Identification and Characterization of m1 Selective Muscarinic Receptor Antagonists[1]

Corinne E. Augelli-Szafran,*,† C. John Blankley,† Juan C. Jaen,† David W. Moreland,† Carrie B. Nelson,‡ Jan R. Penvose-Yi,† Roy D. Schwarz,‡ and Anthony J. Thomas†

Departments of Medicinal Chemistry and Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

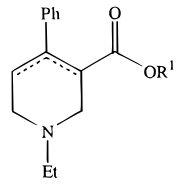
Received January 28, 1998

A series of esters of 1,4-disubstituted tetrahydropyridine carboxylic acids (**I**) has been synthesized and characterized as potential m1 selective muscarinic receptor antagonists. The affinity of these compounds for the five human muscarinic receptor subtypes (Hm1−Hm5) was determined by the displacement of [^{3}H]-NMS binding using membranes from transfected Chinese hamster ovarian cells. One of the most potent and selective compounds of this series is an analogue of **I** [**11**, $R^1 = (CH_2)_5CH_3$], which has an IC_{50} value of 27.3 nM at the m1 receptor and possesses 100-fold (m2), 48-fold (m3), 74-fold (m4), and 19-fold (m5) selectivities at the other receptors. Thus, this analogue appears to be more selective on the basis of binding than the prototypical m1 antagonist, pirenzepine. Functional data, such as the inhibition of carbachol-stimulated phosphatidylinositol hydrolysis, on selected analogues confirmed the muscarinic antagonistic properties of this chemical series.

Introduction

Muscarinic receptors are widely distributed both centrally and peripherally. These receptors play an important role in cognitive function, in the central control of movement, in the peripheral control of gastrointestinal functions, and in bronchodilation. Three muscarinic receptor subtypes (M_1, M_2, and M_3) were initially characterized pharmacologically by the use of subtype selective antagonists.[2] More recently, five distinct muscarinic receptor subtypes (m1−m5) have been characterized at the molecular level.[3] Therefore, it is now possible to specifically target the blockade of one muscarinic receptor subtype. The advantage of a selective antagonist for one muscarinic receptor would be the elimination of the potential side effects that are usually present with nonselective antagonists. Possible therapeutic targets for selective muscarinic antagonists include an m1 antagonist for the inhibition of gastric acid secretion in the treatment of peptic ulcers,[4] an m2 antagonist as an antibradycardiac drug,[5] an m3 agent as a bronchodilator for pulminary obstructive disorder and asthma,[6] an m2 and/or m3 antagonist for urinary incontinence,[7] and an m4 antagonist to combat Parkinsonian movement disorders.[8] Also, an m1 antagonist could be used as a pharmacological tool and aid in the development of selective m1 agonists which, in turn, may be useful in the treatment of Alzheimer's disease. Alzheimer's disease is characterized by the progressive degeneration of cholinergic neurons that project from the basal forebrain to the cerebral cortex and hippocampus.[9] Cholinomimetic therapy is currently viewed as the most promising short-term symptomatic treatment for the disease. The receptors responsible for the central cognition-enhancing effects of muscarinic cholinomimetic agents are generally defined as m1. The activation of the peripheral m2 and m3 receptors is thought to be responsible for the side effects, e.g., sweating, diarrhea, cramps, excessive salivation, of the currently available muscarinic agents. Thus, m1 selective muscarinic receptor agonists hold the best promise for selective improvement of cognitive function without the occurrence of side effects. Therefore, the development of a truly potent and m1 selective muscarinic receptor antagonist may be the optimal tool needed for the development of such a therapeutic agent.

From this series of tetrahydropyridines, several compounds have been identified as m1 selective muscarinic receptor antagonists and some appear more selective than pirenzepine, a known m1 selective muscarinic receptor antagonist. This structure−activity relationship (SAR) study examines the ester functionality of **I**.



(I)

Based on chemical leads from mass screening, it appeared that both the 4-phenyl and the *N*-ethyl groups of **I** were necessary for m1 muscarinic receptor potency and selectivity. Therefore, this study focuses on varying R^1.

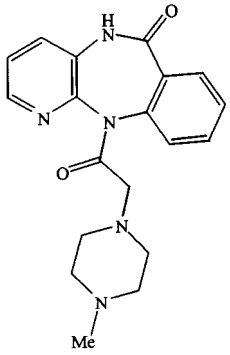
Chemistry

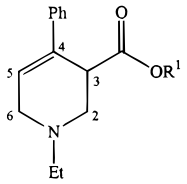
All examples shown in Table 1 were prepared by the synthetic route illustrated in Scheme 1. Treatment of the protected nicotinic acid derivative, 3-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)pyridine[10] (**II**), with phenyllithium yielded the 1,4-dihydrophenylpyridine intermediate **III**.[11] Aromatization of **III** in the presence of sulfur yielded pyridine **IV**.[11] Hydrolysis of the oxazoline ring of **IV** with hydrochloric acid gave carboxylic acid **V**.[11]

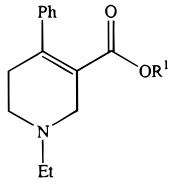
† Department of Medicinal Chemistry.
‡ Department of Neuroscience Therapeutics.

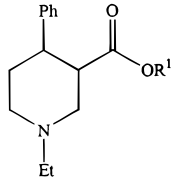
10.1021/jm980067l CCC: $18.00
Published on Web 01/26/1999

