Novel Functional M_1 Selective Muscarinic Agonists. 2. Synthesis and Structure-Activity Relationships of 3-Pyrazinyl-1,2,5,6-tetrahydro-1-methylpyridines. Construction of a Molecular Model for the M_1 Pharmacophore

John S. Ward,*,† Leander Merritt,† V. J. Klimkowski,† M. L. Lamb,§ C. H. Mitch,† F. P. Bymaster,† B. Sawyer,† H. E. Shannon,† Preben H. Olesen,‡ Tage Honoré,‡ Malcolm J. Sheardown,‡ and Per Sauerberg‡

Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, Novo Nordisk CNS Division, Novo Nordisk Park, DK-2760 Måløv, Denmark, and Department of Chemistry, Drew University, Madison, New Jersey 07940

Received April 6, 1992

A series of 3-(3-substituted-pyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridines were synthesized and found to have high affinity for central muscarinic receptors. The ability of some of these compounds to inhibit the electrically stimulated twitch of the guinea pig vas deferens indicated that the compounds were M_1 agonists. M_1 agonist activity was related to the length of the side chain attached to the pyrazine ring, with maximal activity being obtained with the hexyloxy side chain. The (hexyloxy)pyrazine **3f** lacked M_2 agonist activity as it failed to affect the guinea pig atria and was also relatively devoid of M_3 agonist activity as determined by its lack of tremorogenic and sialogogic effects in mice. A comparison of the M_1 agonist efficacy of these pyrazines and related 1,2,5-thiadiazoles and 1,2,5-oxadiazoles suggested that M_1 efficacy was related to the magnitude of electrostatic potential located over the nitrogens of the respective heterocycles. The heteroatom directly attached to the 3 position of the pyrazine or 1,2,5-thiadiazole heterocycle markedly influenced the M_1 efficacy of the compounds by determining the energetically favorably conformers for rotation about the bond connecting the tetrahydropyridyl ring and the heterocycle. A three-dimensional model for the M_1-activating pharmacophore was proposed based on computational studies and the model of the muscarinic pharmacophore proposed by Schulman.

Introduction

The discovery that Alzheimer's disease can be consistently characterized by major deficits in cerebral cholinergic transmission[1-3] has stimulated interest in agents that will enhance central cholinergic transmission.[4] The goal of most of these efforts has been to reverse the memory and learning disruption that has been associated with the loss of cholinergic function.[5] Among those agents enhancing cholinergic function, acetylcholinesterase inhibitors, e.g. tacrine,[6] 9-amino-1,2,3,4-tetrahydroacridinol,[7] and physostigmine,[8] and directly acting agonists,[9] e.g., arecoline, RS-86, and pilocarpine, have shown sufficient promise to proceed to clinical trials. However, the direct-acting agents have not produced promising responses and the acetylcholinesterase inhibitors, although seemingly more efficacious, have severe dose-limiting side effects and their usefulness as an Alzheimer's disease therapy remains to be proven.[6-8] Although the clinical failures of the directly acting agonists have been attributed to their low efficacy at cortical muscarinic receptors compared to acetylcholine,[10] an equally valid reason for their failure is their lack of muscarinic receptor subtype specificity that contributes to their dose-limiting side effects.

Both functional and radioligand binding studies have identified pharmacologically distinct M_1, M_2, and M_3 muscarinic receptor subtypes,[11] and molecular biology has now identified five genetically distinct muscarinic receptor subtypes, m_1-m_5.[12-15] The relationship between pharmacologically defined and genetically defined muscarinic receptor subtypes has been suggested to be $M_1 = m_1$, m_4, and m_5; $M_2 = m_2$; and $M_3 = m_3$.[16] In Alzheimer's disease,

† Lilly Research Laboratories.
‡ Novo Nordisk CNS Division.
§ Department of Chemistry.

(1) Perry, E. K. The cholinergic hypothesis-ten years on. *Br. Med. Bull.* **1986**, *42*, 63-69.
(2) Sims, N. R.; Bowen, D. M.; Smith, C. C. T.; Neary, D.; Thomas, D. J.; Davison, A. N. Presynaptic cholinergic dysfunctions in patients with dementia. *J. Neurochem.* **1983**, *40*, 503-509.
(3) Perry, E. K. Acetylcholine and Alzheimer's disease. *Br. J. Psychiatry* **1988**, *152*, 737-740.
(4) Moos, W. H.; Davis, R. E.; Schwarz, R. D.; Gamzu, E. R. Cognition activators. *Med. Res. Rev.* **1988**, *8*, 353-391.
(5) Archer, T.; Fowler, C. J. Toward an animal model for the cholinergic lesion of Alzheimer's disease. *Trends Pharmacol. Sci.* **1985**, *6*, 61.
(6) Summer, W. K.; Majovski, L. V.; Marsh, G. M.; Tachiki, K.; King, A. Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *N. Engl. J. Med.* **1986**, *315*, 1241-1245.
(7) Shutske, G. M.; Pierrat, F. A.; Cornfeldt, M. L.; Szewcak, M. R.; Huger, F. P.; Bores, G. M.; Haroutunian, V.; Davis, K. L. (±)-9-Amino-1,2,3,4-tetrahydroacridine-1-ol. A potential Alzheimer's disease therapeutic of low toxicity. *J. Med. Chem.* **1988**, *31*, 1278-1279.
(8) Christe, J. E.; Shering, A.; Ferguson, J.; Glen, A. I. M. Physostigmine and arecoline: Effects of intravenous infusions in Alzheimer Presenile Dementia. *Br. J. Psychiatry* **1981**, *138*, 46-50.
(9) Hollander, E.; Mohs, R. C.; Davis, K. L. Cholinergic approaches to the treatment of Alzheimer's disease. *Br. Med. Bull.* **1986**, *42*, 97.
(10) Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, L. L.; Kneen, C.; MacLeod, A.; Merchant, K.; Snow, R. J.; Baker, R. Novel quinuclidine-based ligands for the muscarinic cholinergic receptor. *J. Med. Chem.* **1990**, *33*, 1128-1138.
(11) Mutschler, E.; Moser, U.; Wess, J.; Lambrecht, G. Muscarinic receptor subtypes: agonists and antagonists. *Prog. Pharmacol. Clin. Pharmacol.*; Gustav Fischer Verlag: Stuttgart, 1989; Vol. 7/1, pp 13-31.
(12) Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. Identification of a family of muscarinic acetylcholine receptor genes. *Science* **1987**, *237*, 527-532.
(13) Bonner, T. I.; Young, A. C.; Brann, M. R.; Buckley, N. J. Cloning and expression of the human and rat m_5 muscarinic acetylcholine receptor genes. *Neuron* **1988**, *1*, 403-410.
(14) Peralta, E. G.; Ashkenzai, A.; Winslow, J. W.; Smith, D. H.; Ramachadran, J.; Capon, D. J. Distinct primary structures, ligand binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors. *EMBO J.* **1987**, *6*, 3923-3929.
(15) Kubo, T.; Fukuda, K.; Mikami, A.; Maeda, A.; Takahashi, H.; Mishima, M.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Kojima, M.; Matsuo, H.; Kirosi, T.; Numa, S. Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. *Nature* **1986**, *323*, 411-416.
(16) Buckley, N. J.; Bonner, T. I.; Buckley, C. M.; Brann, M. R. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol. Pharmacol.* **1989**, *35*, 469-476.

0022-2623/92/1835-4011$03.00/0

the basal forebrain muscarinic neurons that predominantly express the M_2 receptor subtype have been found to diminish[17,18] while the cortical neurons expressing the M_1 receptor subtype remain relatively unaffected.[19] It is generally believed that the lack of stimulation of these cortical M_1 receptor sites, due to the degeneration of the basal forebrain cholinergic neurons that synapse with them, is responsible for the cognitive deficits associated with Alzheimer's disease. This suggests that a selective M_1 agonist would be an appropriate therapy for alleviating the memory deficiencies associated with Alzheimer's disease.

