at muscarinic receptors while in the TZTP series the unsubstituted thiadiazole has affinity for Oxo-M receptors higher than that of **2** or **1** and also stimulated m_1 receptors in the rabbit vas deferens, although, not as robustly as **2** or **1**. Aceclidine has almost 4-fold higher affinity for Oxo-M receptors than **30a** and produces 60.5% stimulation of PI hydrolysis while **30a** produces none. Taken together,

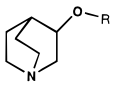
Table 2. Pharmacological Data for the (1-Azabicyclo[2.2.2]octan-3-yloxy)-1,2,5-thiadiazoles

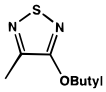
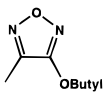
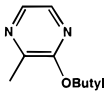


		receptor binding to rat brain membranes IC_{50} nM (range)[a]		PI hydrolysis in A9 L−m_1 cells		salivation in mice
no.	R	[^{3}H]OxoM	[^{3}H]Pz	% max ± SEM	IC_{50}, nM[b] ± SEM	10 mg/kg, ip, score
6g	Cl	98.5 (73−124)	63.5 (41−86)	2.0 ± 0.2	n.d.	0
17a	OMe	201 (161−240)	155 (126−183)	1.7 ± 0.6	n.d.	0
17b	OEt	20 (17−23)	65.5 (32−99)	1.6 ± 0.5	n.d.	0
17c	O-*n*-Pr	12.5 (11−14)	44.5 (26−63)	11.0 ± 5.9	n.d.	0
17d	O-*n*-Bu	10.7 (9−13)	42.3 (27−52)	29.9 ± 4.3	102.0 ± 0.4	0.4
17e	O-*n*-pentyl	12.7 (11−15)	47.7 (25−67)	47.5 ± 11.0	164.8 ± 30.5	0
17f	O-*n*-hexyl	11.7 (6.1−18)	42 (19−85)	64.4 ± 3.9	258.9 ± 89.5	0.2
18a	SMe	17.5 (11−24)	42.5 (41−44)	3.8 ± 1.9	n.d.	0.4
18b	SEt	6.5 (4−9)	20 (18−22)	5.9 ± 1.9	n.d.	0
18c	S-*n*-Pr	2.9 (1.4−4.2)	21 (19−25)	42.5 ± 9.0	430.9 ± 36.4	0.8
18d	S-*n*-Bu	2.5 (3.8−1.3)	5 (1.9−8.1)	50.3 ± 5.3	22.3 ± 9.5	1.0
18e	S-*n*-pentyl	4.4 (1.6−9)	9 (2.3−21)	76.2 ± 11.8	433.3 ± 26.6	0.6
18f	(*S*)-S-*n*-pentyl	6.4 (5.7−7)	3.7 (2−5.1)	15.3 ± 17.2	n.d.	0
18g	(*R*)-S-*n*-pentyl	1.4 (1−2.3)	2.7 (1.9−3.6)	68.9 ± 8.9	135.8 ± 49.5	1.2
18h	S-*n*-hexyl	7.6 (5.2−10)	28.4 (8.8−48)	89.3 ± 13.1	225 ± 16.4	1.0
30a	H	537 (200−873)	833 (819−846)	5.2 ± 3.7	n.d.	0
30b	CH_2SEt	66.5 (46−87)	79 (74−84)	1.1 ± 1.5	n.d.	0
30c	CH_2CH_2SMe	58.7 (39−96)	66 (44−84)	3.1 ± 3.9	n.d.	0
xanomeline		2 ± 0.5[c]	5 ± 1[c]	55 ± 2	200 ± 80	0
aceclidine		120 ± 27[c]	2427 ± 409[c]	60.5 ± 5.3	4466 ± 107	2

[a] All values are the geometric means of results from two to three separate experiments. [b] n.d. means not determined. [c] ± SEM.

Table 3. Effect of Heterocycle on Pharmacological Activity



		receptor binding to rat brain membranes IC_{50}, nM (Range)[a]		PI hydrolysis in A9 L-m_1 cells		salivation in mice
R	no.	[^{3}H]OxoM	[^{3}H]Pz	% max ± SEM	EC_{50}, nM[b] ± SEM	10 mg/kg, i.p. Score
	**17d**	10.7 (9-13)	42.3 (27-52)	29.9 ± 4.3	102.0 ± 0.4	0.4
	**37**	252 (31-339)	91 (56-125)	1.1 ± 0.6	n.d.	0
	**39**	38.2 (19.3-52)	22.3 (14.5-30)	2.3 ± 2.1	n.d.	0

[a] All values are the geometric means of results from two to three separate experiments. [b] n.d. means not determined.

this indicates that the 1,2,5-thiadiazole heterocycle successfully mimics the carbomethoxy group of arecoline but poorly mimics the acetyl group of aceclidine.

The increase in muscarinic affinity of the alkoxy derivatives **17a**−**d** with increasing alkyl chain length is reminiscent of the TZTP series and suggests that the alkyl chain in this series is also accessing a binding domain at the propyloxy and butyloxy chain length that leads to increased affinity. However, m_1 efficacy follows a different pattern from muscarinic affinity in the alkoxythiadiazoles **17c**−**f** but is again similar to the efficacy pattern seen in the TZTP series. Essentially no m_1 efficacy is seen with short alkoxy substituents (**17a,b**), slight efficacy with propyloxy **17c**, and continued increases in efficacy with increasing alkyl chain length through hexyloxy **17f**. The new binding domain that is accessed by the C-3−C-6 alkoxy substituents is not only important for high affinity binding but also even more important for receptor activation since efficacy increases with chain length while affinity is relatively unchanged.

Because parent thiadiazole **30a** has both poor affinity for the agonist affinity state and no efficacy at m_1 receptors compared to **4**, it appears that the 1,2,5-thiadiazole does not sufficiently mimic the carbonyl portion of an ester group. This is somewhat surprising because, as shown in Figure 2, the carbonyl group of aceclidine has an intense area of electrostatic potential over the carbonyl oxygen that is similar to that over the nitrogen proximal to the azabicyclic ring in the 1,2,5-thiadiazole ring. By contrast, the C-4−C-6 alkoxy derivatives **17d**−**f** have higher affinity for the agonist affinity state than **4** and, at least with **17f**, comparable efficacy to **4** at m_1 receptors. Clearly, the alkoxy substituents attached to the thiadiazole ring are intimately involved in conferring high affinity and efficacy through additional binding interactions with the muscarinic receptor.

Additional evidence for the importance of the side chain in conferring high muscarinic affinity and m_1 efficacy is provided by studying the alkylthio side chains in the quinuclidine series **18** (Table 2). The length of the alkylthio substituent in **18** markedly controls the affinity for the Oxo-M binding site and efficacy at m_1 receptors in the same manner as the alkoxy substituents with threshold efficacy occurring at the C-3 chain length, **18c**, maximal affinity and m_1 potency occurring at the C-4 chain length, **18d**, and maximal m_1 efficacy occurring at the C-6 chain length, **18h**.

Resolution of **18e** shows that the highest affinity for the agonist affinity state and highest m_1 efficacy resides with the *R* enantiomer **18g**. This can be contrasted with

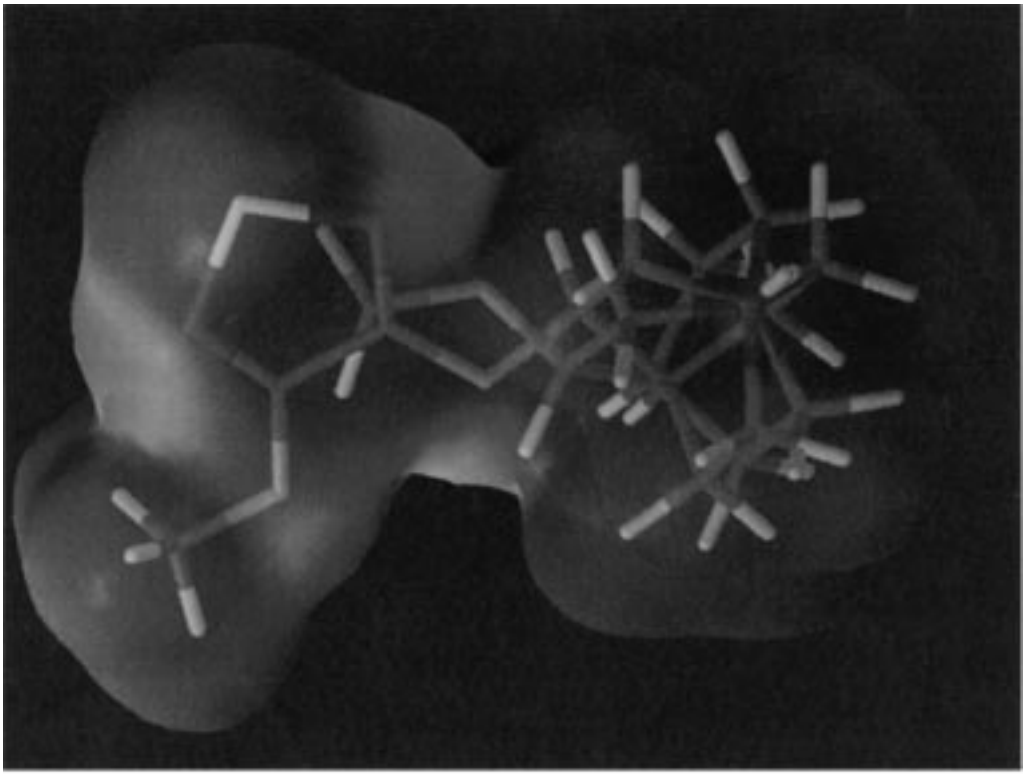


Figure 2. Overlap of electrostatic potentials in (*S*)-aceclidine and **18g** [(*R*)-**18e**].

