Design, Synthesis, and Neurochemical Evaluation of 5-(3-Alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines as M_1 Muscarinic Receptor Agonists

Philip G. Dunbar,† Graham J. Durant,‡ Zheng Fang,§ Yahaya F. Abuh, Afif A. El-Assadi, Dan O. Ngur, Sumudra Periyasamy, Wayne P. Hoss, and William S. Messer, Jr.*

Department of Medicinal and Biological Chemistry, Center for Drug Design and Development, College of Pharmacy, The University of Toledo, 2801 West Bancroft Street, Toledo, Ohio 43606

Received September 30, 1992

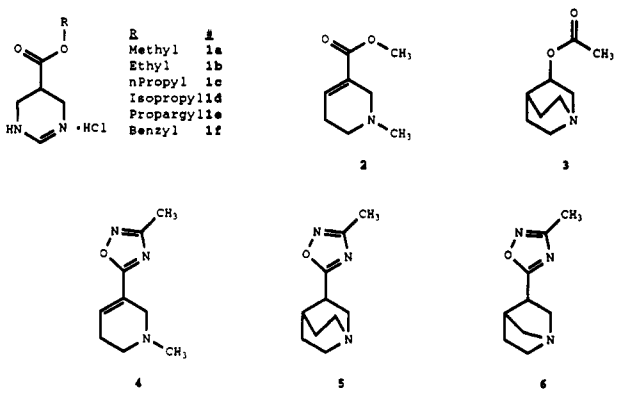
A series of 5-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines (**7a–h**) was synthesized for biological evaluation as selective agonists for M_1 receptors coupled to phosphoinositide (PI) metabolism in the central nervous system. Each ligand bound with high affinity to muscarinic receptors from rat brain as measured by inhibition of [^{3}H]-(*R*)-quinuclidinyl benzilate ([^{3}H]-(*R*)-QNB) binding. 5-(3-Methyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine trifluoroacetate (CDD-0098-J; **7a**) displayed high affinity ($IC_{50} = 2.7 \pm 0.69\ \mu M$) and efficacy at muscarinic receptors coupled to PI metabolism in the rat cortex and hippocampus. Increasing the length of the alkyl substituent increased affinity for muscarinic receptors yet decreased activity in PI turnover assays. The hippocampal PI response of **7a** was blocked by lower concentrations of pirenzepine (**8**) or by higher concentrations of either AF-DX 116 (**9**) or *p*-fluorohexahydrosiladifenidol (**10**), suggesting that at low concentrations **7a** selectively stimulates PI turnover through M_1 receptors.

Introduction

Recent work has focused on the development of M_1-selective agonists for Alzheimer's disease[1,2] based on the localization of M_1 receptors in the cerebral cortex and hippocampus,[3-9] the involvement of these areas in cognition and memory, and studies demonstrating that M_1 antagonists such as pirenzepine produce memory impairments in experimental animals.[10-13] M_1 agonists are expected to bind selectively to M_1 muscarinic receptors and stimulate phosphoinositide (PI) turnover in the hippocampus.[14,15] It is predicted that a centrally-active, M_1-selective agonist will help alleviate the cognitive and memory deficits associated with a loss of cholinergic neurons as found in Alzheimer's disease.

A key factor in the development of centrally active muscarinic agonists is the ability to incorporate a suitable replacement for the quaternary ammonium group in acetylcholine, while still affording penetration into the central nervous system. Previous studies have documented the binding affinity and agonist activity of pilocarpine at M_1 muscarinic receptors in rat brain.[16,17] Several groups have developed carbamate analogs of pilocarpine with muscarinic activity[18] and thiolactone derivatives with a degree of M_1 receptor selectivity.[19] The imidazole system in pilocarpine suggested, by analogy, the utility of amidines in general as suitable ammonium bioisosteres. As a result, a series of 1,4,5,6-tetrahydropyrimidine esters (**1a–f**) has been synthesized and evaluated as muscarinic agonists with some selectivity for activating M_1 receptors in the hippocampus.[20] The tetrahydropyrimidine esters may be seen as analogs of arecoline (**2**) with a potentially labile ester group. To improve the duration of action and the pharmacological profile (potency and efficacy) of these ligands, a replacement for the ester moiety was desirable.

Over the past several years, a number of groups have explored the utility of the 1,2,4-oxadiazole moiety as a suitable ester bioisostere in the development of chemically stable, centrally active muscarinic agonists.[21-25] Based on classical muscarinic agonists arecoline (**2**) and aceclidine (**3**), these compounds range in activity from partial agonists, as in the 1,2,4-oxadiazole derivatives of arecoline (**4**),[21,25] norarecoline,[26] and quinuclidine (**5**),[27] to full muscarinic agonists in the azanorbornane (**6**) series.[22,24,28]



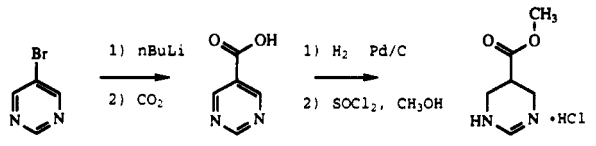
Although many of these compounds are quite potent and efficacious, selectivity for individual muscarinic receptor subtypes has not been established. Indeed, the data suggest that most of these compounds are relatively nonselective agonists with activity at muscarinic receptor subtypes in a variety of tissues and cell types.[28,29] There remains an urgent need for selective muscarinic agonists with activity limited to M_1 receptors in the cerebral cortex and hippocampus, the areas of brain most closely associated with cognition and memory function.

To this end, the series of 1,4,5,6-tetrahydropyrimidines has been developed further, incorporating the 1,2,4-oxadiazole system as an ester bioisostere. In the studies presented here, a series of 5-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines (**7a–h**) was synthesized. Affinity for muscarinic receptors in rat brain was measured

† Present address: Washington State University, Health Research and Education Center, W. 601 1st Avenue, Spokane, WA 99204.
‡ Present address: Cambridge Neuroscience, Inc., One Kendall Square, Building 700, Cambridge, MA 02139.
§ Present address: Synaptic Pharmaceutical Corp., 215 College Road, Paramus, NJ 07652.