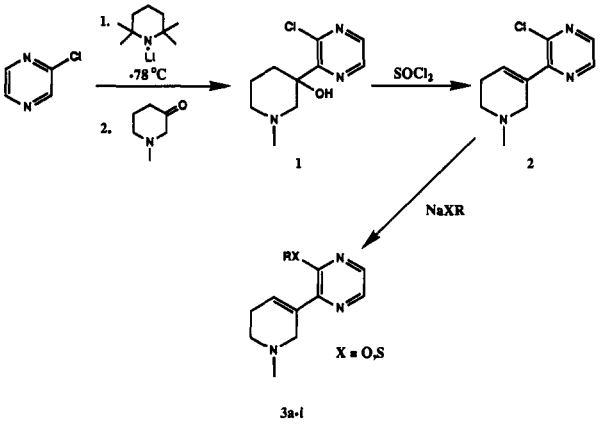
We have described in a recent paper some 3-(3-alkoxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines and 3-[3-(alkylthio)-1,2,5-thiadiazol-4-yl]-1,2,5,6-tetrahydro-1-methylpyridines that are highly efficacious and functionally selective M_1 agonists.[20] By contrast the corresponding iso-π-electronic 1,2,5-oxadiazoles were markedly less active as M_1 agonists. The pyrazine ring is also iso-π-electronic with the 1,2,5-thiadiazole ring and, while pyrazine is more basic, the two ring systems share many physical and chemical properties.[21,22] Like the 1,2,5-thiadiazole and 1,2,5-oxadiazole rings, the nitrogen atoms in the pyrazine ring are ideally located to provide hydrogen-bonding sites that appear to be important for potent muscarinic agonist activity.[10,23]

In this paper, we describe the syntheses and in vitro biological evaluations of 3-(3-alkoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridines and 3-[3-(alkylthio)pyrazinyl]-1,2,5,6-tetrahydro-1-methylpyridines that bear the same substitution pattern on the heteroaromatic moiety as the iso-π-electronic 1,2,5-thiadiazoles and 1,2,5-oxadiazoles described in our previous publication. Some of these pyrazines, like their related 1,2,5-thiadiazole analogs, are M_1 agonists. In addition, we present computational and molecular modeling data that permit the rationalization of the relative M_1 activity of the 1,2,5-thiadiazole, 1,2,5-oxadiazole, and pyrazine compounds that have been studied and propose a 3D model for the M_1 agonist pharmacophore.

Chemistry

The methods of synthesizing the 3-alkoxy- and 3-(alkylthio)pyrazines **3** are summarized in Scheme I. Moderate yields of (3-chloropyrazinyl)piperidinol **1** were obtained from the reaction of 2-chloro-3-lithiopyrazine[24] and 1-methylpiperidin-3-one at −78 °C. Thionyl chloride converted alcohol **1** directly to tetrahydropyridine **2** without isolation of the expected chloropiperidine or potential isomeric tetrahydropyridines. When crude **2** was treated with an appropriate sodium alkoxide or alkanethiolate followed by heating, the desired pyrazines **3** were isolated after chromatography and conversion to their oxalate or hydrochloride salts. The removal of the excess higher boiling alcohols encountered in some of these preparations was aided by distillation of their azeotropes with water under reduced pressure.

Scheme I. Syntheses of Pyrazinyl-1,2,5,6-tetrahydro-1-methylpyridines



Biological Evaluation

The affinities of the compounds for muscarinic receptor sites in rat hippocampus, where the M_1 receptor predominates, were determined using competitive radioligand binding assays employing [^{3}H]oxotremorine-M (Oxo-M) or [^{3}H]pirenzepine (Pz). The ability of a compound to displace Oxo-M, a potent muscarinic agonist lacking subtype selectivity, from hippocampal membranes was interpreted as the compound's affinity for the "agonist conformational state" of the muscarinic receptor sites.[25] Similarly, the displacement of M_1 receptor selective Pz from hippocampal binding sites was interpreted as a compound's affinity for M_1 receptor sites.[26]

In the isolated rabbit vas deferens, M_1 agonists inhibit the electrically stimulated twitch response.[20,27-29] The efficacy of each compound in inhibiting this twitch response was measured up to a concentration of 10 μM

(17) Potter, L. T.; Flynn, D. D.; Hanchett, H. E.; Kalinoski, D. L.; Luber-Narod, J.; Mash, D. C. Independent M_1 and M_2 receptors; ligands, autoradiography and functions. Subtypes of Muscarinic Receptors. *Trends Pharmacol. Sci. Suppl.* **1983**, 22–31.

(18) Meyer, E. M.; Arendash, G. W.; Judkins, J. H.; Ying, L.; Wade; Kem, W. R. Effects of nucleus basalis lesions on the muscarinic and nicotinic modulation of [^{3}H]acetylcholine release in rat cerebral cortex. *J. Neurochem.* **1987**, *49*, 1758–1762.

(19) Narang, P. K.; Cutler, N. R. Pharmacotherapy in Alzheimer's disease: basis and rationale. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1986**, *10*, 519–531.

(20) Sauerberg, P.; Olesen, P. H.; Nielsen, S.; Treppendahl, S.; Sheardown, M. J.; Honoré, T.; Mitch, C. H.; Ward, J. S.; Pike, A. J.; Bymaster, F. P.; Sawyer, B. D.; Shannon, H. E. Novel Functional M_1 Selective Muscarinic Agonists. Synthesis and Structure–Activity Relationships of 3-(1,2,5-Thiadiazolyl)-2,3,5,6-tetrahydro-1-methylpyridines. *J. Med. Chem.* **1992**, *35*, 2274–2283.

(21) Weinstock, L. M.; Pollack, P. I. The 1,2,5-thiadiazoles. *Adv. Heterocycl. Chem.* **1968**, *9*, 107–163.

(22) Weinstock, L. M.; Shinkai, I. 1,2,5-Thiadiazoles and their benzo derivatives. *Comprehensive Heterocyclic Chemistry*, Potts, K. E., Ed.; Pergamon Press: Oxford, 1984; Vol. 6, Chapter 4.26, pp 513–543.

(23) MacLeod, A. M.; Baker, R.; Freedman, S. B.; Patel, S.; Merchant, K. J.; Roe, R.; Saunders, J. Synthesis and muscarinic activities of 1,2,4-thiadiazoles. *J. Med. Chem.* **1990**, *33*, 2052–2059.

(24) Turck, A.; Mojovic, L.; Quequiner, G. A new route to 2,3-disubstituted pyrazines; regioselective metalation of chloropyrazine. *Synthesis* **1988**, 881–884.

(25) Freedman, S. B.; Harley, E. A.; Iversen, L. L. Relative affinities of drugs acting at cholinoreceptors in displacing agonist and antagonist radioligands: the NMS/Oxo-M ratio as an index of efficacy at cortical muscarinic receptors. *Br. J. Pharmacol.* **1988**, *93*, 437–445.

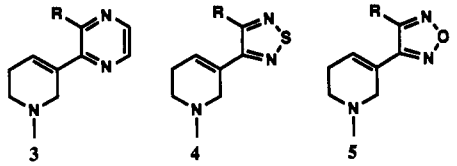
(26) Hammer, R.; Giachetti, A. Muscarinic receptor subtypes: M_1 and M_2, biochemical and functional characterization. *Life Sci.* **1982**, *31*, 2991–2998.

(27) Eltze, M. Muscarinic M_1- and M_2-receptors mediating opposite effects on neuromuscular transmission in rabbit vas deferens. *Eur. J. Pharmacol.* **1988**, *151*, 205–221. Eltze, M.; Gmelin, G.; Weiss, J.; Strohmann, C.; Tacke, R.; Mutschler, E.; Lambrecht, G. Presynaptic muscarinic receptors mediating inhibition of neurogenic contractions in rabbit vas deferens are of the ganglionic M_1-type. *Eur. J. Pharmacol.* **1988**, *158*, 233–242.