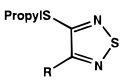
4 where the *S* enantiomer has the highest muscarinic activity.[14,19] We have previously proposed that the electrostatic potential over the nitrogen that is proximal to the azacyclic ring in **2** is involved in binding and activating the m_1 receptor, and others have proposed a similar function for the carbonyl group of **4**.[36,37] The thiadiazole ring in **18** is likely to serve a similar binding function as proposed for **2**. In addition, the tetrahydropyridine nitrogen of **2** and the quinuclidine nitrogens of both **18** and **4** are likely to bind to the same aspartate on muscarinic receptors.[38] Figure 2 shows how the high electrostatic potential regions of the carbonyl group of (*S*)-**4** and the proximal nitrogen of the thiadiazole ring of **18g** can be closely aligned by overlapping the quinuclidine ring of both compounds, rotating the quinuclidine rings along the nitrogen−apical carbon axis, and further rotating the acetyl and 1,2,5-thiadiazole groups. Such a binding arrangement could explain the different enantioselectivities of **4** and **18** where the less active isomer of **4** and **18** are unable to bind and activate muscarinic receptors because they are unable to populate the required binding conformation because of steric interactions. Interestingly, the propynyloxy ether of quinuclidine, WAL 2014 (Figure 1), another preferential m_1 agonist in clinical development,[39] shows the same enantioselectivity as **18**. A comparison of the potential binding motifs among aceclidine, WAL 2014, and the **17** and **18** series may reveal further insights into m_1 receptor activation.[40]

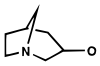
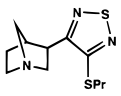
The position of the sulfur atom in the side chain of **18** is very important in binding to the agonist affinity state of the muscarinic receptor and in activating m_1 receptors. Insertion of one or two methylene groups between the sulfur atom in the side chain and the 1,2,5-thiadiazole as in **30b** and **30c**, respectively, while maintaining total side chain length produced a 20-fold decrease in Oxo-M binding site affinity compared to **17c** and an almost total loss of m_1 efficacy (Table 2). Although the methylene groups could be introducing intramolecular steric interactions that preclude populating the ideal conformation of the side chain for optimal receptor interaction, it is more likely that the sulfur atom is directly involved in binding and activating the receptor and that displacement of the sulfur atom by even one methylene unit from the 1,2,5-thiadiazole interferes with the spatial requirements for that binding and receptor activation.

The rigid framework provided by azabicyclic ring systems can control the orientation of the 1,2,5-thiadiazole and its important hydrogen-bonding centers relative to the direction of the N−H bond of the protonated nitrogen when it is complexed to a receptor. This relative orientation can be crucial to the potency and efficacy of the muscarinic agonist so a number of other azabicyclic systems were investigated in our structure−activity study utilizing (propylthio)-1,2,5-thiadiazole as the heterocycle. Muscarinic agonists that contain the azanorbornane and isotropane ring systems such as represented in Table 4, i.e., **25**−**28**, **32**−**34**, have sometimes shown more potent muscarinic activity than derivatives from those series that contain the quinuclidine ring.[14,20,35,41,42] Among these derivatives, highest m_1 efficacy is seen with the azanorbornanes **26** and **32** and the *endo*-6-hydroxyisotropane **28**, suggesting that these azabicycles permit access to the receptor-activating conformation in contrast to the other isotropane isomers studied.

A significant difference between our present studies with hydroxyazabicyclic rings and previous studies of azabicyclic bioisosteres of arecoline can be found in the relative affinities of the isomeric pairs **26** vs **32**, **27** vs **33**, and **28** vs **34**.[14,20,35,41−43] In all of our present cases,

Table 4. Effects of 1-Azabicycles on the Pharmacology of (Propylthio)-1,2,5-thiadiazoles



R	no.	receptor binding to rat brain membranes IC_{50}, nM (Range)[a] [^{3}H]OxoM	[^{3}H]Pz	PI hydrolysis in A9 L-m_1 cells % max ± SEM	EC_{50}, nM[b] ± SEM	salivation in mice 10 mg/kg, i.p. Score
	18c	2.9 (1.4-4.2)	21 (19-25)	42.5 ± 9.0	430.9 ± 36.4	0.8
	25	5.2 (1.7-8.7)	6.8 (2.5-11)	22.8 ± 1.2	n.d.	0
	26 (endo)	0.76 (0.52-1)	4.4 (4.2-4.6)	94.3 ± 1.8	1.9 ± 1.3	2[c]
	32 (exo)	5.5 (4-7)	66.7 (54-87)	82.3 ± 0.9	161.7 ± 8.7	1.8[c]
	27 (endo)	2.8 (2.7-2.9)	7 (5.6-8.3)	6.4 ± 0.3	n.d.	0.2
	33 (exo)	15 (14-16)	20 (17-22)	10 ± 11	n.d.	0
	28 (endo)	0.38 (0.31-0.45)	0.55 (0.5-0.6)	59.3 ± 5.1	1.00 ± 0.01	2[c]
	34 (exo)	179 (135-223)	33 (30-36)	2.0 ± 0	n.d.	0
	40 (endo)	5.8 ± 0.1[d,e]				
	41 (exo)	0.6 ± 0.1[d,e]				
xanomeline		2 ± 0.5[e]	5 ± 1[e]	55 ± 2	200 ± 80	0
aceclidine		120 ± 27[e]	2427 ± 409[e]	60.5 ± 5.3	4466 ± 107	2

[a] All values are the geometric means of results from two to three separate experiments. [b] n.d. means not determined. [c] Tremors were also observed. [d] Reference 52. [e] ±SEM.

the endo isomer shows much higher affinity for muscarinic receptors than does the exo isomer while in the azabicyclic arecoline bioisostere studies the exo isomer always shows much higher affinity. This is even the case for the *endo*- and *exo*-azanorbornyl-1,2,5-thiadiazoles **40** and **41** (Table 4), respectively, that lack the oxygen bridge between the azabicyclic ring and the 1,2,5-thiadiazole ring.[42] Interestingly, the affinities of **40** (endo) and **32** (exo) are very similar, and the affinities of **41** (exo) and **26** (endo) are also very similar, perhaps indicating that the two members of each of these pairs share similar pharmacophoric interactions on the muscarinic receptor. It is difficult to speculate how the two members of each pair compare conformationally in binding to the receptor without knowing the enantioselectivity of the respective azanorbornyl ring systems. This could require a nitrogen overlap and azacyclic ring rotation such as was described in Figure 2.

The affinity of the compounds in our study for muscarinic Pz receptors generally followed the same structure−activity relationship as the for the muscarinic Oxo-M receptors. In addition, the affinities for the Pz receptors was generally less than for the Oxo-M receptors with the exceptions of **6g**, **17a**, **18f**, **37**, **39**, and **34**. With the exception of **34**, the precision of the binding data may be insufficient to justify a claim that these are exceptions to the general observation that Oxo-M receptor affinity is greater than the Pz receptor affinity. However, in all of these cases the compounds lack efficacy at m_1 receptors in the A9 L cells, consistent with their poor affinity for the agonist affinity state of the muscarinic receptor. The higher affinity of these compounds for the Pz receptor relative to the Oxo-M receptor may suggest that they are binding to the m_1 antagonist receptor state.