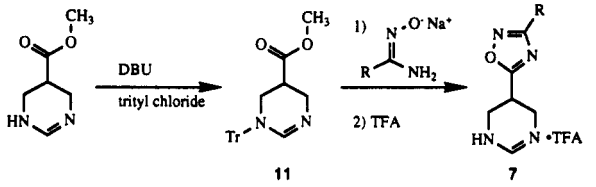
0022-2623/93/1836-0842$04.00/0

Scheme I



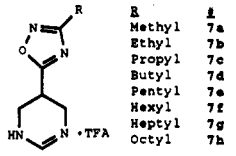
1a

Scheme II

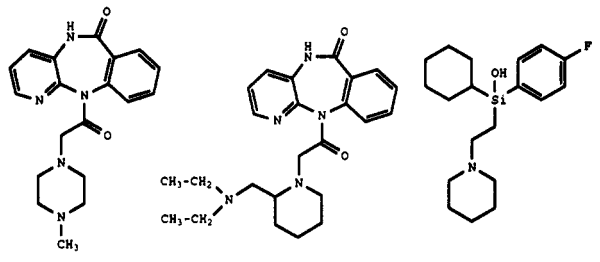


11 7

by inhibition of [^{3}H]-(*R*)-quinuclidinyl benzilate binding. Agonist activity was evaluated by measuring PI metabolism in the rat cortex in preliminary fashion and more fully for **7a** in rat hippocampus. Functional selectivity of **7a** for M_1 receptors coupled to the PI response was demonstrated using the M_1-selective antagonist pirenzepine (**8**), the M_2-selective antagonist AF-DX 116 (**9**), or the M_3-selective antagonist *p*-fluorohexahydrosiladifenidol (**10**).



R	#
Methyl	7a
Ethyl	7b
Propyl	7c
Butyl	7d
Pentyl	7e
Hexyl	7f
Heptyl	7g
Octyl	7h



Pirenzepine 8 AF-DX 116 9 p-F-Hexahydrosiladifenidol 10

Synthetic Chemistry

A series of 5-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines was synthesized from the reduced pyrimidine methyl ester, which had been formed previously by esterification of reduced pyrimidine-5-carboxylic acid (see Scheme I).[20] 5-(Methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (**1a**) served as a useful starting material for the methyl-1,2,4-oxadiazole (**7a**). It was protected as the *N*-trityl free base by reacting the hydrochloride salt with diazabicycloundecene (DBU) and trityl chloride in DMF (see Scheme II). The resulting free base was added to a sodium amidoxime salt generated in situ. The required amidoximes were obtained by reacting nitriles with hydroxylamine.[30] Deprotection of the *N*-tritytetrahydropyrimidine oxadiazole with trifluoroacetic acid (TFA) gave the products (**7a–h**) as TFA salts. Physicochemical data for the 5-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines are presented in Table III.

Pharmacological Results and Discussion

A series of 5-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines was synthesized and evaluated for activity at muscarinic receptors in the rat central nervous system. The binding affinity of each ligand was determined indirectly by assessing the inhibition of specific [^{3}H]-(*R*)-QNB binding to rat brain membranes. Each 3-alkyl derivative bound with high affinity (IC_{50} values less than 10 μM) to muscarinic receptors in rat brain. Hill slopes were generally less than unity, which could be interpreted as reflecting differential interaction with multiple receptor subtypes and/or high and low affinity agonist binding sites. Further studies are necessary to distinguish between these possibilities.

Table I. Inhibition of [^{3}H]-(*R*)-QNB Binding to Rat Brain Membranes by Several Muscarinic Ligands[a]

ligand	IC_{50}, μM	Hill slope	PI at 100 μM, %
carbachol	5.5 ± 1.0	0.32 ± 0.02	470 ± 81
arecoline (**2**)	1.0 ± 0.25	0.76 ± 0.16	110 ± 21
aceclidine (**3**)	0.51 ± 0.10	0.64 ± 0.05	80 ± 17
4	0.69 ± 0.44	0.65 ± 0.18	50 ± 24
5	0.35 ± 0.06	0.48 ± 0.11	100 ± 35
pilocarpine	7.6 ± 4.4	0.74 ± 0.03	58 ± 5.6
7a	2.7 ± 0.69	0.47 ± 0.03	700 ± 99
7b	1.0 ± 0.32	0.67 ± 0.08	130 ± 16
7c	0.55 ± 0.09	0.61 ± 0.08	79 ± 13
7d	0.49 ± 0.13	0.60 ± 0.11	61 ± 17
7e	0.56 ± 0.23	0.84 ± 0.07	29 ± 9.0
7f	2.3 ± 0.06	0.62 ± 0.02	4.3 ± 8.7
7g	2.3 ± 1.9	0.79 ± 0.19	34 ± 13
7h	0.53 ± 0.20	1.0 ± 0.07	28 ± 15

[a] Also shown is the stimulation of PI metabolism in rat cortical slices. Data represent the mean (± sem) from at least three assays each performed in triplicate.

Compound **7a** displayed a moderate affinity (2.7 ± 0.69 μM) for muscarinic receptors in the central nervous system (see Table I). The affinities of other muscarinic agonists also are presented for comparison. Increasing the alkyl chain on the 1,2,4-oxadiazole ring led to compounds with slightly higher affinity for muscarinic receptors, although no significant differences were noted in the IC_{50} values for the 5-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidines ($p > 0.05$, by one factor analysis of variance). These findings are in general agreement with slight increases in affinity for muscarinic receptors labeled with [^{3}H]-(*R*)-QNB associated with increasing the length of the 3-alkyl substituent on 1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methylpyridine.[25]

A direct comparison of the relative potencies of three 3-methyl-1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methylpyridine (**4**), quinuclidine (**5**), and 1,4,5,6-tetrahydropyrimidine (**7**) is shown in Figure 1. As can be seen, both **4** and **5** were more potent than **7a** for binding sites labeled with [^{3}H]-(*R*)-QNB. The higher potency of the 1,2,5,6-tetrahydro-1-methylpyridine derivative also is consistent with previous comparisons of the methyl esters, arecoline (IC_{50} = 1.0 ± 0.25 μM), and **1a** (IC_{50} = 9.2 ± 1.9 μM).[20]

The ability of each ligand to stimulate PI metabolism was examined initially in the cerebral cortex at a single concentration (see Table I). **7a–h** stimulated muscarinic receptors coupled to PI turnover in the rat cerebral cortex to varying degrees. The 3-methyl-1,2,4-oxadiazole derivative **7a** stimulated PI turnover in the rat cerebral cortex to 700% above basal levels at 100 μM and was by far the most active ligand examined, exhibiting agonist activity in the cerebral cortex with a maximal response comparable to that of carbamylcholine.