(28) Shannon, H. E.; Sawyer, B. D.; Bymaster, F. P.; Heath, I.; Mitch, C. H.; Ward, J. S. Characterization of muscarinic receptors in rabbit vas deferens. Submitted for publication.

(29) Dörje, F.; Levey, A. I.; Brann, M. R. Immunological detection of muscarinic receptor subtype proteins (m_1–m_5) in rabbit peripheral tissues. *Mol. Pharm.* **1991**, *40*, 459–462.

Table I. In Vitro Data for 1-Methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazoles, -pyrazines, and -1,2,5-oxadiazoles



3 4 5

R	no.	receptor binding to rat brain membranes: IC_{50} ± SEM, nMs [^{3}H]Oxo-M	[^{3}H]Pz	inhibn of twitch height in rabbit vas deferens % max ± SEM	IC_{50}, nM
OMe	**3a**	850 ± 190	840 ± 90	0	
	4a	22 ± 0.8	148 ± 19	47 ± 22	
OEt	**3b**	200 ± 40	175 ± 30	14 ± 5	
	4b	5.7 ± 0.24	48 ± 1	43 ± 20	
O-*n*-Pr	**3c**	58 ± 11	65 ± 11	4 ± 2	
	4c	1.6 ± 0.06	18 ± 0.2	25 ± 31	
O-*n*-Bu	**3d**	17 ± 3	17 ± 1	0	
	4d	1.4 ± 0.12	5.0 ± 0.7	68 ± 7	3
O-*n*-pentyl	**3e**	19 ± 3	16 ± 2	29 ± 6	
	4e	2.0 ± 0.25	4.0 ± 0.6	93 ± 1	0.004
O-*n*-hexyl	**3f**	20 ± 3	17 ± 1	93 ± 1	266
	4f	9.7 ± 1.1	7.0 ± 0.7	94 ± 1	0.008
	5	84 ± 25	277 ± 31	47 ± 20	
O-*n*-heptyl	**3g**	18 ± 1	27 ± 3	57 ± 14	5100
	4g	6.2 ± 0.02	12 ± 3	69 ± 5	9
S-*n*-pentyl	**3h**	13 ± 3	10 ± 1	18 ± 15	
	4h	4.8 ± 1.4	2.4 ± 0.2	90 ± 5	0.002
S-*n*-hexyl	**3i**	44 ± 19	28 ± 3.0	62 ± 10	224
	4i	6.5 ± 2.2	5 ± 0.7	92 ± 1	0.001
NH-*n*-hexyl	**4k**	60 ± 3.0	105 ± 20	65 ± 10	3000
n-heptyl	**4l**	187 ± 37	73 ± 9	26 ± 6	224
Cl	**2**	276 ± 25	>1000	0	
	4j	10.8 ± 2.1	375 ± 48	84[a]	537
arecoline		77 ± 13	1300 ± 260	95 ± 1	545
McN-A-343		355 ± 40	955 ± 75	91 ± 2	659

[a] $n = 2$.

with IC_{50}'s being determined for each compound that produced at least a 50% inhibition of the twitch response.

Results

The affinity of the alkoxypyrazines for the muscarinic agonist conformational state, as measured by Oxo-M displacement (Table I), increased with increasing carbon chain length with optimum affinity being obtained with the butyloxy derivative **3d**. As the carbon chain length increased beyond four carbons, little change in affinity for this conformational state occurred. Alkoxy chains with greater than seven carbons were not investigated. The (alkylthio)pyrazines, **3h** and **3i**, had slightly greater affinity for the agonist conformation state than their corresponding alkoxypyrazines, **3e** and **3f**. The chloropyrazine **2** had only moderate affinity for the agonist conformational state. With the exception of **3a**, **3b**, and **2**, all of the pyrazines had higher affinity for the Oxo-M binding site than the conceptually antecedent arecoline.

A similar pattern of increasing affinity with increasing chain length was seen in Pz displacement assays. Highest affinity for the M_1 receptor site, as defined by Pz displacement, was obtained with the (pentyloxy)pyrazine **3e**, although, there was little difference in affinity between the butyloxy (**3d**), pentyloxy (**3e**), and hexyloxy (**3f**) compounds. Affinity for the M_1 receptor began to diminish when the alkoxy chain contained more than six carbons (**3g**). Slightly higher affinity for the M_1 receptor was seen with the (alkylthio)pyrazines **3h** and **3i**, than with the alkoxypyrazines of the same chain length (**3e** and **3f**, respectively). The chloropyrazine **2** had comparatively poor affinity for the M_1 receptor site and was the only compound studied lacking higher affinity for Pz binding sites than arecoline.

The efficacy of the alkoxypyrazines at the M_1 receptor, as measured by the ability to inhibit the electrically stimulated twitch of the rabbit vas deferens, was very low until a chain containing at least five carbons was reached (**3e**). Increasing the chain length by another carbon [(hexyloxy)pyrazine **3f**] produced full M_1 efficacy comparable to that obtained with arecoline and McNeil-A-343, but **3f** was at least twice as potent as these two standards (IC_{50} = 266 nM). Further chain lengthening, [(heptyloxy)pyrazine **3g**] significantly diminished both efficacy and potency at the M_1 receptor. In the vas deferens preparation, the (alkylthio)pyrazines **3h** and **3i** were both less efficacious than arecoline, McNeil-A-343, and the corresponding alkoxypyrazines of similar chain length (**3e** and **3f**, respectively). The chloropyrazine **2** did not show any inhibitory activity on the rabbit vas deferens.

Discussion

A comparison of the Oxo-M affinity, Pz affinity, and efficacy and potency on the rabbit vas deferens between the alkoxythiadiazoles **4a–g** from our earlier study[20] and the alkoxypyrazines **3a–g** (Table I) shows that the chain length in both series of compounds has markedly similar effects on these biological parameters. In both series, maximum Oxo-M affinity was obtained with the butyloxy derivatives **3d** and **4d**, maximum Pz affinity was obtained with the pentyloxy derivatives **3e** and **4e**, and maximum efficacy for inhibition of the rabbit vas deferens was obtained with the hexyloxy derivatives **3f** and **4f**. However, maximum potency in the vas deferens preparation in the thiadiazole series was obtained with the (pentyloxy)-thiadiazole 4e while maximum potency in the pyrazine series was obtained with the (hexyloxy)pyrazine **3f**.

Clearly, where comparisons were possible, the alkoxythiadiazoles were much more active than the alkoxypyrazines of the same chain length in all of these biological tests. This relationship also held when comparing the (alkylthio)thiadiazoles (**4h** and **4i**) and (alkylthio)pyrazines (**3h** and **3i**) as well as for comparing the chlorothiadiazole **4j** and chloropyrazine **2**. The (hexyloxy)-1,2,5-oxadiazole **5** that was examined in these tests had less affinity for the muscarinic receptors and was less efficacious in the rabbit vas deferens than the corresponding (hexyloxy)pyrazine **3f** or (hexyloxy)thiadiazole **4f**. This suggests that, when the alkoxy chain length is held constant, the order of M_1 activity for the iso-π-electronic heterocycles examined is 1,2,5-thiadiazole > pyrazine > 1,2,5-oxadiazole.

Certain of the alkoxypyrazines and alkoxythiadiazoles show relatively small differences in affinities for the Oxo-M and Pz binding sites, respectively, similar efficacies on the rabbit vas deferens, and large differences in potencies on the rabbit vas deferens, e.g., **3f** vs **4f**, **3g** vs **4g**. Recent studies have demonstrated a substantial M_1 receptor reserve and highly efficient receptor–effector coupling in the rabbit vas deferens.[30] In tissue or cultured cells having a large receptor reserve, the potency of agonists, particularly highly efficacious agonists, can be substantially

(30) Micheletti, R.; Schiavone, A. Functional determination of McN-A-343 affinity for M_1 receptors. *J. Pharmacol. Exp. Ther.* **1990**, *253*, 310–314.