We had previously proposed that **2** binds to the m_1 receptor through interactions with four pharmacophoric groups.[13] On the m_1 receptor, Asp[105] binds with the nitrogen of the tetrahydropyridine ring of **2**, two hydrogen-bonding groups on the receptor interact with the regions of high electrostatic potential over the nitrogens on the 1,2,5-thiadiazole ring, and a lipophilic region on the receptor binds to the lipophilic hexyl group on the side chain. While our data did not exclude the alternative binding of the electrostatic potential over the heteroatom on the side chain, we did not favor that mode of binding because of the much smaller magnitude of the electrostatic potential compared to the thiadiazole nitrogens and because of previous studies by other groups correlating muscarinic efficacy to the magnitude of the electrostatic potential over two heteroatoms in the heterocyclic ring of arecoline analogues.[44] However, further consideration of our previous data with side chains[10,13] where simple alkyl side chains had poor m_1 efficacy as well as the effects of side chains in our present study where alkyl side chains lack m_1 efficacy (**30b**,**c**) suggests that the heteroatom attached to the 1,2,5-thiadiazole in the side chain could be participating in the hydrogen-bonding interaction with the receptor either alone, in concert with the thiadiazole nitrogen, or hydrogen bonding to a different or additional site on the receptor.

Preliminary m_1 muscarinic pharmacophore generation studies using CATALYST[45] with a proprietary training set provided a pharmacophore model that illustrates how the side chain heteroatom site in **2** and **18g** may be involved in binding to and activating the m_1 receptor (Figure 3). The red hatched sphere represents a positive ionizable center, the large green hatched spheres represent hydrogen-bonding sites on the receptor, the small green hatched spheres represent hydrogen-bonding centers on **2** and **18g,** the blue cones represent hydrogen bond vectors, and the light blue spheres represent lipophilic bonding sites. This model takes into account all of our previously proposed pharmacophoric interactions except that the side chain heteroatom is involved in hydrogen bonding to the receptor instead of the alternative thiadiazole nitrogen.

Salivation in mice is considered a indication of m_3 activity that should be avoided in a selective m_1 agonist. No salivation was observed in the thiadiazole analogues of acetylcholine, **11a,b**, **12**, **15**, and **16** (Table 1), and only slight salivation was detected with the alkoxythiadiazole aceclidine analogues **17d** and **17f** (Table 2). By contrast, many of the alkythiothiadiazole analogues of aceclidine, **18a**,**c**−**e**,**h**, produced more salivation than the alkoxythiadiazole analogues but less than aceclidine. Among the isotropane analogues (Table 4) of **18c**, 3-hydroxy isomer **27** produced slight salivation and 6-hydroxy isomer **28** produced copious salivation that was also accompanied by tremors. Both azanorbornyl analogues of **18c**, **26**, and **32** (Table 4) produced copious salivation in mice accompanied by tremors.

The viability of a m_1 muscarinic agonist as a therapy will be dependent on both m_1 efficacy, m_1 potency, and m_1 selectivity. Xanomeline **2** is only a partial m_1 agonists in A9 L−m_1 cells but shows a high degree of

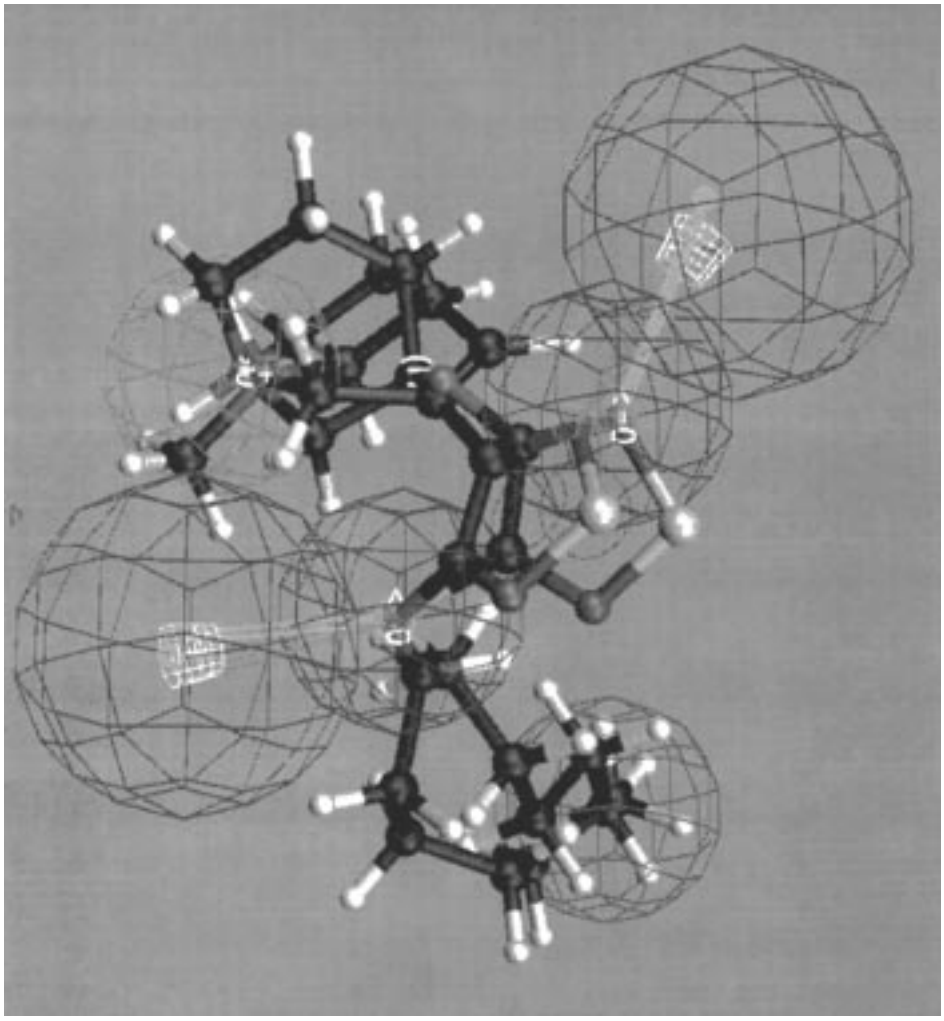


Figure 3. CATALYST m_1 pharmacophore applied to xanomeline and **18g**.

m_1 functional selectivity with a clear separation between m_1- and m_3-mediated effects such as salivation.[11,12] Only a few of the compounds in our present study show similar or superior initial efficacy, potency, and selectivity compared to **2**. Among these are alkoxythiadiazole aceclidine analogues **17e** and **17f** that show roughly comparable m_1 efficacy, potency, and selectivity to **2**. By contrast, the alkylthio derivatives **18c,d,e,g,h** produce comparable or superior m_1 efficacy and potency to **2** but show more m_3 activity as shown by their production of salivation in mice. Similarly, alternative azabicyclic derivatives **26**, **28**, and **32** are both potent and efficacious m_1 agonists but lack selectivity as shown by their production of salivation and even tremor in mice. Further structure−activity studies focusing on improvements in the m_1 efficacy, potency, and selectivity of **17e** and **17f** are in progress, and the potential for alternative therapeutic uses for these muscarinic agonists is also under investigation.

Summary

Several analogues of acetylcholine **3** and aceclidine **4** in which the acetyl function was replaced with a 1,2,5-thiadiazole had high affinity for muscarinic receptors and several were potent and efficacious m_1 agonists. The 1,2,5-thiadiazole alone did not successfully mimic the acetyl function and provide potent muscarinic ligands but the addition of an alkoxy or alkylthio substituent containing three to six carbon atoms to the 1,2,5-thiadiazole generally gave compounds that had both high affinity for muscarinic receptors and m_1 efficacy. The structure−activity data support the participation of the oxygen or sulfur atom in the alkoxy or alkylthio substituent, respectively, in the binding and activating of the m_1 receptor in contrast to the previously proposed activating model in which the nitrogen in the thiadiazole ring distal to the azacycle was a major participant in the binding and activation. Both xanomeline and the highly active members of the compounds in this study fit the newly proposed model. The (pentyloxy)- (**17e**) and (hexyloxy)- (**17f**) (quinuclidinyloxy)-1,2,5-thiadiazoles had m_1 efficacy, potency, and selectivity comparable to those of xanomeline. In general, azacycles attached to alkoxy- or (alkylthio)-1,2,5-thiadiazoles through an oxygen bridge provide promising new series of muscarinic agonists, some of which could be superior to xanomeline as functionally selective m_1 agonists.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. A Waters PrepLC/500A using PrepPAK-500 silica gel cartridges, with the solvents specified, were used for HPLC separations. A Harrison Research Chromatotron model 7924T using Analtech precast silica gel rotors, with the solvents specified, were used for radial chromatography. Merck F254 silica gel plates were used for TLC. All reactions, exclusive of extraction procedures, were conducted under a