Increasing the length of the 3-alkyl chain on the 1,2,4-oxadiazole ring of 1,4,5,6-tetrahydropyrimidine dramatically decreased activity in the phosphoinositide metab-

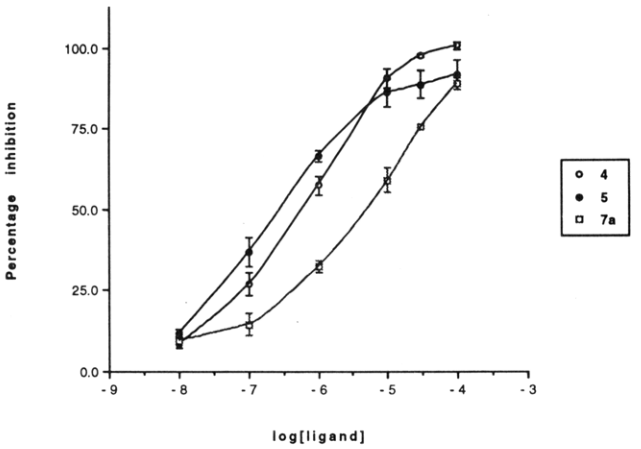


Figure 1. Inhibition of specific [^{3}H]-(*R*)-QNB binding to muscarinic receptors in rat brain membranes by three 3-methyl-1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methylpyridine (**4**), quinuclidine (**5**), and 1,4,5,6-tetrahydropyrimidine (**7a**). The data represent the mean (± sem) inhibition from three experiments each performed in triplicate.

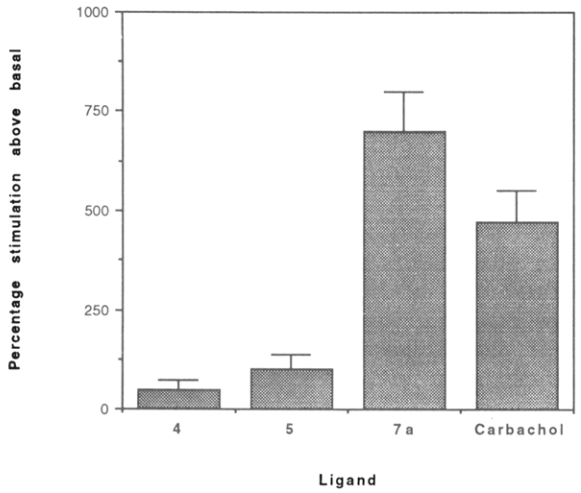


Figure 2. Stimulation of phosphoinositide metabolism in rat hippocampal slices by carbachol and the three 3-methyl-1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methylpyridine (**4**), quinuclidine (**5**), and 1,4,5,6-tetrahydropyrimidine (**7a**). The data represent the mean (± sem) stimulation above basal levels at 100 μM for each ligand as determined from at least three experiments each performed in triplicate. Single-factor analysis of variance followed by a Tukey–Kramer test indicated that the responses elicited by **7a** and carbachol were significantly higher than the responses produced by either **4** or **5** ($p < 0.01$). The same analysis indicated that the response to **7a** was significantly greater than the response to carbachol ($p < 0.05$).

olism assay. Again, these data are consistent with similar observations in 1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methylpyridine[25] and quinuclidine[27] where increasing the length of the 3-alkyl substituent led to compounds with higher affinity yet lower agonist activity. Although activity was generally lower, further evaluation of these compounds is warranted to determine the subtype(s) of muscarinic receptor activated by each ligand. In particular, assays measuring the inhibition of adenylyl cyclase activity will help determine activity at M_2 and/or M_4 receptors.

Figure 2 compares directly the agonist activity of three 3-methyl-1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methyl-pyridine (**4**), quinuclidine (**5**), and 1,4,5,6-tetrahydropyrimidine (**7a**) in the rat cortical PI metabolism assay. The response produced by 100 μM **7a** is significantly higher than that produced by the same concentration of carbachol, **4** or **5**. The maximal response of **7a** appears to approximate that reported previously for the highly efficacious muscarinic agonist 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane (**6**).[22]

Table II. Stimulation of PI Metabolism in Rat Hippocampal Slices by **7a** and Aceclidine (**3**)[a]

ligand	EC_{50}, μM	maximal stimulation, %	IC_{50}, nM		
			pirenzepine	**9**	**10**
3	21 ± 4.6	390 ± 70	120 ± 48	280 ± 23	23 ± 7
7a	5.2 ± 0.89	510 ± 91	38 ± 10	1700 ± 660	1200 ± 480

[a] Also shown is the inhibition of the response to 10 μM **7a** and 50 μM aceclidine by the selective muscarinic antagonists pirenzepine, **9**, and **10**. Data represent the mean (± sem) from three separate assays performed in triplicate for each agonist or antagonist tested.

Table III. Physicochemical Data for 1,4,5,6-Tetrahydro-5-(3-alkyl-1,2,4-oxadiazol-5-yl)pyrimidines[a]

compd	yield, %	mp, °C	formula
7a	62	120–122	$C_7H_{11}N_4O{\cdot}C_2F_3O_2$
7b	49	116–118	$C_8H_{13}N_4O{\cdot}C_2F_3O_2$
7c	43	126–128	$C_9H_{15}N_4O{\cdot}C_2F_3O_2$
7d	27	98–100	$C_{10}H_{17}N_4O{\cdot}C_2F_3O_2$
7e	19	97–99	$C_{11}H_{19}N_4O{\cdot}C_2F_3O_2$
7f	36	89–91	$C_{12}H_{21}N_4O{\cdot}C_2F_3O_2$
7g	49	101–102	$C_{13}H_{23}N_4O{\cdot}C_2F_3O_2$
7h	30	97–99	$C_{14}H_{25}N_4O{\cdot}C_2F_3O_2$

[a] Elemental analyses were within ±0.4% of theoretical values with the exception of **7h**, which contained hexanes.

