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C(8)-Substituted 1-azabicyclo[3.3.1]non-3-enes: a novel scaffold for muscarinic receptor ligands

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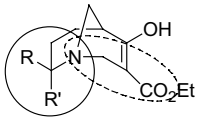
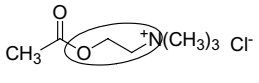
Abstract—The [3.3.1]-bicyclic amine, *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (**1**), has been shown to be a potent competitive antagonist against the hM_1–hM_5 muscarinic receptors. This heterocyclic system has not been extensively evaluated despite the notable activities reported for other bicyclic amines. Synthetic strategies permitted the selective alteration of five structural sites in **1**. Pharmacological evaluation demonstrated that modification of either the C(3) alkoxycarbonyl or the C(4) enol units in **1** gave compounds with high affinity for the hM_1–hM_5 muscarinic receptors with selectivity for the hM_2 receptor.

1. Introduction

Bicyclic amines provide a source of novel pharmacological agents.[1–6] We reported recently that the C(8)-substituted [3.3.1]-bicyclic amine **1** is a potent competitive antagonist against the hM_1–hM_5 muscarinic receptors.[6] Numerous pathophysiological processes have been linked to muscarinic receptor function,[7] and includes Alzheimer and Parkinson diseases, schizophrenia, urinary incontinence, irritable bowel syndrome, chronic obstructive pulmonary disease, and the perception of pain.[7,8]



1 (*exo*) R = CH_2OBn, R' = H
2 (*endo*) R = H, R' = CH_2OBn



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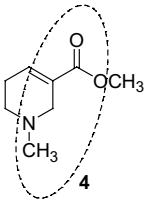

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Compared with other bicyclic amines,[2,5] the [3.3.1]-bicyclic amine system has not been extensively studied because of the low yields produced by their syntheses.[1] In the course of our study, we developed a five-step route to **1** and its C(8) *endo*-isomer **2** that gave a 39% overall yield.[6]

Compound **1** contained structural units similar to two pharmacophores, acetylcholine chloride (**3**), and arecoline (**4**), previously shown important for either muscarinic receptor function or binding.[7] While **3** and **4** have been proposed to exhibit the same principle sites of action,[7a] minor structural changes within these ligands alter their potency and pharmacological profile at the different muscarinic receptor subtypes.[7,9] Thus, we modified each pharmacophore independently in **1** to assess the relative importance of each unit for bioactivity and to learn if specific modifications affected receptor selectivity. We document that structural changes within the projected arecoline pharmacophore provided compounds that retained excellent muscarinic receptor binding affinities while only modestly affecting

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receptor selectivity, compared with **1**, and that alterations of the putative acetylcholine chloride pharmacophore led to diminished binding affinities.

2. Results and discussion

2.1. Choice of compounds

Beginning with **1** we modified three positions unique to the arecoline pharmacophore (Fig. 1, series A–C), one position that corresponded to the acetylcholine structural unit (Fig. 1, series D), and one position common to both (Fig. 1, series E). The number of potential structural changes and the need to assess the bioactivity at all five muscarinic receptors required us to limit the number of compounds slated for synthesis and evaluation. Accordingly, we selected, in many cases, structural modifications for the two pharmacophores that have been shown to provide high muscarinic receptor binding affinity.

The compounds (**5**–**21**) prepared are illustrated in Figure 1. In series A, we modified the C(3) alkoxycarbonyl unit by replacing the ethyl unit with methyl, allyl, propargyl, and hexyl groups to give **5**–**8**, respectively. Similar structural changes in arecoline analogues have provided muscarinic agonists[10,11] and an antagonist.[12] In series B, we converted the C(4) enol unit to the corresponding methyl **9** and propargyl **10** enol ethers. To prepare series C derivatives **11**–**13** we first decarboxylated the β-hydroxy-α,β-unsaturated ester **1** to ketone **11** and then converted **11** to the *anti*-**12** and *syn*-**13** methyl oximes. Impetus for this modification was derived by the pharmacological profiles reported for the M_1 receptor agonists, CI-1017,[4] and sabcomeline.[13]

Three structural modifications were made within the acetylcholine chloride-like pharmacophore in **1** (series D). We converted the C(8) benzyl ether in **1** to the corresponding benzyl alcohol **14**, the acyl derivatives **15** and **16**, the carbamoyl analogues **17** and **18**, and the chloro derivative **19**. The muscaranic agonist aceclidine[14] contains an acetoxy unit, the muscarinic antagonist aprophen[15] a 2,2-diphenylpropionoxy moiety, and the muscarinic agonist WAY-131256,[16] like **17** and **18**, contains a carbamate unit.