Table 5. Physical Data for the 1,2,5-Thiadiazoles

no.	formula[a]	mp/bp, °C	cryst solv
6c	$C_5H_7ClN_2OS$	103−106 at 15 mmHg	
6d	$C_6H_9ClN_2OS$	120−125 at 14 mmHg	
6e	$C_7H_{11}ClN_2OS$	129−135 at 9 mmHg	
6f	$C_8H_{13}ClN_2OS$	114−118 at 7 mmHg	
6g	$C_9H_{12}ClN_3OS{\cdot}C_2H_2O_4$	154−156	MeOH−EtOAc
11a	$C_{10}H_{19}N_3O_2S{\cdot}HCl$	97−98°	EtOAc
11b	$C_{11}H_{22}IN_3O_2S$	137−138	EtOAc
12	$C_{11}H_{19}N_3O_2S{\cdot}HCl$	157−158	EtOAc
15	$C_{10}H_{17}N_3O_2S{\cdot}HCl$	157−158	EtOAc
16	$C_9H_{15}N_3O_2S{\cdot}HCl$	167−168.5	2-propanol
17a	$C_{10}H_{15}N_3O_2S{\cdot}HCl$	197−198	MeOH−EtOAc
17b	$C_{11}H_{17}N_3O_2S{\cdot}HCl$	212−213	2-propanol
17c	$C_{12}H_{19}N_3O_2S{\cdot}HCl$	218−219	2-propanol
17d	$C_{13}H_{21}N_3O_2S{\cdot}HCl$	204−205	$CHCl_3$−EtOAc−ether
17e	$C_{14}H_{23}N_3O_2S{\cdot}HCl$	171−172	EtOAc
17f	$C_{15}H_{25}N_3O_2S{\cdot}HCl$	165−166	EtOAc
18a	$C_{10}H_{15}N_3OS_2{\cdot}HCl$	228 dec	acetone
18b	$C_{11}H_{17}N_3OS_2{\cdot}HCl$	226−228	acetone
18c	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	174−176	$CHCl_3$−EtOAc−ether
18d	$C_{13}H_{21}N_3OS_2{\cdot}HCl$	151−153	$CHCl_3$−EtOAc−ether
18e	$C_{14}H_{23}N_3OS_2{\cdot}HCl$	186−187	$CHCl_3$−EtOAc−ether
18f	$C_{14}H_{23}N_3OS_2{\cdot}HCl$	194−195	$CHCl_3$−EtOAc−ether
18g	$C_{14}H_{23}N_3OS_2{\cdot}HCl$	195−196	$CHCl_3$−EtOAc−ether
18h	$C_{15}H_{25}N_3OS_2{\cdot}HCl$	165−167	$CHCl_3$−EtOAc−ether
19a	$C_4H_5ClN_2S_2$	91−96 at 3 mmHg	
19b	$C_5H_7ClN_2S_2$	84−94 at 1.5 mmHg	
21	$C_{13}H_{21}N_3O_3S_2{\cdot}HCl$	180−181	2-propanol
25	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	183−185	$CHCl_3$−ether
26	$C_{11}H_{17}N_3OS_2{\cdot}1.5C_2H_2O_4$	136−137	acetone
28	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	193−195	EtOAc
27	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	186−187.5	acetone−EtOAc
30a	$C_9H_{15}N_3O_2S{\cdot}HCl$	240 dec	2-propanol
30b	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	135−136	EtOAc
30c	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	177−178	acetone
31	$C_5H_8N_2OS_2$	84−85	heptane
32	$C_{11}H_{17}N_3OS_2{\cdot}HCl$	176−177	$CHCl_3$−ether
33	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	156−157	EtOAc
34	$C_{12}H_{19}N_3OS_2{\cdot}HCl$	189−190	acetone−EtOAc
37	$C_{13}H_{21}N_3O_3{\cdot}HCl$	186−188	$CHCl_3$−EtOAc−ether
38	$C_{11}H_{14}Cl\ N_3O$	112.5−114	ether
39	$C_{15}H_{23}N_3O_2{\cdot}HCl$	150−151	EtOAc

[a] All compounds were correctly analyzed for C, H, and N, ±0.4%.

nitrogen atmosphere. A General Electric QE-300 spectrometer (300 MHz) was employed for ^{1}H NMR measurements using the solvents described. Chemical shifts in ppm are reported with reference to $CDCl_3$ at 7.26 ppm or CD_3OD at 4.80 ppm. Analytical data, melting points, and crystallization solvents are reported in Table 5. No particular attempt was made to optimize reaction conditions for most of the reactions described.

3-Chloro-4-(pentyloxy)-1,2,5-thiadiazole, 6e. A solution of 1-pentanol (60 mL, 0.55 mol) and triethylamine (1.5 mL) was cooled to −8 °C, and cyanogen (36 g, 0.69 mol) was slowly bubbled through the solution while the temperature was maintained below 2 °C. After the addition, the reaction mixture was stirred for another hour at −5 °C and then added dropwise to a solution of DMF (180 mL) and sulfur monochloride (120 mL, 1.5 mol) that was maintained at a temperature below 10 °C. After the addition, the cooling source was removed and the reaction mixture was stirred overnight. The reaction mixture was cooled in an ice−water bath and the excess sulfur monochloride destroyed by careful dropwise addition of H_2O such that the temperature did not exceed 30 °C. The liquid was decanted from the semisolid sulfur precipitant and the sulfur residue triturated with hexane. The aqueous fraction was extracted with hexane, and the combined extracts and triturants were washed with H_2O, aqueous $NaHCO_3$, and brine. After drying, the solvent was evaporated and the yellow liquid residue was distilled to give **6e** as a clear liquid (92.7 g, 82%): ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t), 1.41−1.45 (4H, m), 1.75−1.95 (2H, m), 4.44 (2H, t).

The remaining 3-chloro-4-*n*-alkoxy-1,2,5-thiadiazoles were prepared in a similar fashion.

3-Chloro-4-propyloxy-1,2,5-thiadiazole, 6c. From 1-propanol (40 mL, 0.536 mol), cyanogen (36 g, 0.69 mol), and S_2Cl_2 (120 mL, 1.5 mol) was obtained a clear liquid (79.9 g, 84%): ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t), 1.87 (2H, q), 4.41 (2H, t).

3-Chloro-4-(butyloxy)-1,2,5-thiadiazole, 6d. From 1-butanol (92 mL, 1 mol), cyanogen (58 g, 1.12 mol), and S_2Cl_2 (230 mL, 2.87 mol) was obtained a clear liquid (153 g, 79%): ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t), 1.51 (2H, q), 1.85 (2H, m), 4.45 (2H, t).

3-Chloro-4-(hexyloxy)-1,2,5-thiadiazole, 6f. From 1-hexanol (102.2 g, 1 mol), cyanogen (50 g, 0.96 mol), and S_2Cl_2 (243 mL, 3.03 mol) was obtained a clear liquid (200 g, 94%): ^{1}H NMR ($CDCl_3$) δ 0.88−0.93 (3H, m), 1.31−1.48 (6H, m), 1.79−1.86 (2H, m), 4.41 (2H, t).

(±)-3-Chloro-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Ethanedioate, 6g. A mixture of (±)-1-azabicyclo-[2.2.2]octan-3-ol (12.7 g, 0.1 mol), triethylamine (0.3 mL), and $CHCl_3$ (150 mL) was cooled to 5 °C, and cyanogen (7.25 g, 0.139 mol) was bubbled into the mixture. The reaction mixture was stirred another hour and then allowed to come to ambient temperature overnight. The solvent was evaporated, the residue dissolved in DMF (20 mL), and the solution added dropwise to a solution of sulfur monochloride (47.3 g, 0.35 mol) in DMF (30 mL) that was cooled in an ice−water bath. After addition, the cooling bath was removed, the reaction mixture was stirred 5 h, the reaction mixture was again cooled in ice−water, and excess sulfur monochloride was destroyed by careful addition of H_2O. The mixture was diluted with water (300 mL) and the aqueous solution decanted from the sulfur

residue. The sulfur residue was triturated with H_2O, and the combined aqueous solutions were evaporated to a small volume (150 mL). The solution was washed with ether and then made basic with 50% NaOH while the temperature was maintained below 30 °C. The mixture was extracted with $CHCl_3$, the extracts were dried, and the solvents were thoroughly evaporated. The residue was suspended in ether, dried, and filtered, and the solvent was evaporated to give the free base of **6g** (18.1 g, 75%) as a yellow oil that slowly solidified. A 1.1 g sample was converted to an ethanedioate salt (0.39 g): ^{1}H NMR (CD_3OD) δ 1.87−2.37 (4H, m), 2.68 (1H, m), 3.28−3.55 (5H, m), 3.89 (1H, q), 5.34 (1H, m).