Table 1. Binding Results of 1,4-Disubstituted Tetrahydropyridines





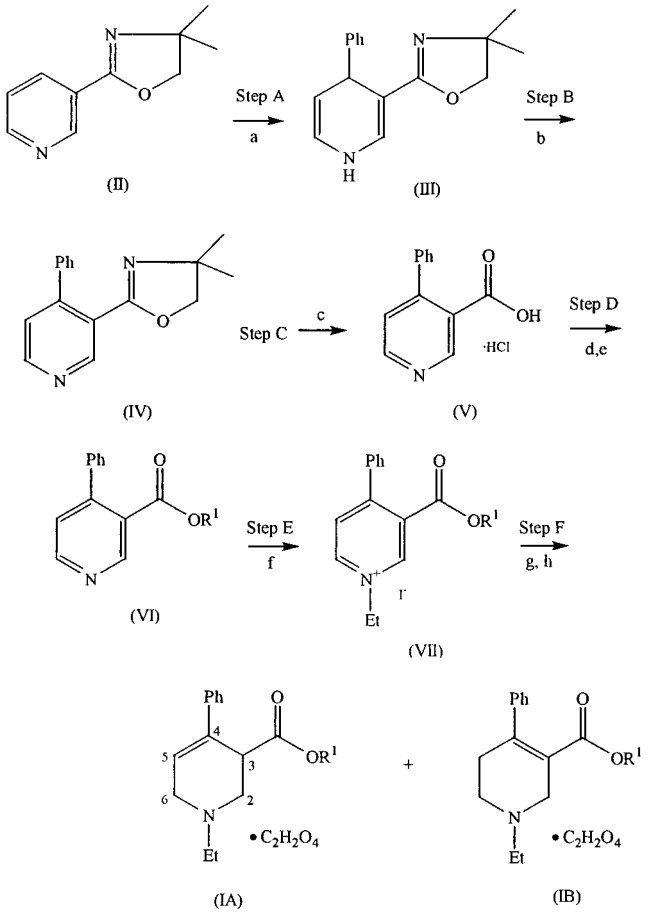




example	R^1	I[a]	IC_{50} (nM)[b] Hm1	Hm2	Hm3	Hm4	Hm5	formula[c]	mp (°C)
pirenzepine			28.2	968.2	955.6	246.8	340.6	$C_{19}H_{21}N_5O_2$	
1	CH_3	A	33117.6	28264.5	88401.3	13042.1	16901.9	$C_{15}H_{19}NO_2{\cdot}C_2H_2O_4$	151−154
2	CH_3	B	2308.4	1727.9	5523.7	13691.8	977.3	$C_{15}H_{19}NO_2{\cdot}C_2H_2O_4$	159−162
3	CH_2CH_3	A	2220.1	2763.7	6874.7	10383.0	4169.5	$C_{16}H_{21}NO_2{\cdot}C_2H_2O_4{\cdot}0.15H_2O$	162−166
4	CH_2CH_3	B	888.1	394.8	1689.3	3226.4	1331.2	$C_{16}H_{21}NO_2{\cdot}C_2H_2O_4{\cdot}0.15H_2O$	130−135
5	$(CH_2)_2CH_3$	A	291.2	1668.5	2312.2	3788.8	1127.9	$C_{17}H_{23}NO_2{\cdot}C_2H_2O_4{\cdot}1.1C_2H_2O_4$	136−142
6	$(CH_2)_2CH_3$	B	278.0	542.3	684.0	1409.3	602.9	$C_{17}H_{23}NO_2{\cdot}C_2H_2O_4$	135−139
7	$(CH_2)_3CH_3$	A	76.8	470.7	290.0	656.3	264.7	$C_{18}H_{25}NO_2{\cdot}C_2H_2O_4$[d]	125−129
8	$(CH_2)_3CH_3$	B	67.9	1865.1	535.5	524.0	254.9	$C_{18}H_{25}NO_2{\cdot}C_2H_2O_4$[e]	131−133
9	$(CH_2)_4CH_3$	A	84.8	1500.6	918.0	701.0	374.0	$C_{19}H_{27}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	130−132
10	$(CH_2)_4CH_3$	B	63.7	600.7	356.9	432.4	341.9	$C_{19}H_{27}NO_2{\cdot}C_2H_2O_4$	110−112
11	$(CH_2)_5CH_3$	A	27.3	2734.2	1308.0	2030.0	508.2	$C_{20}H_{29}NO_2{\cdot}C_2H_2O_4{\cdot}0.76H_2O$	122−124
12	$(CH_2)_5CH_3$	B	35.0	227.0	349.0	367.0	138.9	$C_{20}H_{29}NO_2{\cdot}C_2H_2O_4$	59−61
13	$(CH_2)_6CH_3$	A	161.4	748.5	1095.5	2196.7	537.3	$C_{21}H_{31}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	126−128
14	$(CH_2)_6CH_3$	B	78.0	73.3	162.5	617.1	194.2	$C_{21}H_{31}NO_2{\cdot}C_2H_2O_4{\cdot}0.45H_2O$	98−100
15	$(CH_2)_7CH_3$	A	77.3	773.5	675.6	1250.0	400.6	$C_{22}H_{33}NO_2{\cdot}C_2H_2O_4{\cdot}1.1C_2H_2O_4$	*f*
16	$(CH_2)_7CH_3$	B	22.0	52.5	86.5	258.8	57.9	$C_{22}H_{33}NO_2{\cdot}C_2H_2O_4{\cdot}1.8C_2H_2O_4$	*f*
17	$(CH_2)_8CH_3$	A	30.4	486.1	1600.8	1577.9	328.6	$C_{23}H_{35}NO_2{\cdot}0.5EtOAc$[g]	*h*
18	$(CH_2)_8CH_3$	B	11.5	22.2	201.6	282.3	85.4	$C_{23}H_{35}NO_2{\cdot}0.25EtOAc$[g]	*h*
19	$(CH_2)_9CH_3$	A	484.2	1174.8	983.0	2123.9	1114.8	$C_{24}H_{37}NO_2{\cdot}0.75EtOAc$[g]	*h*
20	$(CH_2)_9CH_3$	B	106.1	20.5	399.5	566.2	235.6	$C_{24}H_{37}NO_2{\cdot}0.31EtOAc$[g]	*h*
21	$CH_2CH(CH_3)_2$	A	128.0	1248.2	844.6	896.9	558.9	$C_{18}H_{25}NO_2{\cdot}C_2H_2O_4{\cdot}1.50H_2O{\cdot}0.02Et_2O$	*f*
22	$CH_2CH(CH_3)_2$	B	120.5	427.5	257.5	742.6	407.3	$C_{18}H_{25}NO_2{\cdot}C_2H_2O_4{\cdot}0.35H_2O{\cdot}0.01Et_2O$	140−142
23	Ph	A	19027.3	14598.2	16753.5	31125.4	31057.6	$C_{20}H_{21}NO_2{\cdot}C_2H_2O_4$[i]	127−130
24	Ph	B	1819.5	954.3	4069.7	8428.6	1568.6	$C_{20}H_{21}NO_2{\cdot}C_2H_2O_4$[j]	185−188
25	CH_2Ph	A	13.4	241.3	403.6	146.3	42.5	$C_{21}H_{23}NO_2{\cdot}HCl$[k,l]	177.5−179
26	CH_2Ph	B	65.4	450.6	211.3	472.8	272.4	$C_{21}H_{23}NO_2{\cdot}HCl$[k]	123−124
27	$(CH_2)_2Ph$	A	233.6	1480.7	1653.8	1740.6	330.9	$C_{22}H_{25}NO_2{\cdot}C_2H_2O_4{\cdot}0.3H_2O$	95−98
28	$(CH_2)_2Ph$	B	9.6	243.6	100.7	69.7	78.1	$C_{22}H_{25}NO_2{\cdot}C_2H_2O_4$	134−135.5
29	$(CH_2)_2Ph$-*p*-CH_3	A	93.5	956.4	1491.0	1756.1	190.3	$C_{23}H_{27}NO_2{\cdot}1.1C_2H_2O_4$	100−103
30	$(CH_2)_2Ph$-*p*-CH_3	B	8.8	136.0	88.3	138.0	24.8	$C_{23}H_{27}NO_2{\cdot}1.1C_2H_2O_4$	141−143
31	$(CH_2)_2Ph$-*p*-OCH_3	A	159.0	734.6	1218.7	2224.5	499.7	$C_{23}H_{27}NO_3{\cdot}1.2C_2H_2O_4$	75−77
32	$(CH_2)_2Ph$-*p*-OCH_3	B	1941.0	38484.0	52495.5	42312.8	865.9	$C_{23}H_{27}NO_3{\cdot}0.55C_2H_2O_4$	*h*
33	$CH_2CH(Ph)_2$	A	4024.4	475.0	871.1	4162.1	3565.8	$C_{28}H_{29}NO_2{\cdot}C_2H_2O_4$[m]	74−80
34	$CH_2CH(Ph)_2$	B	2829.7	2573.4	5051.5	7708.4	3108.6	$C_{28}H_{29}NO_2{\cdot}C_2H_2O_4$[n]	*h*
35	$(CH_2)_3Ph$	A	59.2	2213.8	1244.3	1132.1	255.8	$C_{23}H_{27}NO_2{\cdot}HCl$[k]	158.5−159.5
36	$(CH_2)_3Ph$	B	122.0	1035.2	749.3	1160.8	479.4	$C_{23}H_{27}NO_2{\cdot}HCl$[k]	112.5−114
37	$(CH_2)_4Ph$	A	743.1	3323.1	14309.6	7422.0	5000.0	$C_{24}H_{29}NO_2{\cdot}C_2H_2O_4$[o]	230 (*d*)
38	$(CH_2)_4Ph$	B	69.4	2162.1	1842.0	2775.5	842.0	$C_{24}H_{29}NO_2{\cdot}C_2H_2O_4{\cdot}0.9H_2O$	116−118
39	$(CH_2)_5Ph$	A	670.0	661.1	2046.8	2388.2	1567.9	$C_{25}H_{31}NO_2{\cdot}C_2H_2O_4$	99−101
40	$(CH_2)_5Ph$	B	316.7	120.7	447.9	951.2	472.2	$C_{25}H_{31}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	127−129
41	C_6H_{11}	A	16.9	273.5	299.3	108.7	63.0	$C_{20}H_{27}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	118−120
42	C_6H_{11}	B	18.1	255.2	40.8	62.0	54.1	$C_{20}H_{27}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	91−93
43	$CH_2C_6H_{11}$	A	54.2	666.3	109.6	167.6	214.5	$C_{21}H_{29}NO_2{\cdot}C_2H_2O_4{\cdot}1.2C_2H_2O_4$	110−112
44	$CH_2C_6H_{11}$	B	14.5	564.9	155.8	142.7	115.4	$C_{21}H_{29}NO_2{\cdot}1.6C_2H_2O_4$[p]	94−97
45	$(CH_2)_2C_6H_{11}$	A	105.2	1944.1	529.6	836.1	159.2	$C_{24}H_{33}NO_2{\cdot}C_2H_2O_4{\cdot}0.15H_2O$	138−142
46	$(CH_2)_2C_6H_{11}$	B	38.7	238.2	114.7	134.7	56.2	$C_{24}H_{33}NO_2{\cdot}C_2H_2O_4{\cdot}0.15H_2O$	93−98
47	$(CH_2)_3C_6H_{11}$	A	963.2	10943.7	24861.7	19581.2	10969.8	$C_{23}H_{33}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	85−88
48	$(CH_2)_3C_6H_{11}$	B	610.6	468.0	2801.3	1167.2	1260.5	$C_{23}H_{33}NO_2{\cdot}C_2H_2O_4{\cdot}0.26H_2O$	139−141
49	$(CH_2)_2Ph$	C[q] (*trans*)	62.4	357.1	315.4	145.4	96.8	$C_{22}H_{27}NO_2{\cdot}C_2H_2O_4$[r]	112−114
50	$(CH_2)_2Ph$	C[q] (*cis*)	3285.2	4496.8	3175.4	8122.8	4264.0	$C_{22}H_{27}NO_2{\cdot}C_2H_2O_4$[s]	78−81
51	C_6H_{11}	C[q] (*trans*)	913.1	4100.3	4514.2	7900.0	2971.4	$C_{20}H_{29}NO_2{\cdot}C_2H_2O_4$[t]	*f*
52	C_6H_{11}	C[q] (*cis*)	1141.1	1611.2	2551.1	4246.3	1889.3	$C_{20}H_{29}NO_2{\cdot}C_2H_2O_4$[u]	116−118
53[v]	$(CH_2)_5CH_3$	C[q] (*cis*)	61.4	405.0	220.3	572.7	222.1	$C_{20}H_{31}NO_2{\cdot}C_2H_2O_4$[w]	*h*

