Design, Synthesis, and Neurochemical Evaluation of 2-Amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines and 2-Amino-5-(alkoxycarbonyl)-1,4,5,6-tetrahydropyrimidines as M_1 Muscarinic Receptor Agonists

Philip G. Dunbar,[‡] Graham J. Durant,[§] Taikyun Rho, Babatunde Ojo, James J. Huzl, III, Douglas A. Smith,[†] Afif A. El-Assadi, Sbeih Sbeih, Dan O. Ngur, Sumudra Periyasamy, Wayne Hoss, and William S. Messer, Jr.*

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

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Four regioisomers of 2-amino-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine (**2a–5a**) were synthesized as the racemates to evaluate the utility of exocyclic amidines in the development of novel agonists for M_1 muscarinic receptors. Of the four regioisomers, only racemic 2-amino-5-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine (**4a**; CDD-0075-A) displayed high affinity (IC_{50} = 10 ± 3.0 μM) and activity at muscarinic receptors coupled to PI metabolism in the rat cortex (260 ± 4.5% stimulation above basal levels at 100 μM). A series of 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines then was synthesized for further evaluation as M_1 agonists. Only the propargyl derivative (**4d**) retained substantial agonist activity (120 ± 14% at 100 μM) in this series. On the basis of the activity of the 5-(alkoxycarbonyl)-1,4,5,6-tetrahydropyrimidines (**1a** and **1d**) and the 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines, the corresponding cyclic guanidine derivatives were synthesized and tested. 2-Amino-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (**7a**) displayed a modest affinity for muscarinic receptors in the CNS (22 ± 5.3 μM) and an ability to stimulate PI turnover in rat cerebral cortex (81 ± 16% at 100 μM). The propargyl derivative (**7d**) also had modest binding affinity (31 ± 15 μM) and high activity (150 ± 8.5% at 100 μM), as expected based on the activity of propargyl esters of 1,4,5,6-tetrahydropyrimidine and 2-amino-3,4,5,6-tetrahydropyridine. Computational chemical studies revealed five distinct minimum-energy conformations for **1a**, (*R*)-**4a**, and **7a**, and three for **1d**, (*R*)-**4d**, and **7d**, each with a unique orientation of the ester moiety. Each of the five conformations for **1a** could be superimposed upon a unique conformer of (*R*)-**4a** and **7a**, suggesting that the compounds interact with muscarinic receptors in a similar fashion. Taken together, the data indicate the general utility of amidine systems as suitable replacements for the ammonium group of acetylcholine in developing ligands with activity at M_1 muscarinic receptors in the central nervous system. Such compounds might be useful in the treatment of patients with Alzheimer's disease.

Introduction

Recent work has focused on the development of M_1-selective agonists for Alzheimer's disease.[1,2] M_1 muscarinic receptors play a role in memory function[3–5] and stimulate phosphoinositide (PI) turnover in the mammalian forebrain.[6,7] It has been suggested that centrally-active, M_1-selective agonists may help alleviate the cognitive and memory deficits associated with the loss of cholinergic neurons that is characteristic of Alzheimer's disease.

A number of ligands have been developed recently with reported activity at M_1 muscarinic receptors. There remains, however, 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. A key strategy in the development of centrally active muscarinic agonists has been the incorporation of a suitable replacement for the quaternary ammonium group in acetylcholine, while still affording penetration into the central nervous system.

Over the past few years, a series of 1,4,5,6-tetrahydropyrimidine esters (**1a–d**) and oxadiazoles has been synthesized and evaluated for muscarinic receptor activity in the rat brain.[8,9] To explore further the utility of amidines in the development of selective M_1 agonists, a series of exocyclic amidine derivatives was synthesized and tested. Affinity for muscarinic receptors in rat brain was measured by inhibition of [^{3}H]-(*R*)-quinuclidinyl benzilate ([^{3}H]-(*R*)-QNB) binding. Agonist activity was evaluated by measuring PI metabolism in the rat cortex in preliminary fashion. The data indicate that the 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines (**4a–d**) and the 2-amino-5-(alkoxycarbonyl)-1,4,5,6–tetrahydropyrimidines (**7a–d**) bind to muscarinic receptors in rat brain. In addition, the methoxy and propargyloxy derivatives of each series stimulate phosphoinositide metabolism in rat cerebral cortex.

Synthetic and Computational Chemistry

A new series of tetrahydropyridine and tetrahydropyrimidine esters was synthesized by esterification of the corresponding reduced acids (see Schemes 1 and 2). Four regioisomers of 2-amino-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine (**2a–5a**) were synthesized.

[†] Department of Chemistry.
[‡] 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.
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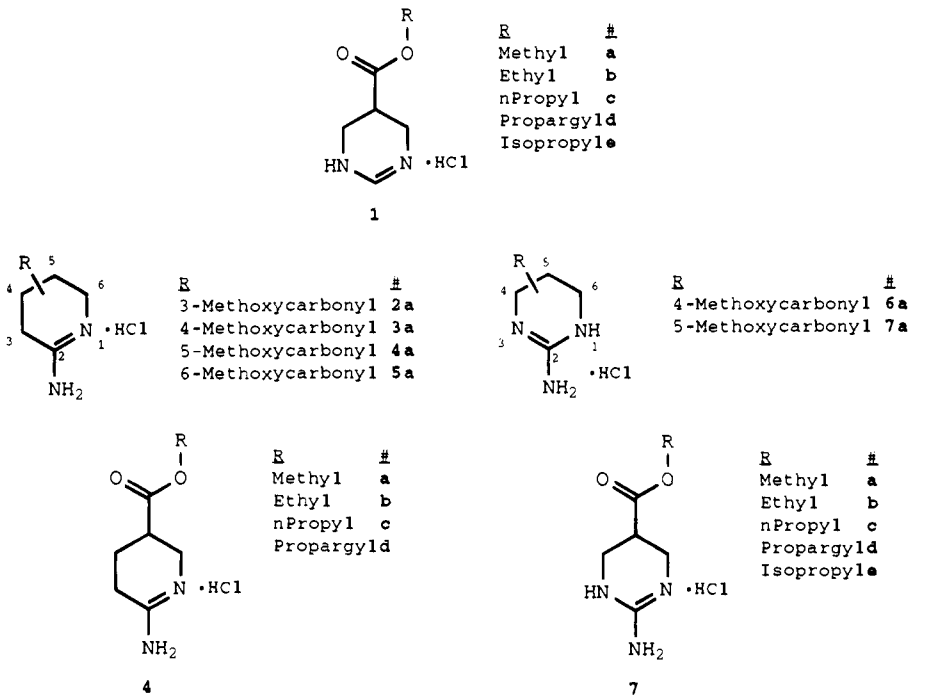
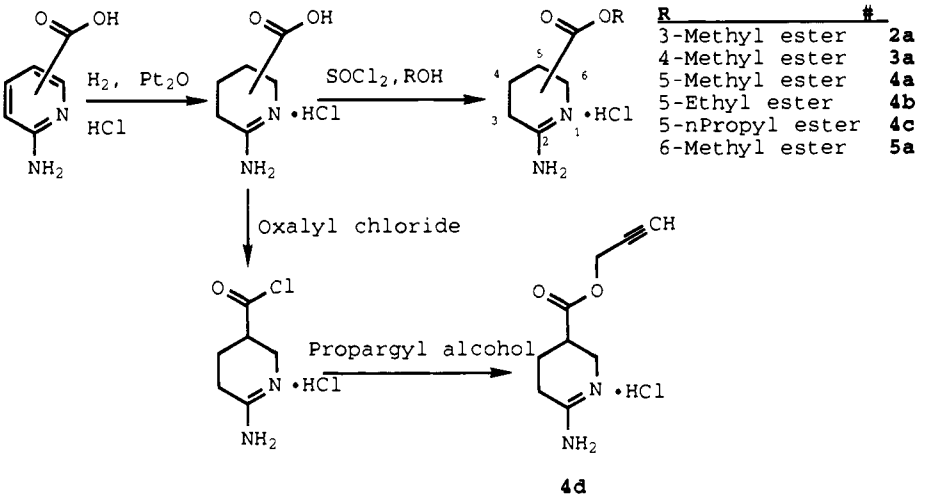
Chart 1