(±)-3-(Butyloxy)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 17d. A solution of 1-azabicyclo-[2.2.2]octan-3-ol (2.2 g, 0.0168 mol) in THF (25 mL) was cooled in ice water and treated with potassium *tert*-butoxide (1.94 g, 0.0173 mol). After 10 min, **6d** (1.86 g, 0.0084 mol) was added, the cooling source was removed, and the reaction mixture stirred for 3.5 h. The solvent was evaporated, the residue acidified with 1 N HCl, and the mixture extracted with ether. The aqueous solution was made basic and extracted with EtOAc. The extracts were washed with H_2O and dried, and the solvent was evaporated to give a clear oil that was purified by radial chromatography, eluting with 10% EtOH−1% NH_4OH−$CHCl_3$ (2.0 g HCl salt, 37%): ^{1}H NMR (CD_3OD) δ 0.97 (3H, t), 1.48 (2H, m), 1.77 (2H, m), 1.87−2.34 (4H, m), 2.61 (1H, m), 3.26−3.54 (5H, m), 3.88 (1H, m), 4.42 (2H, t), 5.30 (1H, m).

Compounds **11a**, **12**, and **17a**−**f** were prepared in a manner analogous to **17d**.

3-(Butyloxy)-4-(2-(dimethylamino)ethoxy)-1,2,5-thiadiazole Hydrochloride, 11a. From 2-(dimethylamino)ethanol (0.67 g, 0.0075 mol), potassium *tert*-butoxide (0.85 g, 0.0075 mol), and **6d** (1.1 g, 0.005 mol) was obtained **11a** (0.94 g, 67%): ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t), 1.44 (2H, m), 1.75 (2H, m), 2.92 (6H, s), 3.53 (2H, m), 4.37 (2H, t), 4.95 (2H, m).

(±)-3-(Butyloxy)-4-((1-methyl-3-pyrrolidinyl)oxy)-1,2,5-thiadiazole Hydrochloride, 12. From (±)-1-methyl-3-pyrrolidinol (0.6 g, 0.0059 mol), potassium *tert*-butoxide (0.6 g, 0.0054 mol), and **6d** (0.93 g, 0.0042 mol) was obtained **12** (0.7 g, 57%): ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t), 1.43 (2H, m), 1.80 (2H, m), 2.38−2.7 (2H, m), 2.96 (3H, d), 3.15 (2H, m), 3.92 (1H, m), 4.28 (1H, m), 4.39 (2H, t), 5.51 (1H, m).

(±)-3-Methoxy-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 17a. From (±)-1-azabicyclo-[2.2.2]octan-3-ol (1.36 g, 0.0104 mol), potassium *tert*-butoxide (1.16 g, 0.0104 mol), and 3-chloro-4-methoxy-1,2,5-thiadiazole[35] (1.77 g, 0.0107 mol) was obtained **17a** (0.85 g, 30%): ^{1}H NMR (CD_3OD) δ 1.86−2.32 (4H, m), 2.62 (1H, m), 3.26−3.52 (5H, m), 3.88 (1H, q), 4.1 (3H, s), 5.28 (1H, m).

(±)-3-Ethoxy-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 17b. From (±)-1-azabicyclo-[2.2.2]octan-3-ol (0.75 g, 0.0059 mol), potassium *tert*-butoxide (0.66 g, 0.0059 mol), and 3-chloro-4-ethoxy-1,2,5-thiadiazole[35] (0.86 g, 0.0048 mol) was obtained **17b** (0.47 g, 33%): ^{1}H NMR ($CDCl_3$) δ 1.46 (3H, t), 1.8−2.4 (4H, m), 2.71 (1H, m), 3.37 (5H, m), 3.76 (1H, q), 4.47 (2H, q), 5.1 (1H, m).

(±)-3-(Propyloxy)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 17c. From (±)-1-azabicyclo-[2.2.2]octan-3-ol (1.1 g, 0.0087 mol), potassium *tert*-butoxide (0.9 g, 0.008 mol), and **5c** (1.06 g, 0.0059 mol) was obtained **17c** (0.59 g, 33%): ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t), 1.73−2.41 (6H, m), 2.72 (1H, m), 3.38 (5H, m), 3.77 (1H, q), 4.35 (2H, t), 5.1 (1H, m).

(±)-3-(Pentyloxy)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 17e. From (±)-1-azabicyclo-[2.2.2]octan-3-ol (0.75 g, 0.0059 mol), potassium *tert*-butoxide (0.66 g, 0.0059 mol), and **6e** (0.83 g, 0.004 mol) was obtained **17e** (0.75 g, 56%): ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t), 1.35 (4H, m), 1.68−2.41 (6H, m), 2.72 (1H, m), 3.38 (5H, m), 3.77 (1H, q), 4.4 (2H, t), 5.1 (1H, m).

(±)-3-(Hexyloxy)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 17f. From (±)-1-azabicyclo-[2.2.2]octan-3-ol (2.2 g, 0.0168 mol), potassium *tert*-butoxide (1.88 g, 0.0168 mol), and **6f** (2.2 g, 0.01 mol) was obtained **17f** (1.75 g, 50%): ^{1}H NMR (CD_3OD) δ 0.92 (3H, m), 1.28−1.52 (6H, m), 1.73−2.34 (6H, m), 2.63 (1H, m), 3.24−3.52 (5H, m), 3.87 (1H, q), 4.41 (2H, t), 5.27 (1H, m).

(±)-3-(Butyloxy)-4-(3-pyrrolidinyloxy)-1,2,5-thiadiazole Hydrochloride, 15. An ice-cooled solution of potassium-*tert*-butoxide (0.3 g, 0.0027 mol) in THF (25 mL) was treated with 1-(*tert*-butylcarbamoyl)-3-hydroxypyrrolidine (0.5 g, 0.0027 mol). After 10 min, **6d** (0.52 g,0.0027 mol) was added, the cooling source was removed, and the reaction mixture was stirred for 2.5 h. The solvent was evaporated, the residue treated with ice−water, and the mixture extracted with ether. The extracts were washed with brine and dried, and the solvent was evaporated. The residue was dissolved in ether (50 mL) and treated with a slow stream of HCl for 5 min. After being stirred overnight, the reaction mixture was extracted with cold water. The aqueous fraction was washed with ether, made basic, and extracted with EtOAc. The extracts were washed with brine and dried, and the solvent was evaporated to give a clear oil. The HCl salt **15** crystallized from EtOAc (0.42 g, 55%): ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t), 1.46 (2H, m), 1.81 (2H, m), 2.28−2.34 (2H, m), 3.4−3.9 (4H, m), 4.4 (2H, t), 5.48 (1H, m).

3-(Butyloxy)-4-(3-azetidinyloxy)-1,2,5-thiadiazole Hydrochloride, 16. This compound was prepared in the same manner as **15** from 1-(*tert*-butylcarbamoyl)-3-hydroxyazetidine (1.1 g, 0.006 mol), potassium *tert*-butoxide (0.67 g, 0.006 mol), and **6d** (0.82 g, 0.0042 mol) to give **16** (0.77 g, 69%): ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t), 1.48 (2H, m), 1.8 (2H, m), 4.2−4.62 (6H, m), 5.48 (1H, m).

3-(Butyloxy)-4-(2-(trimethylamino)ethoxy)-1,2,5-thiadiazole Iodide, 11b. A suspension of **11a** (0.5 g, 0.0018 mol) in ice−water was treated with 1 N NaOH (2 mL) and the solution extracted with EtOAc. The extracts were dried and treated with CH_3I (0.3 mL), and the reaction mixture was stirred overnight. The solid precipitant was collected, washed with EtOAc, and dried to provide **11b** (0.64 g, 91%): ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t), 1.43 (2H, m), 1.76 (2H, m), 3.63 (9H, s), 4.32 (2H, m), 4.39 (2H, t), 4.9 (2H, m).

(±)-3-(Pentylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18e. A solution of **6g** free base (1.67 g, 0.0068 mol) in DMF (25 mL) was treated portionwise with freshly ground, flaked $Na_2S{\cdot}9H_2O$ (1.8 g, 0.0075 mol). After 1 h, 1-bromopentane (1.53 g, 0.010 mol) was added and the reaction mixture stirred overnight. The solvent was evaporated, the residue was acidified with 1 N HCl, and the mixture was extracted with ether. The aqueous fraction was made basic and extracted with ether. The extracts were dried, and the solvent was evaporated to give a straw-colored liquid that was purified by radial chromatography, eluting with 5% EtOH−0.5% NH_4OH−$CHCl_3$ (HCl salt, 1.07 g, 45%): ^{1}H NMR (CD_3OD) δ 0.93 (3H, m), 1.4 (4H, m), 1.75 (2H, m), 1.88−2.33 (4H, m), 2.63 (1H, m), 3.2−3.54 (7H, m), 3.88 (1H, q), 5.32 (1H, m).

Compounds **18c,d,f**−**h** were prepared by the same procedure as **18e**.