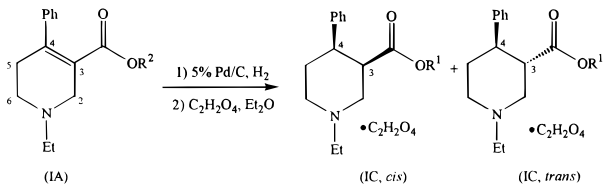
[a] Letters A, B, and C indicate the location of the double bond (i.e., **IA**: 1,2,3,6-tetrahydropyridine analogue, **IB**: 1,2,5,6-tetrahydropyridine analogue; and **IC**: piperidine analogue). [b] The affinity of these compounds for the five human receptor subtypes (m1−m5) was determined by [^{3}H]-NMS binding using membranes from transfected CHO cells. For compounds tested more than once, the value is given as the geometric mean. The standard error of the mean (SEM) for all compounds tested ranged from 6−18%. Complete protocol is described by Dorje et al.[3a] and Buckley et al.[14] [c] All compounds include 1 mol of oxalic acid and have analytical results within ±0.4% of theoretical values unless otherwise noted. Some difficulty was found in obtaining combustion analysis in the indicated compounds due to the propensity of these compounds to retain solvents. [d] High mass calcd, 288.1964; found, 288.1953. [e] High mass calcd, 288.1964; found, 288.1961. [f] A gum. [g] Free base. [h] A liquid. [i] High mass calcd, 308.1651; found, 308.1657. [j] High mass calcd, 308.1651; found, 308.1649. [k] HCl salt. [l] High mass calcd, 322.1807; found, 322.1793. [m] High mass calcd, 412.2276; found, 412.2277. [n] High mass calcd, 412.2277; found, 412.2282. [o] High mass calcd, 364.2277; found, 364.2280. [p] High mass calcd, 328.2277; found, 328.2273. [q] Stereochemistry was determined by proton NMR analysis. [r] High mass calcd, 338.2120; found, 338.2116. [s] High mass calcd, 338.2120; found, 338.2111. [t] High mass calcd, 316.2277; found, 316.2274. [u] High mass calcd, 316.2277; found, 316.2274. [v] No *trans*-**53** was isolated due to minimal amount of sample. [w] High mass calcd, 318.2433; found, 318.2433.

Scheme 1[a]



[a] Reagents and conditions: (a) PhLi, THF, −78 °C; (b) S_8, Δ, $PhCH_3$; (c) conc HCl, D; (d) $SOCl_2$, DMF, D; (e) R^1OH, $[(CH_3)_2CH]_2NEt$, CH_2Cl_2, 0 °C to rt; (f) EtI, CH_3CN, D; (g) $NaBH_4$, MeOH/H_2O; (h) $C_2H_2O_4$, Et_2O.

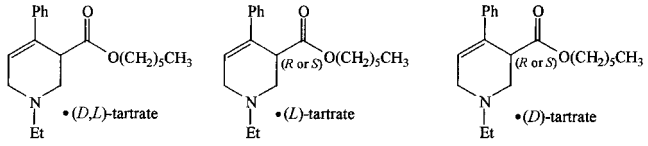
Treatment of **V** with thionyl chloride, followed by different alcohols in the presence of *N*,*N*-diisopropylethylamine, gave the corresponding pyridinyl esters **VI**.[11] Treatment of **VI** with ethyl iodide yielded quaternary salt **VII**. Reduction of **VII** with sodium borohydride afforded 4-substituted tetrahydropyridines **IA** and **IB**, which are separable by chromatography. Catalytic hydrogenation (5% Pd/C, H_2) of 1,2,5,6-tetrahydropyridines (IA) yielded the corresponding piperidines **49**−**53** (eq 1). A mixture of primarily cis (**IC**) versus trans



(1)

(**ID**) piperidine analogues were isolated and separated by chromatography (cis:trans ratio for **50** and **49**, 6:1; **52** and **51**, 7.5:1; **53**, 8:1). Decoupling experiments were done to determine the coupling constant between protons 3 and 4 of **IA**. For a selected cis analogue, the observed coupling constant was 3.5 Hz which indicated that the protons were axial−equatorial (cis) to each other. Similar experiments were done for the corresponding trans analogue(s).

Table 2. Binding Results[a] of **11A**, **11B**, and **11C** (IC_{50}, nM)

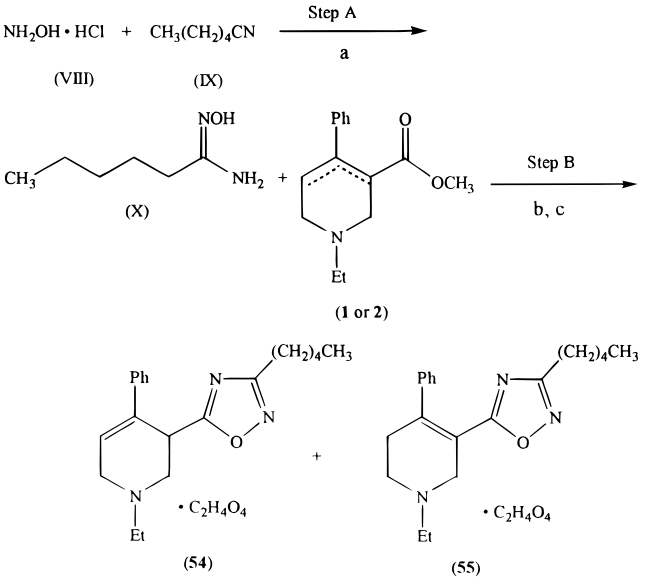


(11A) **(11B)** **(11C)**

example	Hm1	Hm2	Hm3	Hm4	Hm5
11A	30.2	1619.5	457.8	505.6	162.5
11B	21.1	1304.6	521.6	537.6	167.0
11C	40.0	1637.8	630.5	534.5	186.9

[a] Complete protocol for determining the affinity of these compounds is referenced in Table 1, footnote b.

Scheme 2[a]



(**1**) yields (1:2) ratio of (**54:55**)
(**2**) yields (1:14) ratio of (**54:55**)

[a] Reagents and conditions: (a) NEt_3, EtOH, 25 °C, 30%; (b) NaH, THF, 23%; (c) $C_2H_4O_4/Et_2O$.

Most final products were converted to the oxalate salts by treating the free base with 1 mol of oxalic acid in the presence of diethyl ether. The remaining compounds were converted to the hydrochloric salts using standard procedures.

The resolution of **11** was performed utilizing di-*p*-toluoyltartaric acid. A 95% ethanolic solution of free base **11** was added to a 95% ethanolic solution of the appropriate chiral di-*p*-toluoyltartaric acid. Fractional recrystallization yielded **11A**, **11B**, and **11C**, respectively (Table 2).

Synthesis of oxadiazole derivatives[12] (**54** and **55**) of **11** is illustrated in Scheme 2. Hydroxylhexanimidamide X[13] is prepared from hydroxylamine hydrochloride and hexanonitrile. Treatment of **1** with hydroxylhexanimidamide X and sodium hydride yielded oxadiazoles **54** and **55** in a ratio of 1:2. When **2** is treated with X under the same reaction conditions, **54** and **55** were isolated in a ratio of 1:14, favoring equilibration to **55**.

Results

The purpose of this study was to identify compounds that have better m1 selectivity than existing muscarinic antagonists, such as the m1 selective muscarinic recep-

tor antagonist, pirenzepine. The primary biological assay that was used to evaluate these compounds is described by Dorje et al.[3a] and Buckley et al.[14] The affinity of these compounds for the five human receptor subtypes (Hm1−Hm5)[3c] was determined by [^{3}H]-NMS binding using membranes from transfected Chinese hamster ovarian (CHO) cells. Activity is expressed as the micromolar concentration of compound required to displace [^{3}H]-NMS by 50% (IC_{50}). IC_{50} values are calculated using a logit equation.