Scheme 1. Synthesis of 2-Amino-3,4,5,6-tetrahydropyridinecarboxylates



A series of 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines (**4a**–**d**) was synthesized. Catalytic reduction over platinum oxide in 90% ethanol and concentrated HCl was used to form 2-amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid from 6-aminonicotinic acid. The 2-amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid subsequently was esterified by refluxing with the desired alcohols in the presence of thionyl chloride to give the corresponding 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridine hydrochloride derivatives (see Scheme 1).

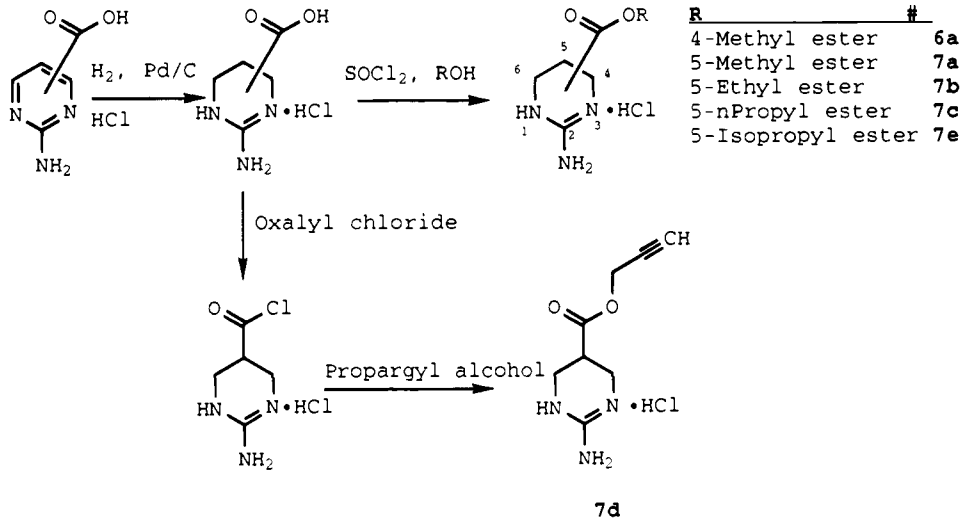
A series of 2-amino-5-(alkoxycarbonyl)-1,4,5,6-tetrahydropyrimidines (**7a**–**d**) was synthesized. 2-Aminopyrimidine-5-carboxylic acid was synthesized using literature procedures.[10] The acid was catalytically reduced over 10% Pd-on-carbon in aqueous acid[11] to form 2-amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid. The carboxylic acid subsequently was esterified utilizing a similar synthetic strategy as above (see Scheme 2). In each of the series, the propargylic esters were prepared by first forming the acid chloride using oxalyl chloride, followed by reaction with propargyl alcohol.

Minimum-energy conformations of **1a**, **1d**, (*R*)-**4a**, (*R*)-**4d**, **7a**, and **7d** were generated using the program MacroModel (version 3.5). High energy and redundant structures were eliminated after minimization using the AMBER force field as implemented in the program. The structures were then ranked in order of increasing energy. The lowest energy conformations for **1a** were compared with corresponding conformations of (*R*)-**4a** and **7a** and the lowest energy conformations of **1d**, (*R*)-**4d**, and **7d**.

Results and Discussion

Four regioisomers of 2-amino-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine (**2a**–**5a**) were synthesized as racemates and evaluated for activity at muscarinic receptors in the rat central nervous system. The binding affinity of each ligand was determined indirectly

Scheme 2. Synthesis of 2-Amino-1,4,5,6-tetrahydropyrimidinecarboxylates



R	#
4-Methyl ester	6a
5-Methyl ester	7a
5-Ethyl ester	7b
5-nPropyl ester	7c
5-Isopropyl ester	7e

Table 1. Physicochemical Data for the 2-Amino-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines and the 2-Amino-(alkoxycarbonyl)-1,4,5,6-tetrahydropyrimidines[a]

compd	% yield	mp (°C)	formula
2a	46.0	138–139	$C_7H_{12}N_2O_2$·HCl
3a	27.0	180–181	$C_7H_{12}N_2O_2$·HCl
4a	95.2	177–179	$C_7H_{12}N_2O_2$·HCl
4b	82.5	175–177	$C_8H_{14}N_2O_2$·HCl
4c	81.8	173–175	$C_9H_{16}N_2O_2$·HCl
4d	19.3	121–123	$C_9H_{12}N_2O_2$·HCl
5a	31.0	132–134	$C_7H_{12}N_2O_2$·HCl
6a	23.0	106–108	$C_6H_{11}N_3O_2$·HCl
7a	81.2	167–168	$C_6H_{11}N_3O_2$·HCl
7b	82.1	154–155	$C_7H_{13}N_3O_2$·HCl
7c	75.2	149–150	$C_8H_{15}N_3O_2$·HCl
7d	53.2	126–127	$C_8H_{11}N_3O_2$·HCl
7e	34.2	145–146	$C_8H_{15}N_3O_2$·HCl

[a] Elemental analyses were within ±0.4% of theoretical values.