The remaining structural site examined was the N(1) position in **1** (site E). This position is shared by both the projected arecoline and the acetylcholine chloride pharmacophores. Quaternization of this amine gave the methyl **20** and the ethyl **21** ammonium salts, respectively. A similar site modification appears in the endogenous transmitter **3** and McN-A-343, a M_1 receptor agonist.[17]

Two additional compounds were included in this study. In **22**, we modified two, rather than just one, sites within the arecoline-like pharmacophore in **1** by converting the C(4) hydroxy and the C(3) ethoxycarbonyl moieties in **1** to a methoxy and a 3-allyloxycarbonyl units, respectively. We also synthesized the *endo*-derivative **23** that was isomeric to *exo*-**9**. In our initial study, we showed that *exo*-**1** binding affinities for hM_1–hM_5 were considerably higher than *endo*-**2**.[6] To provide additional support for this structural feature, we compared **9** with **23**.

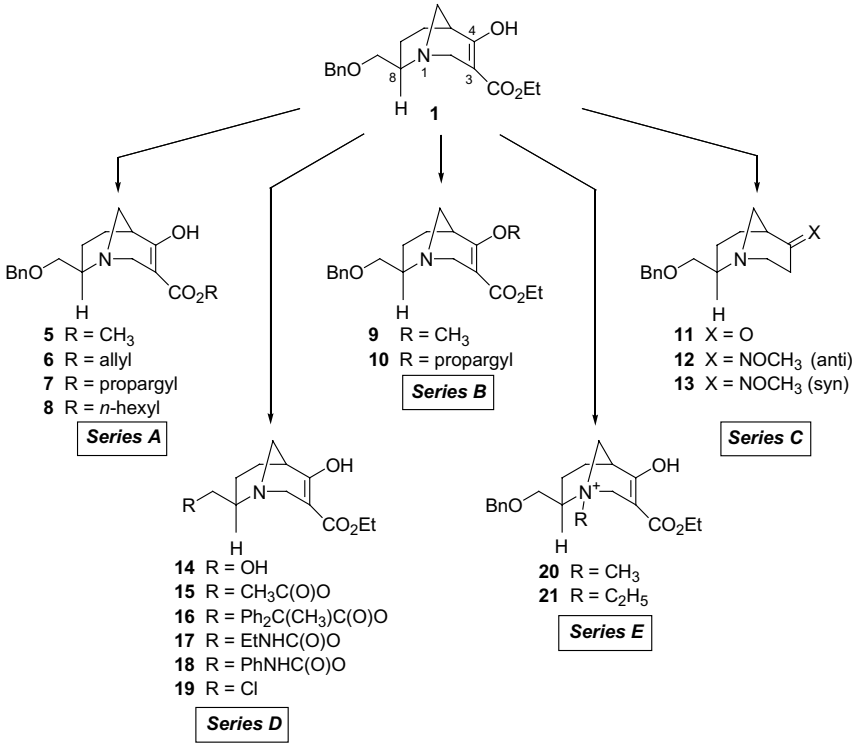
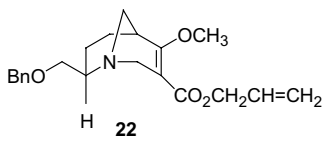


Figure 1. Site modifications in [3.3.1]-bicyclic amine **1**.