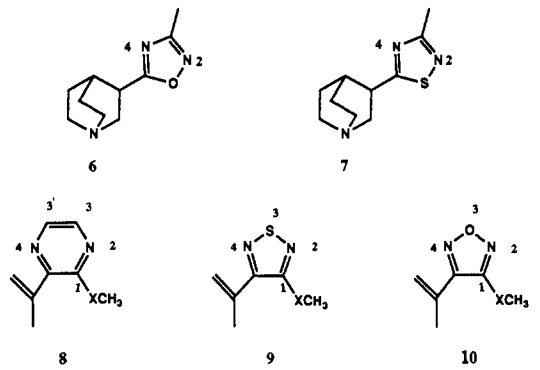


Figure 1. Comparison of N positions in the heterocycles studied.

increased relative to their potency in receptor binding assays. Studies in our laboratories are now underway to compare the efficacies and potencies of such compounds in other functional assays to gain a better understanding of these relationships.

Like the (hexyloxy)thiadiazole **4f**, the (hexyloxy)-pyrazine **3f** appeared to be a functionally selective agonist at M_1 receptors. The isolated guinea pig atria was not affected by **3f** at concentrations up to 80 μM, indicating that the compound lacked M_2 agonist activity.[31] Compound **3f** failed to produce salivation in mice at a dose of 10 mg/kg, ip, also suggesting a lack of M_3 agonist activity,[32] nor did the compound produce tremors at this dose.

Computational Studies

Examination of the biological data on the alkoxypyrazines **3**, alkoxy-1,2,5-oxadiazole **5**, and the 1,2,5-thiadiazoles **4** (Table I) shows that M_1 agonist activity is highly dependent on both the heterocycle attached to the 1,2,5,6-tetrahydropyridine ring and the 3-substituent attached to that heterocycle. We conducted computational studies on **3–5** as well as several model compounds to determine how these molecules activate the M_1 receptor and to aid us in the design of more selective agonists.

Recent studies of muscarinic agonists containing heterocycles that are isomers of those found in **3–5** showed a good correlation between muscarinic efficacy and the ability of the heterocycle to present two regions of negative electrostatic potential as hydrogen-bonding sites to the receptor.[10,23] In these studies, the most potent muscarinic agonists were those containing the 1,2,4-oxadiazole **6** and 1,2,4-thiadiazole **7**, heterocycles that have regions of high electrostatic potential located over N2 and N4. The pyrazine, 1,2,5-thiadiazole, and 1,2,5-oxadiazole heterocycles found in **3**, **4**, and **5**, respectively, have nitrogens atoms located in the same relative positions as in 1,2,4-oxadiazole and 1,2,4-thiadiazole (Figure 1), suggesting that the differences in M_1 efficacy observed for these three compounds may also be related to the magnitude of the electrostatic potential adjacent to the ring nitrogens. Recently, after the completion of our studies, other workers have also drawn attention to the similar electrostatic distributions among the pyrazine, 1,2,4-oxadiazole, and 1,2,4-thiadiazole rings.[33] To investigate this possibility, the molecular electrostatic potential for several 2-propenyl-1,2,5-thiadiazoles (**9**), -1,2,5-oxadiazoles (**10**), and pyrazines (**11**) (Figure 1) was calculated in the plane of the heterocyclic rings using the STO-3G basis set in GAUSSIAN 90. The 2-propenyl group was included in the structures studied to fully account for the electronegativity of the sp^2 carbon atom attached to the heterocyclic ring.

Table II. Magnitude of the Electrostatic Potential Minimum Adjacent to Ring Atoms 2 and 4 in **8**, **9**, and **10**

no.	X	V_2[a]	V_4[a]
8a	O	−72.1	−84.1
8b	S	−73.6	−82.5
9a	O	−71.0	−74.5
9b	S	−73.5	−73.2
9c	CH_2	−74.7	−72.6
9d	NH	−74.9	−76.4
10	O	−68.7	−68.5

[a] V_2 and V_4 (kcal mol^{-1}) refer to the potential minimum located adjacent to ring atom 2 and 4, respectively, in the plane of the heterocyclic ring.

The minimum electrostatic potential values adjacent to the ring nitrogens for the compounds studied are reported in Table II. Similar calculations on dimethyl-1,2,4-oxadiazole produced values of negative electrostatic potential nearly the same as those previously reported (V_2 = −78 kcal mol^{-1}, V_4 = −72 kcal mol^{-1}).[10] The magnitude of the negative electrostatic potential found over N2 and N4 for the alkoxy-1,2,5-thiadiazole **9a**, alkoxypyrazine **8a**, and the alkylthio-1,2,5-thiadiazole **9b**, when compared to that for the 1,2,4-oxadiazole, suggests that the pyrazine and 1,2,5-thiadiazole derivatives **3** and **4**, respectively, should be highly efficacious muscarinic agonists, whereas the lower order of electrostatic potential over N2 and N4 for the alkoxy-1,2,5-oxadiazole **10** suggests that **5** should be a less efficacious M_1 agonist than either **4f**, **3f**, or **4i**. As shown in Table I, **5** was significantly less efficacious as an M_1 agonist on the rabbit vas deferens than **4f**, **3f**, or **4i**.

The magnitude of the negative electrostatic potential over N2 and N4 for the (alkylthio)pyrazine **8b**, alkyl-1,2,5-thiadiazole **9c**,[20] and (alkylamino)-1,2,5-thiadiazole **9d**[20] suggests that **3i**, **4k**, and **4l** should also be at least as efficacious as **3f**, **4f**, and **4i** on the rabbit vas deferens. However, Table I shows that these compounds are only partial M_1 agonists when compared to **3f**, **4f**, and **4i**. As will be discussed later, the diminished M_1 efficacy that is seen with **3i**, **4l**, and **4k** may be a consequence of steric interactions between the side chain on the pyrazine or 1,2,5-thiadiazole ring and the tetrahydropyridine ring.

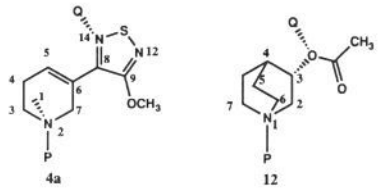
We chose the model of the muscarinic pharmacophore developed by Schulman and co-workers to obtain a three-dimensional picture of how these regions of high electrostatic potential in **3–5** bind to the M_1 receptor.[34] One of the muscarinic agonists used to develop this model was (*S*)-aceclidine (**12**), a structurally rigid, acetylcholine analog. Schulman's model of the activating conformation of aceclidine was chosen as the preliminary M_1 pharmacophore because, like the compounds in our study, it is a

(31) Birdsall, N. J. M.; Chan, S. C.; Eveleigh, P.; Hulme, E. C.; Miller, K. W. The modes of binding of ligands to cardiac muscarinic receptors. Subtypes of muscarinic receptors IV. *Trends Pharmacol. Sci. Suppl.* **1989**, 31–34.

(32) Hulme, E. C.; Birdsall, N. J. M.; Buckley, N. J. Muscarinic receptor subtypes. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 633–673.

(33) Street, L. J.; Baker, R.; Book, T.; Reeve, A. J.; Saunders, J.; Willson, T.; Marwood, R. S.; Patel, S.; Freedman, S. B. Synthesis and muscarinic activity of quinuclidinyl- and (1-azanorbornyl)pyrazine derivatives. *J. Med. Chem.* **1992**, *35*, 295–305.

(34) Schulman, J. M.; Sabio, M. L.; Disch, R. L. Recognition of cholinergic agonists by the muscarinic receptor. 1. Acetylcholine and other agonists with the NCCOCC backbone. *J. Med. Chem.* **1983**, *26*, 817–823.



4a 12

Figure 2. Schulman's model of the activating conformation of (*S*)-aceclidine and the proposed model applied to **4a**.