Table 2. 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	1.0 ± 0.25	0.76 ± 0.16	110 ± 21%
1a	9.2 ± 1.9	0.52 ± 0.077	110 ± 11%
1b	2.2 ± 1.9	0.69 ± 0.029	150 ± 17%
1c	2.4 ± 0.44	0.69 ± 0.039	7.1 ± 2.1%
1d	3.3 ± 0.80	0.51 ± 0.039	230 ± 35%
1e	3.3 ± 0.12	0.60 ± 0.031	7.2 ± 3.0%
2a	320 ± 270	0.40 ± 0.090	−5.5 ± 6.9%
3a	120 ± 39	0.44 ± 0.11	9.0 ± 4.6%
4a	10 ± 3.0	0.44 ± 0.037	260 ± 4.5%
4b	4.3 ± 0.50	0.80 ± 0.034	31 ± 13%
4c	1.7 ± 0.43	0.83 ± 0.050	51 ± 26%
4d	2.3 ± 0.50	0.63 ± 0.024	120 ± 14%
5a	250 ± 76	0.59 ± 0.078	8.1 ± 2.7%
6a	4300 ± 3900	0.40 ± 0.08	–
7a	22 ± 5.3	0.46 ± 0.06	81 ± 16%
7b	8.2 ± 1.9	0.59 ± 0.078	28 ± 14%
7c	4.8 ± 0.86	0.81 ± 0.062	4.3 ± 8.0%
7d	31 ± 15	0.58 ± 0.15	150 ± 8.5%
7e	29 ± 19	0.63 ± 0.10	23 ± 13%

[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. Previously published data for carbachol, arecoline, and **1a–e** are presented here for comparison.[8]

by assessing the inhibition of specific [^{3}H]-(*R*)-QNB binding to rat brain membranes as shown in Table 2. Only the 5-methoxycarbonyl regioisomer (**4a**) bound with high affinity (IC_{50} value less than or equal to 10 μM) to muscarinic receptors in rat brain. The affinity of **4a** was comparable to that found previously for **1a**, as shown in Figure 1. Compound **4a** also stimulated PI metabolism in the rat cerebral cortex (260 ± 4.5% above basal at 100 μM).

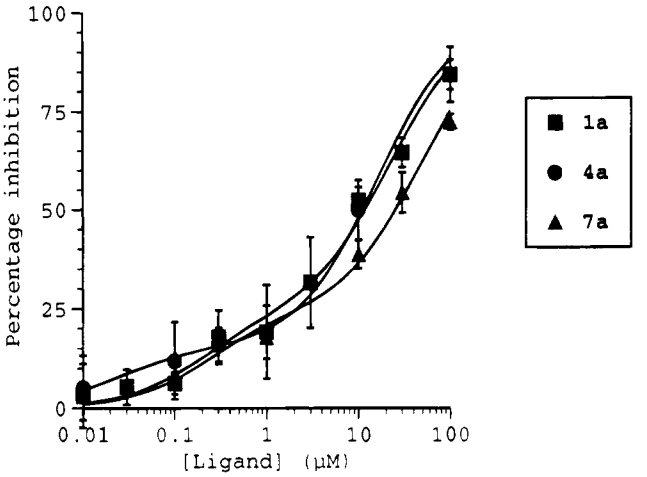


Figure 1. Inhibition of specific [^{3}H]-(*R*)-QNB binding to rat brain membranes by three amidine derivatives. The data represent the mean inhibition from at least three independent assays. The data reflect an interaction with two binding sites for each ligand: (**1a**, 23.8% high affinity, 194 nM IC_{50}H, 22.0 μM IC_{50}L; **4a**, 15.2% high affinity, 23.3 nM IC_{50}H, 15.9 μM IC_{50}L; **7a**, 24.1% high affinity, 240 nM IC_{50}H, 48.4 μM IC_{50}L).

On the basis of the activity of **4a**, a series of 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridines then was synthesized for further evaluation as muscarinic agonists. Increasing the length of the alkyl substituent increased affinity for muscarinic receptors, as was found previously for the 5-(alkoxycarbonyl)-1,4,5,6-tetrahydropyrimidine series.[8] Only the propargyl derivative **4d** retained high agonist activity (120 ± 14% at 100 μM) in this series.

The relatively high activity of 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridine compared with the three other regioisomers revealed an important structural relationship between the amidine moiety and the ester group. The position of the ester relative to the endocyclic nitrogen is similar in both **4a** and **1a**. The relationship was more clearly defined through the molecular modeling studies outlined below.

Table 3. Data from Computational Studies of **1a**, **1d**, (*R*)-**4a**, (*R*)-**4d**, **7a**, and **7d**[a]

ligand	relative energy[b] (kCal/mol)	molecular volume (Å^3)
1a conformation i	0	132
1a conformation ii	1.98	129
1a conformation iii	2.47	130
1a conformation iv	20.1	130
1a conformation v	21.2	131
1d conformation i	0	153
1d conformation ii	1.87	153
1d conformation iii	1.92	154
(*R*)-**4a** conformation i	0	147
(*R*)-**4a** conformation ii	0.0430	147
(*R*)-**4a** conformation iii	0.323	148
(*R*)-**4a** conformation iv	9.92	147
(*R*)-**4a** conformation v	10.4	147
(*R*)-**4d** conformation i	0	170
(*R*)-**4d** conformation ii	0.48	170
(*R*)-**4d** conformation iii	1.08	170
7a conformation i	0	141
7a conformation ii	1.65	142
7a conformation iii	1.70	142
7a conformation iv	2.03	142
7a conformation v	12.8	141
7d conformation i	0	165
7d conformation ii	0.59	164
7d conformation iii	0.81	163

[a] Five minimum energy conformations were observed for each methyl ester, while three conformers were found for each propargyl derivative. The relative energy and molecular volume are shown for each ligand. [b] The relative energies of these conformers may exceed the 50 kJ limit used during the generation and minimization of conformations. This is due to the use of MOPAC-ESP charges rather than the default force field charges during the final minimization sequence.

Since both the 2-amino-5-(alkoxycarbonyl)-3,4,5,6-tetrahydropyridine and the 5-(alkoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (**1a**–**d**) series displayed muscarinic activity, the corresponding cyclic guanidine derivatives were synthesized and tested. 2-Amino-4-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (**6a**) was inactive in binding assays (IC_{50} value greater than 100 μM). As shown in Figure 1, 2-amino-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine (**7a**) displayed a modest affinity (IC_{50} value less than 100 μM) for muscarinic receptors in the CNS (22 ± 5.3 μM) and the ability to stimulate PI turnover in rat cerebral cortex (81 ± 16% at 100 μM). Again, the position of the ester relative to either endocyclic nitrogen in **7a** is similar to that found in **4a** and **1a**.

The ethyl, propyl, and isopropyl derivatives (**7b**, **7c**, and **7e**) displayed high binding affinities, yet were much less active than **7a**. The propargyl derivative **7d** had comparable affinity (31 ± 15 μM) to **7a** and high agonist activity (150 ± 8.5% at 100 μM), as might be expected based on the activity of **1d** and **4d**. A similar spatial orientation of the basic nitrogen and carboxylate functionalities is found in all the active compounds. The similarities between the three types of amidines were explored using computational chemistry and molecular modeling.

A multiconformational search was performed on each methyl ester (**1a**, (*R*)-**4a**, and **7a**) and propargyl ester (**1d**, (*R*)-**4d**, and **7d**). Five low-energy conformations were found for each methyl ester, while only three low-energy conformations were found for each propargyl ester. These were ranked in order of increasing energy (from i to v) as shown in Table 3. The 1,4,5,6-tetrahydropyrimidine methyl ester (**1a**) was the smallest in terms of molecular volume, followed by the guanidine (**7a**) and the exocyclic amidine ((*R*)-**4a**). The propargyl derivatives were larger in molecular volume and more restricted in conformational flexibility.

