Functionally Selective M_1 Muscarinic Agonists. 3. Side Chains and Azacycles Contributing to Functional Muscarinic Selectivity among Pyrazinylazacycles

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In an attempt to improve upon the M_1 agonist activity of the selective M_1 agonist xanomeline and related compounds, the M_1 muscarinic efficacies and potencies of 3- and 6-substituted pyrazinylazacycles were varied by changing both the 3- and 6-substituents as well as the azacycle. Significant improvements in efficacy and potency over the previously prepared [3-(hexyloxy)pyrazinyl]tetrahydropyridine **19** were obtained with the [3-(hexyloxy)pyrazinyl]-quinuclidine **5i.** The M_1 activity of **5i** showed some enantioselectivity with (*S*)-**5i** being ca. 4-fold more potent than (*R*)-**5i.** Like **19** and xanomeline, **5i** was a functionally selective M_1 agonist that showed greater functional selectivity than widely studied pyrazinylquinuclidine **5n** (L-689,660). The improved functional selectivity of **5i** over **5n** could be attributed to the additional binding interactions between the hexyloxy side chain of **5i** and the M_1 receptor that are not available to **5n.** Although **5i** may show M_1 functional selectivity comparable to xanomeliine, **5i** is a less efficacious and potent M_1 agonist than xanomeline.

Introduction

The cholinergic deficits observed in Alzheimer's disease[1–3] have given rise to the cholinergic hypothesis of dementia[4] and spawned many efforts to develop therapies based on enhancement of central cholinergic transmission.[5] The greatest efforts have focused on the use of acetylcholinesterase inhibitors and directly acting cholinomimetic drugs,[6,7] but the results of clinical trials have been generally disappointing, potentially because the side effects seen with these agents prevent therapeutic doses from being employed.[8–10] These intolerable side effects might be avoided by developing drugs that are functionally selective for muscarinic receptor subtypes.[11–16]

Four pharmacologically distinct muscarinic receptor subtypes (M_1–M_4) have been identified, and five genetically distinct human muscarinic receptors (m_1–m_5) have been cloned and characterized with the relationship between these two classifications being $M_1 = m_1$, $M_2 = m_2$, $M_3 = m_3$, and $M_4 = m_4$.[17,18] These muscarinic receptor subtypes are differentially located in the brain with high levels of the m_1–m_3 receptors being located in the cortex and hippocampus, areas of the brain normally associated with cognition and learning.[19–21] The density of the M_1 receptors in the cortex and hippocampus remain relatively unchanged in Alzheimer's patients, while the M_2 receptors in the basal forebrain diminish.[22–25] This has led to the hypothesis that replacement therapy with selective M_1 agonists would alleviate the cognitive deficits of Alzheimer's disease.[25]

We recently described a series of 3-(3-substituted-pyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridines **19** that were functionally selective M_1 agonists.[16] These compounds, although more potent M_1 agonists than their arecoline antecedent, were generally much less active than a series of (1,2,5-thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines from which xanomeline (Figure 1) was chosen for clinical development.[13–15] Several research groups had shown that when the tetrahydropyridyl rings of other cholinergic agonists were replaced by different azacycles, particularly quinuclidinyl and azanorbornyl ring systems, enhancements in cholinergic agonist activity could be achieved.[26–29] The primary goal of the present study was to improve upon the M_1 agonist activity of the pyrazinyltetrahydropyridines and xanomeline by synthesizing pyrazines containing some of these additional azacycles.

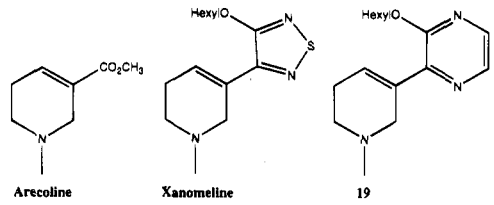

Figure 1. Comparison of the structures of arecoline, xanomeline, and **19**.

Although Street et al.[30] have already described pyrazinylazacycles that encompass parts of our research strategy, those studies focused on 6-substituted pyrazines, relatively small substituents, and the few 3-substituted pyrazines synthesized did not contain the alkoxy and alkylthio 3-substituents used in our tetrahydropyridine series. Our studies of pyrazinyltetrahydropyridines and (1,2,5-thiadiazolyl)tetrahydropyridines had shown that M_1 agonist activity and selectivity were very dependent upon the side chain and length of the 3-alkoxy and 3-alkylthio substituent, so it was possible that these investigators had not included the optimum substitution patterns for M_1 selectivity in their study. In addition, the earlier study of 6-substituted pyrazines did not directly evaluate the M_1 agonist activity of the compounds but instead relied upon the ratio of the affinities of the compounds for the high- and low-affinity states of the cortical muscarinic receptors (NMS/OXO-M ratio) as a measure of cortical efficacy.[31] This NMS/

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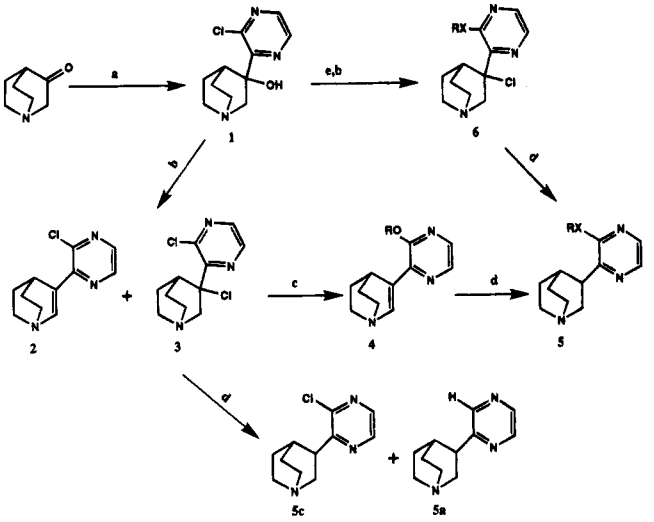
Scheme 1. Preparation of 3-Pyrazinyl-1-azabicyclo[2.2.2]octanes[a]



[a] Reagents: (a) 2-chloro-3-lithiopyrazine; (b) $SOCl_2$; (c) sodium alkoxide; (d) H_2/Pd–C; (e) NaXR (X = O, S).

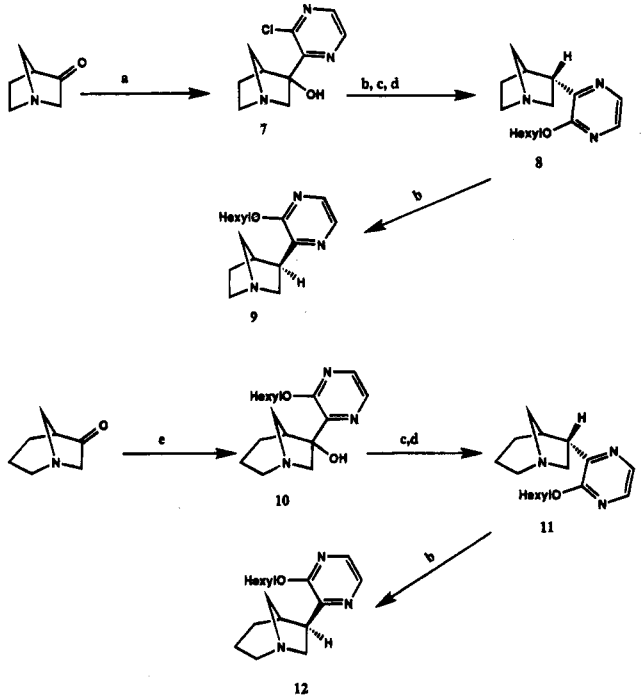
Oxo-M ratio is correlated with the ability of a compound to stimulate phosphoinositol (PI) hydrolysis in rat cortical slices. Because the rat cortex contains both m_1 and m_3 receptors that are coupled to PI hydrolysis,[19] this ratio does not distinguish between m_1 and m_3 agonist activity. Therefore, our strategy was to synthesize 3-alkoxy- and 3-(alkylthio)pyrazine azacycles, directly determine their M_1 agonist activity by measuring their ability to stimulate PI hydrolysis in an A9 L cell line stably expressing the cloned m_1 receptor (A9 L-m_1), [32] and compare their M_1 efficacies and potencies to that of xanomeline and some of the more active pyrazine azacycles described in the study of Street et al.[30]

The quinuclidinyl-6-chloropyrazine **5n** (L-689,660) has been the most widely studied compound in the previous series of pyrazine azacycles.[12,33–35] This compound has been shown to be a functionally selective M_1/M_3 agonist[12] and both the M_1 and M_3 activities are enantioselective, with the *R* enantiomer being much more potent than the *S* enantiomer.[33] Therefore, we were particularly interested in comparing the enantioselectivity and muscarinic receptor subtype selectivity of **5n** to the most potent and selective M_1 agonist in our alkoxypyrazine series, **5i**.

Chemistry

The (3-alkoxypyrazinyl)quinuclidines were prepared by the two methods shown in Scheme 1. Addition of 2-chloro-3-lithiopyrazine[36] to 3-quinuclidinone gave amino alcohol **1**. Early in our investigations, **1** was converted to a separable mixture of enamine **2** and tertiary chloride **3** with thionyl chloride. This mixture of **2** and **3** was treated with sodium alkoxides, the 3-alkoxypyrazine enamine **4** isolated, and the double bond reduced to give the alkoxypyrazines **5**. A more efficient preparation of alkoxypyrazines **5** that worked equally well for the preparation of (alkylthio)pyrazines **5** used the displacement of the chloro group of **1** with either sodium alkoxides or sodium alkylthiolates, respectively, and subsequent conversion to tertiary chlorides **6**. Only small amounts of the enamines analogous to **2** were produced by this method. The tertiary chlorides **6** were

Scheme 2. Preparation of Pyrazinyl-1-azabicycloheptanes and -octanes[a]



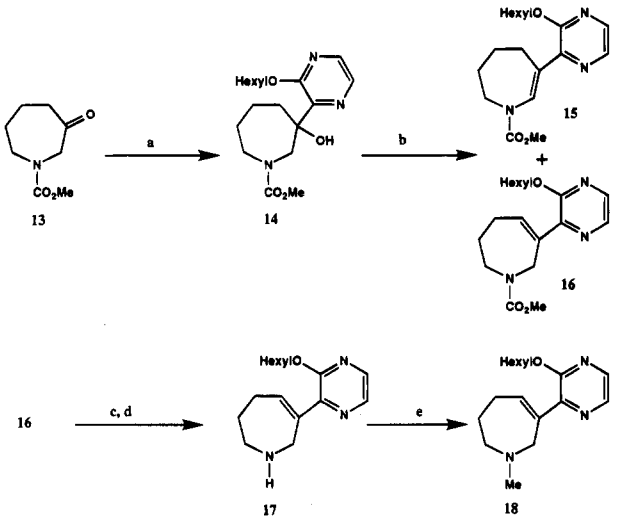
[a] Reagents: (a) 2-chloro-3-lithiopyrazine; (b) NaOhexyl; (c) $SOCl_2$; (d) H_2/Pd–C; (e) 2-(hexyloxy)-3-lithiopyrazine.

catalytically reduced to the desired alkoxy- and (alkylthio)pyrazines **5**. Catalytic reduction of a mixture of **2** and **3** gave a separable mixture of 3-chloropyrazine **5c** and the previously described parent pyrazine **5a**. The remaining pyrazinylquinuclidines **5b,n** were prepared according to published procedures.[30]