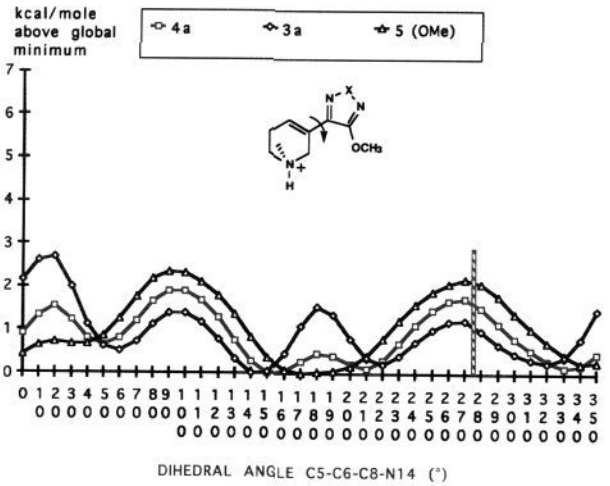


Figure 3. Conformational analysis of 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazoles, -pyrazines, and -1,2,5-oxadiazoles.

nonquarternary muscarinic agonist capable of penetrating the blood–brain barrier and has been shown to have affinity and efficacy at the M_1 receptor in the same biological tests employed in our studies.[28] The Schulman model of the muscarinic pharmacophore, as applied to **12** with its requisite binding sites P and Q, |PQ| of 6.6 Å, and dihedral angle P–N–O–Q of 112.5°, was constructed and manipulated with SYBYL (Figure 2).

To fit **3–5** to Schulman's model of the activating conformation of **12**, we assumed that the carboxylate binding point P on the M_1 receptor interacted with the nitrogen of the tetrahydropyridine ring in the same manner as for the quinuclidine ring of **12**. This binding mode was best simulated by requiring that the N-methyl group of the tetrahydropyridine ring be in an axial position. A minimum-energy conformation for protonated **4a** with the *N*-methyl group constrained in an axial position was calculated using MOPAC and AM1 parameters. Dihedral driver calculations were then carried out in 10° increments about the C5–C6–C8–N14 dihedral angle to yield local minimum conformations at 48°, 153°, 209° and 334°, respectively (Figure 3). There was essentially free rotation about the C5–C6–C8–N14 dihedral angle with less than a 2 kcal mol^{-1} separation between the global maximum and global minimum conformation of **4a**. When these same calculations were performed on **4f**, virtually identical results were obtained.

A least-squares fit of the tetrahydropyridine ring in the global minimum conformation of **4a** described above with the quinuclidine ring of **12** was performed where **4a**-N2 was matched to **12**-N1, **4a**-C7 to **12**-C2, **4a**-C3 to **12**-C6, **4a**-C1 to **12**-C7, **4a**-C6 to **12**-C3, and **4a**-C4 to **12**-C5, respectively. Within this composite structure, the thiadiazole ring of **4a** was rotated about the C6–C8 bond to bring a region of high negative electrostatic potential over

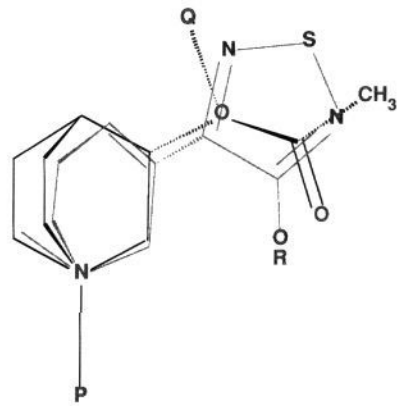


Figure 4. Overlay of Schulman's model of the muscarinic pharmacophore and the 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazoles.

N14 or N12 proximal to the Q binding point in **12**. The conformer that produced the closest approach of one of these nitrogens in the thiadiazole ring was that shown in Figure 4, where N14 was closest to Q. No conformer could be obtained in which the region of electrostatic potential over N12 could be reasonably aligned with Q.

The conformation of **4a** shown in Figure 4 is probably very close to that responsible for efficiently activating the M_1 receptor. The |N14Q| is 1.59 Å in this composite compared to the 1.2 Å used as the |OQ| in Schulman's model and the P–N2–N14–Q dihedral angle is 119° compared to the P–N–O–Q dihedral range of 100–117° required by the Schulman model of the muscarinic pharmacophore. This particular conformer of **4a** has a C5–C6–C8–N14 dihedral angle of 274° and occurs near a global maximum for rotation about this bond (See bar in Figure 3). However, because there is a very small barrier to rotation about this bond, this particular conformation should be energetically accessible. Even closer fits to the Schulman model are obtainable by rotation about the N–P axis of **4a**.

A comparison of the calculated heats of formation of several energetically minimized conformers bearing axial *N*-methyl groups with those same conformers where the axial *N*-methyl group had been converted to the equatorial position shows that the axial conformers are less than 2 kcal mol^{-1} higher in energy than the equatorial isomers. This suggests that the imposition of an axial *N*-methyl group does not produce energetically disfavored conformers that could be precluded from serving as the M_1 activating pharmacophore. Recently, similar results have been obtained comparing the axial and equatorial NCH_3 conformers of arecoline.[35]

When **3a** and **5** were treated in the same manner as described for **4a**, comparably good fits to the Schulman model were produced. As was the case for **4a**, **3a** and **5** do not present high energy barriers for rotation about the bond connecting the two heterocyclic rings, although, their barriers are higher than for **4a** (Figure 3).

The M_1 efficacy of the tetrahydropyridylthiadiazoles and -pyrazines is also dependent on the heteroatom that is directly attached to the thiadiazole or pyrazine (Table

(35) Orlek, B. S.; Blaney, F. E.; Brown, F.; Clark, M. S. G.; Hadley, M. S.; Hatcher, J.; Riley, G. J.; Rosenberg, H. E.; Wadsworth, H. J.; Wyman, P. Comparison of azabicyclic esters and oxadiazoles as ligands for the muscarinic receptor. *J. Med. Chem.* **1991**, *34*, 2726–2735.

I). This heteroatom could markedly influence the amount of electrostatic potential that has been shown to be important for receptor activation. However, the order of the electron-donating ability of the groups directly attached to the thiadiazole (CH_2 > NH > S > O) and the pyrazine (S > O) that would influence relative electrostatic potential is almost opposite to the order of M_1 efficacy for the thiadiazoles (O = S > NH > CH_2) and pyrazines (O > S). The reversal in the predicted order of efficacy could be due to the effect of these attaching heteroatoms or groups on the barrier to rotation about the bond connecting the tetrahydropyridine ring and the thiadiazole or pyrazine heterocycle (Figure 5). As shown previously in Figure 5, there is less than a 3 kcal mol^{-1} barrier to rotation about this bond for the highly efficacious alkoxypyrazine **3f**, alkoxythiadiazole **4f**, and (alkylthio)thiadiazole **4i**, while for the less efficacious compounds **3i**, **4k**, and **4l** significantly higher barriers to rotation occur near 10° and a new barrier to rotation of almost 3 kcal mol^{-1} and higher begins to occur between 180° and 200°. These barriers to free rotation could prevent these less efficacious compounds from efficiently populating the conformer near 275° that is required for M_1 receptor activation.