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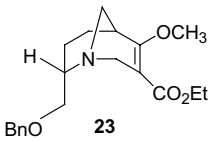

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3. Synthesis

3.1. Series A

Two transesterification methods were employed to prepare **5**–**8** in moderate-to-excellent yields without evidence of decarboxylation. Beginning with **1** and either methanol or allyl alcohol and using DMAP and 4 Å molecular sieves in refluxing toluene[18] we obtained **5** (96% yield) and **6** (40% yield), respectively. A similar procedure was employed for **7** except that **5** was used as the starting material rather than **1**. This change was necessitated by the similar TLC R_f values observed for **1** and **7** and our need to separate unreacted starting material from products upon workup. For **8**, we employed the method of Otera and co-workers,[19] beginning with **1**, hexyl alcohol, and catalytic amounts of 1-hydroxy-3-chlorotetrabutyldistannoxane (35% yield).

3.2. Series B

Compounds **9** and **10** were prepared by Mitsunobu coupling (1,1′-azobis(*N*,*N*-dimethylformamide) (TMAD), *n*-Bu_3P)[20] of **1** with MeOH (49% yield) and propargyl alcohol (36% yield), respectively.

3.3. Series C

anti-**12** and *syn*-**13** Methyl oximes were prepared from **11**. We learned that heating **1** with DMAP in toluene provided **11** in 58% yield. Subsequent treatment of **11** with *O*-methoxylamine hydrochloride and Et_3N gave a ~2:1 ratio of **12** and **13**, respectively, that was separable by PTLC. ^{13}C NMR analysis of **12** and **13** allowed their stereochemical assignment as *anti*-**13** and *syn*-**13**, respectively. In agreement with ^{13}C NMR patterns reported by Roberts and co-workers, the C(3) carbon signal for *anti*-**12** appeared upfield (4.5 ppm) from the corresponding resonance for the *syn*-**13** isomer.[21]

3.4. Series D

Five [3.3.1]-bicyclic amine derivatives were prepared using alcohol **14**, which was prepared by catalytic hydrogenation (10% Pd–C, 20 atm of H_2) of **1** at 80% yield. Treatment of **14** with either acetyl chloride or diphenylpropionyl chloride in the presence of Et_3N gave **15** (62% yield) and **16** (54% yield). Similarly, addition of either ethyl isocyanate or phenyl isocyanate to a CH_2Cl_2 solution of **14** provided **17** (80% yield) and **18** (37% yield), respectively. The C(8) chloromethyl derivative **19** was prepared in 74% yield by treatment of **14** with methanesulfonyl chloride and Et_3N in CH_2Cl_2. In the conversion of **14** to **19**, we did not detect the intermediate formation of the C(8) methylenemesylate (TLC analysis).

3.5. Series E

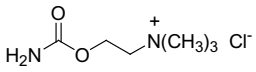
The N(1) quaternary ammonium salts **20** (63% yield) and **21** (77% yield) were prepared by treating **1** with iodomethane and ethyl trifluoromethanesulfonate, respectively.

4. Compound characterization

Satisfactory spectral data (IR, ^1H NMR, ^{13}C NMR, microanalysis, low- and high-resolution MS) were obtained for bicyclic amines **5**–**21**. The expected molecular ion was observed for all compounds. Series A and D modifications did not alter the [3.3.1]-bicyclic amine system for these compounds. Thus, it was not surprising that we observed a consistent set of ^1H and ^{13}C NMR ring resonances for **5**–**8** and **14**–**19** that were similar to **1**. Conversion of enol **1** to enol ethers **9** and **10** (series B) led to characteristic upfield ^{13}C NMR shifts for C(4) (6.0–6.3 ppm) and C(3) (1.8–3.2 ppm) and a downfield shift for C(2) (2.7–2.8 ppm). Similar ^{13}C NMR differences have been reported for enols and enol ethers.[22] Distinguishing oximes **12** and **13** (series C) was the ^{13}C NMR C(4) resonance at 160.3 and 161.0 ppm, respectively, the appearance of the C(3) methylene hydrogens in the ^1H NMR (**12**: δ 2.35–2.99; **13**: δ 2.31–2.63), and a 4.5 ppm difference for the C(3) ^{13}C NMR signals for *anti*-**12** (δ 24.1) and *syn*-**13** (δ 28.6).[21] N(1)-Quaternization of **1** to give **20** and **21** led to the expected downfield ^1H NMR shifts[22] for the C(2) (0.43–0.47 ppm) and the C(9) (0.74–0.89 ppm) methylene protons, and the C(8) (0.48–0.50 ppm) methine hydrogen. Similarly, in the ^{13}C NMR we detected that the C(2) (9.3–9.7 ppm), C(8) (7.4–10.0 ppm), and C(9) (9.7–11.1 ppm) signals resonated downfield from **1**.[23]