Addition of 2-chloro-3-lithiopyrazine to 1-azabicyclo-[2.2.1]heptan-3-one[28] produced tertiary alcohol **7** that was ultimately converted to the the *endo*-(hexyloxy)-pyrazine **8** using procedures similar to those described by Street et al. and as outlined in Scheme 2.[30] The stereochemistry of the 2-chloropyrazine **7** was not investigated but was assumed to be *exo* due to the addition of the lithiopyrazine on the least hindered side of the ketone. After conversion of **7** to the analogous hexyloxy derivative, chlorination of the tertiary alcohol intermediate probably led to inversion of the configuration at the 3-position, and subsequent hydrogenolysis of the chloro group with retention of configuration provided predominantly the *endo* product **8**. Only a small amount of *exo* product **9** could be detected in the crude hydrogenolysis mixture. The thermodynamically more stable *exo* product **9** was separated from a 1:9 mixture of **8** and **9** after heating the hydrogenolysis mixture with sodium hexyl oxide. The configurations of **8** and **9** at the 3-position were determined by comparing their NMR spectra to that of related 1-azabicyclo[2.2.1]heptanes (azanorbornanes) reported in the literature. Thus, the C-5 protons of **8** (above 1.95 ppm) are shifted upfield from those in **9** (1.98 and 2.25 ppm) as in previous literature examples of this ring system.[27,28,30] In addition, the base-catalyzed conversion of **8** to thermodynamically more stable **9** is consistent with the configurational assignments.[30]

The syntheses of *endo*- and *exo*-pyrazinyl-1-azabicyclo-[3.2.1]octanes (pyrazinylisotropanes) **11** and **12** are also depicted in Scheme 2. Addition of 2-(hexyloxy)-3-

Scheme 3. Preparation of Pyrazinyltetrahydroazepines[a]



[a] Reagents: (a) 2-(hexyloxy)-3-lithiopyrazine; (b) Burgess's reagent; (c) $ISi(CH_3)_3$; (d) H^+/MeOH; (f) formaldehyde/formic acid.

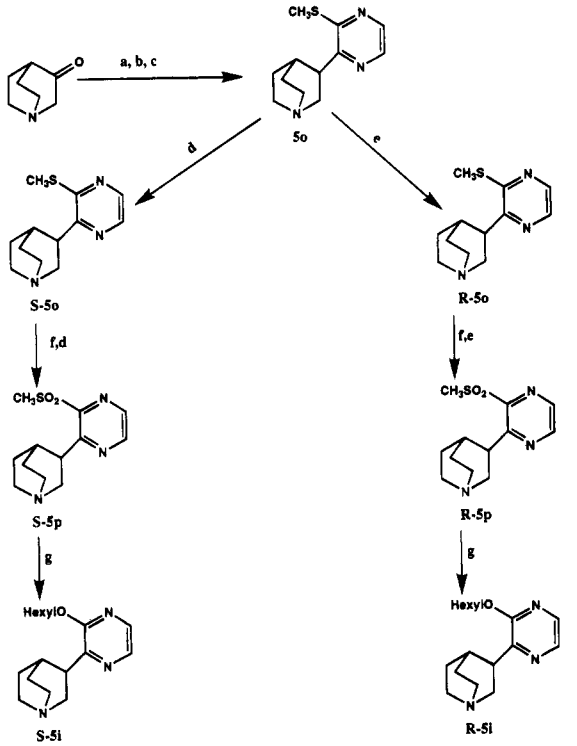
lithiopyrazine to 1-azabicyclo[3.2.1]octan-5-one[37] produced tertiary alcohol **10** that was subsequently chlorinated and subjected to hydrogenolysis to give predominantly the *endo* (hexyloxy)pyrazine **11**. Equilibration of **11** in the presence of sodium hexyl oxide gave a 1:9 mixture of **11** and **12** from which the *exo*-pyrazine **12** was isolated. Configurational assignments were again made with reference to the NMR spectra of related pyrazinylisotropanes.[28]

As shown in Scheme 3, 2-(hexyloxy)-3-lithiopyrazine was added to tetrahydroazepin-3-one **13**[38] to give alcohol **14**. Dehydration of **14** gave a 4:1 separable mixture of what appeared to be enamide **15** and allylic amine **16**. These assignments were based on the chemical shift and coupling of the vinylic protons in the NMR spectra of **15** and **16**. As would be expected, the enamide proton of **15** was a broad singlet at 7.61 ppm significantly down field from the olefinic proton of **16** which was a sharp triplet at 6.68 ppm similar to the chemical shift and olefinic coupling of the vinyl proton in **19**. The four protons α to the azepine amine function (3.7 ppm, m; 4.45 ppm, doublet) also confirmed the allylic amine structure of **16**. Hydrolysis of **16** gave the unsubstituted azepine **17** that was then converted to the 1-methylazepine **18**.

The enantiomers of **5i** could be separated on an analytical HPLC column containing a chiral support, but numerous attempts to resolve **5i** directly using chiral acids failed, primarily because of the poor crystallinity of most of the salts. The salts of the quinuclidinylpyrazines with shorter pyrazinyl side chains appeared to form crystals with many different acids, and in the cases studied, better separation of the enantiomers of the compounds could be obtained on a chiral HPLC column than could be obtained with compounds containing longer side chains. These observations suggested that the enantiomers of **5i** might be obtained indirectly using a shorter chained pyrazine **5** as an intermediate. Because the sulfone group attached to a pyrazine has been shown to be easily displaceable by nucleophiles, the strategy for the synthesis of the enantiomers of **5i** depicted in Scheme 4 appeared to be practical.

Addition of 2-(methylthio)-3-lithiopyrazine[39] to 3-quinuclidinone followed by chlorination and hydrogenolysis gave (methylthio)pyrazine **5o**. Greater than 90% ee material was obtained by resolving **5o** with the (2*S*,3*S*)- and (2*R*,3*R*)- tartaric acids to give (*S*)-**5o** and (*R*)-**5o**, respectively. The resolved **5o**'s were converted to their respective sulfones by oxidation with Oxone. Both (*S*)-**5p** and (*R*)-**5p** were further resolved by conversion to the (2*S*,3*S*)- and (2*R*,3*R*)-tartaric acid salts, respectively, to give better than 95% ee material. The absolute configuration of position 3 in (*S*)-**5p** was determined by comparison to the known absolute configuration of the (2*S*,3*S*)-tartaric acid counterion in the X-ray crystal structure (Figure 2). Finally, (*S*)-**5p** was converted to (*S*)-**5i**, and (*R*)-**5p** was converted to (*R*)-**5i** by treatment with sodium hexyloxide. The (*S*)-**5i** obtained contained less than 3% of (*R*)-**5i**, and no (*S*)-**5i** could be detected in the (*R*)-**5i**.

Scheme 4. Resolution of Pyrazinylquinuclidines **5**[a]



[a] Reagents: (a) 2-(methylthio)-3-lithiopyrazine; (b) $SOCl_2$; (c) H_2/Pd–C; (d) (2*S*,3*S*)-tartrate; (e) (2*R*,3*R*)-tartrate; (f) Oxone; (g) NaOhexyl.

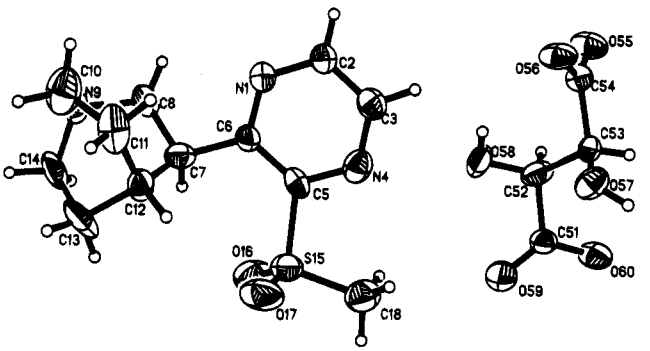
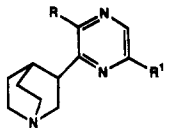


Figure 2. ORTEP representation of the X-ray crystal structure of (*S*)-**5p** (2*S*,3*S*)-tartaric acid.

Biological Evaluation

Competitive radioligand-binding assays using [^{3}H]-oxotremorine-M (Oxo-M) or [^{3}H]pirenzepine (Pz) in rat

Table 1. Pharmacological Data for the 3-Pyrazinyl-1-azabicyclo[2.2.2]octane



no.	R	R^1	receptor binding to rat brain membranes IC50 ± SEM, nM [^{3}H]OxoM	receptor binding to rat brain membranes IC50 ± SEM, nM [3H]Pz	PI hydrolysis in A9 L-m1 cells % max ± SEM	PI hydrolysis in A9 L-m1 cells EC50 mM[a]	salivation in mice 10 mg/kg ip score	salivation in mice MED[b] mg/kg ip
5a	H	H	4.0 ± 0.6	74 ± 11	80 ± 14	0.26 ± 0.08	1.4	0.1
5b	Me	H	23 ± 3	80 ± 4	4 ± 1	nd	2.0	3
5c	Cl	H	56 ± 7	180 ± 22	13 ± 2	1.59 ± 0.11	1.2	10
5d	OMe	H	72 ± 6	111 ± 14	1 ± 9	nd	0	nd
5e	OEt	H	23 ± 2	38 ± 2	1 ± 0	nd	0	nd
5f	O-*n*-Pr	H	9.0 ± 0.8	19 ± 1	4 ± 1	nd	0	nd
5g	O-*n*-Bu	H	5.0 ± 0.9	10 ± 2	15 ± 5	24.1 ± 0.2	0	nd
5h	O-*n*-pentyl	H	6.0 ± 0.7	7.0 ± 0.7	32 ± 5	9.13 ± 0.46	0.2	nd
5i	O-*n*-hexyl	H	4.5 ± 0.5	6.0 ± 0.4	40 ± 6	0.52 ± 0.03	0	nd
(*R*)-**5i**	O-*n*-hexyl	H	11.3 ± 0.8	10.6 ± 0.4	46 ± 8	1.73 ± 0.70	0	nd
(*S*)-**5i**	O-*n*-hexyl	H	2.5 ± 0.1	3.7 ± 0.1	33 ± 4	0.36 ± 0.02	0	nd
5j	O-*n*-heptyl	H	10 ± 1	13 ± 12	35 ± 3	0.40 ± 0.03	0	nd
5k	*S*-*n*-Bu	H	0.5 ± 0.1	0.4 ± 0.07	5 ± 1	nd	0	nd
5l	*S*-*n*-pentyl	H	0.7 ± 0.2	1.1 ± 0.2	7 ± 3	nd	0	nd
5m	*S*-*n*-hexyl	H	2.0 ± 0.3	2.2 ± 0.3	16 ± 4	0.27 ± 0.05	0	nd
5n	H	Cl	4.6 ± 0.4	4.7 ± 0.3	8 ± 1	nd	1	3
arecoline			77 ± 13	1300 ± 260	73 ± 3	3.08 ± 1.80	0	nd
McN-A-343			355 ± 40	955 ± 75	52 ± 6	4.15 ± 1.40	nd	nd
xanomeline			2 ± 0.5	5 ± 1	55 ± 2	0.20 ± 0.08	0	nd

[a] nd means not determined. [b] MED means minimum effective dose.

hippocampus membranes, where M_1 receptors predominate, were used to determine the the affinities of compounds for muscarinic receptors. The affinities of the compounds for the receptors labeled by Pz were considered to be the compounds' affinities for M_1 receptors,[40] while affinities for the receptors labeled by Oxo-M were considered to be the compounds' affinities for the "agonist conformational state"[31] of the muscarinic receptor sites.