(±)-3-(Propylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18c. From the free base of **6g** (1.67 g, 0.0068 mol), flaked $Na_2S{\cdot}9H_2O$ (1.8 g, 0.0075 mol), and 1-bromopropane (1.25 g, 0.01 mol) was obtained **18c** (1.28 g, 58%): ^{1}H NMR (CD_3OD) δ 1.06 (3H, t), 1.8 (2H, m), 1.88−2.33 (4H, m), 2.64 (1H, m), 3.25 (2H, t), 3.28−3.54 (5H, m), 3.89 (1H, m), 5.32 (1H, m).

(±)-3-(Butylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18d. From the free base of **6g** (1.8 g, 0.0073 mol), flaked $Na_2S{\cdot}9H_2O$ (1.94 g, 0.0081 mol), and 1-iodobutane (2 g, 0.011 mol) was obtained **18d** (1.82 g, 74%): ^{1}H NMR (CD_3OD) δ 0.95 (3H, t), 1.48 (2H, m), 1.73 (2H, m), 1.88−2.33 (4H, m), 2.64 (1H, m), 3.22−3.53 (7H, m), 3.89 (1H, m), 5.32 (1H, m).

(*S*)-3-(Pentylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18f. From the free base of **(*S*)-6g** (1.7 g, 0.0069 mol) (prepared by method B using (*S*)-1-azabicyclo[2.2.2]octan-3-ol,[46] see below), flaked $Na_2S{\cdot}9H_2O$

(1.83 g, 0.0076 mol), and 1-bromopentane (1.58 g, 0.0105 mol) was obtained **18f** (1.0 g, 41%): $[\alpha]_D$ 25.41 (EtOH, c = 1, 20 °C); ^{1}H NMR (CD_3OD) δ 0.93 (3H, t), 1.4 (4H, m), 1.75 (2H, m), 1.88−2.33 (4H, m), 2.63 (1H, m), 3.2−3.54 (7H, m), 3.88 (1H, q), 5.32 (1H, m).

(*R*)-3-(Pentylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18g. From the free base of **(*R*)-6g** (2.35 g, 0.0096 mol) (prepared by method B using (*R*)-1-azabicyclo[2.2.2]octan-3-ol,[46] see below), flaked $Na_2S\cdot 9H_2O$ (2.53 g, 0.0105 mol), and 1-bromopentane (2.19 g, 0.0145 mol) was obtained **18g** (1.68 g, 50%): $[\alpha]_D$ −24.6 (EtOH, c = 1, 20 °C); ^{1}H NMR (CD_3OD) δ 0.93 (3H, t), 1.4 (4H, m), 1.75 (2H, m), 1.88−2.33 (4H, m), 2.63 (1H, m), 3.2−3.54 (7H, m), 3.88 (1H, q), 5.32 (1H, m).

(±)-3-(Hexylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18h. From the free base of **6g** (1.8 g, 0.0073 mol), flaked $Na_2S\cdot 9H_2O$ (1.94 g, 0.0081 mol), and 1-iodohexane (2.3 g, 0.011 mol) was obtained **18h** (1.0 g, 38%): ^{1}H NMR (CD_3OD) δ 0.92 (3H, t), 1.24−1.52 (6H, m), 1.74 (2H, m), 1.88−2.32 (4H, m), 2.63 (1H, m), 3.2−3.53 (7H, m), 3.88 (1H, q), 5.32 (1H, m).

3-Chloro-4-(ethylthio)-1,2,5-thiadiazole, 19a. Cyanogen (36 g, 0.69 mol) was bubbled into ether (250 mL) maintained at −10 °C. To the solution was added dropwise diethylamine (3 mL) followed by dropwise addition ethanethiol (47 mL, 0.64 mol) at such a rate that the temperature did not exceed −5 °C. The reaction mixture was maintained below 0 °C for 5 h and then stirred at ambient temperature overnight. Ether was distilled from the reaction until the pot temperature reached 50 °C. The reaction mixture was cooled to ambient temperature and then added dropwise to a solution of sulfur monochloride (125 mL, 1.56 mol) in DMF (150 mL) that was cooled to 5 °C. Cooling was removed and reaction was stirred overnight. The reaction mixture was cooled in an ice−water bath and excess sulfur monochloride destroyed by careful addition of H_2O while the temperature was maintained below 40 °C. The liquid was decanted from the semisolid sulfur precipitant and the sulfur residue triturated with hexane. The aqueous fraction was extracted with hexane, and the combined extracts and triturants were washed with H_2O, aqueous $NaHCO_3$, and brine and dried, and the solvent was evaporated. The residue was distilled to give a yellow liquid (80.2 g, 69%): ^{1}H NMR ($CDCl_3$) δ 1.47 (3H, t), 3.28 (2H, t).

3-Chloro-4-(1-propylthio)-1,2,5-thiadiazole, 19b. This compound was prepared by the same procedure as **19a**. From cyanogen (34 g 0.65 mol), propanethiol (57 mL, 0.63 mol), and sulfur monochloride (125 mL, 1.56 mol) was obtained a yellow liquid (98 g, 80%): ^{1}H NMR ($CDCl_3$) δ 1.03 (3H, t), 1.8 (2H, m), 3.22 (2H, t).

3-Chloro-4-(ethylsulfonyl)-1,2,5-thiadiazole, 20. A solution of Oxone (84 g, 0.137 mol) in H_2O (400 mL) was rapidly stirred as 3-chloro-4-(ethylthio)-1,2,5-thiadiazole (12.2 g, 0.067 mol) in THF (200 mL) was added. After the mixture was stirred overnight, the THF was evaporated and the residue extracted with ether. The extracts were washed with H_2O, aqueous $NaHCO_3$, and brine. Drying and thorough evaporation of the solvent gave **20** as a clear liquid (13.6 g, 95%): ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t), 3.56 (2H, t).

Alternative Synthesis of 6g (Method B). A solution of l-azabicyclo[2.2.2]octan-3-ol (1.2 g, 0.0092 mol) in THF (25 mL) was treated dropwise with 1.6 M *n*-butyllithium in hexane (5.9 mL, 0.0095 mol). The solution was cooled to −8 °C, and a solution of **20** (1.83 g, 0.0086 mol) in THF (15 mL) was added dropwise. After 15 min, cooling was removed and the reaction mixture stirred overnight. The reaction mixture was treated with water (10 mL), diluted with ether (100 mL), and extracted with 1 N HCl (25 mL). The aqueous solution was washed with ether, made basic, and extracted with ether. The extracts were dried and the solvent was evaporated to give the free base of **6g** (1.05 g, 50%) as a brownish liquid. This material was adequate for subsequent reactions but could be further purified by conversion to **6g** and recrystallization.

(±)-3-(Butylsulfonyl)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 21. A solution of **18d** (2.18 g, 0.0065 mol) in H_2O (30 mL) was cooled in ice−water as Oxone (6 g, 0.0097 mol) in H_2O (25 mL) was added dropwise. The cooling bath was removed, and the reaction mixture was stirred for 4 h. Excess oxidant was destroyed with $NaHSO_3$, the solution was cooled in ice−water, and the reaction mixture was made basic with 5 N NaOH. The mixture was extracted with EtOAc, the extracts were washed with brine and dried, and the solvent was evaporated to give a yellow oil. Purification by radial chromatography eluting with 10% EtOH−1% NH_4OH−$CHCl_3$ gave the free base of **21** as a yellow oil (HCl salt, 1.6 g, 61%): ^{1}H NMR (CD_3OD) δ 1.06 (3H, t), 1.8 (2H, m), 1.88−2.33 (4H, m), 2.64 (1H, m), 3.25 (2H, t), 3.28−3.54 (5H, m), 3.89 (1H, m), 5.32 (1H, m).

(±)-3-(Methylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18a. A suspension of 60% NaH in oil (0.12 g, 0.003 mol) in THF (10 mL) was treated with 6.19 M methanethiol in DMF (0.6 mL, 0.0037 mol). After 1 h, **21** (0.165 g, 0.0005 mol) was added, and the reaction mixture was stirred overnight. The solvent was evaporated, the residue treated with ice−water, and the mixture extracted with EtOAc. The extracts were washed with brine and dried, and the solvent was evaporated (HCl salt, 0.07 g, 47%): ^{1}H NMR ($CDCl_3$) δ 1.8−2.38 (4H, m), 2.68 (3H, s), 2.71 (1H, m), 3.24−3.46 (5H, m), 3.73 (1H, m), 5.23 (1H, m).

(±)-3-(Ethylthio)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 18b. A solution of ethanethiol (0.2 mL) in THF (5 mL) was cooled in ice−water as potassium *tert*-butoxide (0.13 g, 0.0012 mol) was added. After 10 min, a solution of the free base of **21** (0.165 g, 0.0005 mol) in THF (2 mL) was added, the cooling source was removed, and the reaction mixture was stirred for 1 h. The solvent was evaporated, the residue treated with ice−water, and the mixture was extracted with EtOAc. The extracts were washed with brine and dried, and the solvent was evaporated (HCl salt, 0.09 g, 58%): ^{1}H NMR ($CDCl_3$) δ 1.43 (3H, t), 1.8−2.36 (4H, m), 2.7 (1H, m), 3.2−3.45 (7H, m), 3.74 (1H, m), 5.23 (1H, m).