Following the initial screen for activity in the cerebral cortex, **7a** was examined further for activity in the hippocampus, an area known to be enriched in M_1 muscarinic receptors[3-9] and important for memory function.[10-13] Compound **7a** also stimulated PI turnover in hippocampal slices as did the quinuclidine derivative, aceclidine (see Table II). A maximal stimulation of 510 ± 91% above basal levels was observed at 1.0 mM, and the EC_{50} was 5.2 ± 0.89 μM. The response elicited by **7a** was blocked by atropine (data not shown), indicating the activation of muscarinic cholinergic receptors.

The selectivity of the response elicited by **7a** was examined using selective antagonists to discriminate between activation of M_1, M_2, or M_3 receptors.[31-36] The M_1 antagonist pirenzepine (**8**) was more effective in blocking the response elicited by 10 μM of **7a** than either the M_2 antagonist **9** or the M_3 antagonist **10** (see Figure 3A). The hippocampal response elicited by **7a** can be compared with the response elicited by aceclidine. The inhibition of the aceclidine response by pirenzepine and the M_2 antagonist **9** is approximately equal, while the M_3 antagonist **10** is 1 order of magnitude more potent (see Figure 3B). These data suggest that low concentrations of **7a** activate M_1 receptors in the hippocampus in a functionally selective manner, although it is possible that other subtypes of muscarinic receptor that couple to PI metabolism (e.g., M_3 receptors) are activated at higher concentrations. Further studies will be necessary to assess the selectivity of the tetrahydropyrimidine derivatives at each of the five muscarinic receptor subtypes. The availability of cell lines expressing each subtype of receptor will help make this assessment possible.

It should be noted that selective M_1 receptor activity has been reported by several groups. In these cases, selectivity for activating M_1 receptors has been achieved by modification of the ester bioisostere. For example, the replacement of the lactone ring with a thiolactone moiety

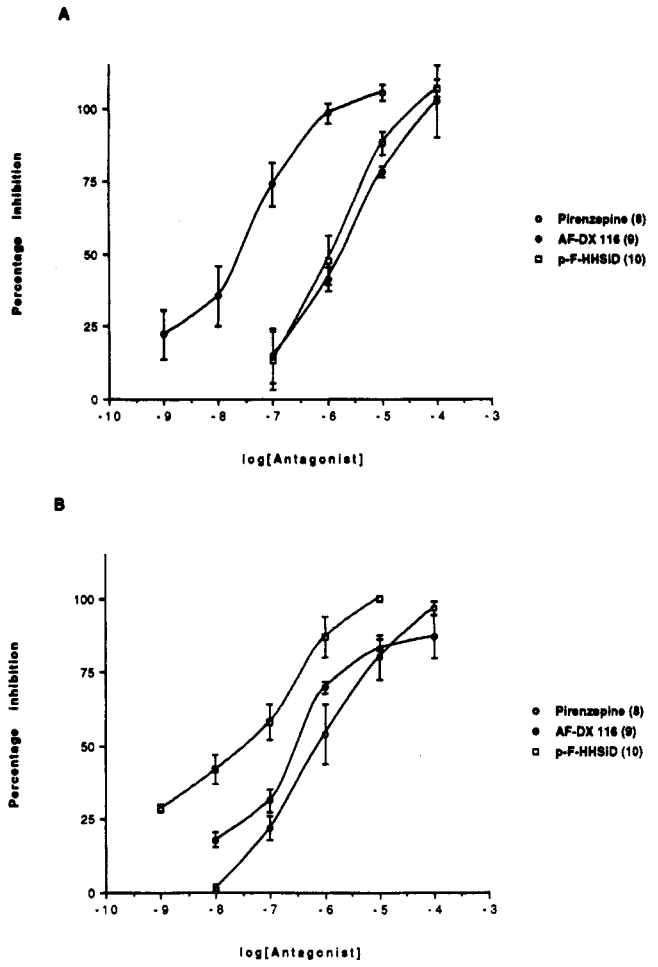


Figure 3. (A) Inhibition by three selective muscarinic antagonists of the PI response elicited by 10 μM **7a**. (B) Inhibition by three selective muscarinic antagonists of the PI response elicited by 50 μM aceclidine (**3**). Data represent the mean percentage inhibition (± sem) from three separate experiments, each performed in triplicate.

increases M_1 selectivity of pilocarpine derivatives.[19] Use of a 1,2,5-thiadiazole or 1,3-oxathiolane moiety as ester bioisosteres also has been reported to improve M_1 selectivity in the 1,2,5,6-tetrahydropyridine and quinuclidine derivatives.[37,38] In addition, replacement of the 3-methyl group with a 3-cyclopropyl moiety limits activity to M_1 receptors in the superior cervical ganglion in the 1,2,4-oxadiazole derivatives of 1-azabicyclo[2.2.1]heptane.[39] These studies address M_1 selectivity through the ester moiety, while the present work shows that 1,4,5,6-tetrahydropyrimidine is a suitable ammonium bioisostere.

Significance

In summary, the data indicate that the 1,2,4-oxadiazole moiety is useful as an ester bioisostere in the 1,4,5,6-tetrahydropyrimidine series of muscarinic agonists. The relative activity of **7a** as compared to the corresponding 1,2,4-oxadiazole derivatives of 1,2,5,6-tetrahydro-1-methylpyridine and quinuclidine suggests that the 1,4,5,6-tetrahydropyrimidine system is a very useful replacement for the ammonium group of acetylcholine in the design of selective agonists for M_1 receptors.