Stimulation of phosphoinositol (PI) hydrolysis in the A9 L cell line transfected with the m_1 receptor[32] is mediated through m_1 receptors.[17] 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 10% 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.[17] Mice were injected with compound ip and visually scored for the appearance of salivation using a scale of 0–2.[14] When an average score of 1 or more was obtained, the compound was retested at lower doses until an average score of less than 1 was obtained. The lowest dose producing at least a score of 1 was designated the minimum effective dose (MED).

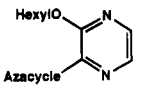
Results

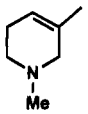
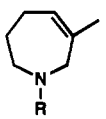
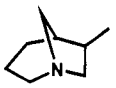
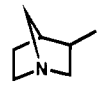
For the quinuclidinylpyrazines containing small 3-substituents (**5a–d**), the order of affinity for the Oxo-M binding site (Table 1), was H > CH_3 > Cl > OCH_3. A small substituent such as Cl in the 6-position, e.g., **5n**, did not diminish affinity for the Oxo-M binding site relative to the unsubstituted pyrazine **5a**. Among the alkoxypyrazines **5d–j**, affinity for the Oxo-M binding site began to increase rapidly with chain length extension until the butyloxy side chain (**5g**) was reached, plateaued for the butyloxy to hexyloxy side chains (**5g–i**), and then began to decline for longer side chains (**5j**). The binding to the Oxo-M receptor was modestly enantioselective with (*S*)-**5i** being more potent than (*R*)-**5i**. Replacing the oxygen in the side chain of **5g–j** with sulfur, **5k–m**, significantly enhanced binding to the Oxo-M binding site, maximum affinity being obtained with the butylthio derivative **5k**. All of the quinuclidinylpyrazines studied had higher affinity for the agonist conformational state than arecoline.

Because the hexyloxy side chain in **5i** conferred the highest affinity for the agonist conformational state in the alkoxypyrazine quinuclidine series, this substituent was used in comparing the remaining azacycles studied. The order of affinity (Table 2) for the Oxo-M binding site among these azacycles was quinuclidine **5i** ≥ *exo*-isotropane **12** ≥ *exo*-azanorbornane **9** > tetrahydropyridine **19** > *endo*-azanorbornane **8** > tetrahydroazepine **18** > *endo*-isotropane **11**. These azacycles could be grouped into three categories based on their affinity: a high-affinity group (4–6 nM), quinuclidine, *exo*-isotropane, and *exo*-azanorbornane; a middle affinity group (20–50 nM), tetrahydropyridine and *endo*-azanorbornane; and a low-affinity group (100–200 nM), tetrahydroazepine and *endo*-isotropane. Only the *endo*-isotropane **11** and the two tetrahydroazepines, **17** and **18**, had lower affinity for the Oxo-M receptor than arecoline.

The order of affinity for the receptor binding Pz in the 3-substituted pyrazines **5a–m** generally paralleled their order of affinities for the Oxo-M receptor. Affinity for the Pz receptor increased with increasing chain length until an optimum length was obtained (**5i**) and then decreased with chain extension. The modest enantioselectivity seen for the Oxo-M receptor ((*S*)-**5i** > (*R*)-**5i**) was also seen for the Pz receptor. Replacing

Table 2. Effects of Azacycles on the Pharmacology of Azacyclic(Hexyloxy)pyrazines



Azacycle	no.	receptor binding to rat brain membranes IC_{50} ± SEM, nM		PI hydrolysis in A9 L-m_1 cells		salivation in mice	
		[^{3}H]OxoM	[^{3}H]Pz	% max ± SEM	EC_{50}, μM[a] ± SEM	10 mg/kg, i.p. Score	M.E.D.[b] mg/kg,i.p.
	19	20 ± 3	17 ± 1	12 ± 2	42.7 ± 0.2	0	n.d.
	17 (R = H)	167 ± 27	44 ± 5	5 ± 2	n.d.	0	n.d.
	18 (R = Me)	117 ± 20	26 ± 2	2 ± 1	n.d.	0	n.d.
	5i	4.5 ± 0.5	6.0 ± 0.4	40 ± 6	0.52 ± 0.03	0	n.d.
	11 (endo)	180 ± 18	120 ± 12	4 ± 1	n.d.	0	n.d.
	12 (exo)	4.0 ± 0.4	2.5 ± 0.3	12 ± 5	0.40 ± 0.04	0	n.d.
	8 (endo)	46 ± 5	58 ± 7	31 ± 1	13.1 ± 0.1	0.6	n.d.
	9 (exo)	5.6 ± 0.7	7.0 ± 1	40 ± 1	0.73 ± 0.19	1.6	1
arecoline		77 ± 13	1300 ± 260	73 ± 3	3.08 ± 1.80	0	n.d.
xanomeline		2 ± 0.5	5 ± 1	55 ± 2	0.20 ± 0.08	0	n.d.

[a] nd means not determined. [b] MED means minimum effective dose.

the oxygen in the side chain with sulfur increased affinity for the Pz receptor. The 6-chloro group in **5n** appeared to markedly enhance affinity for the Pz receptor compared to **5a**, corroborating similar results reported by others using other antagonist ligands.[30]

Among the other azacycles, the order of affinity for Pz receptors was *exo*-isotropane **12** > quinuclidine **5i** ≥ *exo*-azanorborane **9** > tetrahydropyridine **19** > tetrahydroazepine **18** > *endo*-azanorbornane **8** > *endo*-isotropane **11**, with all compounds having higher affinity than arecoline for the Pz receptor.

The unsubstituted quinuclidinylpyrazine **5a** was the most potent and efficacious compound studied in this series in stimulating PI hydrolysis in the A9 L-m_1 cell line (Table 1) and was also more potent and efficacious than arecoline and McNiel-A-343. Substitution of the 3-position of the pyrazine ring with small groups, e.g., CH_3 (**5b**), Cl (**5c**), or OCH_3 (**5d**), diminished efficacy as did substitution of the 6-position with Cl (**5n**). Within the alkoxypyrazine series **5d–j**, increasing efficacy was clearly related to increasing side chain length until the hexyloxy chain (**5i**) was reached, after which efficacy plateaued with chain extension. Efficacy in PI hydrolysis was slightly stereoselective with (*R*)-**5i** producing 13% greater stimulation than (*S*)-**5i**. The (alkylthio)pyrazines **5k–m** were less efficacious in stimulating PI hydrolysis than the alkoxypyrazines **5g–i** of corresponding carbon chain length. None of the alkoxy- or (alkylthio)pyrazines were as efficacious as arecoline, McNiel-A-343, or **5a** in stimulating PI hydrolysis.

The potency of the alkoxypyrazines **5g–j** in stimulating PI hydrolysis increased with chain length. Chain lengths beyond heptyloxy were not studied because **5j**'s efficacy in stimulating PI hydrolysis and affinity for muscarinic receptors had begun to decline compared to that of shorter chain lengths. Potency was also enantioselective with (*S*)-**5i** being almost 5 times more potent than (*R*)-**5i**. The (hexylthio)pyrazine **5m** had the highest potency among the alkoxy- and (alkylthio)pyrazines, but **5m** showed rather low efficacy. Among the quinuclidinylpyrazines, **5a,c,i,j,m** were more potent than arecoline and McNiel-A-343.

Among the other azacycles that had been attached to the (hexyloxy)pyrazine ring, highest efficacy in stimulating PI hydrolysis was shared by the quinuclidine **5i** and *exo*-azanorbornane **9** (Table 2). The *endo*-azanorbornane **8** was only slightly less efficacious followed by *exo*-isotropane **12** and tetrahydropyridine **19**, while tetrahydroazepines **17** and **18** as well as *endo*-isotropane **11** were almost inactive. The *exo*-isotropane **12** was the most potent stimulator of PI hydrolysis among these azacycles followed by quinuclidine **5i**, *exo*-azanorbornane **9**, *endo*-azanorbornane **8**, and tetrahydropyridine **19**.

The quinuclidinylpyrazines **5a–c,n** all produced substantial salivation in mice at the 10 mg/kg ip screening dose (Table 1). By contrast, the only alkoxy- or (alkylthio)pyrazine in this series producing any salivation was **5h**. The order of potency for producing salivation for the quinuclidinylpyrazines containing small 3-substit-

uents was H > CH_3 > Cl > OCH_3. The unsubstituted pyrazine **5a** was a particularly potent sialogog, producing significant salivation at a dose of 0.1 mg/kg. Although arecoline does not produce salivation at the screening dose, tremors were observed and a higher dose of 30 mg/kg ip did produce a salivation score of 1. The lack of sialogogic activity for arecoline at the screening dose is probably due to the well-known rapid metabolism of the compound.[41]

In the (hexyloxy)prazine series employing different azacycles, only the azanorbornanes **8** and **9** produced salivation in mice with the *exo*-azanorbornane **9** being more efficacious than **8** (Table 2). The sialogogic potency of **9** was second only to the unsubstituted pyrazine **5a** in the compounds studied.

Discussion

Among the quinuclidinylpyrazines **5a–d** with small 3-substituents, the order of affinity for the Oxo-M binding site is inversely related to the size of the substituent. This suggests that larger groups create internal energy barriers that impede the formation of conformations that result in efficient binding or that these groups occupy an area in the receptor subject to rigorous steric constraints. Street et al. previously suggested that internal energy barriers created by 3-substituents could explain the decrease in Oxo-M binding seen with **5b** as well as for a series of 3-(alkylpyrazinyl)-1-azanorbornanes when compared to their unsubstituted pyrazine parents.[30]

Regardless of whether the decrease in Oxo-M binding seen with the 3-substitutents in **5b–d** is due to internal or external steric interactions, it was surprising to find that the lengthening of the alkoxy side chain of **5d**, e.g., **5e–j**, actually restores affinity for the Oxo-M binding site. This dependence of affinity on side chain length is similar to that previously reported for a series of 3-(alkoxypyrazinyl)-1-methyl-1,2,5,6-tetrahydropyridines and alkoxy(1,2,5-thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines and indicates that lengthening the side chain brings into play another favorable lipophilic interaction with the receptor that eventually, e.g., with **5i**, entirely overcomes the loss of affinity produced by smaller substituents.[16,42] In addition, replacing the oxygen in the side chain with a sulfur, a larger but more lipophilic atom that could impose more steric congestion, e.g., **5k–m**, actually increases affinity for the Oxo-M binding site demonstrating how important this lipophilic interaction can be in determining affinity. Earlier work on pyrazinylquinuclidines did not place longer lipophilic groups in the 3-position and, therefore, did not take advantage of this important lipophilic binding domain of the receptor.[30]

The enantioselectivity of **5i** for the Oxo-M binding site is the same as that observed with 3-acetoxyquinuclidine (aceclidine) where the *S* isomer shows higher affinity than the *R* isomer.[43]

The additional lipophilic binding site may also have a role in determining the order of Oxo-M binding site affinity of the bicyclic azacycles containing the 3-(hexyloxy)pyrazine moiety. The order of affinity for the Oxo-M binding site for these bicyclic azacycles is quinuclidine **5i** ≥ *exo*-isotropane **12** ≥ *exo*-azanorbornane **9** > *endo*-azanorbornane **8** > *endo*-isotropane **11**, which is different from the order described for either the unsubstituted pyrazine or 6-methoxypyrazine series reported by Street et al.[30] Similar to the unsubstituted pyrazine series, the *exo* isomers of the isotropane and azanorbornane (hexyloxy)pyrazines have much higher Oxo-M binding affinity than the respective *endo* isomers. By contrast, in the 6-methoxypyrazine series, the *endo* isomers of the isotropane and azanorbornane have higher affinity for the Oxo-M binding site than the respective *exo* isomers.