In this discussion, the IC_{50} values of Hm1 versus Hm2 are routinely compared. Since there are many side effects mediated by the m2 receptors, it is desirable to eliminate affinity at the m2 receptors. This m1 versus m2 receptor selectivity would add to the attractiveness of a potent m1 antagonist. In addition to the selectivity observed for the m1 receptor versus the m2 receptor for this series of compounds, some of these compounds also exhibited selectivity for the m1 receptor versus the m3, m4, and m5 receptors as illustrated in Table 1. From the first group of compounds in Table 1 (**1**−**22**), which contains various alkyl chains, **11** [$R^1 = (CH_2)_5CH_3$; 1,2,3,6-tetrahydropyridine analogue] was identified as one of the most m1 selective muscarinic receptor antagonists known to date. Both **11** and pirenzepine are about as equally potent at the m1 receptor. However, **11** is at least 3 times more selective for m1 versus m2 and 8 times more selective for m1 versus m4 compared to pirenzepine. This is truly an advantage over this prototypical m1 antagonist. Receptor selectivities at the m3 and m5 muscarinic receptors are about equal for **11** and pirenzipine. This pronounced selectivity of m1 versus m2 and m4 for **11** was not observed for any other example shown in Table 1. As the chain length was shortened (**1**−**10**), potency and selectivity were decreased. However, when the chain length was lengthened, (**12**−**20**), potency was maintained at the m1 receptor except for C_7 (**13**, $IC_{50} = 161.4$ nM), C_8 (**15**, $IC_{50} = 77.3$ nM), and C_{10} (**19**, $IC_{50} = 484.2$ nM) derivatives. Selectivity versus the m2 receptor was either reduced or lost for all of these compounds when compared to **11.** For many of these examples (**1**−**22**), the 1,2,3,6-tetrahydropyridines (**IA**) are more selective for m1 than m2 than their corresponding 1,2,5,6-analogues (**IB**) (i.e., **11** versus **12**, respectively) but are similar in their m1 affinity.

To further study the structure−activity relationship of the ester functionality of these tetrahyropyridines, various benzyl esters were synthesized (**23**−**40**). The most m1 selective muscarinic receptor analogue from this group of compounds was **35** [$R^1 = (CH_2)_3Ph$, Hm1, $IC_{50} = 59.2$ nM, versus Hm2, $IC_{50} = 2213.8$ nM]. Longer alkyl tethers [**37** ($R^1 = (CH_2)_4Ph$) and **39** ($R^1 = (CH_2)_5$-Ph] exhibited a decrease in potency and selectivity. However, by shortening the alkyl tether (**23**−**28**), additional potent and m1 selective antagonists ([**25**, $R^1 = CH_2Ph$, Hm1, $IC_{50} = 13.4$ nM, Hm2, $IC_{50} = 241.3$ nM] and [**28**, $R^1 = (CH_2)_2Ph$, Hm1, $IC_{50} = 9.6$ nM, Hm2, $IC_{50} = 243.6$ nM]) were identified. However, selectivity for the m1 receptor versus the m2 receptor was decreased at least 4-fold when compared to **11**. Cyclohexyl esters (**41**, **42**, **44**, and **46**) were quite potent at the m1 receptor ($IC_{50} = 16.9$, 18.1, 14.5, 38.7 nM, respectively). However, none were as selective as **11**.

Table 3. Effects of Selected Compounds of **IA** and **IB** on PI Turnover in CHO Hm1 Cells

example	% basal control[a]
1	108
2	89
11	98
12	109
25	79
26	99
28	90
42	108

[a] 100 μM of each compound was used. Test run in triplicate.

Table 4. Inhibition of Carbachol Stimulation of PI Turnover in CHO Hm1 Cells by Selected Compounds of **IA** and **IB**

example	% inhibition[a]
1	0
2	40
11	100
12	99
26	100
28	89
42	97

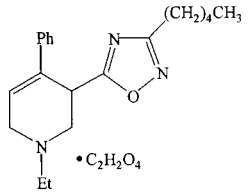
[a] 100 μM concentration, triplicate tubes.

To examine the effect of the double bond of the tetrahydropyridines on potency and selectivity, piperidines **49**−**53** were synthesized. Catalytic reduction of **27**, **41**, and **11** yielded **49** and **50**, **51** and **52**, and **53**, respectively. When comparing the affinity and the selectivity for the m1 receptor of these reduced compounds to the m1 receptor affinity and selectivity of the corresponding 1,2,3,6- and 1,2,5,6-tetrahydropyridines, the tetrahydropyridines appear to be the more potent and, generally, more m1 selective.

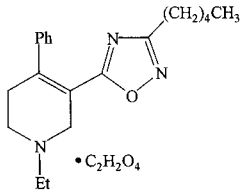
The functional activity of a selected group of compounds was determined.[15] It is known that the m1, m3, and m5 muscarinic receptors are preferentially coupled to the stimulation of phosphoinositide metabolism via phospholipase C activation whereas the m2 and m4 muscarinic receptors are negatively coupled to adenylate cyclase.[16] Therefore, compounds then can be classified as receptor agonists or antagonists based on the compounds' affinities for particular receptor subtypes. The data illustrated in Tables 3 and 4 indicate that none of the selected compounds produce any significant stimulation of phosphatidylinositol (PI) hydrolysis at high concentration (100 μM) in CHO Hm1 cells. These results indicate that none of the tested compounds are as efficacious as muscarinic agonists (Table 3). However, the blockade of carbachol-stimulated PI turnover produced by selected compounds in CHO Hm1 cells (Table 4) is convincing evidence that the preferred compounds are indeed muscarinic antagonists. For example, the weak binding (Table 4) of **1** and **2** (i.e., methyl esters) correlates well with their weak inhibition of carbachol's effects. However, **11**, **12**, **26**, **28**, and **42** are potent antagonists and inhibit the effects of the muscarinic agonist, carbachol (89−100%).

The resolution of **11** was performed with (D)- and (L)-di-*p*-toluoyltartaric acid utilizing standard conditions. Initially, **11** was treated with both the (D)- and (L)-isomers to give **11A**. This racemic salt was synthesized to determine if the tartaric acid had any effect on the muscarinic binding results versus free base **11.** In addition, **11** was treated separately with the (D)- and (L)-tartaric acids to yield enantiomers **11B** and **11C**.

Table 5. Binding Results[a] of Oxadiazole Analogues (**54** and **55**) (IC_{50}, nM)



(**54**)



(**55**)

example	Hm1	Hm2	Hm3	Hm4	Hm5
54	888.6	10788.8	6712.1	6337.5	2075.4
55	1087.7	1222.1	1927.8	8392.3	1467.3

[a] Complete protocol for determining the affinity of these compounds is referenced in Table 1, footnote b.

HPLC analysis of **11A**, **11B**, and **11C** confirmed **11A** as a racemic mixture and, within experimental error, confirmed **11B** and **11C** as the corresponding enantiomers. The binding results of (D,L)-(**11A**), (D)-(**11B**), and (L)-(**11C**) showed no significant difference in their affinity for the m1 receptor versus **11**. However, selectivity for the m1 receptor versus the m2 receptor decreased about 2-fold for each isomer (Table 2).

Muscarinic antagonists, such as scopolamine, have been shown to alter spontaneous motor activity in rodents.[17] Additionally, scopolamine has been shown to impair performance in a test of spatial working memory.[18] When **11** was tested in a mouse water maze, no behavioral activity or gross side effects were observed with **11**, following peripheral administration in rats or mice at doses up to 178 mg/kg, s.c. These preliminary in vivo studies[15] of **11** indicate that this compound may be rapidly metabolized in rodents. However, inhibition of spontaneous motor activity was observed with icv (intracerebroventricular) injection in mice (30 μg/mouse, 38% inhibition), showing an effect with this compound when delivered directly to the central nervous system.[15] A possible remedy to this speculated metabolism was to synthesize a bioisostere of the ester group of **11**. The selected bioisostere was an oxadiazole ring. Oxadiazoles **54** and **55** exhibited at least a 30-fold decrease in their affinity for the m1 receptor versus **11** and **12**, respectively (Table 5). With respect to m1 versus m2 selectivity, at least an 8-fold decrease was observed.