5. Pharmacological analysis

Our initial studies documented that *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]-non-3-ene (**1**) efficiently bound to the human M_1–M_5 receptors and functioned as an antagonist.[6] Structural inspection of **1** showed that this [3.3.1]-bicyclic amine contained structural units similar to two pharmacophores, acetylcholine chloride (**3**) and arecoline (**4**), that are commonly found in muscarinic ligands.[1,7,10–12] We determined that the K_i of **1** for binding to hM_1–hM_5 was 10–50 times lower than carbachol (**24**) and 30–230 times lower than **4**.[6] In this study, we modified each pharmacophore independently and assessed the effect of these modifications on binding affinity and receptor selectivity against the hM_1–hM_5 muscarinic receptors.



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We tested **5**–**21** first at 100 μM and then at 1 μM concentrations in competition binding assays using [^{3}H]QNB and membranes prepared from COS-7 cells independently expressing each of the five human muscarinic receptors.[24,25] At 100 μM concentration, most compounds inhibited [^{3}H]QNB by at least 80% (data not shown). Only series C and D compounds showed diminished receptor binding, with **16** being the least effective (hM_1: 8%; hM_2: 11%; hM_3: 22%; hM_4: 21%; hM_5: 35%). At 1 μM concentrations of competing drug, differences in binding affinities among the five series were observed and differences in the binding affinities for **5**–**21** at the five different receptors were revealed (Fig. 2). Series A (**1**, **5**–**8**) and B (**9**, **10**) proved to be the most effective while series C (**11**–**13**) and D (**14**–**19**) compounds were the least effective in this assay. The series E quaternary salts, **20** and **21**, had moderate binding affinities at 1 μM.

Inspection of the binding data (Fig. 2) for **1** and **5**–**21** revealed structural patterns that governed C(8)-substituted 1-azabicyclo[3.3.1]non-3-ene bioactivity. First, structural modifications of the arecoline pharmacophore (series A, B) that retained the α,β-unsaturated alkoxycarbonyl provided compounds with excellent affinity, which typically exceeded **4** at the five muscarinic receptors. Second, alteration of the 2-aminoethyl ether unit of the acetylcholine chloride-like pharmacophore in **1** to give **14**–**19** (series D) led to reduced binding affinities for the hM_1–hM_5 muscarinic receptors. Surprisingly, **14** and **15** were ineffective in inhibiting [^{3}H]QNB binding at 1 μM. Compound **14** is the debenzylated analogue of **1**, and **15** is the corresponding acetyl derivative that bears structural resemblance to **3**.

Compounds from the most active series, **7** (series A) and **9** (series B), were selected for further testing in the binding assays using a wider range of concentrations. Representative binding curves are given in Figure 3 and the calculated K_i values for hM_1–hM_5 determined from multiple experiments listed in Table 1. The values are compared with **1**.

The 1 μM binding affinity showed that the C(8)-substituted 1-azabicyclo[3.3.1]non-3-enes **5**–**21** exhibited modest receptor selectivity with the highest affinity observed for the hM_2 and hM_4 receptors compared with the hM_1, hM_3, and hM_5 receptors (Fig. 2). A similar result was observed for **1**. Significantly, M_2 and M_4 selective antagonists have gained attention for their use in movement and cardiac disorders, dementia, and pain[2–5,26] and recent studies have provided striking examples of novel M_2-selective antagonists for the treatment of Alzheimer disease.[27] Thus, we were interested to determine the extent to which structural changes affected receptor site selectivity. Table 1 shows that the 20-fold M_2 versus M_3 selectivity ($p < 0.01$) for **1** was increased to 29-fold ($p < 0.01$) for the methyl enol ether