After superimposition of each conformation upon each different molecular (see Table 4) it became apparent that there were corresponding conformations for each amidine methyl ester. For example, the lowest energy conformations of **1a**, (*R*)-**4a**, and **7a** were superimposable (with RMS values less than 0.08 Å). The superimposition of each set of methyl esters is shown in Figure 3. The propargyl esters adopted three low-energy conformations comparable to the lowest energy conformations of the methyl esters, as shown in Figure 4 and Table 4. These data suggest that all of the compounds interact with muscarinic receptors in a similar fashion, although the propargyl derivatives have limited conformational flexibility. Such restricted conformational flexibility may limit the interaction of propargyl derivatives with muscarinic receptor subtypes, as suggested by other groups.[12]

Taken together, the data indicate the general utility of amidine systems in developing ligands with activity at muscarinic receptors coupled to phosphoinositide metabolism in the central nervous system. Furthermore, the location of the ester substituent relative to the amidine system was consistent in each series examined, as confirmed by molecular modeling studies. The high agonist activity in the series of ester derivatives warrants further development of the compounds as selective muscarinic agonists. Accordingly, it will be important to examine the activities of these ligands at cloned muscarinic receptor subtypes expressed in cell lines to verify that the PI response elicited in rat cerebral cortex is mediated through activation of M_1 muscarinic receptors. In addition, structural modifications to the ester moiety might yield compounds with higher stability, affinity, activity, and/or selectivity at muscarinic receptor subtypes. It will be important to address the ability of these (and similar) ligands to penetrate into the central nervous system, stimulate M_1 receptors, and improve memory deficits associated with a loss of cholinergic activity. Such compounds might be useful in the treatment of the symptoms of Alzheimer's disease.

Significance

The data indicate the utility of amidine systems as suitable replacements for the ammonium group of acetylcholine in developing ligands with activity at M_1 muscarinic receptors in the central nervous system. Further studies of these compounds are warranted to assess functional selectivity for muscarinic receptor subtypes and examine *in vivo* activity. Ultimately, such compounds might be of clinical utility in the treatment of the cognitive impairments and memory deficits found in Alzheimer's disease.

Materials and Methods

Chemistry. Compounds were synthesized utilizing reagents commercially available from Aldrich Chemical Co. and Fisher Scientific without further purification. 2-Amino-5-chloropyrimidine-4-carboxylic acid was obtained from Pfaltz and Bauer, Inc. NMR spectra were obtained on a Bruker ACF 300-MHz NMR in deuteriochloroform, deuteriomethanol, or deuterium oxide, using either TMS or TSP as an internal

Table 4. Superimposition RMS (Angstroms)

	1a					(*R*)-4a					7a					1d			(*R*)-4d			7d		
	i	ii	iii	iv	v	i	ii	iii	iv	v	i	ii	iii	iv	v	i	ii	iii	i	ii	iii	i	ii	iii
1a i	–	1.0	1.0	1.4	1.4	*0.07*	1.1	1.0	1.3	1.4	*0.03*	1.0	1.0	1.4	1.4	*0.01*	1.0	1.0	1.0	1.0	*0.07*	*0.03*	0.98	0.98
ii		–	0.76	0.63	0.93	1.0	0.74	*0.08*	0.63	0.90	1.2	*0.04*	0.78	0.92	0.63	1.0	0.77	*0.01*	0.74	*0.08*	1.0	1.0	0.79	*0.05*
iii			–	0.92	0.63	1.0	*0.08*	0.80	0.97	0.64	1.0	0.79	*0.04*	0.63	0.92	1.0	*0.01*	0.77	*0.08*	0.79	1.0	1.0	*0.05*	0.80
iv				–	0.59	1.4	0.90	0.64	*0.09*	0.55	1.4	0.64	0.94	0.58	*0*	1.4	0.93	0.66	0.90	0.64	1.4	1.4	1.0	0.64
v					–	1.4	0.62	0.95	0.65	*0.08*	1.4	1.0	0.64	*0*	0.59	1.4	0.63	0.93	0.63	0.95	1.4	1.4	0.64	0.95
(*R*)-4a i						–	1.1	1.0	1.3	1.4	*0.08*	1.0	1.0	1.4	1.4	*0.07*	1.0	1.0	1.1	1.0	*0.00*	*0.08*	1.0	0.99
ii							–	0.76	0.94	0.63	1.1	0.76	*0.10*	0.62	0.90	1.0	*0.1*	0.74	*0.00*	0.76	1.1	1.1	*0.11*	0.77
iii								–	0.63	0.92	1.0	*0.08*	0.81	0.94	0.64	1.0	0.80	*0.1*	0.76	*0.00*	1.0	1.0	0.82	*0.1*
iv									–	0.61	0.99	0.64	0.99	0.64	*0.10*	1.3	0.97	0.63	0.94	0.40	1.3	1.3	0.99	0.64
v										–	1.4	0.92	0.65	*0.1*	0.55	1.4	0.68	0.91	0.63	0.92	1.4	1.4	0.66	0.93
7a i											–	0.99	1.0	1.4	1.4	*0.03*	1.0	1.0	1.1	1.0	*0.08*	*0.00*	0.99	0.98
ii												–	0.81	0.94	0.63	1.0	*0.80*	*0.04*	0.76	*0.08*	1.0	1.0	0.82	*0.01*
iii													–	0.63	0.94	1.0	*0.03*	0.79	*0.10*	0.82	1.0	1.0	*0.01*	0.82
iv														–	0.58	1.4	0.63	0.92	0.62	0.94	1.4	1.4	0.64	0.95
v															–	1.4	0.92	0.63	0.90	0.64	1.4	1.4	0.94	0.64
1d i																–	1.2	1.2	1.2	1.2	*0.07*	*0.03*	1.1	1.2
ii																	–	0.98	*0.09*	1.2	1.1	1.2	*0.04*	1.0
iii																		–	1.1	*0.08*	1.2	1.2	1.0	*0.05*
(*R*)-4d i																			–	1.1	1.1	1.2	*0.11*	1.1
ii																				–	1.3	1.2	1.2	*0.09*
iii																					–	*0.08*	1.1	1.2
7d i																						–	1.1	1.2
ii																							–	1.2
iii																								–

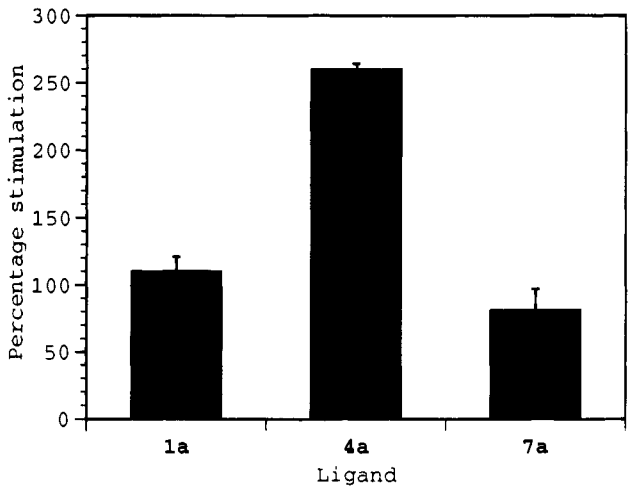


Figure 2. Stimulation of phosphoinositide metabolism in the rat cerebral cortex by three amidine derivatives (**1a**, **4a**, and **7a**). Data represent the mean (±SEM) stimulation above basal levels at 100 μM of each ligand. The results are from three independent experiments for each ligand.

standard. IR spectra were obtained on a 1600 series Perkin-Elmer FTIR. Mass spectral data were recorded on a Hewlett-Packard 5890 spectrometer. TLC was performed on Kodak Chromatogram sheet 13181 silica gel with a fluorescent indicator (F254). 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 (data available upon request).