Discussion

A structure−activity study which examines the effects of various R^1 groups of **I** on m1 muscarinic receptor affinity and selectivity is reported. Approximately 50% of analogues **1**−**22**, which contain various alkyl chain lengths (C_1−C_{10}), are potent m1 muscarinic receptor antagonists (IC_{50} values less than 100 nM). The most selective m1 muscarinic receptor antagonist identified from this study is **11**, which also showed high affinity for the m1 muscarinic receptor (**11**, Hm1: IC_{50} = 27 nM). This compound has an m1 receptor affinity similar to that of the prototypical m1 selective muscarinic receptor antagonist, pirenzepine (IC_{50} = 28.2 nM). However, **11** is more selective for m1 versus the m2 (3-fold) and m4 (8-fold) receptors than pirenzepine. Therefore, **11** could be claimed as the most m1 selective muscarinic receptor antagonist known to date.

Benzyl ester analogues (**23**−**40**) did not improve the selectivity of **11**. For this group of compounds, the optimal distance between the oxygen atom and the phenyl ring apparently necessary to maintain reasonable affinity and selectivity for the m1 receptor is three carbon atoms (**35**). To study the effect of a benzyl ester versus a cyclohexyl ester, **41**−**48** were examined. Many of these derivatives (**41**, **42**, **44**, **46**) were potent m1 muscarinic antagonists, but none were as selective as **11**.

The significance of the double bond of **I** was investigated by studying the piperidines derivatives (**49**−**53**). The most potent and selective piperidine was **53**, the direct analogue of **11**. This compound was about 2-fold less potent and about 16-fold less selective versus the m2 receptor. These data may indicate that the configuration of the 1,2,3,6-tetrahydropyridine contributes to the m1 receptor affinity and selectivity.

Preliminary functional studies on a select group of compounds indicated that these compounds possess muscarinic antagonist properties. The data in Table 3 show that none of the tested compounds produced any significant stimulation of PI hydrolysis at a high concentration (100 μM) in CHO Hm1 cells (98% of basal control). Therefore, these compounds are not efficacious as m1 selective muscarinic receptor agonists, despite their affinity for the receptor. The larger esters such as hexyl, benzyl, and cyclohexyl versus methyl inhibited carbachol stimulation of PI turnover (Table 4) in CHO Hm1 cells. These data indicate that these particular compounds are truly muscarinic antagonists, whereas the methyl ester is less effective. These data correlate well with the binding data illustrated in Table 1.

Data obtained from the isomers of **11** (Table 4) indicate that the racemate is as potent as the (*R*)- or the (*S*)-isomer. Apparently, the configuration at this chiral center is not important for affinity or selectivity at the m1 receptor.

Preliminary in vivo studies, i.e., spontaneous motor activity and spatial working memory, were examined with **11**. When **11** was administered icv, a slight inhibition of spontaneous motor activity was observed. If this compound were to get into the brain, in vivo activity may be observed. However, since no in vivo activity was noted in the mouse water maze study, it can only be speculated that the ester group of **11** may be readily metabolized. Therefore, oxadiazoles **54** and **55**, bioisosteres of **11** in which the ester group is masked by the oxadiazole ring, were synthesized and tested for m1 affinity and selectivity. Neither compound showed an improvement over **11**, but instead, a 30-fold decrease in affinity and an 8-fold decrease in selectivity for the m1 receptor (Table 5) were observed.

In summary, this study has generated **11**, a very potent and m1 selective muscarinic receptor antagonist, which could be used as a pharmacological tool to assist in understanding muscarinic receptor subtypes. From the analogues of **I** that were studied, the binding site of the m1 receptor does seem to tolerate other R_1 substituents, such as longer chain alkyl esters, benzyl esters, and cyclohexyl esters, since these analogues show a strong affinity for the m1 muscarinic receptor. However, the m1 selectivity of these compounds is not as pronounced as that of **11**. Therefore, it is **11** that

possesses m1 affinity and the best m1 selectivity. Even though **11** is not the most potent compound from this series, it is indeed the most m1 selective. Detailed pharmacological studies of **11** will be the topic of future communications from these laboratories.

Experimental Section

High-field nuclear magnetic resonance (NMR) spectra were recorded in deuteriochloroform ($CDCl_3$) or deuteriomethyl sulfoxide (DMSO-d_6) as solvent on a Varian Unity 400 MHz spectrometer. All chemical shifts are reported in ppm downfield from internal tetramethysilane. Infrared spectra were determined on a Mattson Galaxy FT-IR spectrophotometer. Elemental analysis for carbon, hydrogen, and nitrogen were determined on a Control Equipment Corporation CEC440 elemental analyzer and are within 0.4% of theory unless noted otherwise. Mass spectra were obtained by using a VG Masslab Trio-2A, Finnigan TSQ-70, or VG Analytical 7070E/HF mass spectrometer. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. All chemicals and reagents used were of commercial purity unless otherwise specified.

1-Ethyl-4-phenyl-1,2,3,6-tetrahydropyridine-3-carboxylic Acid Hexyl Ester Ethanedioate (11) and 1-Ethyl-4-phenyl-1,2,5,6-tetrahydropyridine-3-carboxylic Acid Hexyl Ester Ethanedioate (12). Step A: Preparation of 3-(4,5-Dihydro-4,4-dimethyl-2-oxazolyl)-1,4-dihydro-4-phenylpyridine. To a cooled (−78 °C) solution of 3-(4,5-dihydro-4,4-dimethyl-2-oxazolylpyridine[10] (140.0 g, 0.79 mol) in 500 mL of dry THF was added phenyllithium (1.42 mol, 1.8 equiv) under a nitrogen atmosphere until GC analysis indicated no more starting material present. The reaction mixture was stirred for 10 min and then quenched with 500 mL water at −78 °C. After the reaction mixture was warmed to room temperature with a water bath, 700 mL of ether was added to the mixture. The resulting solid was filtered, rinsed one time with chilled methanol, and then dried at 40 °C in a vacuum oven overnight to yield 166.6 g (0.65 mol, 82%) of the desired product. 400 MHz NMR (DMSO-d_6): 0.96 (s, 3H); 1.10 (s, 3H); 3.74 (s, 2H); 4.42−4.44 (d, 1H, J = 4.8 Hz); 4.63−4.67 (m, 1H); 6.14−6.17 (m, 1H); 6.94−6.95 (d, 1H, J = 5.2 Hz); 7.06−7.11 (m, 1H); 7.19−7.24 (m, 4H); 7.98−8.00 (m, 1H, NH). MS: 255 (MH^+), 254 (M^+).

Step B: Preparation of 3-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)-4-phenyl-pyridine. 3-(4,5-Dihydro-4,4-dimethyl-2-oxazolyl)-1,4-dihydro-4-phenylpyridine (80.6 g, 0.31 mol) and sulfur (10.6 g, 0.33 mol, 1.04 equiv) were heated to reflux in 750 mL of toluene for 3.5 h. GC analysis then indicated that the reaction was complete. The mixture was cooled to room temperature and filtered. The mother liquor was concentrated in vacuo and then kept under vacuum overnight at room temperature. The crude material was distilled (bp 163−166 °C at 2.8−3.2 Torr) to yield 65.3 g (0.25 mol, 81%) of the desired product. 400 MHz NMR (DMSO-d_6): 1.21 (s, 6H); 3.85 (s, 2H); 7.41−7.50 (m, 6H); 8.71−8.72 (d, 1H, J = 5.2 Hz); 8.78 (s, 1H). MS: 253 (MH^+), 252 (M^+).

Step C: Preparation of 4-Phenyl-3-pyridine Carboxylic Acid Monohydrochloride. 3-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)-4-phenylpyridine (108.0 g, 0.42 mol) and 220 mL of concentrated HCl were heated to reflux for 16 h. The mixture was then cooled to room temperature, filtered, and rinsed with 300 mL of 1 *N* HCl. The resulting white solid was dried in a vacuum oven at 40 °C for 6 h to give 95.0 g (0.40 mol, 96%) of the desired product. 400 MHz NMR (DMSO-d_6): 7.47−7.55 (m, 5H); 7.79−7.81 (d, 1H, J = 5.6 Hz); 8.89−8.90 (d, 1H, J = 5.6 Hz); 9.06 (s, 1H). MS: 200 (MH^+), 199 (M^+).