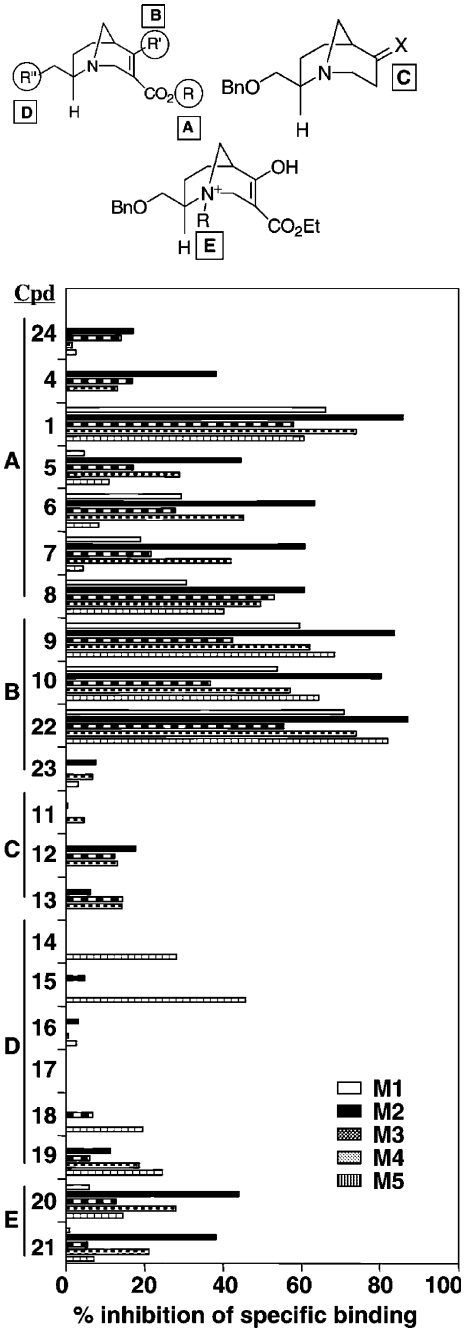

Figure 2. Compound screen for binding at the human M_1–M_5 receptors. The indicated compounds (1 μM) were incubated with [^{3}H]QNB and receptor expressing COS-7 cell membranes. Data are presented as the percent inhibition of specific binding, which was defined as [^{3}H]QNB binding inhibited by 1 μM atropine. The data shown are the result of an experiment performed in duplicate.

9. We also noted a modest increase in selectivity of **9** for M_2 versus M_4 when compared with **1**, but a decrease in selectivity when the M_2/M_1 and M_2/M_5 values were compared for these two compounds. Correspondingly, when compared with **1** exchange of the ethyl ester in **1** for the propargyl unit in **7** led to diminished receptor selectivities at M_1 and M_3–M_5 when compared with M_2. These findings indicated that structural changes within the arecoline pharmacophore affected substrate binding at the different muscarinic receptors, but that the