(±)-*endo*-3-(1-Propylthio)-4-(1-azabicyclo[3.2.1]octyl-6-oxy)-1,2,5-thiadiazole Hydrochloride, 28. A solution of potassium *tert*-butoxide (5.4 g, 0.048 mol) in THF (120 mL) was treated with (±)-*endo*-1-azabicyclo[3.2.1]octan-6-ol[25,26] (5.1 g, 0.04 mol). After 15 min, the reaction mixture was cooled in ice−water, **19b** (8.0 g, 0.041 mol) was added, the cooling source was removed, and the reaction mixture was stirred for 3 h. The reaction was quenched with H_2O, the mixture was diluted with EtOAc, and the organics were separated. The organic extract was washed with H_2O, the extracts were dried, and the solvent was evaporated. The residue was purified by HPLC, eluting with 5% ethanol−0.5% NH_4OH−$CHCl_3$ to give the free base of **28** (8 g, 70%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t), 1.72−2.08 (5H, m), 2.3 (1H, m), 3.06 (1H, m), 3.16−3.6 (7H, m), 4.23 (1H, m), 5.5 (1H, m).

Compounds **25**−**27** were prepared in the same manner as **28**.

(±)-3-(1-Propylthio)-4-(1-azabicyclo[3.2.1]octyl-5-oxy)-1,2,5-thiadiazole Hydrochloride, 25. From potassium *tert*-butoxide (0.85 g, 0.0072 mol), (±)-1-azabicyclo[3.2.1]octan-3-ol[47] (0.91 g, 0.0072 mol), and **19b** (1.53 g, 0.0079 mol) was obtained **25** (0.82 g, 35%) after purification by radial chromatography, eluting with 5% EtOH−0.5% NH_4OH−$CHCl_3$: ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t), 1.82 (2H, m), 2.04−2.72 (6H, m), 3.15−3.52 (6H, m), 3.73−4.01 (2H, m).

(±)-3-(1-Propylthio)-4-(endo-1-azabicyclo[2.2.1]hept-3-yloxy)-1,2,5-thiadiazole Sesquiethanedioate, 26. From potassium *tert*-butoxide (0.26 g, 0.002 mol), (±)-*endo*-1-azabicyclo[2.2.1]heptan-3-ol[24] (0.23 g, 0.002 mol), and **19b** (0.43 g, 0.002 mol) were obtained white crystals of **26** (0.24 g, 29%): ^{1}H NMR (DMSO-d_6) δ 0.97 (3H, t), 1.7 (2H, m), 1.86−2.1 (2H, m), 3.05−3.42 (8H, m), 3.75 (1H, t), 5.34 (1H, m).

(±)-3-(1-Propylthio)-4-(*endo*-1-azabicyclo[3.2.1]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 27. From potassium *tert*-butoxide (0.65 g, 0.00055 mol), (±)-*endo*-1-azabicyclo-[3.2.1]octan-3-ol[25] (0.64 g, 0.005 mol), and **19b** (1.1 g, 0.0056 mol) was obtained **27** (0.55 g, 34%) after purification by radial

chromatography, eluting with 10% EtOH−1% NH_4OH−$CHCl_3$: ^1H NMR ($CDCl_3$) free base δ 1.06 (3H, t), 1.83 (3H, m), 2.12 (2H, m) 2.25 (2H, m), 2.66 (1H, m), 2.84 (1H, m), 2.98 (1H, m), 3.22 (2H, t), 3.28−3.46 (3H, m), 4.88 (1H, m).

3-Hydroxy-4-(propylthio)-1,2,5-thiadiazole, 31. A mixture of **19b** (10 g, 0.052 mol), 2 N NaOH (100 mL), and DMSO (10 mL) was heated to reflux for 24 h. The solution was cooled and extracted with ether. The aqueous fraction was acidified with concentrated HCl and cooled in ice−water for 3 h. The resulting solid was collected and washed with a small amount of cold water to give a white solid (8.15 g, 89%): ^1H NMR ($CDCl_3$) δ 1.07 (3H, t), 1.8 (2H, m), 3.21 (2H, t).

(±)-3-(2-(Methylthio)ethyl)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 30c. A solution of 3-(2-(methylthio)ethyl)-4-hydroxy-1,2,5-thiadiazole (0.45 g, 0.0026 mol) and triphenylphosphine (0.7 g, 0.0027 mol) was cooled in ice−water as diethyl diazodicarboxylate (0.4 mL, 0.0026 mol) was added dropwise. After addition, (±)-1-azabicyclo[2.2.2]octan-3-ol (0.33 g, 0.0026 mol) was added, the cooling source was removed, and the reaction mixture was stirred for 1 h. The solvent was evaporated, the residue was suspended in H_2O, and the mixture was acidified and washed with ether. The aqueous solution was made basic and extracted with EtOAc. The extracts were dried, the solvent was evaporated, and the residue was purified by radial chromatography, eluting with 10%−EtOH−1% NH_4OH−$CHCl_3$ (HCl salt, 0.6 g, 71%): ^1H NMR ($CDCl_3$) (free base) δ 1.37−1.93 (4H, m), 2.15 (3H, s), 2.27 (1H, m), 2.71−3.00 (7H, m), 3.08 (2H, t), 3.37 (1H, q), 4.93 (1H, m).

Compounds **30a**, **30b**, and **32**−**34** were prepared in the same manner as **30a.**

(±)-3-(1-Azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 30a. White crystals of **30a** (0.36 g, 50%) were obtained starting with hydroxy-1,2,5-thiadiazole (0.28 g, 0.0027 mol), triphenylphosphine (0.7 g, 0.0027 mol), diethyl diazodicarboxylate (0.4 mL, 0.0026 mol), and (±)-1-azabicyclo-[2.2.2]octan-3-ol (0.33 g, 0.0026 mol): ^1H NMR ($CDCl_3$) δ 1.81−2.38 (4H, m), 2.7 (1H, m), 3.23−3.5 (5H, m), 3.82 (1H, q), 5.22 (1H, m), 8.03 (1H, s).

(±)-3-((Ethylthio)methyl)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 30b. Light yellow crystals of **30b** (0.46 g, 55%) were obtained starting with 3-((ethylthio)methyl)-4-hydroxy-1,2,5-thiadiazole (0.45 g, 0.0026 mol), triphenylphosphine (0.7 g, 0.0027 mol), diethyl diazodicarboxylate (0.4 mL, 0.0026 mol), and (±)-1-azabicyclo[2.2.2]-octan-3-ol (0.33 g, 0.0026 mol): ^1H NMR ($CDCl_3$) δ 1.25 (3H, t), 1.8−2.4 (4H, m), 2.51 (2H, q), 2.72 (1H, m), 3.23−3.3.47 (5H, m), 3.69−3.86 (3H, m + s), 5.25 (1H, m).

(±)-3-(1-Propylthio)-4-(exo-1-azabicyclo[2.2.1]hept-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 32. The free base of **32** (2.69 g, 60%) was obtained starting with **31** (2.62 g, 0.0149 mol), triphenylphosphine (3.96 g, 0.0151 mol), diethyl diazodicarboxylate (2.27 mL, 0.0145 mol), and (±)-*endo*-1-azabicyclo[2.2.1]heptan-3-ol (**22**)[24] (1.64 g, 0.0145 mol): ^1H NMR ($CDCl_3$) δ 1.07 (3H, t), 1.8 (3H, m), 2.3 (1H, m), 3.12−3.33 (5H, m), 3.5−3.77 (4H, m), 5.07 (1H, m).

(±)-3-(1-Propylthio)-4-(*exo*-1-azabicyclo[3.2.1]oct-3-yloxy)-1,2,5-thiadiazole Hydrochloride, 33. White crystals of **33** (0.19 g, 32%) were obtained starting with **31** (0.3 g, 0.0017 mol), triphenylphosphine (0.47 g, 0.0018 mol), diethyl diazodicarboxylate (0.28 mL, 0.0018 mol), and (±)-*endo*-1-azabicyclo[3.2.1]octan-3-ol (**23**)[25] (0.23 g, 0.0018 mol): ^1H NMR ($CDCl_3$) free base δ 1.05 (3H, t), 1.67−1.9 (5H, m), 2.29 (1H, m), 2.46 (1H, m), 2.59 (1H, m), 2.74 (1H, q), 2.87 (1H, d), 3.01 (2H, m), 3.19 (2H, t), 3.39 (1H, q), 5.12 (1H, m).