2-Amino-3,4,5,6-tetrahydropyridine-3-carboxylic Acid Hydrochloride. 2-Aminonicotinic acid (0.912 g, 6.6 mmol) was dissolved in 90% ethanol (137 mL), and concentrated HCl (3.5 mL, 40 mM) was added. The solution was hydrogenated over PtO_2 (200 mg) in a Parr shaker apparatus at room temperature and 29 psig for 2 h. Filtration and evaporation gave 1.22 g (100%) of the oily product identified by IR 3300–2500, 1724 cm^{-1}.

2-Amino-3-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine Hydrochloride (2a). 2-Amino-3,4,5,6-tetrahydropyridine-3-carboxylic acid hydrochloride (1.2 g, 6.6 mmol) was suspended in anhydrous methanol (100 mL), and thionyl chloride (0.5 mL, 7 mmol) was added dropwise with stirring at room temperature. The solution was refluxed overnight and then evaporated to dryness *in vacuo*. The resulting crude white solid was recrystallized from methanol/ether to give 613 mg (46%) of white crystals: mp 138–139 °C; ^1H NMR (D_2O) 1.9 (m, 2H), 2.1 (t, 1H), 2.2 (d, 1H), 3.4 (m, 3H), 3.8 (s, 3H) and IR 1737 cm^{-1}. Anal. ($C_7H_{12}N_2O_2$·HCl) C, H, N.

2-Amino-3,4,5,6-tetrahydropyridine-4-carboxylic Acid Hydrochloride. 2-Aminopyridine-4-carboxylic acid[13] (1.38 g, 10 mmol) was hydrogenated over PtO_2. Filtration and evaporation gave 1.57 g (88%) of crude white crystals identified as the product: ^1H NMR (D_2O) 1.7 (m, 1H), 1.9 (m, 1H), 2.6 (d, 2H), 2.7 (m, 1H), 3.2 (t, 2H); IR 1728 cm^{-1}.

2-Amino-4-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine Hydrochloride (3a). 2-Amino-3,4,5,6-tetrahydropyridine-4-carboxylic acid hydrochloride (1.54 g, 8.6 mmol) was esterified employing a method similar to that used for **2a**. The resulting crude white solid was recrystallized from methanol/ether to give 452 mg (27%) of white crystals: mp 180–181 °C; ^1H NMR (D_2O) 1.7 (m, 1H), 2.0 (m, 1H), 2.6 (d, 2H), 2.8 (m, 1H), 3.2 (t, 2H), 3.5 (s, 3H); IR 1733 cm^{-1}. Anal. ($C_7H_{12}N_2O_2$·HCl) C, H, N.

2-Amino-3,4,5,6-tetrahydropyridine-5-carboxylic Acid. A mixture of 6-aminonicotinic acid (5 g, 36.2 mmol), 90% ethanol, and concentrated HCl was hydrogenated over PtO_2. Filtration and evaporation was followed by recrystallization from ethanol–diethyl ether to give white crystals (5.8 g, 89.7%) of 2-amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid as the hydrochloride salt: mp 261–263 °C; ^1H NMR (CD_3OD) 2.00 (m, 1H), 2.15 (m, 1H), 2.70 (t, 2H), 2.94 (m, 1H), 3.55 (m, 2H).

2-Amino-5-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine (4a). 2-Amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid hydrochloride (3.0 g, 16.8 mmol) was esterified utilizing a method similar to that used for **2a**. Recrystallization of the crude product from methanol–diethyl ether yielded white crystals (3.08 g, 95.2%) of 2-amino-5-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine as the hydrochloride salt: mp 181–183 °C; ^1H NMR (D_2O) 1.76 (m, 1H), 1.94 (m, 1H), 2.47 (t, 2H), 2.72 (m, 1H), 3.35 (m, 2H), 3.52 (s, 3H); MS *m/z* 193.6 (MH^+). Anal. ($C_7H_{12}N_2O_2$·HCl) C, H, N.

2-Amino-5-(ethoxycarbonyl)-3,4,5,6-tetrahydropyridine Hydrochloride (4b). 2-Amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid hydrochloride (0.5 g, 2.80 mmol) was esterified in absolute ethanol (40 mL) by a method similar to that used for **2a**. The crude product was recrystallized from ethanol–diethyl ether to give white crystals (0.48 g, 82.5%) of 2-amino-5-(ethoxycarbonyl)-3,4,5,6-tetrahydropyridine as the hydrochloride salt: mp 175–177 °C; ^1H NMR (CD_3OD) 1.1 (t,