The (hexyloxy)pyrazinyl monocyclic azacycles **19**, **17**, and **18** have substantially lower affinity for the Oxo-M binding site than the *exo*-azabicycles **5i**, **12**, and **9**. This lower affinity is consistent with the model for the M_1 agonist conformation state we described in an earlier publication in which the N-Me group of **19** assumed an axial conformation.[16] Monocyclic pyrazines **19**, **17**, and **18** must overcome some energy barrier to achieve this axial conformation, whereas **5i**, **12**, and **9** constituitively incorporate an axial *N*-alkyl group and more easily achieve the required agonist conformation state.

Like affinity for the Oxo-M binding site, affinity for the Pz binding site is also highly dependent upon the alkoxy chain length in the pyrazinylquinuclidines **5**, suggesting that the two binding sites take advantage of the same lipophilic interaction. In addition, the enantioselectivity (*S* > *R*) and the order of affinity for the Pz binding site for the different [(hexyloxy)pyrazinyl]azabicycles (**12** > **5i** ≥ **9** > **8** > **11**) are very similar to the enantioselectivity and the order of affinity for the Oxo-M binding site, suggesting that these molecules also take advantage of this lipophilic site.

The potent and efficacious stimulation of PI hydrolysis in A9 L-m_1 cells by **5a** complements earlier reports of **5a** producing PI hydrolysis in rat cortical tissue.[30] Like binding to Oxo-M and Pz receptors, stimulation of PI hydrolysis is very sensitive to steric constraints imposed by small substituents in the pyrazine 3-position, e.g., **5b–d**, as these compounds are significantly less efficacious than **5a**. The poor efficacy of **5n** in A9 L-m_1 cells supports the prediction of low cortical efficacy for **5n** based on antagonist/agonist binding ratios and is also consistent with the low partial agonist activity observed with **5n** in stimulating PI hydrolysis in rat cortex and hippocampus as well as in CHO-hm_1 cells.[33,34]

Systematic lengthening of the alkoxy or alkylthio side chain up to six carbon atoms, **5d–m**, increases efficacy at the m_1 receptor just as it increases Oxo-M and Pz affinity. Maximum efficacy is obtained with the (hexyloxy)pyrazine **5i** and diminishes with further chain lengthening (**5j**). This demonstrates that not only affinity but also efficacy are highly dependent on the lipophilic binding site occupied by the alkoxy and alkylthio side chains. This dependence of m_1 efficacy on alkoxy and alkylthio chain length is comparable to that observed with a series of tetrahydropyridylpyrazines where m_1 efficacy was measured using the rabbit vas deferens.[16] Although the (alkylthio)pyrazines have higher affinity for both Oxo-M and Pz receptors than the alkoxypyrazines, the efficacy of the alkoxypyrazines at the m_1 receptor is greater in magnitude than that of the (alkylthio)pyrazines with similar carbon chain lengths showing that efficacy is much more sensitive to steric effects imposed by the larger sulfur atom near the pyrazine ring than is affinity for either receptor.

The *R* enantiomer of **5n** has been shown to be an M_1

agonist in the rat cervical ganglion and an M_3 agonist in the guinea pig ileum and is more potent than the *S* enantiomer in these tests.[33] By contrast, while (*R*)-**5i** was slightly more efficacious in stimulating PI hydrolysis in A9 L-m_1 cells than (*S*)-**5i**, (*S*)-**5i** was almost 5-fold more potent than (*R*)-**5i**. The enantioselectivity of the M_1 agonist action of **5i** appears to be like that of the cholinergic agonist aceclidine (3-acetoxyquinuclidine) in which the *S* enantiomer is much more potent than the *R* enantiomer in a number of measures of cholinergic activity, including the ability to stimulate PI hydrolysis in A9 L-m_1 cells.[44,45] The differences in enantioselectivity seen with **5n,i** could be due to differences between the coupling of second-messenger systems in the A9 L cell line and rat cervical ganglion or to stimulation of multiple muscarinic subtypes in the cervical ganglion.[46] However, because **5n** does not appear to utilize all of the same receptor binding points as **5i**, i.e., the lipophilic binding site employed by the hexyloxy side chain of **5i** is not used by **5n**, the same enantioselectivity in stimulating the m_1 receptor might not be expected.

The quinuclidine (**5i**) and azanorbornane (**8**, **9**) rings confer the highest efficacy in stimulating PI hydrolysis in the (hexyloxy)pyrazine series, but even these compounds are only partial agonists producing 40% or less of the stimulation produced by carbachol. They are also less efficacious than arecoline and McN-A-343. However, **5i** and *exo*-azanorbornane **9** were at least 5.9- and 4.2-fold more potent than arecoline or McNiel-A-343, respectively.

In A9 L-m_1 cells, **5i** is more than 3-fold more efficacious and 82-fold more potent than tetrahydropyridine **19**. Thus, our goal of obtaining much more potent and efficacious M_1 agonists among the azabicyclic pyrazines was achieved, but none of these compounds exceeded the potency and selectivity of xanomeline.[14] In an alternative M_1 assay, the rabbit vas deferens preparation,[47] **5i**, **19**, and xanomeline are almost full agonists (81%, 93%, and 94% of carbachol, respectively) with IC_{50}'s of 9.8, 266,[16] and 0.008 nM,[13] respectively. The relative order of these potencies is consistent with the PI hydrolysis data and shows that the vas deferens is a much more sensitive M_1 agonist assay for this class of compounds than PI hydrolysis in the A9 L-m_1 cell line.

In contrast to the pyrazines bearing small substituents (**5a–c,n**), the (alkoxypyrazinyl)- and [(alkylthio)-pyrazinyl]quinuclidines **5d–m** produced almost no salivation in mice at the maximum doses tested. Alkoxy and alkylthio substituents in the 3-position of these pyrazines clearly diminish salivation, an M_3 agonist effect, even though the affinity of these compounds for muscarinic receptors is comparable to or higher than that of **5a–c,n**. Although the lengthening of the alkoxy and alkylthio side chains in **5d–m** fully or partially restored muscarinic affinity and efficacy at m_1 receptors to the levels seen with **5a**, the M_3 agonist activity was not restored by chain lengthening. This demonstrates that the lipophilic binding site on the receptor that is accessed by the longer 3-alkoxy and 3-alkythio side chains is capable of differentially affecting M_1 and M_3 functional activity. Interestingly, the M_3 agonist activity is restored to the alkoxypyrazines by the azanorbornane azacycle (**8**, **9**) but not by the other azacycles evaluated. Even though **9** has efficacy and potency in M_1 tests similar to **5i**, the increased M_3 activity of **9** made it less suitable than **5i** for further development.

The observation of salivation in mice with **5a–c,n** suggests that there is poor separation between M_1 and M_3 agonist activity with these compounds. Other studies support this conclusion for **5n.**[12,34] For instance, the muscarinic agonist potency of **5n** in the rat cervical ganglion (M_1 test) was similar to that in the guinea pig ileum and trachea (M_3 tests), and PI hydrolysis efficacy and potency in CHO-hm_1 and CHO-hm_3 cells were similar. This poor separation between M_1 and M_3 agonist activity may account for the poor efficacy of **5n** in memory enhancement tests in rats because sufficiently high doses of **5n** could not be employed before side effects obscured memory-enhancing effects.[35]

Our data indicate that **5i** is a partial M_1 agonist that is almost 5-fold more efficacious than **5n**. By contrast, **5n** is a more efficacious M_3 agonist than **5i**. Clearly, there is a significantly higher separation between M_1 and M_3 activity with **5i** than with **5n,** and the failure of **5i** to produce salivation in mice at the doses tested suggests that it is a functionally selective M_1 agonist. This functional selectivity is supported by a lack of significant stimulation of the guinea pig bladder by **5i** even at a concentration of 10 μM, corroborating the lack of M_3 activity in mice.[48] The lack of a negative inotropic effect in isolated guinea pig atria (M_2 activity)[49] with **5i** at concentrations up to 75 μM also shows that **5i** lacks M_2 agonist activity. Taken together, these data show that **5i** is a functionally selective partial M_1 agonist with a wide separation between M_1 and M_2/M_3 agonist activity. Furthermore, ex-vivo binding experiments in rats[15] show that **5i** readily crosses the blood–brain barrier and produces neurochemical effects consistent with its M_1 agonist activity. Therefore, **5i** is a centrally acting, functionally selective M_1 agonist that would be an improvement over **5n** as a therapy for cognitive disorders because it is both a more efficacious and more selective M_1 agonist. By contrast, the more efficacious and potent M_1 activity of xanomeline compared to **5i** suggests that xanomeline could be a superior clinical candidate for the treatment of Alzheimer's disease. Further pharmacological characterization of **5i** will be reported in future publications.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. A Waters PrepLC/500A column using PrepPAK-500 silica gel cartridges, with the solvents specified, were used for HPLC separations. Analytical HPLC analyses were performed on a Waters Associates Model 510 with a Spectroflow 757 detector using a Chiralcel OJ column (4.6 × 250 mm) and the solvents specified. A Harrison Research Chromatotron Model 7924T instrument 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 an argon atmosphere. A General Electric QE-300 spectrometer (300 MHz) was employed for ^1H NMR measurements using the solvents described. Chemical shifts in ppm are reported with reference to $CDCl_3$ at 7.26 ppm or D_2O at 4.80 ppm. Analytical data, melting points, and crystallization solvents are reported in Table 3. No particular attempt was made to optimize reaction conditions for most of the reactions described.