Step D: Preparation of 4-Phenylnicotinic Acid Hexyl Ester. To a suspension of 4-phenyl-3-pyridinecarboxylic acid monohydrochloride (50.0 g, 0.212 mol) in thionyl chloride (499 mL, 32.2 equiv) was slowly added dimethylformamide (25.72 mL, 1.56 equiv), and the reaction mixture was heated to reflux for 16 h. After the mixture was cooled to room temperature, the clear liquid was concentrated in vacuo to yield an opaque white oil. This oil was diluted with diethyl ether and then concentrated in vacuo two times. This acid chloride was then diluted in dichloromethane (215 mL) and added dropwise to a cooled (0 °C) solution of hexanol (29.30 mL, 1.1 equiv) and diisopropylethylamine (40.65 mL, 1.1 equiv) in dichloromethane (536 mL). The resulting dark brown solution was allowed to gradually warm to room temperature and stir for 20 h. The reaction mixture was then quenched with saturated $NaHCO_3$ (500 mL, pH 8) and extracted two times with dichloromethane. The layers were separated, and the combined organic layers were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford 64 g of crude product. Purification by flash chromatography (silica gel, 25% EtOAc/hexane) yielded 34.4 g (57%) of the desired product. 400 MHz NMR (DMSO-d_6): 0.80−0.84 (t, 3H, J = 7.2 Hz); 0.96−1.01 (m, 2H); 1.07−1.22 (m, 4H); 1.31−1.37 (m, 2H); 4.02−4.06 (t, 2H, J = 6.4 Hz); 7.35−7.38 (m, 2H); 7.45−7.50 (m, 4H); 8.76−8.77 (d, 1H, J = 5.2 Hz); 8.88 (s, 1H). MS: 284 (MH^+).

Step E: Preparation of 1-Ethyl-3-hexyloxycarbonyl-4-phenylpyridinium Iodide. 4-Phenylnicotinic acid hexyl ester (34.4 g, 0.121 mol) was combined with ethyl iodide (58.3 mL, 6 equiv) and heated to reflux for 23 h. The reaction mixture was then cooled to room temperature and concentrated in vacuo to yield 52.7 g (99%) of the desired product. 400 MHz NMR (DMSO-d_6): 0.11−0.84 (t, 3H, J = 7.2 Hz); 0.95−1.00 (m, 2H); 1.09−1.27 (m, 4H); 1.34−1.41 (m, 3H); 1.57−1.61 (t, 3H, J = 7.2 Hz); 4.126−4.158 (t, 2H, J = 6.4 Hz); 4.693−4.747 (q, 2H, J = 7.2 Hz); 7.52−7.57 (m, 2H); 7.58−7.62 (m, 2H); 8.30−8.32 (d, 1H, J = 6.8 Hz); 9.27−9.29 (m, 1H); 9.50−9.50 (d, 1H, J = 1.2 Hz). MS: 284 (M^+-CH_3CH_2I).

Step F: Preparation of 1-Ethyl-4-phenyl-1,2,3,6-tetrahydropyridine-3-carboxylic Acid Hexyl Ester Ethanedioate (11) and 1-Ethyl-4-phenyl-1,2,5,6-tetrahydro-pyridine-3-carboxylic Acid Hexyl Ester Ethanedioate (12). To a cooled solution of 1-ethyl-3-hexyloxycarbonyl-4-phenylpyridinium iodide (52.7 g, 0.12mol) in MeOH (280 mL) was added water (280 mL), followed by the portionwise addition of $NaBH_4$ (44.0 g, 1.16 mol, 9.7 equiv). This bright orange reaction mixture was stirred at 0 °C for 1.5 h. The reaction was then slowly quenched with concentrated HCl (150 mL, pH 1), and the mixture was stirred at room temperature for 10 min and neutralized with NH_4OH (150 mL, pH 10−11). The mixture was extracted 3 times with EtOAc, and the combined organic layers were dried (MgSO4), filtered, and concentrated in vacuo to afford 33.48 g of crude product, an orange oil with solids. Purification by flash chromatography (silica gel, 25% EtOAc/hexane, 2 times) yielded the free bases of **11** (2.83 g, 8%) and **12** (2.51 g, 7%).

1-Ethyl-4-phenyl-1,2,3,6-tetrahydropyridine-3-carboxylic Acid Hexyl Ester (**11**, free base). 400 MHz NMR (DMSO-d_6): 0.81−0.84 (t, 3H, J = 7.0 Hz); 1.00−1.03 (t, 3H, J = 7.0 Hz); 1.08−1.23 (m, 6H); 1.35−1.39 (m, 2H); 2.36−2.46 (m, 3H); 2.53−2.57 (m, 1H); 2.86−2.92 (m, 1H); 3.10−3.14 (m, 1H); 3.27−3.32 (m, 1H); 3.79−3.86 (m, 2H); 3.93−3.99 (m, 1H); 6.18−6.19 (m, 1H); 7.19−7.29 (m, 1H); 7.30−7.35 (m, 2H); 7.37−7.37 (m, 2H). MS: 316 (MH^+). HPLC conditions: Diacel ChiralPak OD, 0.46 × 250 × 10 μm 200 IPA:700 hexane: 0.1% DEA; 1.0 mL/min; 20 min. A 1:1 ratio of enantiomers; R_T = 5.79 and 6.63.

1-Ethyl-4-phenyl-1,2,5,6-tetrahydropyridine-3-carboxylic Acid Hexyl Ester (**12**, free base). 400 MHz NMR (DMSO-d_6): 0.80−0.88 (m, 3H); 0.90−0.99 (m, 2H); 1.01−1.11 (m, 6H); 1.13−1.27 (m, 4H); 2.46−2.50 (m, 3H); 2.57−2.60 (m, 2H); 3.33 (s, 2H); 3.74−3.78 (t, 2H, J = 6.4 Hz); 7.12,−7.14 (m, 2H); 7.26−7.327(m, 3H). MS: 316 (MH^+).

To a solution of each of the free bases of **11** and **12** in diethyl ether was added 1 equiv of oxalic acid in diethyl ether. Solid immediately precipitated out of the mixture which was allowed to stir at room temperature for 16 h. The resulting white solids were filtered and dried at room temperature to give the oxalate salts of the corresponding products.

1-Ethyl-4-phenyl-1,2,3,6-tetrahydropyridine-3-carboxylic Acid Hexyl Ester, Ethanedioate (**11**). Anal. ($C_{20}H_{29}NO_2 \cdot C_2H_2O_4 \cdot 0.76\ H_2O$): C, H, N. 1-Ethyl-4-phenyl-1,2,3,6-tetrahydro-pyridine-3-carboxylic Acid Hexyl Ester, Ethanedioate (**12**). Anal. ($C_{20}H_{29}NO_2 \cdot C_2H_2O_4$): C, H, N.

Hexyl (±)-4-Phenyl-1-ethyl-1,2,3,6-tetrahydro-3-pyridinecarboxylate, (±)-2,3-Bis[(4-methylbenzoyl)oxy]butanedioic (1:1) (Salt) (11A). To a solution of the free base of **11** (200 mg, 0.634 mmol) in 10 mL 95% ethanol was added (D)- and (L)-di-*p*-toluoyl-tartaric acid (0.5 equiv of each) in 1 mL 95% ethanol. The white precipitated solid (**11A**) was filtered and dried at room temperature. Anal. ($C_{20}H_{29}NO_2 \cdot C_{20}H_{18}O_8 \cdot 0.5\ H_2O$): C, H, N. HPLC conditions: Diacel ChiralPak OD, 0.46 × 250 × 10 μm; 200 IPA:700 hexane:0.1% DEA; 1.0 mL/min; 20 min. A 1:1 ratio of enantiomers; $R_T = 5.79$ and 6.62.

(*R*) or (*S*) Hexyl-4-phenyl-1-ethyl-1,2,3,6-tetrahydro-3-pyridinecarboxylate, [*R*-(*R,*R**)]-2,3-Bis[(4-methylbenzoyl)oxy]butanedioic (1:1) (Salt) (11B).** The same procedure as that described for **11A** was followed, using only (L)-di-*p*-toluoyltartaric acid and recrystallized two times. Anal. ($C_{20}H_{29}NO_2 \cdot C_{20}H_{18}O_8 \cdot 0.5H_2O$): C, H, N. HPLC conditions: Diacel ChiralPak OD, 0.46 × 250 × 10 μm; 200 IPA:700 hexane:0.1% DEA; 1.0 mL/min; 20 min. $R_T = 5.92$.

(*R*) or (*S*) Hexyl-4-phenyl-1-ethyl-1,2,3,6-tetrahydro-3-pyridinecarboxylate, [*S*-(*R,*R**)]-2,3-Bis[(4-methylbenzoyl)oxy]butanedioic (1:1) (Salt)(11C).** The same procedure as that described for **11A** was followed, using only (D)-di-*p*-toluoyltartaric acid and recrystallized two times. Anal. ($C_{20}H_{29}NO_2 \cdot C_{20}H_{18}O_8 \cdot H_2O$): C, H, N. HPLC conditions: Diacel ChiralPak OD, 0.46 × 250 × 10 μm; 200 IPA:700 hexane:0.1% DEA; 1.0 mL/min; 20 min. $R_T = 5.69$.