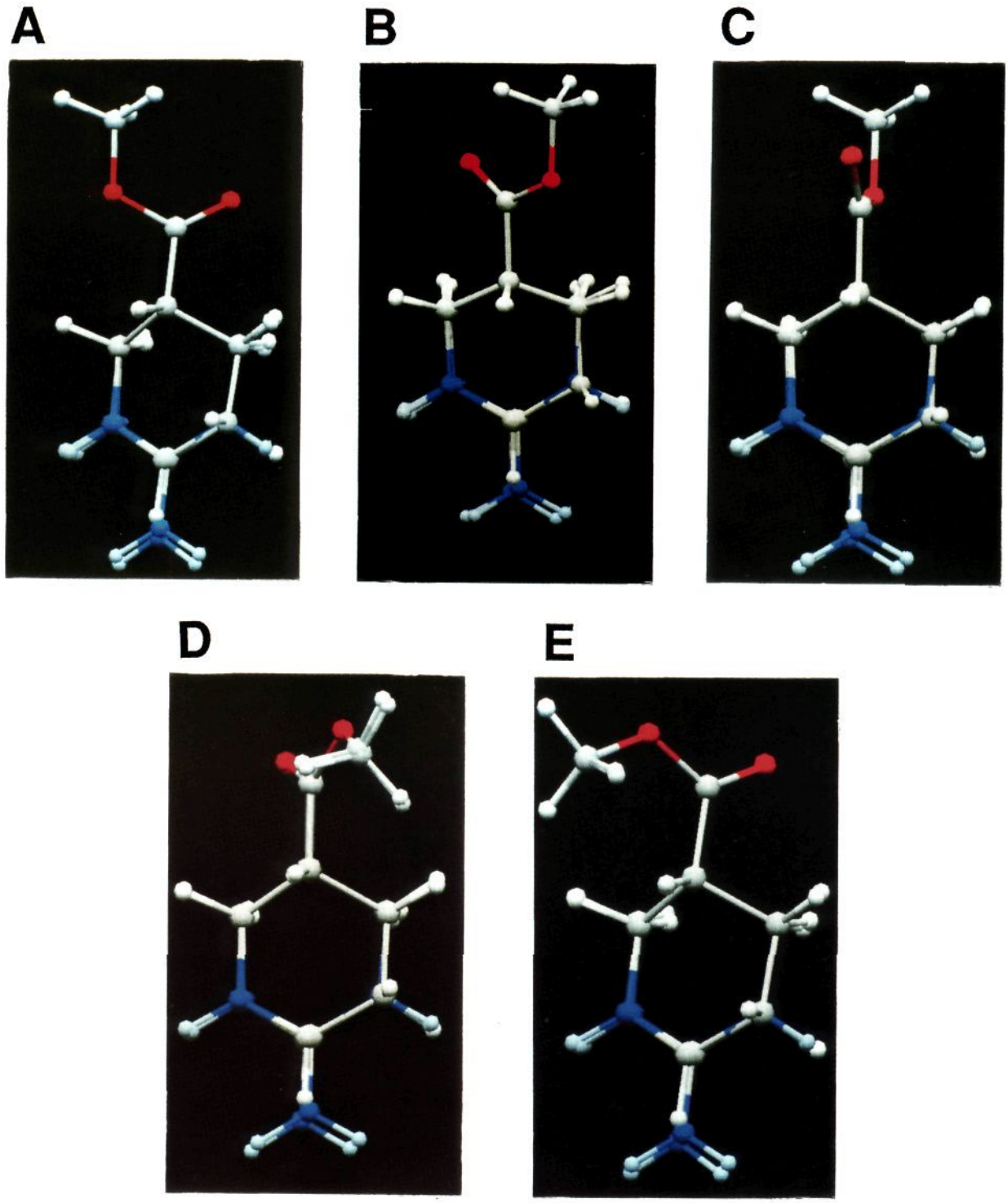


Figure 3. Superimposition of the five low-energy conformations for **1a**, (*R*)-**4a**, and **7a**. (A) Superimposition of conformation iii of **1a** with conformation ii of (*R*)-**4a** and conformation iii of **7a**. (B) Superimposition of conformations ii of **1a** and **7a** with conformation iii of (*R*)-**4a**. (C) Superimposition of the lowest energy conformations (i) of **1a**, (*R*)-**4a**, and **7a**. (D) Superimposition of conformations iv of **1a** and (*R*)-**4a** with conformation v of **7a**. (E) Superimposition of conformations v of **1a** and (*R*)-**4a**, with conformation iv of **7a**.

3H), 2.0 (m, 2H), 2.6 (d, 2H), 2.8 (s, 1H), 3.5 (d, 2H), 4.0 (q, 2H); MS *m/z* 171.2 (MH^+). Anal. ($C_8H_{14}N_2O_2$·HCl) C, H, N.

2-Amino-5-(propyloxycarbonyl)-3,4,5,6-tetrahydropyridine Hydrochloride (4c). 2-Amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid hydrochloride (0.4 g, 2.23 mmol) was esterified in normal propanol (40 mL) by a method similar to that utilized for **2a**. Recrystallization of the crude residue from propanol–diethyl ether yielded white crystals (0.4 g, 81.8%) of 2-amino-5-(propyloxycarbonyl)-3,4,5,6-tetrahydropyridine as the hydrochloride salt: mp 173–175 °C; ^1H NMR (CD_3OD) 0.9 (t, 3H), 1.5 (m, 2H), 2.0 (m, 2H), 2.6 (d, 2H), 2.9 (s, 1H), 3.5 (d, 2H), 4.0 (5, 2H); MS *m/z* 185.2 (MH^+). Anal. ($C_9H_{16}N_2O_2$·HCl) C, H, N.

2-Amino-5-(propargyloxycarbonyl)-3,4,5,6-tetrahydropyridine Hydrochloride (4d). 2-Amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid hydrochloride (0.5 g, 2.79 mmol) was suspended in a solution of oxalyl chloride (10 mL, 114 mmol) in benzene (25 mL), heated under reflux for 2.5 h, and then evaporated to dryness *in vacuo* to give a pale green residue of the acid chloride (0.65 g). A mixture of the acid chloride (0.6

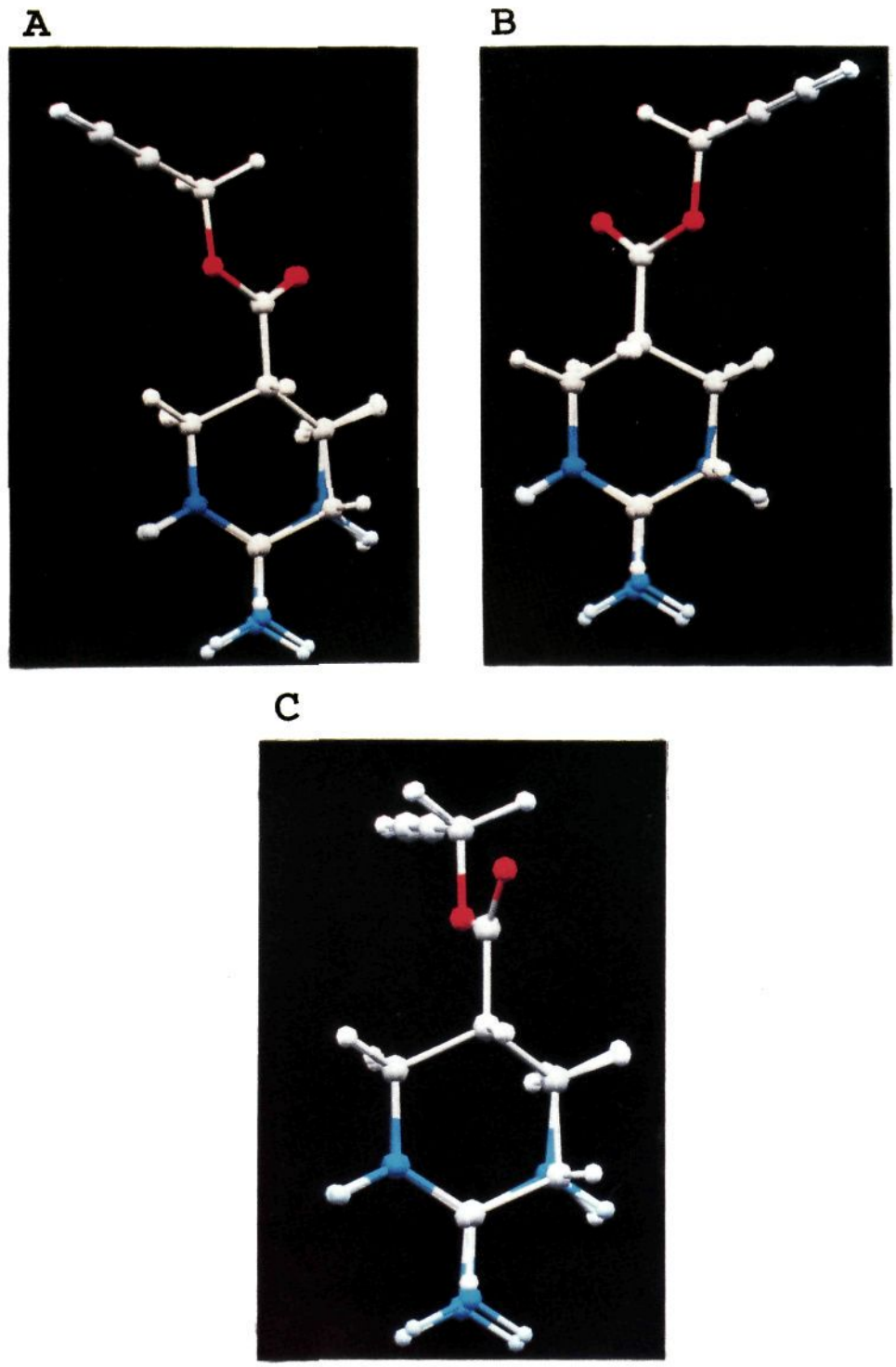


Figure 4. Superimposition of the three low-energy conformations for **1d**, (*R*)-**4d**, and **7d**. (A) Superimposition of conformations ii of **1d** and **7d** with conformation i of (*R*)-**4d**. (B) Superimposition of conformations iii of **1d** and **7d** with conformation ii of (*R*)-**4d**. (C) Superimposition of the lowest energy conformations (i) of **1d** and **7d** with conformation iii of (*R*)-**4d**.