Experimental Section

Chemistry. Compounds were synthesized utilizing reagents commercially available from Aldrich Chemical Co. and Fisher Scientific without further purification. NMR spectra were obtained on a Bruker ACF 300-MHz NMR in deuteriochloroform, deuteriomethanol, or deuterium oxide, on the δ scale using either TMS or TSP as an internal standard. Melting points were taken on an Electrothermal digital melting point apparatus and are presented uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and were within ±0.4% of calculated values.

1-(Triphenylmethyl)-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (11). 5-(Methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine hydrochloride[20] (1.49 g, 8.3 mmol), diazabicycloundecene (DBU, 2.5 mL, 16.6 mmol), and trityl chloride (2.32 g, 8.3 mmol) were suspended in anhydrous DMF (20 mL) under nitrogen at room temperature. After 18 h of stirring, the suspension was evaporated in vacuo and the residue chromatographed (silica, chloroform/methanol, 9:1) to yield 2.28 g (71%, R_f = 0.15) white crystalline solid: mp 93–95 °C; ^{1}H NMR ($CDCl_3$) δ 2.48 (1 H, m), 3.11 (1 H, m), 3.39 (2 H, m), 3.66 (3 H, s), 3.69 (1 H, m), 7.34 (15 H, m), 7.91 (1 H, s); MS *m/z* 384 (M^+ of free base).

1-(Triphenylmethyl)-5-(3-methyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine. Sodium hydride (60% dispersion in mineral oil, 26 mg, 0.65 mmol) and acetamidoxime[30] (48 mg, 0.65 mmol) were suspended in dry THF (4 mL) in an oven-dried round-bottom flask under nitrogen at 0 °C. After 15 min of stirring, the ice bath was removed and the gray suspension refluxed for 45 min to give a white suspension. 1-(Triphenylmethyl)-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (250 mg, 0.65 mmol) was added dissolved in dry THF (3 mL) and reflux continued for 18 h. The solvents were evaporated in vacuo, and the residue was taken up in water (10 mL). The aqueous suspension was extracted exhaustively with chloroform, and the organics were dried over $MgSO_4$. After evaporation, the organic residue was chromatographed (silica, chloroform/methanol, 9:1) to yield 93 mg (35%, R_f = 0.26) of white crystals: mp 72–74 °C; ^{1}H NMR ($CDCl_3$) δ 2.75 (1 H, m), 3.22 (1 H, m), 3.49 (2 H, m), 3.75 (1 H, m), 7.30 (15 H, m), 7.72 (1 H, s); MS *m/z* 408 (M^+ of the free base). Anal. ($C_{26}H_{24}N_4O$) C, H, N.

5-(3-Methyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine Trifluoroacetate (7a). 1-(Triphenylmethyl)-5-(3-methyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine (254 mg, 0.6 mmol) was dissolved in trifluoroacetic acid (1 mL) with stirring at room temperature for 24 h. The resulting dark solution was evaporated in vacuo, and the residue was recyrstallized from methanol/ether to yield 105 mg (62%) of white crystals: mp 120–122 °C; ^{1}H NMR (CD_3OD) δ 2.35 (3 H, s), 3.29 (1 H, s), 3.76–3.89 (4 H, s), 8.10 (1 H, s). Anal. ($C_7H_{11}N_4O\cdot C_2F_3O_2$) C, H, N.

5-(3-Ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine Trifluoroacetate (7b). 5-(3-Ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine trifluoroacetate (**7b**) was prepared by a procedure similar to **7a**, with the exception that sodium hydride (60% dispersion in mineral oil, 56 mg, 1.4 mmol), propionamide oxime (123 mg, 1.4 mmol), and 1-(triphenylmethyl)-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (540 mg, 1.4 mmol) were used. After formation of the trifluoroacetate salt, the residue was recrystallized from methanol/ether to yield 144 mg (49%) of white crystals: mp 116–118 °C; ^{1}H NMR (D_2O) δ 1.28 (3 H, t, J = 7.6 Hz), 2.79 (2 H, q, J = 7.6 Hz), 3.80–3.96 (5 H, m), 8.06 (1 H, s). Anal. ($C_8H_{13}N_4O\cdot C_2F_3O_2$) C, H, N.

5-(3-Propyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine Trifluoroacetate (7c). 5-(3-Propyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine trifluoroacetate was prepared by a procedure similar to **7a**, with the exception that sodium hydride (60% dispersion in mineral oil, 108 mg, 2.7 mmol), butyramide oxime (276 mg, 2.7 mmol), and 1-(triphenylmethyl)-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (1.04 g, 2.7 mmol) were used. After formation of the trifluoroacetate salt, the residue was recrystallized from methanol/ether to yield 240 mg (43%) of white crystals: mp 126–128 °C; ^{1}H NMR (D_2O) δ 0.93 (3 H, t, J = 7.4 Hz), 1.74 (2 H, sext, J = 7.4 Hz), 2.76 (2 H, t, J = 7.4 Hz), 3.80–3.96 (5 H, m), 8.06 (1 H, s). Anal. ($C_9H_{15}N_4O\cdot C_2F_3O_2$) C, H, N.

5-(3-Butyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine Trifluoroacetate (7d). 5-(3-Butyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine trifluoroacetate was prepared by a procedure similar to **7a**, with the exception that sodium hydride

(95%, 40 mg, 1.7 mmol), valeramide oxime (193 mg, 1.7 mmol), and 1-(triphenylmethyl)-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (640 mg, 1.7 mmol) were used. After formation of the trifluoroacetate salt, the residue was recrystallized from methanol/ether to yield 150 mg (27%) of white crystals: mp 98–100 °C; ^{1}H NMR (D_2O) δ 0.66 (3 H, t, J = 7.4 Hz), 1.07 (2 H, m), 1.45 (2 H, m), 2.54 (2 H, t, J = 7.4 Hz), 3.62 (5 H, m), 7.80 (1 H, s). Anal. ($C_{10}H_{17}N_4O\cdot C_2F_3O_2$) C, H, N.