The length of the alkyl chain attached to the thiadiazole or pyrazine ring clearly has a dramatic effect on M_1 efficacy, with five to six carbon atoms conferring maximum efficacy. This side chain probably fits into a hydrophobic pocket in the receptor or lies along a lipophilic section of one of the α helices in a transmembrane segment of the receptor, stabilizing the conformer responsible for receptor activation.[36] The steric limitations of this hydrophobic pocket have been described in our previous publication.[20]

Previous investigations in series of muscarinic ligands containing 3-substituted 1,2,4-oxadiazoles attached to 1,2,5,6-tetrahydropyridyl or quinuclidinyl rings found that increasing the lipophilicity and size of the 3-substituent diminished muscarinic agonist activity and converted the compounds to muscarinic antagonists.[10,37,38] Similarly, substitution of the 5- or 6-position of the pyrazine ring in a series of quinuclidinyl- and azanorbornylpyrazine muscarinic agonists also diminished agonist activity.[33] This clearly shows that this region of the muscarinic receptor is sterically very demanding for agonist activity. Fitting the muscarinic tetrahydropyridyl-1,2,4-oxadiazoles to our model of the M_1 pharmacophore in the same manner as was done with the 1,2,5-thiadiazoles in Figure 4 places the 3-position of the 1,2,4-oxadiazole in the same relative position as the sulfur atom in the 1,2,5-thiadiazole and shows that the 1,2,5-thiadiazole avoids these potentially

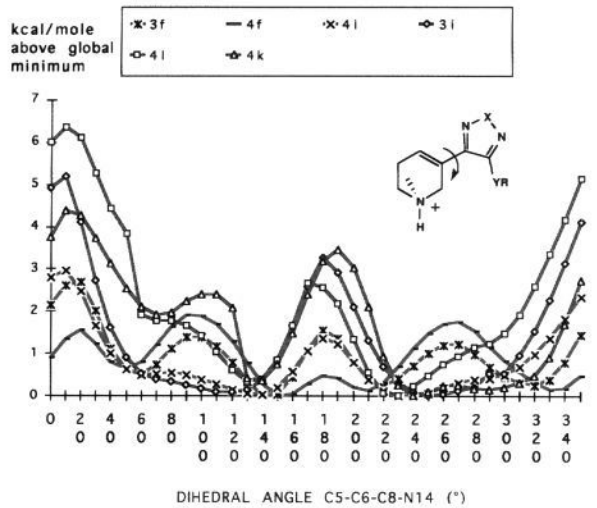


Figure 5. Conformational analysis of substituted 1-methyl-1,2,5,6-tetrahydropyridyl-1,2,5-thiadiazoles and -pyrazines.

deleterious steric interactions. Analogous observations can be made for the pyrazines **3**. By contrast, applying this model to the M_1 agonist data suggests that the region of the receptor occupied by the 3-substituent of the 1,2,5-thiadiazole or pyrazine is, within limits, fairly accommodating of lipophilicity and steric bulk and can actually be used to stabilize the agonist conformation. This latter region of the receptor cannot be accessed by the 1,2,4-oxadiazoles in this model.

A proposed model for the binding of the activating conformation of **4f** to the M_1 receptor is shown in Figure 6. In this model the protonated cationic head group of the tetrahydropyridine ring with an axial *N*-methyl group binds in a centrosymmetric fashion with Asp^{105} on the third transmembrane-spanning helix of the M_1 receptor.[39-42] Two hydrogen-bonding groups on other transmembrane-spanning helices of the receptor form hydrogen bonds to N12 and N14 of the thiadiazole ring, with the hydrogen bond with N14 corresponding to that described by Schulman, et al. The hydrogen bonding with N12 is not accounted for in the Schulman model and it is not clear that this hydrogen bond is employed in the binding of acetylcholine. However, the importance of the electrostatic potential at N12 in determining M_1 efficacy suggests that a residue on the M_1 receptor does interact with N12. The formation of these hydrogen bonds at N12 and N14 induces conformational changes in the M_1 receptor that eventually lead to activation of the second messenger system. Tyrosines on transmembrane-spanning helices 6 and 7 have been suggested as candidates for

(36) In the X-ray crystal structure of the photoreaction center of *Rhodopseudomonas viridis*, the carbon chain of a *N,N*-dimethyldodecylamine *N*-oxide detergent molecule can be seen to lie along a hydrophobic section of one of the α helices on the receptor just in the manner we suggest as a possibility for the interaction of the side chain with the α helix in the M_1 receptor. Dieisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3Å resolution. *Nature* **1985**, *318*, 618–624. Roth, M.; Lewit-Bentley, A.; Michel, H.; Deisenhofer, J.; Huber, R.; Oesterhelt, D. Detergent structure in crystals of a bacterial photosynthetic reaction centre. *Nature* **1989**, *340*, 659–662. Hartmut Michel, Max Planck Institute of Biophysics, Frankfort, Germany, private communication.

(37) Sauerberg, P.; Kindtler, J. W.; Nielsen, L.; Sheardown, M. J.; Honoré, T. Muscarinic cholinergic agonists and antagonists of the 3-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydropyridine type. Synthesis and structure–activity relationships. *J. Med. Chem.* **1991**, *34*, 687–692.

(38) Showell, G. A.; Gibbons, T. L.; Kneen, C. O.; MacLeod, A. M.; Merchant, K.; Saunders, J.; Freedman, S. B.; Patel, S.; Baker, R. Tetrahydropyridyloxadiazoles: Semirigid muscarinic ligands. *J. Med. Chem.* **1991**, *34*, 1086–1094.

(39) Curtis, C. A. M.; Wheatley, M.; Bansal, S.; Birdsall, N. J. M.; Eveleigh, P. Propylbenzilylcholine mustard labels on acidic residue in transmembrane helix 3 of the muscarinic receptor. *J. Biol. Chem.* **1989**, *264*, 489–495.

(40) Hulme, E. C.; Curtis, C. A. M.; Wheatley, M.; Harris, A. C. M.; Aitken, A. Muscarinic receptors: structure and localization of the ligand binding site. *Trends Pharmacol. Sci.* **1989**, *10* (Suppl. Subtypes of Muscarinic Receptors IV), 22–25.

(41) Fraser, C. M.; Wang, C.; Robinson, D. A.; Gocayne, J. D.; Ventner, J. C. Site-directed mutagenesis of m_1 muscarinic acetylcholine receptors; Conserved aspartic acids play important roles in receptor function. *Mol. Pharmacol.* **1990**, *36*, 840–847.

(42) Asp^{71} has also been proposed as the ultimate binding site of the cationic head group of muscarinic agonists with other Asp serving to relay the agonist to this binding site.[10] Asp^{71} appears to be very important for receptor activation,[39] but the evidence that the cationic head group binds to this residue is less convincing than the evidence that Asp^{105} is responsible for cationic head group binding.

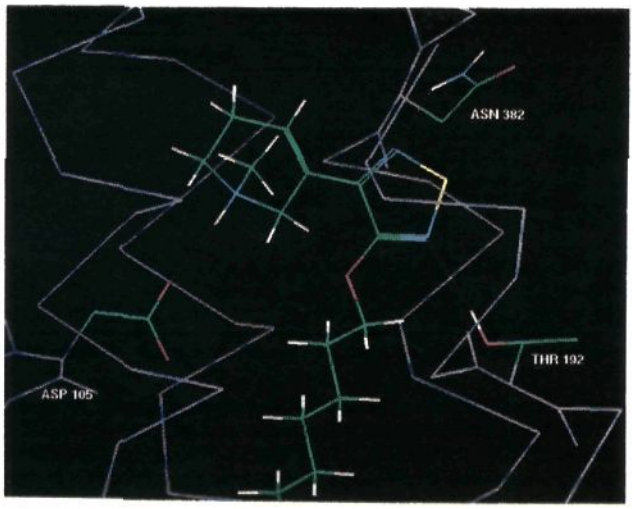


Figure 6. Proposed model for the binding of the activating conformation of **4f** to the M_1 muscarinic receptor.

this hydrogen-bonding function, but our modeling studies have not found these residues to be ideally placed for this role.[32]

The Asp^{105}–N2–N14–OH dihedral angle of ca. 119° is further stabilized by the lipophilic C_6 side chain fitting into a hydrophobic cavity among the transmembrane-spanning helices or by lying along a lipophilic section of one of the transmembrane segments of one of the α helices. Further molecular modeling studies are planned that could clarify the role of this important determinant of M_1 efficacy and selectivity.

Summary

Examples of selective M_1 agonists can be found among a series of 3-(3-alkoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridines that are iso-π-electronic with the potent M_1 selective agonists 3-(3-alkoxy-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridines. Among these iso-π-electronic M_1 agonists, M_1 efficacy appears to be dependent on the negative electrostatic potential over the nitrogen atoms in the iso-π-electronic heterocycles, the heteroatom directly attached to the iso-π-electronic heterocycle, and the length of the side chain attached to the iso-π-electronic heterocycle. These M_1 agonists can be shown to fit the 3D model of the muscarinic pharmacophore proposed by Schulman et al., but additional binding points are proposed to account for the potency and selectivity of these compounds. This model may now be used to design additional M_1 selective 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 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 III. No particular attempt was made to optimize reaction conditions for most of the reactions described.