g, 3.28 mmol) and propargyl alcohol (15 mL, 258 mmol) was stirred at room temperature overnight and then evaporated *in vacuo* to give a greenish residue. The residue was suspended in water (50 mL), stirred for 2 h, and filtered. The filtrate was evaporated to dryness under reduced pressure to give a brown gummy residue of the crude product. Recrystallization of the crude residue from methanol–diethyl ether yielded pale green crystals (0.117 g, 19.3%) of 2-amino-5-(propargyloxycarbonyl)-3,4,5,6-tetrahydropyridine as the hydrochloride salt: mp 121–123 °C; ^{1}H NMR (D_2O) 1.1 (t, 1H), 2.0 (m, 2H), 2.5 (t, 2H), 2.9 (m, 1H), 3.4 (m, 2H), 4.0 (s, 2H); MS *m/z* 182.1 (MH^+). Anal. ($C_9H_{12}N_2O_2$·HCl) C, H, N.

2-Amino-3,4,5,6-tetrahydropyridine-6-carboxylic Acid Hydrochloride. 2-Aminopyridine-6-carboxylic acid[14] (2.01 g, 14.5 mmol) was hydrogenated over PtO_2. Filtration and evaporation gave 2.08 g (80%) of crude white crystals identified as the product: ^{1}H NMR (D_2O): 1.8 (m, 3H), 2.2 (m, 1H), 2.6 (t, 2H), 4.1 (t, 1H); IR 1724 cm^{-1}.

2-Amino-6-(methoxycarbonyl)-3,4,5,6-tetrahydropyridine Hydrochloride (5a). 2-Amino-3,4,5,6-tetrahydropyri-

dine-6-carboxylic acid hydrochloride (1.99 g, 11.1 mmol) was esterified using a method similar to that employed for **2a**. The resulting crude white solid was recrystallized from ethanol to give 656 mg (31%) of white crystals: mp 132–134 °C; ^{1}H NMR (D_2O) 1.8 (m, 2H), 2.0 (m, 1H), 2.2 (m, 1H), 2.7 (t, 2H), 3.8 (s, 3H), 4.4 (t, 1H); IR 1753 cm^{-1}. Anal. ($C_7H_{12}N_2O_2$·HCl) C, H, N.

2-Amino-1,4,5,6-tetrahydropyrimidine-4-carboxylic Acid Hydrochloride. A mixture of 2-amino-5-chloropyrimidine-4-carboxylic acid (0.75 g, 4.35 mmol), concentrated HCl (1.0 g, 9.86 mmol), and 10% palladium-on-carbon (1.0 g, 0.94 mmol) in 50 mL of H_2O was shaken at room temperature under an atmosphere of hydrogen (29 psig). After the theoretical amount of hydrogen was absorbed (4 h), the catalyst was removed by filtration, the filtrate was evaporated to dryness, and the residue was recrystallized from methanol–diethyl ether to yield white crystals (0.65 g, 80.5%) of 2-amino-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid as the hydrochloride salt: mp 199–200 °C; ^{1}H NMR (D_2O) 2.18 (m, 2H), 3.37 (m, 2H), 4.37 (t, 1H).

2-Amino-4-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine Hydrochloride (6a). 2-Amino-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid hydrochloride (0.65 g, 3.50 mmol) was esterified in anhydrous methanol (50 mL) by a method similar to that used for **2a**. The yellow residue was recrystallized from methanol–tetrahydrofuran to give white needles (155 mg; 23%) of 2-amino-4-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine as the hydrochloride salt: mp 106–108 °C; ^{1}H NMR (D_2O) 2.02 (m, 2H), 3.2 (m, 2H), 3.62 (s, 3H), 4.18 (m, 1H). Anal. ($C_6H_{11}N_3O_2$·HCl) C, H, N.

2-Amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic Acid. A mixture of 2-aminopyrimidine-5-carboxylic acid[10] (2.19 g, 15.76 mmol) and aqueous HCl was hydrogenated over 10% palladium-on-carbon. The residue was recrystallized from ethanol–diethyl ether to yield white crystals (3.1 g, 95%) of 2-amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid as the hydrochloride salt: mp 190–192 °C; ^{1}H NMR (D_2O) 3.01 (m, 1H), 3.40 (d, 4H). Anal. ($C_5H_9N_3O_2$·HCl) C, H, N.

2-Amino-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine Hydrochloride (7a). 2-Amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid hydrochloride (1.3 g, 7.40 mmol) was esterified in anhydrous methanol (70 mL) employing a method similar to that used for **2a**. The crude product was recrystallized from methanol–diethyl ether to yield white crystals (1.47 g, 81.2%) of 2-amino-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine as the hydrochloride salt: mp 167–168 °C; ^{1}H NMR (CD_3OD) 3.09 (m, 1H), 3.45 (d, 4H), 3.65 (s, 3H). Anal. ($C_6H_{11}N_3O_2$·HCl) C, H, N.

2-Amino-5-(ethoxycarbonyl)-1,4,5,6-tetrahydropyrimidine Hydrochloride (7b). 2-Amino-5-(methoxycarbonyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (0.2 g, 1.03 mmol) was esterified in absolute ethanol (100 mL) by a method similar to that employed to synthesize **2a**. The crude product was recrystallized from ethanol–diethyl ether to give white crystals (0.175 g, 82.1%) of 2-amino-5-(ethoxycarbonyl)-1,4,5,6-tetrahydropyrimidine as the hydrochloride salt: mp 154–155 °C; ^{1}H NMR (CD_3OD) 1.2 (t, 3H), 3.0 (m, 1H), 3.5 (d, 4H), 4.1 (q, 2H); MS *m/z* 172.1 (MH^+). Anal. ($C_7H_{13}N_3O_2$·HCl) C, H, N.

2-Amino-5-(propyloxycarbonyl)-1,4,5,6-tetrahydropyrimidine Hydrochloride (7c). 2-Amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid hydrochloride (0.2 g, 1.11 mmol) was esterified in normal propanol (70 mL) employing a method similar to that used for **2a**. Recrystallization of the crude product from propanol–diethyl ether yielded white crystals (0.150 g, 75.2%) of 2-amino-5-(propyloxycarbonyl)-1,4,5,6-tetrahydropyrimidine as the hydrochloride salt: mp 149–150 °C; ^{1}H NMR (CD_3OD) 0.9 (t, 3H), 1.5 (m, 2H), 3.0 (m, 1H), 3.5 (d, 4H), 4.0 (t, 2H); MS *m/z* 186.2 (MH^+). Anal. ($C_8H_{15}N_3O_2$·HCl) C, H, N.