1,4,5,6-Tetrahydro-5-(3-pentyl-1,2,4-oxadiazol-5-yl)pyrimidine Trifluoroacetate (7e). 1,4,5,6-Tetrahydro-5-(3-pentyl-1,2,4-oxadiazol-5-yl)pyrimidine trifluoroacetate also was prepared by a procedure similar to **7a**, with the exception that sodium hydride (95% 40 mg, 1.7 mmol), hexanamide oxime (217 mg, 1.7 mmol), and 1-(triphenylmethyl)-1,4,5,6-tetrahydro-5-(methoxycarbonyl)pyrimidine (640 mg, 1.7 mmol) were used. After formation of the trifluoroacetate, the residue was recrystallized from methanol/ether to yield 110 mg (19%) of white crystals: mp 97–99 °C; ^{1}H NMR (D_2O) δ 0.62 (3 H, t, J = 7.4 Hz), 1.05 (4 H, m), 1.47 (2 H, m), 2.53 (2 H, t, J = 7.4 Hz), 3.65 (5 H, m), 7.82 (1 H, s). Anal. ($C_{11}H_{19}N_4O\cdot C_2F_3O_2$) C, H, N.

1,4,5,6-Tetrahydro-5-(3-hexyl-1,2,4-oxadiazol-5-yl)pyrimidine Trifluoroacetate (7f). 1,4,5,6-Tetrahydro-5-(3-hexyl-1,2,4-oxadiazol-5-yl)pyrimidine trifluoroacetate also was prepared by a procedure similar to **7a**, with the exception that sodium hydride (60% dispersion in mineral oil, 104 mg, 2.6 mmol), heptanamide oxime (375 mg, 2.6 mmol), and 1-(triphenylmethyl)-1,4,5,6-tetrahydro-5-(methoxycarbonyl)pyrimidine (1 g, 2.6 mmol) were used. After formation of the trifluoroacetate salt, the residue was recrystallized from methanol/ether to yield 92 mg (36%) of brown crystals: mp 89–91 °C; ^{1}H NMR (D_2O) δ 0.65–0.69 (3 H, t, J = 7.0 Hz), 1.09–1.15 (6 H, m), 1.51–1.56 (2 H, m), 2.57–2.62 (2 H, t, J = 7.4 Hz), 3.62–3.79 (5 H, m), 7.87 (1 H, s). Anal. ($C_{12}H_{21}N_4O\cdot C_2F_3O_2$) C, H, N.

1,4,5,6-Tetrahydro-5-(3-heptyl-1,2,4-oxadiazol-5-yl)pyrimidine Trifluoroacetate (7g). 1,4,5,6-Tetrahydro-5-(3-heptyl-1,2,4-oxadiazol-5-yl)pyrimidine trifluoroacetate was prepared by a procedure similar to **7a**, with the exception that sodium hydride (60% dispersion in mineral oil, 96 mg, 2.4 mmol), octanamide oxime (380 mg, 2.4 mmol), and 1-(triphenylmethyl)-1,4,5,6-tetrahydro-5-(methoxycarbonyl)pyrimidine (910 mg, 2.4 mmol) were used. After formation of the trifluoroacetate salt, the residue was recrystallized from methanol/ether to yield 285 mg (49%) of white crystals: mp 101–102 °C; ^{1}H NMR (D_2O) δ 0.83–0.85 (3 H, m), 1.26–1.31 (8 H, m), 1.72 (2 H, m), 2.78 (2 H, t, J = 7.4 Hz), 3.80–3.96 (5 H, m), 8.06 (1 H, s). Anal. ($C_{13}H_{23}N_4O\cdot C_2F_3O_2$) C, H, N.

1,4,5,6-Tetrahydro-5-(3-octyl-1,2,4-oxadiazol-5-yl)pyrimidine Trifluoroacetate (7h). 1,4,5,6-Tetrahydro-5-(3-octyl-1,2,4-oxadiazol-5-yl)pyrimidine trifluoroacetate was prepared by a procedure similar to **7a**, with the exception that sodium hydride (60% dispersion in mineral oil, 104 mg, 2.6 mmol), nonanamide oxime (448 mg, 2.6 mmol), and 1-(triphenylmethyl)-1,4,5,6-tetrahydro-5-(methoxycarbonyl)pyrimidine (1.0 g, 2.6 mmol) were used. After formation of the trifluoroacetate, the residue was recrystallized from methanol/ether/hexane to yield 182 mg (30%) of white crystals: mp 97–99 °C; ^{1}H NMR (D_2O) δ 0.86 (3 H, m), 1.27–1.29 (10 H, m), 1.72 (2 H, m), 2.78 (2 H, t, J = 7.2 Hz), 3.84–3.96 (5 H, m), 8.06 (1 H, s). Anal. ($C_{14}H_{25}N_4O\cdot C_2F_3O_2$) C, H, N.

Receptor Binding. Binding to muscarinic receptors was carried out essentially as described previously.[20,40] Binding was determined indirectly by the ability of compounds to compete with 50 pM [^{3}H]-(*R*)-quinuclidinyl benzilate ([^{3}H]-(*R*)-QNB) in a suspension of brain membranes for 2 h. Nonspecific binding was evaluated by the inclusion of 1000-fold excess atropine in a separate set of samples. IC_{50} values were determined from Hill plots of the inhibition data and are reported as means ± sem of three independent experiments each performed in triplicate.

Phosphoinositide Metabolism. The methods were modified from those described by Brown and associates[41] as reported previously.[17,20,42] The cerebral cortex and hippocampus were dissected according to the method of Glowinski and Iversen.[43] Compounds were screened for activity at 100 μM in the cortical assay, and active compounds were evaluated further in the hippocampus. In these studies, [^{3}H]inositol was purified prior to use by passing over a Dowex AG1-X8 anion-exchange column to remove charged degradation products of [^{3}H]inositol. The amount of [^{3}H]inositol phosphates formed in the assay was determined essentially according to Wreggett and Irvine[44] except that the separation of inositol phosphates was accomplished using an Amersham Super Separator Manifold.

Acknowledgment. The work was supported by Lilly Research Laboratories, by grants NS 23929 (W.P.H.), NS 25765 (W.S.M.), and NS 01493 (W.S.M.) from the National Institutes of Health, and by the Ohio Department of Aging. The authors thank Harlan E. Shannon, Frank P. Bymaster, Charles Mitch, and John Ward for their helpful discussions of the studies outlined here and Sandy Tiell for her help in preparing the manuscript.