(*trans*)-1-Ethyl-4-phenylpiperidine-3-carboxylic Acid Phenethyl Ester Ethanedioate (1:2) (49) and (*cis*)-1-Ethyl-4-phenylpiperidine-3-carboxylic Acid Phenethyl Ester Ethanedioate (1:2) (50). To a solution of 1-ethyl-4-phenyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid 2-phenylethyl ester ethanedioate (1:1) **(28)** (0.75 g, 0.0017 mol) in 75 mL of tetrahydrofuran was added 0.2 g of 5% Pd/C, and the mixture was shaken under 52 psi H_2 for at least 23 h. The reaction mixture was filtered and concentrated in vacuo to give crude desired product. To 3 mL of 1 M K_2CO_3 and 75 mL of ethyl acetate was added 820 mg of this crude reduced oxalate salt, and the mixture was stirred. The layers were separated, and the organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a 6:1 ratio of cis:trans isomers. Purification by medium-pressure liquid chromatography (5% MeOH/dichloromethane, the trans isomer elutes first) yielded 30 mg of the free base of **49** and 180 mg of the free base of **50**, for an overall yield of 31%.

(*trans*)-1-Ethyl-4-phenyl-piperidine-3-carboxylic Acid Phenethyl Ester (**49**). 400 MHz NMR ($CDCl_3$): 0.98−1.06 (m, 1H); 1.17−1.20 (t, 3H, $J = 7.0$ Hz); 1.38−1.54 (m, 2H); 1.83−1.86 (m, 1H); 2.46−2.48 (m, 1H); 2.55−2.61 (m, 2H); 2.63−2.65 (m, 1H); 2.67−2.68 (m, 0.5H); 2.89−2.90 (m, 0.5H); 3.00−3.09 (m, 0.5H); 3.10−3.18 (m, 0.5H); 3.34−3.35 (m, 2H); 4.01−4.06 (m, 1H); 4.55−4.59 (m, 1H); 7.06−7.32 (m, 10H). MS: 338 (MH^+), 337 (M^+).

(*cis*)-1-Ethyl-4-phenyl-piperidine-3-carboxylic Acid Phenethyl Ester (**50**). 400 MHz NMR (DMSO-d_6): 0.91−0.94 (t, 3H, $J = 7.0$ Hz); 1.71−1.75 (m, 1H); 2.02−2.07 (m, 1H); 2.22−2.33 (m, 3H), 2.40−2.50 (m, 1H); 2.66−2.76 (m, 2H); 2.78−2.81 (m, 1H); 2.82−2.95 (m, 1H); 3.00−3.06 (m, 1H); 3.10−3.19 (m, 1H); 3.99−4.10 (m, 2H); 7.11−7.26 (m, 10H). MS: 338 (MH^+), 337 (M^+).

Treatment of each isomer separately with 1 mol of oxalic acid in diethyl ether, followed by filtration, yielded **49** and **50**.

(*trans*)-1-Ethyl-4-phenylpiperidine-3-carboxylic Acid Phenethyl Ester Ethanedioate (1:2) **(49)**. 400 MHz NMR (DMSO-d_6): 0.91−0.99 (m, 1H); 1.18−1.25 (m, 3H); 1.33−1.48 (m, 2H); 1.85−1.95 (m, 0.5H); 1.96−2.10 (m, 0.5H); 2.55−2.60 (m, 1H); 2.69−2.71 (m, 1H); 2.88−2.94 (m, 1H); 3.01−3.14 (m, 2H); 3.21−3.39 (m, 1H); 3.42−3.45 (m, 0.5H); 3.50−3.53 (m, 0.5H); 3.79−3.85 (m, 1H); 3.96−4.02 (m, 1H); 4.49−4.53 (m, 1H); 7.05−7.38 (m, 10H). mp: 112−144 °C. High mass calcd, 338.2120; found, 338.2116.

(*cis*)-1-Ethyl-4-phenylpiperidine-3-carboxylic Acid Phenethyl Ester Ethanedioate (1:2) **(50)**. 400 MHz NMR (DMSO-d_6): 1.18−1.21 (t, 3H, $J = 7.0$ Hz); 2.05−2.08 (m, 1H); 2.27−2.29 (m, 1H); 2.49−2.60 (m, 2H); 3.06−3.39 (m, 7H); 3.56−3.61 (m, 1H); 3.95−4.06 (m, 2H); 7.04−7.34 (m, 10H). mp 78−81 °C. High mass calcd, 338.2120; found, 338.2111.

1-Ethyl-3-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine Ethanedioate (1:1) (54) and 1-Ethyl-5-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine Ethanedioate (1:1) (55). Step A: Preparation of *N*′-Hydroxyhexanimidamide. To a solution of hydroxylamine hydrochloride (20.0 g, 0.287 mol) and triethylamine (29.04 g, 0.287 mol) in methanol (120 mL) was added dropwise hexanonitrile (27.96 g, 0.287 mol) in 30 mL methanol. The reaction mixture was stirred at room temperature for 16 h. The mixture was then concentrated in vacuo, and the residue was triterated with 250 mL of $CHCl_3$. The white solid was filtered, and the filtrate was concentrated in vacuo and triterated with diethyl ether. This white precipitate was filtered, and the filtrate was concentrated in vacuo to yield crude product. Purification by chromatography (silica gel, 5% MeOH/CH_2Cl_2) yielded *N*′-hydroxyhexanimidamide (11.0 g 30%). 400 MHz NMR (DMSO-d_6): 0.84−0.87 (t, 3H, $J = 7.2$ Hz); 1.19−1.32 (m, 4H); 1.43−1.58 (m; 2H); 1.92−1.96 and 2.13−2.19 (m, 2H); 5.37−5.74 (br.s, 1H); 10.3 (br.s, 1H). MS: 131 (MH^+); 130 (M^+).

Step B: Preparation of 1-Ethyl-3-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine Ethanedioate (1:1) (54) and 1-Ethyl-5-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine Ethanedioate (1:1) (55).[11] A suspension of 1-ethyl-4-phenylpiperidinyl-3-carboxylic acid methyl ester ethanedioate (**1**) (2.78 g) in 200 mL of ethyl acetate and 10 mL 1 M K_2CO_3 was stirred for 15 min. The layers were separated, and the organic layer was dried (MgSO4), filtered, and concentrated in vacuo to give 2.06 g of the free base of **1**. To a green suspension of *N*′-hydroxyhexanimidamide (1.31 g, 0.010 mol, 1.2 equiv) and sodium hydride (60% dispersion in mineral oil, rinsed with hexane, 1.2 equiv) was added 2.0 g of 4 Å molecular sieves, and the mixture was heated to reflux for 1 h. After the heating source was removed, 1-ethyl-4-phenylpiperidinyl-3-carboxylic acid methyl ester (**1**, 2.06 g, 0.008 mol) was added, and the reaction mixture was heated again to reflux for 2.5 h and then at room temperature for 16 h. The suspension was filtered, and the filtrate was concentrated in vacuo to give an orange residue. This residue was diluted with water and extracted 4 times with dichloromethane. The combined organic layers were dried (NaSO4), filtered, and concentrated in vacuo to give crude product. Purification by medium-pressure liquid chromatography (silica gel, 20% ethyl acetate/hexane) yielded 0.2 g of **54** and 0.41 g of **55**, in the free base forms. To a solution of free base **54** (0.15 g) in 50 mL of diethyl ether was added oxalic acid (0.04 g) in 5 mL of diethyl ether, and the mixture was stirred at room temperature for 16 h. The diethyl ether was removed, and the solid was filtered, rinsed with additional ether, and dried to give 1-ethyl-3-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine ethanedioate (1:1) (**54**) (0.06 g, 31%). To a solution of free base **55** (0.35 g) in 140 mL of diethyl ether was added oxalic acid (0.97 g) in 10 mL of diethyl ether, and the mixture was stirred at room temperature for 16 h. The diethyl ether was removed, and the solid was filtered, rinsed with additional ether, and dried to give 1-ethyl-5-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine ethanedioate (1:1) (**55**) (0.32 g, 72%).

1-Ethyl-3-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine Ethanedioate (1:1) (**54**). 400 MHz NMR (DMSO-d_6): 0.78−0.82 (t, 3H, $J = 7.2$ Hz); 1.05−1.28 (m, 7H); 1.51−1.58 (m, 2H); 2.50−2.59 (m, 2H); 2.71−2.76 (m, 2H); 3.12−3.14 (m, 1H); 3.32−3.41 (m, 2H); 3.59−3.63 (m, 1H); 4.78 (br. s., 1H); 6.35 (br. s., 1H); 7.22−7.41 (m, 5 H). Anal. ($C_{20}H_{27}N_3O \cdot 0.5H_2O_4 \cdot C_2H_2O_4$): C, H, N.