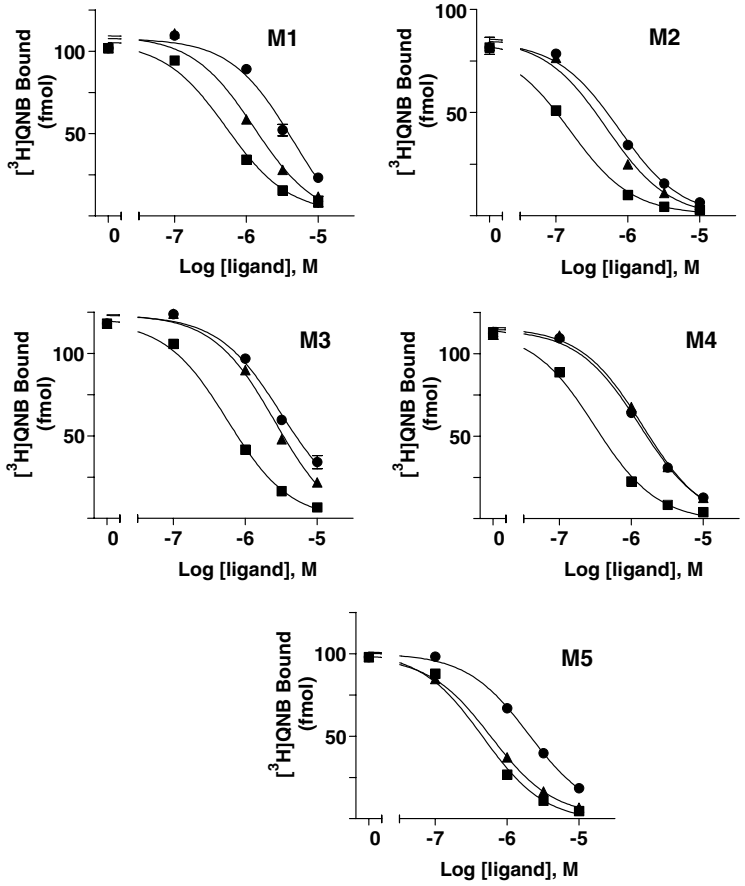

Figure 3. Competition binding assay at the human M_1–M_5 receptors: Increasing amounts of the indicated analogue were incubated with [^{3}H]QNB and COS-7 cell membranes expressing the indicated receptor. Data are shown for compound **1** (■), **7** (●), and **9** (▲). The data shown are typical curves of three independent experiments carried out in duplicate for each receptor. Bars represent the range of duplicate values.

Table 1. Binding affinities of **1**, **7**, and **9** at human muscarinic receptors

Compd	hM_1 K_i[a]	hM_2 K_i[a]	hM_3 K_i[a]	hM_4 K_i[a]	hM_5 K_i[a]
1	0.18 ± 0.02	0.06 ± 0.01	1.2 ± 0.3	0.16 ± 0.003	0.32 ± 0.03
7	1.2 ± 0.2	0.47 ± 0.2	6.0 ± 1	0.78 ± 0.2	1.5 ± 0.6
9	0.44 ± 0.02	0.18 ± 0.02	5.2 ± 1	0.75 ± 0.04	0.46 ± 0.05

[a] K_i values (μM) were calculated according to the formula:

$$K_i = \frac{IC_{50}}{1 + \frac{[[^3H]QNB]}{K_D}}$$

where IC_{50} is the concentration of competing analogue that inhibited [^{3}H]QNB binding by 50%, [[^{3}H]QNB] is the concentration of [^{3}H]QNB in the binding assay, and K_D is the K_D of [^{3}H]QNB. The K_D determined for [^{3}H]QNB for hM_1 was 203 pM, hM_2 was 179 pM, hM_3 was 1010 pM, hM_4 was 362 pM, hM_5 was 723 pM. Values are mean ± SEM ($n = 3$). The binding affinities (K_i) for arecoline (**4**) and carbachol (**24**) are provided in Ref. 6.

observed changes were modest for the compounds examined.

Two additional compounds were evaluated in this study. We tested **22**, a bicyclic amine in which both the C(3) carboalkoxy and the C(4) hydroxy groups in **1** were altered. Our initial study showed that structural changes can be independently made in these two sites without significant changes in binding. We observed that the binding affinities of **22** for the muscarinic receptors were similar to the methyl enol ether **5** and slightly better than the allyloxycarbonyl derivative **6**. Compound **23** is the *endo*-isomer of *exo*-**9**. We learned that *exo*-**1** bound to the five muscarinic receptors between 5- and 50-fold greater than *endo*-**2** did.[6] A similar structural dependency of C(8) stereochemistry on bioactivity held for the isomer C(4) enol ethers **9** and **23** (Fig. 2, panel B).