References

(1) Hollander, E.; Mohs, R. C.; Davis, K. L. Cholinergic approaches to the treatment of Alzheimer's disease. *Br. Med. Bull.* **1986**, *42*, 97–100.
(2) Moos, W.; Davis, R. E.; Schwarz, R. D.; Gamzu, E. R. Cognition activators. *Med. Res. Rev.* **1988**, *8*, 353–391.
(3) Buckley, N. J.; Bonner, T. I.; Brann, M. R. Localization of a family of muscarinic receptor mRNAs in rat brain. *J. Neurosci.* **1988**, *8*, 4646–4652.
(4) Cortés, R.; Palacios, J. M. Muscarinic cholinergic receptor subtypes in the rat brain. I. Quantitative autoradiographic studies. *Brain Res.* **1986**, *362*, 227–238.
(5) Mash, D. C.; Potter, L. T. Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. *Neuroscience* **1986**, *19*, 551.
(6) Messer, W. S., Jr.; Hoss, W. P. Selectivity of pirenzepine in the central nervous system. I. Direct autoradiographic comparison of the regional distribution of pirenzepine and carbamylcholine binding sites. *Brain Res.* **1987**, *407*, 27–36.
(7) Messer, W. S., Jr.; Ellerbrock, B. R.; Smith, D. A.; Hoss, W. Regional differences in the binding of selective muscarinic antagonists to rat brain: Comparison with minimum energy conformations. *J. Med. Chem.* **1989**, *32*, 1164–1172.
(8) Wamsley, J. K.; Gehlert, D. R.; Roeske, W. R.; Yamamura, H. I. Muscarinic antagonist binding site heterogeneity as evidenced by autoradiography after direct labeling with [^{3}H]-QNB and [^{3}H]-pirenzepine. *Life Sci.* **1984**, *34*, 1395–1402.
(9) Wall, S. J.; Yasuda, R. P.; Hory, F.; Flagg, S.; Martin, B. M.; Ginns, E. I.; Wolfe, B. B. Production of antisera selective for m1 muscarinic receptors using fusion proteins: Distribution of m1 receptors in rat brain. *Mol. Pharmacol.* **1991**, *39*, 643–649.
(10) Caulfield, M. P.; Higgens, G. A.; Straughan, D. W. Central administration of the muscarinic receptor subtype-selective antagonist pirenzepine selectively impairs passive avoidance learning in the mouse. *J. Pharm. Pharmacol.* **1983**, *35*, 131–132.
(11) Hagan, J. J.; Jansen, J. H. M.; Broekkamp, C. L. E. Blockade of spatial learning by the M_1 muscarinic antagonist pirenzepine. *Psychopharmacology* **1987**, *93*, 470–476.
(12) Messer, W. S., Jr.; Thomas, G. J.; Hoss, W. P. Selectivity of pirenzepine in the central nervous system. II. Differential effects of pirenzepine and scopolamine on performance of a representational memory task. *Brain Res.* **1987**, *407*, 37–45.
(13) Messer, W. S., Jr.; Bohnett, M.; Stibbe, J. Evidence for a preferential involvement of M_1 muscarinic receptors in representational memory. *Neurosci. Lett.* **1990**, *116*, 184–189.
(14) Gil, D. W.; Wolfe, B. B. Pirenzepine distinguishes between muscarinic receptor-mediated phosphoinositide breakdown and inhibition of adenylate cyclase. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 608–616.
(15) Fisher, S. K.; Agranoff, B. W. Receptor activation and inositol lipid hydrolysis in neural tissues. *J. Neurochem.* **1987**, *48*, 999–1017.
(16) Messer, W. S., Jr.; Ellerbrock, B.; Price, M. Autoradiographic analyses of agonist binding to muscarinic receptor subtypes. *Biochem. Pharmacol.* **1989**, *38*, 837–850.
(17) Hoss, W.; Woodruff, J. M.; Ellerbrock, B. R.; Periyasamy, S.; Ghodsi-Hovsepian, S.; Stibbe, J.; Bohnett, M.; Messer, W. S., Jr. Biochemical and behavioral responses of pilocarpine at muscarinic receptor subtypes in the CNS. Comparison with receptor binding and low-energy conformations. *Brain Res.* **1990**, *533*, 232–238.
(18) Sauerberg, P.; Chen, J.; Woldemussie, E.; Rapoport, H. Cyclic carbamate analogues of pilocarpine. *J. Med. Chem.* **1989**, *32*, 1322–1326.
(19) Shapiro, G.; Floersheim, P.; Boelsterli, J.; Amstutz, R.; Bolliger, G.; Gammenthaler, H.; Gmelin, G.; Supavilai, P.; Walkinshaw, M. Muscarinic activity of the thiolactone, lactam, lactol and thiolactol analogues of pilocarpine and a hypothetical model for the binding of agonists to the m1 receptor. *J. Med. Chem.* **1992**, *35*, 15–27.