(±)-3-(1-Propylthio)-4-(*exo*-1-azabicyclo[3.2.1]oct-6-yloxy)-1,2,5-thiadiazole Hydrochloride, 34. White crystals of **34** (0.27 g, 46%) were obtained starting with **31** (0.3 g, 0.0017 mol), triphenylphosphine (0.47 g, 0.0018 mol), diethyl diazodicarboxylate (0.28 mL, 0.0018 mol), and (±)-*endo*-1-azabicyclo[3.2.1]octan-6−ol (**24**)[25,26] (0.23 g, 0.0018 mol): ^1H NMR ($CDCl_3$) free base δ 1.06 (3H, t), 1.38−1.87 (6H, m), 2.38 (1H, m), 2.8 (3H, m), 2.99 (2H, m), 3.19 (2H, t), 3.48 (1H, m), 5.15 (1H, m).

(±)-3-(Butyloxy)-4-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,2,5-oxadiazole Hydrochloride, 37. A suspension of 3,4-bis-(phenylsulfonyl)furoxan (4.6 g, 0.0126 mol) in 1-butanol (400 mL) was heated to 60 °C as a solution of sodium butoxide (0.3 g Na, 40 mL of 1-butanol) was added dropwise over 15 min. After 1 h, the solvent was evaporated, the residue was treated with H_2O, and the mixture was extracted with ether. The extracts were washed with H_2O and dried, and the solvent was evaporated to give a white solid (3.15 g). A mixture of this white solid and $P(OMe)_3$ was heated to reflux overnight. The reaction mixture was cooled and poured into ice−water containing 5 N HCl (6 mL). The mixture was extracted with ether, the extracts were washed with brine and dried, and the solvent was evaporated. The residue was purified by radial chromatography, eluting with 15% EtOAc−hexane to give a clear liquid **36** (1.85 g). A solution of **36** in THF (30 mL) was added dropwise to the lithium salt of (±)-1-azabicyclo[2.2.2]-octan-3-ol (1.85 g, 0.014 mol + 1.6 M *n*-butyllithium (8.4 mL, 0.0135 mol)) in THF (20 mL). The reaction mixture was warmed to 50 °C for 5 h. After being cooled to ambient, the reaction mixture was treated with dilute HCl, and the mixture was extracted with ether. The aqueous phase was made basic and extracted with EtOAc. The extracts were washed with H_2O and dried, and the solvent was evaporated to give a clear liquid (HCl salt, 1.4 g, 33%): ^1H NMR (CD_3OD) δ 0.98 (3H, t), 1.5 (2H, m), 1.82 (2H, m), 1.86−2.33 (4H, m), 2.68 (1H, m), 3.22−3.5 (4H, m), 3.55 (1H, m), 3.84 (1H, q), 4.35 (2H, t), 5.15 (1H, m).

(±)-2-Chloro-3-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,4-diazine, 38. A solution of (±)-1-azabicyclo[2.2.2]octan-3-ol (2.5 g, 0.02 mol) in THF (40 mL) was treated dropwise with 1.6 N 1-butyllithium in hexane (12.5 mL, 0.02 mol). The solution was then added in a steady stream to an ice-cold solution of 2,3-dichloropyrazine (3.3 g, 0.022 mol) in THF (100 mL). The cooling source was removed, and 2 h later the reaction mixture was heated to reflux for 1 h. The solvent was evaporated and the residue suspended in dilute HCl. The mixture was extracted with ether, the aqueous layer made basic, and the mixture extracted with again with ether. The basic extracts were washed with H_2O and dried, and the solvent was evaporated to give a brown solid that was recrystallized (0.75 g, 15%): ^1H NMR ($CDCl_3$) δ 1.4−1.87 (3H, m), 2.04 (1H, m), 2.2 (1H, m), 2.71−3.12 (5H, m), 3.4 (1H, q), 5.15 (1H, m), 7.93 (1H, d), 8.0 (1H, d).

(±)-2-(Butyloxy)-3-(1-azabicyclo[2.2.2]oct-3-yloxy)-1,4-diazine, 39. A 0.47 M sodium butoxide solution in 1-butanol (30 mL) was treated with **38** (0.48 g, 0.002 mol), and the reaction mixture was heated to reflux for 4 h. The solution was acidified and the butanol evaporated. The residue was suspended in H_2O and extracted with ether. The aqueous phase was made basic and extracted with EtOAc. The extracts were washed with brine and dried, and the solvent was evaporated to give a yellow oil (HCl salt, 0.32 g, 51%): ^1H NMR ($CDCl_3$) δ 0.98 (3H, t), 1.46 (2H, m), 1.72−2.18 (5H, m) 2.38 (1H, m), 2.63 (1H, m), 3.33 (4H, m), 3.74 (1H, q), 4.35 (2H, t), 5.3 (1H, m), 7.53 (1H, d), 7.69 (1H, d).

Salivation in Mice. Male Cr1:CF1RBR mice (Charles River Laboratories, Portage, MI) weighing 20−30 g were used for salivation testing. Mice, in groups of five, were injected ip with 10 mg/kg doses of compound dissolved in distilled water. After 30 min, salivation and tremor were scored on a scale of 0, 1, or 2, where 0 = no effect, 1 = moderate salivation or tremor, and 2 = marked salivation or tremor.

Radioligand Binding Assays. The brain from male Sprague−Dawley rats was homogenized in 10 volumes of 0.32 M sucrose and centrifuged at 1000*g* for 10 min, and the supernatant was centrifuged at 17000*g* for 20 min. The synaptosomal fraction (P2) pellet was homogenized in 50 volumes of 20 mM Tris-Cl buffer, pH 7.4, and centrifuged at 50000*g* for 10 min. After resuspension in buffer, the suspension was preincubated for 30 min at 4 °C and centrifuged again. The pellet was resuspended in 3 volumes of buffer and frozen at −70 °C until used.

The inhibition of binding of pirenzepine to rat brain membranes was determined by adding unlabeled drug, 1 nM [^{3}H]pirenzepine (87 Ci/mmol, New England Nuclear, Boston, MA), and rat brain membranes equivalent to 10 mg tissue wet weight (about 0.1 mg protein) in 1 mL total volume of 20 mM Tris-Cl buffer, pH 7.4, containing 1 mM $MnCl_2$.[48] The inhibition of binding of oxotremorine-M to rat brain membranes was determined by adding unlabeled drug, 3 nM [^{3}H]oxotremorine-M (87 Ci/mmol, New England Nuclear), and rat brain membranes equivalent to 10 mg tissue wet weight (about 0.1 mg protein) in 1 mL total volume of 20 mM Tris-Cl buffer, pH 7.4, containing 1 mM $MnCl_2$. For pirenzepine and oxotremorine-M binding, the homogenates were incubated at 25 °C for 60 and 15 min, respectively. After incubation, the homogenates were filtered through Whatman GF/C filters with vacuum. The filters were rinsed three times with 1 mL of cold buffer and placed in scintillation vials containing Ready Protein+ (Beckman) scintillation fluid. Radioactivity trapped on the filters was determined by liquid scintillation spectrometry. Nonspecific binding was determined using 1 μM atropine.

The concentration of compound required to inhibit binding 50% (IC_{50}) was calculated using the ALLFIT program.[49]

Stimulation of Phosphoinositol Hydrolysis in A9 L−m_1 Cells. A9 L−m1 cells were cultured to confluence in 75 mL flasks containing Dubecco's modified essential media. Cells were prelabeled with 1 μCi/mL of myo[2-^{3}H]inositol (Amersham Inc, 16.3 Ci/mmol) for 48 h prior to assay. On the day of assay, cells were detached using a 30 s exposure to 0.25% trypsin in 1 mM EDTA. The cells were collected by centrifugation (300*g* for 5 min) and resuspended in oxygenated HEPES buffer containing 10 mM LiCl, 142 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5.6 mM D-glucose, and 30 mM sodium HEPES at pH 7.4. Cells were incubated at 37 °C for 45 min in the presence of varying concentrations of drug. The reaction was terminated by the addition of 3 mL of ice-cold 10 mM LiCl, sonicated, and centrifugated at 20000*g*. The supernatant was decanted over a Accell QMA anion exchange SEP−PAK cartridge in the formate form (Waters Associates, Milford, MA). The cartridges were washed with 10 mL of H_2O followed by 10 mL of 5 mM sodium borate. [^{3}H]PI was eluted directly into scintillation vials for counting with 4 mL of 0.1 ammonium formate/0.01 mM formic acid/5 mM sodium borate.[50] Data are expressed as the percent of total [^{3}H]PI stimulated in the presence of 1 mM carbachol. Half-maximal values (EC_{50}) were determined from the mean of seven point curves using a four-parameter logistic model (Prism-GraphPad Software, Inc. San Diego, CA).

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