Table III. Analytical Data for the Pyrazines

no.	formula[a]	mp, °C	cryst solv
1	$C_{10}H_{14}ClN_3O$	111–112	hexane
2	$C_{10}H_{12}ClN_3 \cdot C_2H_2O_4$	110–111	2-propanol
3a	$C_{11}H_{15}N_3O \cdot C_2H_2O_4$	150–151	EtOAc
3b	$C_{12}H_{17}N_3O \cdot C_2H_2O_4 \cdot 0.25H_2O$	*133–135*	*EtOAc*
3c	$C_{13}H_{19}N_3O \cdot C_2H_2O_4$	146–147	EtOAc
3d	$C_{14}H_{21}N_3O \cdot C_2H_2O_4$	160–161.5	EtOAc
3e	$C_{15}H_{23}N_3O \cdot C_2H_2O_4$	151–152	EtOAc
3f	$C_{16}H_{25}N_3O \cdot C_2H_2O_4$	154–155	EtOAc
3g	$C_{17}H_{27}N_3O \cdot C_2H_2O_4$	125–127	EtOAc
3h	$C_{15}H_{23}N_3S \cdot HCl$	123–124	EtOAc
3i	$C_{16}H_{25}N_3S \cdot HCl$	120.5–122	EtOAc

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

3-(3-Chloropyrazinyl)-1-methyl-3-piperidinol, 1. A solution of 2,2,6,6-tetramethylpiperidine (7.2 mL, 0.042 mmol) in dry THF (300 mL) was cooled to −8 °C as 1.6 M 1-butyllithium in hexane (25 mL, 0.04 mol) was added dropwise. After 20 min, the reaction 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, 1-methylpiperidin-3-one (3.5 g, 0.035 mol) in THF (10 mL) was added dropwise. After 1.5 h, a solution of 8 mL of concentrated HCl and 4 mL of EtOH 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 extracted (4×) with CH_2Cl_2 (30 mL), the extracts were washed with brine, and the solvent was evaporated to give a brown solid. The solid was triturated with hot ether (300 mL), the solution evaporated, and the residue recrystallized to give 3.77 g of **1** (53% yield): ^1H NMR ($CDCl_3$) δ 1.7–2.2 (5 H, m), 2.4 (3 H, s), 2.7 (1 H, d), 2.85–3.05 (2 H, m), 4.4 (1 H, bs), 8.35 (1 H, d), 3.5 (1 H, d).

3-(3-Chloropyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 2. Thionyl chloride (10 mL) was cooled in an ice–water bath with stirring as **1** (1 g, 0.0044 mol) was added. Cooling was removed and, after 2 h, the solvent was evaporated. The residue was treated with ice–water, made basic with saturated K_2CO_3, and extracted (3×) with CH_2Cl_2 (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 starting with CH_2Cl_2 and ending with 7.5% methanol–1% NH_4OH–CH_2Cl_2 to give an orange liquid (0.43 g) that was converted to the ethanedioate salt **2** (0.26 g): ^1H NMR (D_2O) δ 2.75 (2 H, m), 3.05 (3 H, s), 3.35 (1 H, m), 3.65 (1 H, m), 4.05 (1 H, m), 4.25 (1 H, d), 6.62 (1 H, bs), 8.38 (1 H, d), 8.55 (1 H, d).

General Procedure for the Syntheses of 3-(3-Alkoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridines, 3. A solution of the sodium alkoxide was generated by adding Na (0.5 g, 0.02 mol) to the appropriate alcohol and heating the mixture, if necessary. To the reaction was added a solution of crude free base of **2** (0.0022 mol) in the alcohol (5 mL), and the mixture was heated to the boiling point of the alcohol or to 100 °C for 2 h. The reaction was cooled, the alkoxide was neutralized with 5 N HCl (4 mL), and the solvent was evaporated. With higher boiling alcohols, the evaporation of the alcohol was assisted by adding more water to the reaction mixture and evaporating the azeotrope. The residue was suspended in water and extracted (3×) with CH_2Cl_2 (25 mL), the extracts were dried, and the solvent was evaporated. The residue was purified by chromatography and the product isolated as a salt.

3-(3-Methoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3a. From the free base of **2** (0.3 g, 0.0014 mol) and sodium methoxide (0.015 mol) in MeOH (25 mL) was obtained **3a** (0.05 g, 12% yield): ^1H NMR (D_2O) δ 2.6–2.9 (2 H, m), 3.1 (3 H, s), 3.3 (1 H, m), 3.65 (1 H, m), 4.0 (3 H, s), 4.05 (1 H, dd), 4.35 (1 H, d), 6.95 (1 H, bs), 8.07 (1 H, d), 8.1 (1 H, d).

3-(3-Ethoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3b. From the free base of **2** (1 g, 0.0048 mol) and sodium ethoxide (0.03 mol) in EtOH (35 mL) was obtained a brown liquid that was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ and converted to the ethandioate salt **3b** (0.55 g, 36% yield): ^1H NMR ($CDCl_3$) δ 1.45 (3 H, t), 2.62 (1 H, bm), 2.75–3.2 (5 H, m), 3.5–3.95

(2 H, m), 4.3–4.75 (3 H, m), 4.8–5.3 (2 H, m), 7.38 (1 H, m), 7.98 (1 H, d), 8.05 (1 H, d).

3-(3-Propoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3c. From the free base of **2** (0.92 g, 0.0044 mol) and sodium 1-propoxide (0.03 mol) in 1-propanol (35 mL) was obtained a brown liquid that was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ and converted to the ethandioate salt **3c** (0.38 g, 27% yield): ^1H NMR ($CDCl_3$) δ 1.05 (3 H, t), 1.87 (2 H, m), 2.75–2.92 (2 H, m), 3.0 (3 H, s), 3.2–3.55 (2 H, m), 4.0–4.4 (4 H, m), 7.38 (1 H, m), 7.97 (1 H, d), 8.06 (1 H, d).

3-(3-Butoxypyrazinyl)-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3d. From the free base of **2** (0.46 g, 0.0022 mol) and sodium 1-butoxide (0.022 mol) in 1-butanol (25 mL) was obtained a brown liquid that was purified by HPLC eluting with an 8-L gradient beginning with CH_2Cl_2 and ending with 10% MeOH–CH_2Cl_2 and converted to the ethanedioate salt **3d** (0.08 g, 11% yield): ^1H NMR ($CDCl_3$) δ 1.0 (3 H, t), 1.55 (2 H, m), 1.85 (2 H, m), 2.5 (5 H, bs + s), 2.63 (2 H, t), 3.5 (2 H, m), 4.4 (2 H, t), 7.1 (1 H, m), 7.9 (1 H, d), 8.1 (1 H, d).

3-[3-(Pentyloxy)pyrazinyl]-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3e. From the free base of **2** (0.92 g, 0.0044 mol) and sodium 1-pentyl oxide (0.03 mol) in 1-pentanol (35 mL) was obtained a brown liquid that was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ and converted to the ethandioate salt **3e** (0.52 g, 34% yield): ^1H NMR ($CDCl_3$) δ 0.94 (3 H, t), 1.43 (4 H, m), 1.84 (2 H, m), 2.7–2.9 (2 H, m), 2.99 (3 H, s), 3.2–3.55 (2 H, m), 4.0–4.43 (4 H, m), 7.38 (1 H, m), 7.97 (1 H, d), 8.07 (1 H, d).