3-(3-Chloro-1,4-diazin-2-yl)-1-azabicyclo[2.2.2]octan-3-ol, 1. A solution of 2,2,6,6-tetramethylpiperidine (7.2 mL, 0.042 mol) in dry THF (300 mL) was cooled to −8 °C as 1.6 M

Table 3. Physical Data for the Pyrazines

no.	formula[a]	mp, °C	cryst solv
1	$C_{11}H_{14}ClN_3O$	215–216	2-propanol
2	$C_{11}H_{12}ClN_3$	66–67	hexane
3	$C_{11}H_{13}Cl_2N_3$	120.5–122	hexane
4i	$C_{17}H_{25}N_3O{\cdot}C_2H_2O_4$	127–128	EtOAc
5a	$C_{11}H_{15}N_3{\cdot}HCl{\cdot}0.25H_2O$	208–209	2-propanol
5b	$C_{12}H_{17}N_3{\cdot}HCl{\cdot}0.75H_2O$	208–210	2-propanol
5c	$C_{11}H_{14}ClN_3{\cdot}HCl{\cdot}0.25H_2O$	223	2-propanol
5d	$C_{12}H_{17}N_3O{\cdot}C_2H_2O_4$	183–184	2-propanol
5e	$C_{13}H_{19}N_3O{\cdot}HCl$	237 dec	2-propanol
5f	$C_{14}H_{21}N_3O{\cdot}HCl$	215–216	2-propanol
5g	$C_{15}H_{23}N_3O{\cdot}HCl$	138.5–139.5	EtOAc
5h	$C_{16}H_{25}N_3O{\cdot}HCl$	166–167	EtOAc
5i	$C_{17}H_{27}N_3O{\cdot}C_2H_2O_4$	137–138	EtOAc
(*S*)-5i	$C_{17}H_{27}N_3O{\cdot}HCl$	128–130	EtOAc
(*R*)-5i	$C_{17}H_{27}N_3O{\cdot}HCl$	133–134	EtOAc
5j	$C_{18}H_{29}N_3O{\cdot}HCl$	147–148.5	EtOAc
5k	$C_{15}H_{23}N_3S{\cdot}HCl$	193–194	EtOAc
5l	$C_{16}H_{25}N_3S{\cdot}HCl$	219–220	EtOAc
5m	$C_{17}H_{27}N_3S{\cdot}HCl$	181–183	EtOAc
5o	$C_{12}H_{17}N_3S{\cdot}HCl$	>250	2-propanol
(*S*)-5p	$C_{12}H_{17}N_3O_2S{\cdot}C_4H_6O_6$	177–178	EtOH
(*R*)-5p	$C_{12}H_{17}N_3O_2S{\cdot}C_4H_6O_6$	179–180	EtOH
6d	$C_{12}H_{15}N_3O$	95–96	hexane
7	$C_{10}H_{12}ClN_3O$	220	2-propanol
8	$C_{16}H_{25}N_3O{\cdot}HCl{\cdot}0.5H_2O$	117–118	ether
9	$C_{16}H_{25}N_3O{\cdot}HCl$	153–154	EtOAc
10	$C_{17}H_{27}N_3O_2$	86–87	ether
11	$C_{17}H_{27}N_3O{\cdot}HCl$	166–167	EtOAc
12	$C_{17}H_{27}N_3O{\cdot}HCl$	203–204	EtOAc
17	$C_{16}H_{25}N_3O{\cdot}HCl{\cdot}0.5H_2O$	145–147	EtOAc
18	$C_{17}H_{27}N_3O{\cdot}HCl$	97–98	EtOAc

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

1-butyllithium in hexane (25 mL, 0.04 mol) was added dropwise. After 20 min, the reaction mixture was cooled to −77 °C followed by dropwise addition of a solution of 2-chloropyrazine (2.9 mL, 0.031 mol) in THF (5 mL). After another 15 min, a solution of 1-azabicyclo[2.2.2]octan-3-one (4.4 g, 0.035 mol) in THF (10 mL) was added dropwise. After 1.5 h, a solution of concentrated HCl (8 mL) and EtOH (4 mL) was added, and the cooling was removed. When the temperature reached −15 °C, 5 N NaOH (20 mL) was added, and the volatile organics were evaporated. The residue was treated with 50 mL of cold water and the solid collected. The aqueous filtrate was extracted with $CHCl_3$ (3 × 150 mL), the extracts were combined with the previously collected solid, and the mixture was warmed to obtain a solution. The solution was dried and the solvent evaporated to give a brown solid that was recrystallized to give **1** (5.8 g): ^{1}H NMR ($CDCl_3$) δ 1.25 (1 H, m), 1.5 (2 H, m), 2.2 (1 H, m), 2.65–3.1 (6 H, m), 3.6 (1 H, bs), 4.05 (1 H, d), 8.3 (1 H, d), 8.5 (1 H, d).

3-Chloro-3-(3-chloro-1,4-diazin-2-yl)-1-azabicyclo[2.2.2]-octane, 3, and 3-(3-Chloro-1,4-diazin-2-yl)-1-azabicyclo-[2.2.2]oct-2-ene, 2. Thionyl chloride (12 mL) was cooled in an ice–water bath with stirring as **1** (1 g, 0.0042 mol) was added. Cooling was removed, and after the reaction mixture was stirred overnight, the solvent was evaporated. The residue was treated with ice–water, made basic with saturated aqueous K_2CO_3, and extracted with CH_2Cl_2 (3 × 25 mL). The extracts were washed with brine and dried, and the solvent was evaporated. The residue was purified by HPLC eluting with an 8 L gradient beginning with CH_2Cl_2 and ending with 10% MeOH–1% NH_4OH–CH_2Cl_2. Compound **3** (yellow crystals, 0.44 g) eluted first followed by **2** (yellow crystals, 0.26 g). **3**: ^{1}H NMR ($CDCl_3$) δ 1.55 (1 H, m), 1.7 (2 H, m), 2.37 (1 H, m), 2.7 (2 H, m), 2.95–3.3 (3 H, m), 3.9 (2 H, m), 8.35 (1 H, d), 8.5 (1H, d). **2**: ^{1}H NMR ($CDCl_3$) δ 1.75 (4 H, m), 2.75 (2 H, m), 3.1 (2 H, m), 3.4 (1 H, bs), 7.35 (1 H, d), 8.25 (1 H, d), 8.5 (1 H, d).

General Procedures for the Syntheses of 3-(3-Alkoxypyrazinyl)-1-azabicyclo[2.2.2]octanes. Method A: 3-(3-Methoxy-1,4-diazin-2-yl)-1-azabicyclo[2.2.2]oct-2-ene, 6d, and 3-(3-Methoxy-1,4-diazin-2-yl)-1-azabicyclo[2.2.2]octane Ethanedioate, 5d. To a solution of sodium methoxide (Na, 1 g, 0.043 mol; MeOH, 40 mL) was added an alcoholic solution of the crude mixture of **2** and **3** that had been generated from **1** (1.2 g, 0.005 mol) as described above. The mixture was heated to reflux for 1 h, the solvent evaporated, the residue treated with ice–water, and the mixture extracted with CH_2Cl_2 (3 × 25 mL). The extracts were washed with brine and dried, and the solvent was evaporated. The residue was purified by HPLC eluting with an 8 L gradient beginning with CH_2Cl_2 and ending with 10% MeOH–1% NH_4OH–CH_2Cl_2 to give solid **6d** (0.17 g): ^{1}H NMR ($CDCl_3$) δ 1.57 (2 H, m), 1.8 (2 H, m), 2.7 (2 H, m), 3.05 (2 H, m), 3.8 (1 H, bs), 4.05 (3 H, s), 7.6 (1 H, s), 7.95 (1 H, d), 8.15 (1 H, d).

A mixture of **6d** (0.17 g, 0.00078 mol) and PtO_2 (0.00075 mol) in EtOH (50 mL) was treated with H_2 (60 psi) over night. The catalyst was removed, the solvent evaporated, and the residue converted to the ethanedioate salt **5d** (0.07 g). ^{1}H NMR (D_2O) δ 1.7 (2 H, m), 2.12 (2 H, m), 2.5 (1 H, m), 3.2–3.6 (5 H, m), 4.85 (1 H, t), 4.0 (3 H, s), 4.05 (1 H, m), 8.05 (1 H, d), 8.1 (1 H, d).

Method B: 3-[3-(Pentyloxy)-1,4-diazin-2-yl]-1-azabicyclo-[2.2.2]octane Hydrochloride, 5h. To a solution of sodium pentyl oxide (Na, 0.5 g, 0.022 mol; 1-pentanol, 30 mL) was added **1** (1.7 g, 0.0071 mol), and the mixture was heated to 100 °C for 2 h. The reaction mixture was cooled and acidified with 1 N HCl (30 mL) and the solvent evaporated. Distillation of the water azeotrope was used to remove most of the alcohol. The residue was made basic and extracted with CH_2Cl_2 (3 × 25 mL), the extracts were washed with brine and dried, and the solvent was evaporated. The residue was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ to give 1.68 g of 3-[3-(pentyloxy)pyrazinyl]-1-azabicyclo-[2.2.2]octan-3-ol: ^{1}H NMR ($CDCl_3$) δ 0.97 (3 H, t), 1.2 (1 H, m), 1.45 (6 H, m), 1.87 (2 H, m), 2.3 (1 H, m), 2.4 (1 H, t), 2.8 (1 H, m), 2.93 (3 H, m), 3.1 (1 H, m), 3.98 (1 H, s), 4.28 (1 H, d), 4.42 (2 H, m), 8.01 (1 H, d), 8.11 (1 H, d).

A solution of this alcohol (1.48 g, 0.0051 mol) in CH_2Cl_2 (50 mL) was cooled to 5 °C as thionyl chloride (1.1 mL) in CH_2Cl_2 (5 mL) was added dropwise. The cooling was removed, and after 1.5 h, the reaction mixture was heated to reflux for 0.5 h. After cooling to ambient temperature, ice–water was added and the reaction mixture made basic with aqueous K_2CO_3. The mixture was extracted with CH_2Cl_2 (3 × 25 mL), the extracts were washed with brine and dried, and the solvent was evaporated. The residue was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ to give **6h** (1.27 g): ^{1}H NMR ($CDCl_3$) δ 0.99 (3 H, t), 1.35–1.8 (7 H, m), 1.9 (2 H, m), 2.37 (1 H, m), 2.6–2.82 (2 H, m), 3.0 (1 H, t), 3.15 (2 H, m), 3.6–4.1 (2 H, m), 4.42 (2 H, t), 8.06 (2 H, m).

A mixture of **6h** (1.05 g, 0.0034 mol), 10% Pd–C (0.4 g), and EtOH (50 mL) was treated with H_2 (60 psi) for 1 h. The catalyst was removed, the solvent evaporated, and the residue converted to an HCl salt to give **5h** (0.28 g): ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.41 (4 H, m), 1.57–1.9 (5 H, m), 2.13 (2 H, m), 2.45 (1 H, m), 3.12 (1 H, m), 3.3–3.46 (3 H, m), 3.55 (1 H, m), 3.7 (1 H, m), 4.3 (1 H, q), 4.37 (2 H, t), 8.03 (1 H, d), 8.1 (1 H, d).

3-(3-Ethoxy-1,4-diazin-2-yl)-1-azabicyclo[2.2.2]octane Hydrochloride, 5e. By method B, from **1** (1.5 g, 0.0063 mol) and sodium ethoxide (0.75 g, 0.033 mol; EtOH, 75 mL) was obtained 3-(3-ethoxypyrazinyl)-1-azabicyclo[2.2.2]octan-3-ol (1.46 g) as a yellow solid. Without further purification, the intermediate was treated with thionyl chloride (1.15 mL) to give **6e** (0.68 g) as a straw-colored liquid. Hydrogenation of this intermediate, purification of the product by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$), and conversion to an HCl salt gave **5e** (0.23 g): ^{1}H NMR ($CDCl_3$) δ 1.39 (3 H, t), 1.65 (2 H, m), 2.0–2.23 (2 H, m), 2.41 (1 H, m), 3.15–3.58 (5 H, m), 3.65–3.75 (1 H, m), 4.18–4.28 (1 H, m), 4.4 (2 H, q), 8.0 (1 H, d), 8.07 (1 H, d).