(20) Messer, W. S., Jr.; Dunbar, P. G.; Rho, T.; Periysamy, S.; Ngur, D.; Ellerbrock, B. R.; Bohnett, M.; Ryan, K.; Durant, G. J.; Hoss, W. Synthesis, biochemical activity and behavioral effects of a series of 1,4,5,6-tetrahydropyrimidines as novel ligands for M_1 receptors. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 781–786.
(21) Saunders, J.; MacLeod, A. M.; Merchant, K.; Showell, G. A.; Snow, R. J.; Street, L. J.; Baker, R. Ester bioisosteres: Synthesis of oxadiazolyl-1-azabicyclo[2.2.1]heptanes as muscarinic agonists. *J. Chem. Soc., Chem. Commun.* **1988**, 1618–1619.
(22) Freedman, S. B.; Harley, E. A.; Patel, S.; Newberry, N. R.; Gilbert, M. J.; McKnight, A. T.; Tang, J. K.; Maguire, J. J.; Mudunkotuwa, N. T.; Baker, R.; Street, L. J.; MacLeod, A. M.; Saunders, J.; Iversen, L. L. A novel series of non-quaternary oxadiazoles acting as full agonists at muscarinic receptors. *Br. J. Pharmacol.* **1990**, *101*, 575–580.
(23) Street, L. J.; Baker, R.; Book, T.; Kneen, C. O.; MacLeod, A. M.; Merchant, K. J.; Showell, G. A.; Saunders, J.; Herbert, R. H.; Freedman, S. B.; Harley, E. A. Synthesis and biological activity of 1,2,4-oxadiazole derivatives: Highly potent and efficacious agonists for cortical muscarinic receptors. *J. Med. Chem.* **1990**, *33*, 2690–2697.
(24) 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.
(25) Sauerberg, P.; Kindtler, J. W.; Nielsen, L.; Sheardown, M. J.; Honore, 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.
(26) Showell, G. A.; Gibbons, T. L.; Kneen, C. O.; MacLeod, A. M.; Merchant, K. J.; Saunders, J.; Freedman, S. B.; Patel, S.; Baker, R. Tetrahydropyridyloxadiazoles: Semirigid muscarinic ligands. *J. Med. Chem.* **1991**, *34*, 1086–1094.
(27) Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, L. L.; Kneen, C.; MacLeod, A. M.; Merchant, K. J.; Snow, R. J.; Baker, R. Novel quinuclidine-based ligands for the muscarinic cholinergic receptor. *J. Med. Chem.* **1990**, *33*, 1128–1138.
(28) Showell, G. A.; Baker, R.; Davis, J.; Hargreaves, R.; Freedman, S. B.; Hoogsteen, K.; Patel, S.; Snow, R. J. Synthesis and in vitro biological profile of all four isomers of the potent muscarinic agonist 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane. *J. Med. Chem.* **1992**, *35*, 911–916.
(29) Clark, M. S. G.; Hatcher, J.; Brown, F. Relative aversive potency of muscarinic agonists. In *Fourth International Symposium on Subtypes of Muscarinic-Receptors*; Elsevier Trends Journals: Wiesbaden, FRG, 1989; p 100.
(30) Hirotsu, T.; Katoh, S.; Sugasaka, K.; Seno, M.; Itagaki, T. Binding ability of acetamide oxime with proton, copper (II) and dioxouranium (VI) in aqueous solutions. *J. Chem. Soc., Dalton Trans.* **1986**, 1609.
(31) Doods, H. N.; Mathy, M.-J.; Davidsko, D.; van Charldorp, K. J.; de Jonge, A.; van Zwieten, P. A. Selectivity of muscarinic antagonists in radioligand and in vivo experiments for the putative M_1, M_2 and M_3 receptors. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 257–262.
(32) Hammer, R.; Giachetti, A. Muscarinic receptor subtypes: M1 and M2 biochemical and functional characterization. *Life Sci.* **1982**, *31*, 2991–2998.
(33) Hammer, R.; Giraldo, E.; Schiavi, G. B.; Monferini, E.; Ladinsky, H. Binding profile of a cardioselective muscarine receptor antagonist, AF-DX 116, to membranes of peripheral tissues and brain in the rat. *Life Sci.* **1986**, *38*, 1653–1662.
(34) Lambrecht, G.; Feifel, R.; Forth, B.; Strohmann, C.; Tacke, R.; Mutschler, E. p-Fluoro-hexahydro-sila-difenidol: The first $M_{2,3}$-selective muscarinic antagonist. *Eur. J. Pharmacol.* **1988**, *152*, 193–194.
(35) Waelbroeck, M.; Gillard, M.; Robberecht, P.; Christophe, J. Muscarinic receptor heterogeneity in rat central nervous system. I. Binding of four selective antagonists to three muscarinic receptor subclasses: A comparison with M_2 cardiac muscarinic receptors of the C type. *Mol. Pharmacol.* **1987**, *32*, 91–99.
(36) Giachetti, A.; Micheletti, R.; Montagna, E. Cardioselective profile of AF-DX 116, a muscarine M_2 receptor antagonist. *Life Sci.* **1986**, *38*, 1663–1672.
(37) Fisher, A.; Brandies, R.; Pittel, Z.; Karton, I.; Sapir, M.; Dachir, S.; Levy, A.; Heldman, E. (±)cis-2-Methylspiro(1,3-oxathiolane-5,3′)-quinuclidine (AF102B): A new M_1 agonist attenuates cognitive dysfunctions in AF64A-treated rats. *Neurosci. Lett.* **1989**, *102*, 325–331.
(38) Sauerberg, P.; Olesen, P. H.; Nielsen, S.; Treppedahl, 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 functionally selective M_1 muscarinic agonists. Synthesis and structure-activity relationships of 3-(1,2,5-thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines. *J. Med. Chem.* **1992**, *35*, 2274–2283.
(39) Freedman, S. B.; Patel, S.; Harley, E. A.; Iversen, L. L.; Baker, R.; Showell, G. A.; Saunders, J.; McKnight, A.; Newberry, N.; Scholey, K.; Hargreaves, R. L-687,306: a functionally selective and potent muscarinic M_1 receptor agonist. *Eur. J. Pharmacol.* **1992**, *215*, 135–136.
(40) Farrar, J. R.; Hoss, W.; Herndon, R. M.; Kuzmiak, M. Characterization of muscarinic cholinergic receptors in the brains of copper deficient rats. *J. Neurosci.* **1985**, *5*, 1083–1089.
(41) Brown, E.; Kendall, D. A.; Nahorski, S. R. Inositol phospholipid hydrolysis in rat cerebral cortical slices: I. Receptor characterisation. *J. Neurochem.* **1984**, *42*, 1379–1387.
(42) Periyasamy, S.; Hoss, W. Kappa opioid receptors stimulate phosphoinositide turnover in rat brain. *Life Sci.* **1990**, *47*, 219–225.
(43) Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in rat brain I: the disposition of [^{3}H]norepinephrine, [^{3}H]-dopamine and [^{3}H]dopa in various regions of the brain. *J. Neurochem.* **1966**, *13*, 655–669.
(44) Wreggett, K. A.; Irvine, R. F. A rapid separation method for inositol phosphates and their isomers. *Biochem. J.* **1987**, *245*, 655–660.