1-Ethyl-5-(3-pentyl-[1,2,4]oxadiazol-5-yl)-4-phenyl-1,2,3,6-tetrahydropyridine Ethanedioate (1:1) (**55**). 400 MHz NMR (DMSO-d_6): 0.82−0.86 (t, 3H, J = 7.0 Hz); 1.17−1.31 (m, 7H); 1.54−1.61m, 2H); 2.58−2.62 (m, 2H); 2.82 (m, 2H); 3.06−3.11 (m, 2H); 3.24−3.38 (m, 2H); 3.98 (m, 2H); 7.17−7.19 (m, 2 H); 7.36−7.37 (m, 3 H). Anal. ($C_{20}H_{27}N_3O \cdot C_2H_2O_4$): C, H, N.

Biological Methods. Receptor binding at the five muscarinic receptors (Hm1−Hm5) is performed by the methods described by Dorje et al.[3a] and Buckley et al.[14] with modifications as described in ref 16. PI turnover assays are performed by the methods described by Berridge et al.[19] with modifications as also described in ref 16.

Acknowledgment. We thank Dr. G. McClusky and staff for spectral and analytical determinations, V. Hoard, E. Getachew, M. Marlatt, D. Johnson, N. Colbry, J. Dodd, and J. McKee for their synthetic assistance of various intermediates and final compounds, B. Tobias for special NMR studies, J. Loo and D. DeJohn for the high-resolution mass spectral data, B. Leja for spectral analysis of **11**, and M. Callahan, C. Christofferson, J. Wiley, and L. Meltzer for the preliminary in vivo studies.

References

(1) Presented in part at the Sixth International Symposium on Subtypes of Muscarinic Receptors, Fort Lauderdale, FL, 1994; Abstract 30. Augelli-Szafran, C. E.; Blankley, J. C.; Jaen, J. C.; Moreland, D. W.; Nelson, C. B.; Penvose, J. R.; Schwarz, R. D.; Thomas, A. J. Identification and Characterization of m1 Selective Muscarinic Antagonists.

(2) (a) Mitchelson, F. Muscarinic Receptor Differentiation. *Pharmacol. Ther.* **1988**, *37*, 357−423. (b) Goyal, R. K. Muscarinic Receptor Subtypes: Physiology and Clinical Implications. *N. Engl. J. Med.* **1989**, *321*, 1022−1029. (c) Hulme, E. C.; Birdsall, N. J.; Buckley, N. J. Muscarinic Receptor Subtypes. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 633−673.

(3) (a) Dorje, F.; Wess, J.; Lambrecht, G.; Tacke, R.; Mutscher, E.; Brann, M. R. Antagonist Binding Profiles of Five Cloned Human Muscarinic Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 727−733. (b) Bolden, C.; Cusack, B.; Richelson, E. Antagonism By Antimuscarinic and Neuroleptic Compounds at the Five Cloned Human Muscarinic Cholinergic Receptors Expressed in Chinese Hamster Ovary Cells. *J. Pharmacol. Exp. Ther.* **1992**, 260, 576−580. (c) Abbreviations for each of the human muscarinic receptors, m1 through m5, are Hm1, Hm2, Hm3, Hm4, and Hm5. The latest reference on muscarinic nomenclature is: Caulfield, M. P.; Birdsall, N. J. International *U*nion of Pharmacology. XVII. Classification of Muscarinic Acetylcholine Receptors. *Pharm. Rev.* **1998**, *50*, 279−290.

(4) Stockbruegger, R. W. Antimuscarinic Drugs. *Methods Find. Exp. Clin. Pharmacol.* **1989**, *11* (Suppl. 1), 79−86. (b) Kromer, W.; Goenne, S. An Acid-Independent Antiulcer Effect of M1 Antimuscarinics in the Rat. *Pharmacol*ogy **1988**, *37* (Suppl. 1), 48−53.

(5) Doods, H. N.; Engel, W.; Su, C. A. P. F. Tanswell, P. AF-DX 116: A Cardioselective Muscarinic Antagonist. *Cardiovasc. Drug Rev.* **1991**, *9*, 30−40.

(6) (a) Maclagan, J. Barnes, P. Muscarinic Pharmacology of the Airways. *Trends Pharmacol. Sci.* **1989**, *10* (Supplement), 88−92. (b) Barnes, P. J.; Minette, P.; Maclagan, J. Muscarinic Receptor Subtypes in Airways. *Trends Pharmacol. Sci.* **1988**, *9*, 412−16.

(7) Kaiser, C.; Audia, V. H.; Carter, J. P.; McPherson, D. W.; Waid, P. P.; Lowe, V. C.; Noronha-Blob, L. Synthesis and Antimuscarinic Activity of Some 1-Cycloalkyl-1-hydroxy-1-phenyl-3-(4-substituted piperazinyl)-2-propanones and Related Compounds. *J. Med. Chem.* **1993**, *36*, 610−16.

(8) Hutt, C. S.; Snider, S. R.; Fahn, S. Interaction Between Bromocriptine and Levodopa. Biochemical Basis for an Improved Treatment for Parkinsonism. *Neurology* **1977**, *27*, 505−10.

(9) Price, D. L.; Whitehouse, P. J.; DeLong, M. R.; Kitt, C. A.; Hedreen, J. C.; Mitchell, S. J.; Struble, R. G.; Price, D. L., Jr.; Cork, L. C.The Basal Forebrain Cholinergic System and Alzheimer's Disease. *Mod. Aging Res.* **1984**, *4*, 385−99.

(10) (a) Hauck, A. E.; Giam, C.-S. Regioselective Nucleophilic Addition of Organolithium Compounds to 3-(4,4-dimethyloxazolin-2-yl)pyridine. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2070−2076. (b) Moos, W. H.; Bermeier, S. C.; Coughenour, L. L.; Davis, R. E.; Hershenson, F. H.; Kester, J. A.; McKee, J. S.; Marriott, J. G.; Schwarz, R. D.; Tecle, H.; Thomas, A. J. Cholinergic Agents: Effect of Methyl Substitution in a Series of Arecoline Derivatives on Binding to Muscarinic Acetylcholine Receptors. *J. Pharm. Sci.* **1992**, *81*, 1015−1019. (c) US 5,446,057, August 29, 1995.

(11) (a) Meyers, A. I.; Gabel, R. A. Oxazolines as Activating Groups in Aromatic Substitution. Selective Metalation or Addition to the 4-Position of Pyridine. A New Synthesis of 1,4-Dihydropyridines. *Heterocycles* **1978**, *11*, 133−138. (b) Comins, D. L.; Stroud, E. D.; Herrick, J. J. Regioselective Addition of Grignard Reagents to the 1-Phenoxycarbonyl Salts of Alkyl Nicotinates. *Heterocycles* **1984**, *22*, 151−157.

(12) Saunders, J.; Cassidy, M. Freedman, S. B.; Harley, E. A.; Iverson, 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.

(13) Hirotsu, T.; Katoh, S.; Shunsaku, 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**, 1609−1611.

(14) 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.

(15) Complete functional studies and detailed analysis of **11** and pirenzepine and preliminary in vivo results of **11** will be described by Schwarz, R. D., et al., in a future communication.

(16) Peralta, E. G.; Ashkenazi, A.; Winslow, J. W.; Ramachandran, J.; Capon, D. J. Differential Regulation of PI Hydrolysis and Adenylyl Cyclase by Muscarinic Receptor Subtypes. *Nature* **1988**, *334*, 434−437.

(17) Geyer, M. A.; Russo, P. V.; Masten, V. L. Multivariate Assessment of Locomotor Behavior: Pharmacolgoical and Behavioral Analyses. *Pharmacol. Biochem. Behav.* **1986**, *25*, 277−288.

(18) Saucier, D.; Hargreaaves, E. L.; Boon, F.; Vanderwolf, C. H.; Cain, D. P. Detailed Behavioral Analysis of Water Maze Acquisition Under Systemic NMDA or Muscarinic Antagonism: Nonspatial Pretraining Eliminates Spatial Learning Deficits. *Behav. Neurosci.* **1996**, *110*, 103−116.

(19) (a) Berridge, M. J.; Downes, C. P.; Hanley, M. R. Lithium Amplifies Agonist-Dependent Phosphatidylinositol Responses in Brain and Salivary Glands. *Biochem. J.* **1982**, *206*, 587−595. (b) Berridge, M. J.; Irvine, R. F. Inositol Triphosphate, A Novel Second Messenger in Cellular Signal Transduction. *Nature* **1984**, *312*, 315−321.

JM980067L