3-[3-(Propyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, 5f. By method B, from **1** (1.5 g, 0.0063 mol) and sodium propyl oxide (0.75 g, 0.033 mol; 1-propanol, 75 mL) was obtained 3-[3-(propyloxy)pyrazinyl]-1-azabicyclo[2.2.2]octan-3-ol (1.67 g) as a white solid. Without further purification, this intermediate was treated with thionyl

chloride (1.2 mL) to give **6f** (1.29 g) as a straw-colored liquid. Hydrogenation of this intermediate, purification of the product by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$), and conversion to an HCl salt gave **5f** (0.57 g): ^1H NMR ($CDCl_3$) δ 1.05 (3 H, t), 1.59–1.7 (2 H, m), 1.8 (2 H, m), 2.0–2.23 (2 H, m), 2.42 (1 H, m), 3.1–3.59 (5 H, m), 3.65–3.75 (1 H, m), 4.2–4.35 (4 H, m), 8.0 (1 H, d), 8.06 (1 H, d).

3-[3-(Butyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, 5g. By method B, from **1** (3.1 g, 0.0132 mol) and sodium butyl oxide (2 g, 0.087 mol; 1-butanol, 150 mL) was obtained 3-[3-(butyloxy)pyrazinyl]-1-azabicyclo-[2.2.2]octan-3-ol (2.2 g) as a yellow solid. Without further purification, this intermediate was treated with thionyl chloride (1.6 mL) to give **6g** (1.95 g) as a straw-colored liquid. Hydrogenation of 1.7 g of this intermediate, purification of the product by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$), and conversion to an HCl salt gave **5g** (1.0 g): ^1H NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.45 (2 H, m), 1.6–1.9 (5 H, m), 2.1 (2 H, m), 2.45 (1 H, m), 3.15–3.8 (6 H, m), 4.28 (1 H, q), 4.35 (2 H, t), 8.02 (1 H, d), 8.1 (1 H, d).

3-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]oct-2-ene Ethanedioate, 4i, and 3-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]octane Ethanedioate, 5i. By method A, from a mixture of **2** and **3** generated from **1** (2 g, 0.0088 mol) and a solution of sodium hexyl oxide (1.5 g of Na, 0.065 mol; 100 mL of 1-hexanol) was obtained 0.55 g of the free base of **4i** as a yellow liquid: ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.25–1.9 (12 H, m), 2.7 (2 H, m), 3.05 (2 H, m), 3.75 (1 H, bs), 4.4 (2 H, t), 7.6 (1H, s), 7.92 (1 H, d), 8.15 (1 H, d).

Hydrogenation of the free base of **4i** (0.29 g, 0.001 mol) using 0.15 g of PtO_2 and conversion to the ethanedioate salt gave 0.15 g of **5i**: ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.3–1.55 (6 H, m), 1.6–1.9 (4 H, m), 2.1 (2 H, m), 2.45 (1 H, m), 3.25 (1 H, m), 3.35–3.65 (4 H, m), 3.72 (1 H, t), 4.25 (1 H, q), 4.35 (2 H, t), 8.05 (1 H, d), 8.1 (1 H, d).

3-[3-(Heptyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, 5j. By method A, from a mixture of **2** and **3** generated from **1** (1.5 g, 0.0066 mol) and a solution of sodium heptyl oxide (1.5 g of Na, 0.065 mol; 100 mL of 1-heptanol) was obtained **4j** (0.59 g) as a yellow liquid: ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.25–2.0 (14 H, m), 2.95 (2 H, m), 3.3 (2 H, m), 4.07 (1 H, bs), 4.4 (2 H, t), 7.65 (1 H, s), 8.0 (1 H, d), 8.15 (1 H, d).

Hydrogenation of this material using 0.3 g of PtO_2 and conversion to the hydrochloride salt gave **5j**, 0.115 g: ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.25–2.9 (12 H, m), 2.12 (2 H, m), 2.45 (1 H, m), 3.22 (1 H, m), 3.37 (3 H, m), 3.55 (1 H, m), 3.72 (1 H, m), 4.3 (1 H, q), 4.35 (2 H, t), 8.02 (1 H, d), 8.1 (1 H, d).

General Procedure for the Syntheses of 3-[3-(Alkylthio)pyrazinyl]-1-azabicyclo[2.2.2]octanes. 3-[3-(Butylthio)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]octane Hydrochloride, 5k. A suspension of NaH (0.6 g, 0.025 mol) in THF (300 mL) was treated with 1-butanethiol (5 mL), and after 30 min the mixture was heated to reflux for 45 min. After cooling the mixture to ambient temperature, **1** (1.5 g, 0.0063 mol) was added, and the reaction mixture was heated to reflux for 2 h. The solvent was removed by distillation, the residue suspended in H_2O (50 mL), and the mixture extracted with $CHCl_3$ (3 × 25 mL), the extracts were washed with brine, and the solvent was evaporated. The brown solid residue (1.2 g) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C as thionyl chloride (0.95 mL) in CH_2Cl_2 (10 mL) was added dropwise. The cooling was removed, and after 1 h the reaction mixture was heated to reflux for 45 min. After cooling the solution to ambient temperature, the reaction mixture was treated with ice–water and the mixture made basic with 1 N NaOH and extracted with CH_2Cl_2 (3 × 25 mL). The extracts were washed with brine and dried, and the solvent was evaporated to give a brown liquid. The liquid was purified by radial chromatography (5% EtOH–0.5% NH_4OH–$CHCl_3$ and then 10% EtOH–1% NH_4OH–$CHCl_3$) to give 3-chloro-3-[3-(butylthio)pyrazinyl]-1-azabicyclo[2.2.2]octane (1 g): ^1H NMR ($CDCl_3$) δ 0.99 (3 H, t), 1.5 (3 H, m), 1.7 (4 H, m), 2.38 (1 H, m), 2.7 (2 H, m), 2.98 (1 H, m), 3.05–3.4 (4 H, m), 3.76 (1 H, d), 4.19 (1 H, d), 8.18 (1 H, d), 8.3 (1 H, d).

A mixture of this chloride (1 g, 0.0032 mol) and 10% Pd on carbon (0.4 g) in EtOH (50 mL) was hydrogenated (1 h, 60 psi of H_2), the catalyst removed, and the solvent evaporated. The residue was suspended in H_2O (5 mL) and made basic with saturated aqueous K_2CO_3 and the mixture extracted with CH_2Cl_2 (3 × 25 mL). The extracts were dried, the solvent was evaporated, the residue was purified by radial chromatography (5% EtOH–0.5% NH_4OH–$CHCl_3$ and then 10% EtOH–1% NH_4OH–$CHCl_3$), and the product was converted to HCl salt **5k** (0.44 g): ^1H NMR ($CDCl_3$) δ 0.99 (3 H, t), 1.5 (2 H, m), 1.7 (4 H, m), 2.15 (2 H, m), 2.5 (1 H, m), 3.23 (3 H, m), 3.4 (3 H, m), 3.64 (2 H, m), 4.38 (1 H, q), 8.21 (1 H, d), 8.31 (1 H, d).

3-[3-(Pentylthio)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, 5l. From NaH (0.6 g), 1-pentanethiol (5 mL), and **1** (1.6 g, 0.00625 mol) was obtained 1.99 g of a yellow solid. A solution of of this solid (1.5 g) in CH_2Cl_2 was treated with thionyl chloride (1.15 mL) and processed as in the general reaction to give a yellow liquid (1.26 g). Hydrogenation of this liquid using 10% Pd on carbon (0.4 g) followed by purification by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$) and conversion to the HCl salt gave **5l** (0.5 g): ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.4 (4 H, m), 1.70 (4 H, m), 2.05–2.3 (2 H, m), 2.5 (1 H, m), 3.1–3.3 (3 H, m), 3.32–3.47 (3 H, m), 3.75–3.9 (2 H, m), 4.22–4.35 (1 H, m), 8.19 (1 H, d), 8.29 (1 H, d).

3-[3-(Hexylthio)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, 5m. From NaH (0.6 g), 1-hexanethiol (5 mL), and **1** (1.5 g, 0.0063 mol) was obtained 1.98 g of a yellow solid. A solution of of this solid (1.5 g) in CH_2Cl_2 was treated with thionyl chloride (1.15 mL) and processed as in the general reaction to give a yellow liquid (1.265 g). Hydrogenation of this liquid using 10% Pd on carbon (0.4 g) followed by purification by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$) and conversion to the HCl salt gave **5m** (0.65 g): ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.3 (4 H, m), 1.45 (2 H, m), 1.70 (4 H, m) 2.05–2.25 (2 H, m), 2.5 (1 H, m), 3.1–3.3 (3 H, m), 3.3–3.5 (3 H, m), 3.55–3.7 (2 H, m), 4.2–4.3 (1 H, m), 8.19 (1 H, d), 8.29 (1 H, d).

3-(1,4-Diazin-2-yl)-1-azabicyclo[2.2.2]octane Hydrochloride, 5a, and 3-(3-chloro-1,4-diazin-2-yl)-1-azabicyclo-[2.2.2]octane Hydrochloride, 5c. A mixture of **3** and **2** (1.175 g, ca. 0.0046 mol) and 10% Pd on carbon (0.4 g) in EtOH (50 mL) was treated with H_2 (60 psi) for 1 h. The catalyst was removed, the solvent evaporated, the residue suspended in H_2O (5 mL), and the mixture made basic with saturated aqueous K_2CO_3. The mixture was extracted with CH_2Cl_2 (3 × 25 mL), the extracts were dried, the solvent was evaporated, and the residue was purified by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$ and then 12.5% ethanol–1.25% NH_4OH–$CHCl_3$). The less polar component was converted to HCl salt **5c** (0.12 g): ^1H NMR (free base, $CDCl_3$) δ 1.3 (1 H, m), 1.55 (1 H, m), 1.81 (2 H, m), 2.13 (1 H, m), 2.74–3.28 (5 H, m), 3.55 (1 H, t), 3.68 (1 H, q), 8.22 (1 H, d), 8.48 (1 H, d)

The more polar component was also converted to HCl salt **5a** (0.23 g): ^1H NMR (free base, $CDCl_3$) δ 1.38 (1 H, m), 1.57–1.88 (3 H, m), 2.08 (1 H, m), 2.77–3.3 (6 H, m), 3.55 (1 H, q), 8.42 (1 H, d), 8.5 (1 H, s), 8.56 (1 H, d).

3-(3-Chloro-1,4-diazin-2-yl)-1-azabicyclo[2.2.1]heptan-3-ol, 7. A solution of 2,2,6,6-tetramethylpiperidine (7.2 mL, 0.042 mol) in THF (300 mL) was cooled to −5 °C as 1.6 M 1-butyllithium (25 mL, 0.04 mol) in hexane was added dropwise. After 10 min, the reaction mixture was cooled to −77 °C followed by dropwise addition of 2-chloropyrazine (2.9 mL, 0.031 mol) in THF (7 mL). After another 15 min, 1-azabicyclo-[2.2.1]heptan-3-one (4.4 g, 0.0396 mol) in THF (10 mL) was added dropwise. After 1 h, a solution of of concentrated HCl (8 mL) in EtOH (4 mL) was added, and cooling was removed. When the temperature reached −10 °C, H_2O (100 mL) was added, the volatile organics were evaporated, and the residue was treated with of 5 N NaOH (11 mL). The solvent was reduced to a small volume (ca. 50 mL), the mixture cooled in ice–water, and the solid collected by filtration. The aqueous fraction was extracted with $CHCl_3$ (3 × 50 mL), the extracts were added to the solid filtrate, and the total volume was increased to 500 mL with more $CHCl_3$. The mixture was heated to dissolve the solid, the solution dried and filtered,

and the solvent evaporated to give a brown solid. Recrystallization of the solid gave tan crystalline **7** (4.1 g): ^{1}H NMR ($CDCl_3$) δ 1.65 (1 H, m), 1.75 (1 H, m), 2.30 (1 H, m), 2.52 (2 H, m), 2.75 (2 H, m), 2.98 (1 H, m), 3.48 (1 H, d), 3.60 (1 H, d), 8.30 (1 H, d), 8.45 (1 H, d).