2-Amino-5-(propargyloxycarbonyl)-1,4,5,6-tetrahydropyrimidine Hydrochloride (7d). 2-Amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid hydrochloride (0.4 g, 2.23 mmol) was suspended in a solution of oxalyl chloride (10 mL, 114 mmol) in benzene (25 mL), heated under reflux for 2.5 h, and then evaporated to dryness *in vacuo* to give a yellow residue of the acid chloride (0.53 g). A mixture of the acid chloride (0.5 g, 2.52 mmol) and propargyl alcohol (15 mL, 258 mmol) was stirred at room temperature overnight and then evaporated *in vacuo* to give a pale brown residue. The residue was suspended in water (50 mL), stirred for 2 h, and filtered. The filtrate was evaporated to dryness *in vacuo* to give a yellow oily residue of the crude product. Recrystallization of the crude product from ethanol–diethyl ether yielded crystals (0.265 g, 53.2%) of 2-amino-5-(propargyloxycarbonyl)-1,4,5,6-tetrahydropyrimidine as the hydrochloride salt: mp 126–127 °C; ^{1}H NMR (D_2O) 1.0 (t, 1H), 2.9 (m, 1H), 3.5 (d, 4H), 4.0 (s, 2H). Anal. ($C_8H_{11}N_3O_2$·HCl) C, H, N.

2-Amino-5-(Isopropyloxycarbonyl)-1,4,5,6-tetrahydropyrimidine Hydrochloride (7e). 2-Amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid hydrochloride (0.25 g, 1.39 mmol) was esterified in 2-propanol (80 mL) by a method similar to that used for **2a**. The crude product was recrystallized from 2-propanol–diethyl ether to give pale yellow crystals (0.0095 g, 34.2%) of 2-amino-5-(isopropyloxycarbonyl)-1,4,5,6-tetrahydropyrimidine as the hydrochloride salt: mp 145–146 °C; ^{1}H NMR (CD_3OD) 1.2 (d, 6H), 3.09 (m, 1H), 3.5 (d, 4H), 4.0 (s, 1H). Anal. ($C_8H_{15}N_3O_2$·HCl) C, H, N.

Computational Chemistry. Molecular modeling was performed utilizing molecular mechanics with charge scaling in order to account for the effects of the electrostatic environment. This involved the generation and minimization of structures using MacroModel 3.5x[15] on an HP 720 or Personal Iris 4D/35 and the calculation of MNDO electrostatic potential (ESP) charges[16] using MOPAC 6.0[17] on a DEC Alpha 3000/500. Structures were drawn using MacroModel and minimized using the AMBER united atom force field.[18,19] Conformational searching in torsional space was performed using the multiconformer method;[20] the angle between the ester and tetrahydropyrimidine rings was varied in 30° increments while the freely rotatable torsional angles for the exocyclic substituents also were varied in 30° increments. Duplicate structures, based on the RMS deviation of heavy atoms, were eliminated as were structures not within 50 kJ of the lowest energy minimum found. The remaining structures were minimized first by the Polak-Ribiere conjugate gradient method, then the full matrix Newton-Raphson method, to a final gradient of less than 0.01 kJ/(mol/Å). Conformations thus obtained were confirmed as minima by calculating the second derivative of the force matrix and checking for the absence of negative Eigenvalues.

Charge scaling was performed on the minimum-energy conformations by converting the MacroModel files to MOPAC format then performing a single point ESP calculation using the MNDO parameters and Hamiltonian.[21] Charges for neutral species were scaled by a factor of 0.9 while calculated charges for protonated species were used directly. These charges were imported into MacroModel, and the minimization procedure described above was performed. This sequence of operations, molecular mechanics minimization using ESP atomic charges in place of the default force field charges, was repeated until both the charges and the atomic positions converged. Compounds were ranked in order of increasing energy and superimposed using a least-squares superimposition in MacroModel.

Receptor Binding. Binding to muscarinic receptors was carried out essentially as described previously.[8,22] Binding was determined indirectly by the ability of compounds to compete with 50 pM [^{3}H]-(*R*)-quinuclidinyl benzilate ([^{3}H]-QNB) in a suspension of brain membranes. Each sample contained approximately 10 pM receptors (or 4 mg/mL of protein) in 40 mM sodium/potassium phosphate buffer (pH 7.4) and varying concentrations of each compound in a final volume of 10 mL. Samples were incubated for 2.0 h at room temperature and then filtered through glass fiber filters using a Brandell cell harvester adapted for receptor binding work. The filters were washed twice with two 5-mL portions of cold buffer. 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[23] as reported previously.[8,24,25] Compounds were screened for activity at 100 μM in rat cortical slices. Rats were sacrificed by cervical dislocation, and their brains were removed and placed in Krebs-Hensleit buffer (KHB) equilibrated previously with 95% O_2/5% CO_2 to a final pH of 7.4 at room temperature. The rat cerebral cortex was dissected according to the method of Glowinski and Iversen[26] and cross-chopped at 300 μm using a McIlwain tissue chopper. The tissue slices from one rat were resuspended in 40 mL of KHB and incubated at 37 °C in a shaking water bath for 45 min. The tissue was washed three times in this manner. At the end of the incubation, the slices were centrifuged at 300*g* for 15 s at room temperature.

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. Aliquots of tissue slices were added to 0.3 mM [^{3}H]inositol (15 Ci/mmol) and 10 mM LiCl in KHB. Vials then were incubated at 37 °C in a shaking water bath for 30 min to label inositol phosphates. Agonist (or buffer for the determination of basal levels) then was added, and slices were incubated for an additional 45 min. The incubations were stopped by the addition of $CHCl_3$/CH_3OH (1:2) followed by $CHCl_3$ and H_2O (1:1). The samples were mixed and spun at 1000*g* for 10 min to separate the phases. Aliquots of the upper phase were removed for determination of [^{3}H]inositol phosphates.

The amount of [^{3}H]inositol phosphates formed in the assay was determined essentially according to Wreggett and Irvine[27] except that the separation of inositol phosphates was accomplished using an Amersham Super Separator Manifold. Total labeled inositol phosphates were determined by the "batch" method, in which aliquots of the aqueous phase were diluted with 2.25 mL of distilled H_2O. The entire amount (3 mL) was loaded onto ACCELL QMA anion-exchange SEP-PAK cartridges, previously converted into the formate form by washing with 10 mL of 1.0 M ammonium formate in 0.1 M formic acid, followed by two 10-mL washes with distilled H_2O. The loaded cartridges then were washed with 10 mL of distilled water followed by 10 mL of 5 mM disodium tetraborate. [^{3}H]Inositol phosphates were eluted with 0.6 M ammonium formate/60 mM formic acid/5 mM disodium tetraborate (pH 4.75), and the eluate was counted in 5 mL of Liquiscint scintillation cocktail.

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