3-[3-(Hexyloxy)pyrazinyl]-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3f. From the free base of **2** (0.69 g, 0.0033 mol) and sodium 1-hexyl oxide (0.022 mol) in 1-hexanol (25 mL) was obtained a brown liquid that 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 and converted to the ethanedioate salt **3f** (0.34 g, 28% yield): ^1H NMR of free base ($CDCl_3$) δ 0.95 (3 H, t), 1.3–1.55 (6 H, m), 1.85 (2 H, m), 2.5 (5 H, bs + s), 2.65 (2 H, t), 4.4 (2 H, t), 7.1 (1 H, m), 7.9 (1 H, d), 8.1 (1 H, d).

3-[3-(Heptyloxy)pyrazinyl]-1,2,5,6-tetrahydro-1-methylpyridine Ethanedioate, 3g. From the free base of **2** (0.82 g, 0.0039 mol) and sodium 1-heptyl oxide (0.022 mol) in 1-heptanol (35 mL) was obtained a brown liquid that was purified by HPLC eluting with an 8-L gradient beginning with CH_2Cl_2 and ending with 10% MeOH–CH_2Cl_2 and converted to the ethanedioate salt **3g** (0.41 g, 28% yield): ^1H NMR of the free base ($CDCl_3$) δ 0.9 (3 H, t), 1.22–1.52 (8 H, m), 1.82 (2 H, m), 2.5 (5 H, bs), 2.62 (2 H, t), 3.5 (2 H, m), 4.4 (2 H, t), 7.1 (1 H, m), 7.9 (1 H, d), 8.05 (1 H, d).

3-[3-(Pentylthio)pyrazinyl]-1,2,5,6-tetrahydro-1-methylpyridine Hydrochloride, 3h. A solution of the free base of **2** (0.92 g, 0.0044 mol) in THF (10 mL) was added to a suspension of sodium pentanethiolate (0.0078 mol) prepared from NaH (0.18 g, 0.0078 mol), 1-pentanethiol (2 mL, 0.016 mol), and THF (30 mL). The reaction was heated to reflux for 20 min followed by distillation of most of the solvent at atmospheric pressure. The residue was cooled and treated with ice–water and the mixture extracted (3×) with CH_2Cl_2 (25 mL). The extracts were dried, and the solvent was evaporated to give a brown oil that was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ and converted to the HCl salt **3h** (0.26 g, 19% yield): ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.38 (4 H, m), 1.7 (2 H, m), 2.58–2.7 (1 H, m), 3.0 (3 H, d), 3.05–3.2 (4 H, m), 3.5–3.6 (1 H, m), 3.7–3.82 (1 H, m), 4.4–4.47 (1 H, d), 6.8 (1 H, m), 8.15 (1 H, d), 8.28 (1 H, d).

3-[3-(Hexylthio)pyrazinyl]-1,2,5,6-tetrahydro-1-methylpyridine Hydrochloride, 3i. A solution of the free base of **2** (1 g, 0.0048 mol) in THF (10 mL) was added to a suspension of sodium hexanethiolate (0.0091 mol) prepared from NaH (0.21 g, 0.0091 mol), 1-hexanethiol (2 mL, 0.014 mol), and THF (30 mL). The reaction was heated to reflux for 20 min followed by distillation of most of the solvent at atmospheric pressure. The residue was cooled and treated with ice–water and the mixture extracted (3×) with CH_2Cl_2 (25 mL). The extracts were dried and the solvent evaporated to give a brown oil that was purified by radial chromatography eluting with 5% EtOH–0.5% NH_4OH–$CHCl_3$ and converted to the HCl salt **3i** (0.33 g, 21% yield): ^1H NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.31 (4 H, m), 1.44 (2 H, m), 1.67 (2 H, m), 2.6–2.7 (1 H, m), 2.96 (3 H, d), 3.05–3.22 (4 H, m), 3.54–3.64 (1 H, m), 3.72–3.8 (1 H, m), 4.4–4.45 (1 H, d), 6.8 (1 H, m), 8.15 (1 H, d), 8.28 (1 H, d).

Radioligand Binding Assays. The hippocampus 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 (P_2) 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 hippocampal membranes was determined by adding unlabeled drug, 1 nM [^3H]pirenzepine (87 Ci/mmol, New England Nuclear, Boston, MA), and hippocampal membranes equivalent to 10 mg of 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 $MnCl_2$.[43] The inhibition of binding of oxotremorine-M to hippocampal membranes was determined by adding unlabeled drug, 3 nM [^3H]-oxotremorine-M (87 Ci/mmol, New England Nuclear), and hippocampal membranes equivalent to 10 mg of tissue wet weight (about 0.1 mg protein) in 1-mL total volume of 20 mM Tris-Cl buffer, pH 7.4. 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 (3×) 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.[44]

Electrically Stimulated Rabbit Vas Deferens. The methods are a slight modification of those of Eltze.[27] Briefly, rabbits were anesthetized with pentobarbital sodium (60 mg/kg, iv) and the vasa deferentia removed. The organs were carefully dissected free of surrounding tissue and divided into two segments, a prostatic and an epididymal segment. Each tissue segment was suspended between an electrode and a force-displacement transducer in a 10-mL organ bath in modified Krebs–Ringer solution consisting of (mM) NaCl (134.0), KCl (3.4), $CaCl_2$ (2.8), $MgSO_4$ (0.6), KH_2PO_4 (1.3), $NaHCO_3$ (16.0), and glucose (7.7) (1 μM yohimbine was included to block a_2 adrenoceptors). The solution was maintained at 31 °C and was continuously bubbled with 95% O_2–5% CO_2. The tension of the preparations was set at 0.75 g and left to equilibrate for at least 30 min before continuous field stimulation (0.5 ms, 40 V, 0.05 Hz) was started. In this preparation, M_2 agonists increase twitch height whereas putative M_1 agonists decrease twitch height.

Cumulative concentration–effect curves were determined in two tissues (one prostatic and one epididymal) with dose additions at 20-min intervals. If the compound inhibited twitch height by at least 50%, then concentration–effect curves were determined in a minimum of six tissues.

Molecular Modeling Details. All investigations were set up and results analyzed using either SYBYL 5.0[45] (running on a Evans and Sutherland PS330 color vector graphics terminal linked to a VAX 11/785) or QUANTA 3.0 (running on a Silicon Graphics 4D/85GTB workstation). Geometry optimization and torsional scans were performed using the AM1 Hamiltonian in MOPAC 5.01.[46] Torsional scans were from 0° to 360° in 10° intervals. At each point in the scan, all defined internal coordinates were refined while the dihedral angle being investigated were constrained. SCF convergence criteria was set to

(43) Potter, L. T.; Ferrendelli, C. A. Two affinity states of M_1 muscarinic receptors. *Cell. Mol. Neurobiol.* **1988**, *8*, 181–191.

(44) De Lean, A.; Munson, P. J.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.* **1978**, *235*, 97–102.

(45) TRIPOS Associates, St. Louis, MO 63117.

(46) Dewar, M. J. S.; University of Texas, Houston, TX. The MOPAC program is available from the Quantum Chemistry Program Exchange.

1.0×10^{-14} using the keyword SCFCRT=1.D-14. Geometry optimization termination criteria was set to a gradient norm of 1×10^{-4} using the keyword GNORM=0.0001. For systems containing sulfur, MNDO parameters were utilized for this atom using the keyword PARASOK. All AM1 calculations were performed using the methyl alkyl side chain. A torsional scan of **4f** resulted in virtually the same energy profile as obtained with **4a** in significantly less CPU time.

To calculate the molecular electrostatic potential, the heterocyclic ring of each system was first orientated so as to lie in the x,y plane with the ring atoms centered about the origin. The electrostatic potential was then evaluated from the STO-3G-derived wavefunction using GAUSSIAN 90, on a 5×5 Å grid utilizing a 0.2-Å interval. This resulted in 51 data points which were subsequently plotted and analyzed using NCSA DataScope. All electrostatic potential calculations were performed using model systems constructed from the AM1-optimized geometry by reducing the 1,2,5,6-tetrahydro-1-methylpyridine ring to the 2-propenyl substructure.

Acknowledgment. The authors thank Mr. Reginald Thomas for assisting in with HPLC separations.