***endo*-3-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.1]-heptane Hydrochloride Hemihydrate, 8.** A mixture of **7** (4.8 g, 0.021 mol) and sodium hexyl oxide (1.5 g of Na, 0.065 mol; 125 mL of hexanol) was heated to 80 °C for 45 min. The reaction mixture was cooled to ambient temperature, 1 N HCl (100 mL) was added, and the hexanol was azeotroped off with water. The residue was made basic with 1 N NaOH and extracted with CH_2Cl_2 (3 × 50 mL). The extracts were washed with brine and dried and the solvent evaporated to give a dark solid. Recrystallization of the solid from ether gave 3-[3-(hexyloxy)pyrazinyl]-1-azabicyclo[2.2.1]heptan-3-ol (3.83 g) as a floculant yellow solid: ^{1}H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.25–1.65 (7 H, m), 1.85 (2 H, m), 2.25–3.07 (6 H, m), 3.1 (1 H, d), 3.75 (2 H, d), 4.45 (2 H, t), 8.01 (1 H, d), 8.07 (1 H, d).

A solution of this alcohol (1.5 g, 0.0052 mol) in CH_2Cl_2 (75 mL) was cooled to 0 °C as thionyl chloride (1.1 mL) in CH_2Cl_2 (10 mL) was added dropwise. The cooling was removed and, after 1.5 h, the reaction mixture heated to reflux for 45 min. The reaction mixture was cooled to ambient temperature, ice–water was added, and the reaction mixture was made basic with saturated aqueous K_2CO_3. The mixture was extracted with CH_2Cl_2 (3 × 25 mL), the extracts were washed with brine and dried, and the solvent was evaporated to give a yellow oil. Radial chromatography (2.5% EtOH–0.25% NH_4OH–$CHCl_3$) gave 3-chloro-3-[3-(hexyloxy)pyrazinyl]-1-azabicyclo-[2.2.1]heptane (1.2 g) as a pinkish liquid: ^{1}H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.25–1.95 (10 H, m), 2.25–3.9 (7 H, m), 4.4 (2 H, t), 8.0–8.1 (2 H, m).

A mixture of the chloride (1.2 g, 0.0039 mol) and 10% Pd on carbon (0.5 g) in EtOH (50 mL) was treated with H_2 (60 psi) for 1 h. The catalyst was removed, the solvent evaporated, the residue suspended in H_2O, and the mixture made basic with saturated aqueous K_2CO_3. The mixture was extracted with CH_2Cl_2 (3 × 25 mL), the extracts were dried, and the solvent was evaporated. The residue was purified by radial chromatography (5% EtOH–0.5% NH_4OH–$CHCl_3$ and then 10% EtOH–1% NH_4OH–$CHCl_3$). The more polar of the two major components was isolated as the HCl salt **8** (0.15 g): ^{1}H NMR ($CHCl_3$) δ 0.9 (3 H, t), 1.25–1.55 (8 H, m), 1.85 (3 H, m), 3.2–3.75 (6 H, m), 4.05 (2 H, m), 4.38 (2 H, m), 8.05 (1 H, d), 8.08 (1 H, d).

***exo*-3-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.1]-heptane Hydrochloride, 9.** A mixture of the components from the preparation of **8** (0.35 g, 0.0013 mol) and sodium hexyl oxide (0.2 g, 0.0087 mol of Na; 12 mL of hexanol) was heated to 120 °C overnight. The reaction mixture was cooled to ambient temperature, 1 N HCl (20 mL) added, and the hexanol azeotroped off with water. The residue was made basic with 1 N NaOH and the mixture extracted with CH_2Cl_2 (3 × 25 mL). The extracts were dried, the solvent was evaporated, the residue was purified by radial chromatography (5% EtOH–0.5% NH_4OH–$CHCl_3$), and the less polar component was isolated as the HCl salt **9** (0.15 g): ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.25–1.55 (7 H, m), 1.82 (2 H, m), 1.98 (1 H, m), 2.25 (1 H, m), 3.05 (1 H, d), 3.15 (1 H, d), 3.2–3.7 (7 H, m), 4.22 (1 H, m), 4.35 (2 H, t), 8.05 (2 H, m).

5-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[3.2.1]-heptan-5-ol, 10. A solution of 2,2,6,6-tetramethylpiperidine (4 mL, 0.024 mol) in THF (125 mL) was cooled to −8 °C as 1.6 M 1-butyllithium (14 mL, 0.022 mol) in hexane was added dropwise. After 10 min, the reaction mixture was cooled to −77 °C followed by dropwise addition of 2-(hexyloxy)pyrazine (3.6 g, 0.02 mol) in THF (15 mL). After another 20 min, 1-azabicyclo[3.2.1]octan-5-one[37] (3.1 g, 0.025 mol) in THF (20 mL) was added dropwise. After 1 h, 1 N HCl (20 mL) was added, and cooling was removed. When the temperature reached −10 °C, H_2O (75 mL) was added, and the solvents were evaporated. The residue was suspended in H_2O and extracted with CH_2Cl_2 (3 × 25 mL), the extracts were dried, and the solvent was evaporated. Recrystallization of the residue provided **10** (3.45 g): ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, t), 1.28–1.60 (7 H, m), 1.82 (3 H, m), 2.21 (1 H, m), 2.38 (1 H, m), 2.68 (1 H, m), 2.75–3.14 (5 H, m), 3.96 (1 H, d), 4.15 (1 H, bs), 4.45 (2 H, t), 8.01 (1 H, d), 8.04, (1 H, d).

***endo*-5-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[3.2.1]-octane Hydrochloride, 11.** A solution of **10** (1.7 g, 0.0056 mol) in CH_2Cl_2 (50 mL) was cooled in an ice–water bath as thionyl chloride (1.15 mL) in CH_2Cl_2 (5 mL) was added dropwise. The cooling was removed, the reaction mixture heated to reflux for 1 h, and the solvent evaporated. The residue was treated with ice–water and the mixture made basic with 5 N NaOH and extracted with CH_2Cl_2 (3 × 25 mL). The extracts were dried, the solvent was evaporated, and the residue was purified by radial chromatography (5% EtOH–0.5% NH_4OH–$CHCl_3$) to give a yellow oil (1.01 g): ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.19 (2 H, m), 1.39 (5 H, m), 1.50 (2 H, m), 1.68–1.90 (3 H, m), 2.98 (2 H, m), 3.16 (2 H, m), 3.79 (2 H, m), 4.41 (2 H, t), 4.88 (1 H, m), 8.10 (2 H, m).

A mixture of the oil and 10% Pd on carbon (0.2 g) in EtOH (50 mL) was treated with H_2 (60 psi) for 1 h. The catalyst was removed, the solvent evaporated, and the residue recrystallized (3×) to give **11** (0.39 g): ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.15–1.50 (7 H, m), 1.53–1.85 (4 H, m), 2.10 (1 H, m), 3.17–3.48 (4 H, m), 3.65 (1 H, d), 3.93 (2 H, m), 4.28–4.46 (3 H, m), 8.05 (2 H, m).

***exo*-5-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[3.2.1]-octane Hydrochloride, 12.** The residue from evaporation of the recrystallization liquids of **11** (0.4 g) was treated with sodium hexyl oxide (0.3 g of Na, 0.013 mol; 12 mL of hexanol) and heated to 100 °C over night. The reaction mixture was cooled, water added, and the hexanol azeotroped from the mixture. The residue was extracted with CH_2Cl_2 (3 × 25 mL), the extracts were dried, and the solvent was evaporated. Purification of the residue by radial chromatography (10% EtOH–1.5% NH_4OH–$CHCl_3$) and conversion to the HCl salt gave **12** (0.088 g): ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.29–1.50 (6 H, m), 1.73 (1 H, s), 1.75–2.23 (6 H, m), 2.88 (1 H, bs), 3.08 (1 H, d), 3.32 (1 H, m), 3.40–3.59 (2 H, m), 3.76 (1 H, m), 4.22–4.41 (3 H, m), 8.05 (2 H, m).

3-[3-(Hexyloxy)-1,4-diazin-2-yl]-2,5,6,7-tetrahydroazepine Hydrochloride Hemihydrate, 17. A solution of 2,2,6,6-tetramethylpiperidine (2.4 mL, 0.014 mol) in THF (125 mL) was cooled to −8 °C as 1.6 M 1-butyllithium (8 mL, 0.013 mol) in hexane was added dropwise. After 10 min, the reaction mixture was cooled to −77 °C followed by dropwise addition of 2-(hexyloxy)pyrazine (2.34 g, 0.013 mol) in THF (5 mL). After another 20 min, hexahydroazepin-3-one methylcarbamate[34] (1.7 g, 0.01 mol) in THF (5 mL) was added dropwise. After 0.5 h, 1 N HCl (13 mL) was added, and the cooling was removed. When the temperature reached 15 °C, the solvents were evaporated, the residue was suspended in H_2O and extracted with CH_2Cl_2 (3 × 25 mL), the extracts were dried, and the solvent was evaporated. The residue was purified by flash chromatography (40% EtOAc–hexane) to give yellow liquid **14** (2.06 g): ^{1}H NMR ($CDCl_3$) δ 0.92 (3 H, t), 1.22–2.10 (14 H, m), 2.44 (1 H, q), 3.30–3.57 (1 H, m), 3.60–3.95 (6 H, m), 4.41 (2 H, 7), 8.06 (2 H, m).

A mixture of Burgess' reagent[50] (2.85 g, 0.012 mol) and **14** (2.06 g, 0.0059 mol) in toluene (100 mL) was heated at 60 °C for 1 h. The solvent was evaporated, the residue treated with H_2O, and the mixture extracted with CH_2Cl_2 (3 × 25 mL). The extracts were dried, the solvent was evaporated, and the residue was purified by flash chromatography (30% EtOAc–hexane and then 50% EtOAc–hexane). The major component, **15** (1.43 g), appeared to be the enamide by ^{1}H NMR [($CDCl_3$) δ 0.95 (3 H, t), 1.37 (4 H, m), 1.50 (2 H, m), 1.80–1.95 (6 H, m), 2.78 (2 H, bs), 3.81 (3 H, s), 3.88 (2 H, bs), 4.35 (2 H, t), 7.61 (1 H, bs), 7.90 (1 H, d), 8.08 (1 H, d)], while the second component, **16** (0.35 g), was the desired allylic amine: ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.38 (4 H, m), 1.48 (2 H, m), 1.82 (2 H, m), 1.96 (2 H, m), 2.48 (2 H, q), 3.57–3.85 (5 H, m), 4.35 (2 H, m), 4.45 (2 H, d), 6.68 (1 H, t), 7.92 (1 H, m), 8.08 (1 H, d).

A mixture of the second component (0.14 g, 0.0004 mol) and iodotrimethylsilane (0.1 mL, 0.0007 mol) in CH_2Cl_2 (7 mL) was heated to reflux for 3 h. The solvent was evaporated, the residue treated with MeOH–HCl (5 mL) for 0.5 h, and the solvent evaporated. The residue was treated with ice–water

and the mixture made basic with 1 N NaOH and extracted with CH_2Cl_2 (3 × 15 mL). The extracts were dried, the solvent was evaporated, the residue was purified by radial chromatography (5% EtOH–0.5% NH_4OH–$CHCl_3$), and the product was converted to the HCl salt **17** (0.1 g): ^1H NMR ($CDCl_3$) δ 0.95 (3 H, t), 1.29–1.5 (6 H, m), 1.86 (2 H, m), 2.11 (2 H, m), 2.67 (2 H, m), 3.55 (2 H, m), 4.42 (4 H, m), 7.3 (1 H, m), 8.0 (1 H, d), 8.07 (1 H, d).

3-[3-(Hexyloxy)-1,4-diazin-2-yl]-2,5,6,7-tetrahydro-1-methylazepine Hydrochloride, 18. A mixture of the free base of **17** (0.14 g, 0.0005 mol), 96% formic acid (1.5 mL), and 37% aqueous formaldehyde (1.5 mL) was heated to reflux for 0.5 h. The solvent was evaporated, the residue suspended in H_2O, and the mixture made basic with 5 N NaOH and extracted with ether (3 × 15 mL). The extracts were dried, and the solvent was evaporated, the residue purified by radial chromatography (10% EtOH–1% NH_4OH–$CHCl_3$), and the product converted to the HCl salt **18** (0.068 g): ^1H NMR (free base, $CDCl_3$) δ 0.95 (3 H, t), 1.28–1.55 (6 H, m), 1.82 (4 H, m), 2.5 (5 H, m + s), 3.03 (2 H, t), 3.95 (2 H, bs), 4.35 (2 H, t) 6.96 (1 H, t), 7.93 (1 H, d), 8.07 (1 H, d).

3-[3-(Methylthio)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, 5o. A solution of 2,2,6,6-tetramethylpiperidine (14.5 mL, 0.086 mol) in dry THF (500 mL) was cooled to −8 °C as 1.6 M 1-butyllithium in hexane (50 mL, 0.08 mol) was added dropwise. After 20 min, the reaction mixture was cooled to −77 °C followed by dropwise addition of a solution of 2-(methylthio)pyrazine (8.5 g, 0.067 mol) in THF (20 mL). After another 25 min, a solution of 1-azabicyclo-[2.2.2]octan-3-one (8.8 g, 0.07 mol) in THF (25 mL) was added dropwise. After 1.5 h, a solution of concentrated HCl (8 mL) and EtOH (4 mL) was added, and the cooling was removed. When the temperature reached −5 °C, 5 N NaOH (20 mL) was added, and the volatile organics were evaporated. The residue was treated with H_2O (150 mL), the solvent evaporated, the residue suspended in cold H_2O (100 mL), and the solid collected (14.4 g): ^1H NMR ($CDCl_3$) δ 1.18 (1 H, m), 1.42 (2 H, m), 2.22 (1 H, m), 2.53 (3 H, s), 2.53–3.0 (6 H, m), 4.2 (1 H, d), 4.7 (1 H, bs), 8.18 (1 H, d), 8.3 (1 H, d).

A mixture of the solid (12.4 g, 0.0494 mol) and CH_2Cl_2 (250 mL) was cooled in an ice–water bath as $SOCl_2$ (7 mL, 0.096 mol) in CH_2Cl_2 (20 mL) was added dropwise. Cooling was removed, and 30 min later the solution was heated to reflux for 45 min. After cooling to ambient temperature, the solvent was evaporated, the residue treated with ice–H_2O, the solution made basic, and the mixture extracted with EtOAc. The extracts were washed with brine and dried, and the solvent was evaporated to give a brown oil (13.3 g): ^1H NMR ($CDCl_3$) δ 1.45 (1 H, m), 1.75 (2 H, m), 2.45 (1 H, m), 2.62 (3 H, s), 2.82 (2 H, m), 3.1 (1 H, m), 3.25 (1 H, m), 3.42 (1 H, m), 3.76 (1 H, m), 4.42 (1 H, m), 8.2 (1 H, d), 8.38 (1 H, d).

A mixture of the oil (13.3 g, 0.049 mol) and 10% Pd on carbon (1.75 g) in EtOH (200 mL) was hydrogenated (1 h, 60 psi of H_2), the catalyst removed, and the solvent evaporated to give a tan solid. The residue was treated with ether (25 mL) and the mixture chilled and filtered. The solid was suspended in H_2O (25 mL) and made basic with saturated aqueous K_2CO_3 and the mixture extracted with EtOAc. The extracts were dried, and the solvent was evaporated, the residue treated with cold ether (25 mL), and the mixture filtered. Evaporation of the solvent gave a clear oil (8.1 g) that was converted to a hydrochloride salt: ^1H NMR (D_2O) δ 1.71 (2 H, m), 2.1 (1 H, m), 2.21 (1 H, m), 2.48 (1 H, m), 2.55 (3 H, s), 3.3 (1 H, m), 3.42 (2 H, t), 3.57 (2 H, m), 3.82 (1 H, m), 4.1 (1 H, q), 8.28 (1 H, d), 8.32 (1 H, d).

(*S*)-3-[3-(Methylsulfonyl)-1,4-diazin-2-yl]-1-azabicyclo-[2.2.2]octane (2*S*,3*S*)-tartrate, (*S*)-5p. The free base of **5o** (7.8 g) in EtOH (75 mL) was added to (2*S*,3*S*)-tartaric acid dissolved in hot EtOH (700 mL). After cooling to ambient temperature overnight, a straw-colored solid was collected (5.5 g). Two more recrystallizations from EtOH provided a tan solid (2.96 g) that had greater than 90% ee by HPLC analysis (2% 2-propanol/hexane). The free base of this salt (1.7 g, 0.0072 mol) in 0.22 N HCl (32.2 mL) was treated dropwise with Oxone (6.8 g, 0.011 mol) in H_2O (30 mL) and the reaction mixture stirred over night. The solution was cooled in an ice–water bath and 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 brown oil (0.9 g). The (2*S*,3*S*)-tartaric acid salt had greater than 95% ee by HPLC analysis (15% 2-propanol/hexane): ^1H NMR (D_2O) δ 1.78 (2 H, m), 2.12 (2 H, m), 3.22–3.6 (8 H, m + s), 4.17 (1 H, q), 4.43 (3 H, m), 8.63 (1 H, d), 8.92 (1 H, d).

The absolute configuration at position 3 in (*S*)-**5p** was determined by comparing the configuration to that of the configuration at positions 2 and 3 in the (2*S*,3*S*)-tartaric acid in the X-ray crystal structure.

(*R*)-3-[3-(Methylsulfonyl)-1,4-diazin-2-yl]-1-azabicyclo-[2.2.2]octane (2*R*,3*R*)-Tartrate, (*R*)-5p. The free base of **5o** (3.83 g) recovered from the initial recrystallization fluid used in the preparation of (*S*)-**5p** was converted to the (2*R*,3*R*)-tartaric acid salt and recrystallized from EtOH three times to give a tan solid (2.73 g). HPLC analysis showed greater than 90% ee. The free base of this salt (1.6 g, 0.0068 mol) in 0.22 N HCl (31.8 mL) was treated dropwise with Oxone (6.4 g, 0.0104 mol) in H_2O (30 mL) and the reaction mixture stirred over night. The solution was cooled in an ice–water bath and 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 brown oil (0.6 g). The (2*R*,3*R*)-tartaric acid salt had greater than 95% ee by HPLC analysis.

(*S*)-3-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane Hydrochloride, (*S*)-5i. A mixture of 60% NaH in oil (0.144 g, 0.0036 mol), 1-hexanol (0.6 mL), and THF (30 mL) was heated to reflux for 30 min. The solution was cooled to ambient temperature and treated with (*S*)-**5p** (0.32 g, 0.0012 mol) in THF (5 mL). After stirring overnight, 1 N HCl (6 mL) was added, and the solvents were 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 an oil (0.3 g) that was converted to the hydrochloride salt (*S*)-**5i**. HPLC analysis (2% 2-propanol/hexane) of the free base of (*S*)-**5i** showed less than 3% of the *R* enantiomer.

(*R*)-3-[3-(Hexyloxy)-1,4-diazin-2-yl]-1-azabicyclo[2.2.2]-octane hydrochloride, (*R*)-5i. This compound was obtained in the same manner as (*S*)-**5i** using (*R*)-**5p** as starting material. HPLC analysis of the free base of (*R*)-**5i** could not detect the presence of any (*S*)-**5i**.

X-ray Crystallographic Structure Analysis of (*S*)-5p. Crystals of (*S*)-**5p** formed in the monoclinic space group $P2_1$ with a unit cell having the dimensions a = 7.591(2) Å, b = 8.457(2) Å, c = 14.415(3) Å, and β = 96.93(2)° with a calculated density of 1.502 g cm^{-3}. A total of 1474 reflections with 2θ less than 116.0° was measured on an automated four-circle diffractometer using monochromatic copper radiation, and the structure was solved using direct methods. Full-matrix least-squares refinement was conducted with anisotropic temperature factors for all atoms except hydrogen, which were included at calculated positions with isotropic temperature factors. The final *R*-factor of 0.06 was obtained for 1305 observed reflections, and no significant features on the final difference Fourier map were noted (largest difference peak and hole were 0.32 and −0.38 eÅ^{-3}, respectively).

Salivation in Mice. Male Crl:CF1^{R}BR 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. Those compounds producing an average score of 1 were tested at one-half log lower doses until a score lower than 1 was achieved. The lowest dose of compound producing a score of 1 was expressed as the minimum effective dose (MED).

Radioligand-Binding Assays. The hippocampus from male Sprague–Dawley rats was homogenized in 10 vol 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 (P_2) pellet was homogenized in 50 vol

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 vol of buffer and frozen at −70 °C until used.

The inhibition of binding of pirenzepine to hippocampal membranes was determined by adding unlabeled drug, 1 nM [^{3}H]pirenzepine (87 Ci/mmol; New England Nuclear, Boston, MA), and hippocampal membranes equivalent to 10 mg of tissue wet weight (about 0.1 mg of protein) in 1 mL total volume of 20 mM Tris-Cl buffer, pH 7.4, containing 1 mM $MnCl_2$.[51] The inhibition of binding of oxotremorine-M to hippocampal membranes was determined by adding unlabeled drug, 3 nM [^{3}H]oxotremorine-M (87 Ci/mmol; New England Nuclear), and hippocampal membranes equivalent to 10 mg of tissue wet weight (about 0.1 mg of 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.[52]

Stimulation of Phosphoinositol Hydrolysis in A9 L-m1 Cells. A9 L-m_1 cells were cultured to confluence in 75 mL flasks containing Dubecco's modified essential media. Cells were prelabeled with 1 μCi/mL *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 and the mixture sonicated and centrifugated at 20000*g*. The supernatent was decanted over an Accell QMA anion exchange SEP-PAK cartridge in the formate form (Waters Assoc., 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. 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.

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Supporting Information Available: X-ray crystallographic data for (*S*)-**5p** (8 pages). Ordering information is given on any current masthead page.

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