Previous work from our lab has demonstrated that derivatives of 8-substituted aza-bridged [3.3.1]-bicyclic amine act as antagonists at all muscarinic receptor subtypes when assessed by functional assays.[6] To

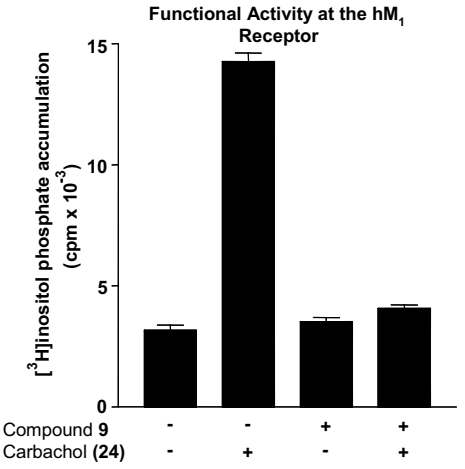

Figure 4. Quantification of functional activity at the human M_1 receptor. The accumulation of [^{3}H]inositol phosphates in COS-7 cells expressing the M_1 receptor was measured in response to 100 μM **24** and/or 100 μM compound **9**. The data shown are the result of an experiment performed in triplicate.

further support this finding with the current series of compounds, we tested the structurally divergent analogue, compound **9**. As shown in Figure 4, the known agonist **24** promoted a greater then 4-fold increase in [^{3}H]inositol phosphate accumulation in hM_1 transfected COS-7 cells. Compound **9**, however, did not stimulate [^{3}H]inositol phosphate accumulation at 100 μM concentration. In fact, 100 μM compound **9** inhibited the action of 100 μM **24** to nearly unstimulated levels. Thus, compound **9** and almost certainly the other analogues in this series act as antagonists at the muscarinic receptor.

6. Conclusion

exo-C(8)-Substituted 1-azabicyclo[3.3.1]non-3-enes have been shown to bind to muscarinic receptors with higher affinities than either **4** or **24**. Synthetic strategies were developed to modify five sites within this scaffold. Seventeen compounds were evaluated in competition binding assays against hM_1–hM_5 muscarinic receptors, and they provided useful guidelines for future studies. Modification of either the C(3) alkoxycarbonyl or C(4) enol units in **1** gave compounds with high affinity for muscarinic receptors. Correspondingly, deleting the C(3) alkoxycarbonyl unit (series C), replacing the C(8) ether group (series D), alkylating the N(1) position (series E), and incorporating a C(8) *endo*-substituent (i.e., **23**) led to compounds with decreased binding affinities. These findings indicate that maintenance of both the acetylcholine-like and the arecoline-like pharmacophores within C(8)-substituted 1-azabicyclo[3.3.1]non-3-enes are necessary for maximal binding affinity at muscarinic receptors. Future studies will be directed toward optimizing the C(8) ether substituent and then systematically evaluating the effects of the arecoline-like pharmacophore C(3) carboxy and C(4) alkoxy substituents on binding affinity and receptor selectivity.

7. Experimental

7.1. General methods

FT-IR spectra were run on a Mattson Galaxy Series FT-IR 5000 spectrometer. ^{1}H and ^{13}C NMR were recorded on Varian VXR 300 and Bruker DRX 500 NMR instruments. Low- and high-resolution (CI) mass spectral investigations were conducted at the University of Texas at Austin by Dr. M. Moini. The low-resolution mass spectra were run on a Finnegan MAT-TSQ-70 instrument and the high-resolution mass spectra were recorded on a Micromass ZAB-E spectrometer. Microanalyses were provided by Atlantic Microlab, Inc. (Norcross, GA).

7.1.1. Synthesis of *exo*-8-benzyloxymethyl-3-methoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (5). A mixture of **1** (33 mg, 0.1 mmol), MeOH (61 μL, 1.5 mmol), and 4-(dimethylamino)pyridine (12 mg, 0.1 mmol) were dissolved in anhydrous toluene (4 mL), and then 0.50 g of molecular sieves (4 Å) was added to the vessel. The mixture was heated to reflux (36 h), cooled to room temperature, and then filtered. The filtrate was concentrated in vacuo and the residue was purified by PTLC (2.5% MeOH–$CHCl_3$) to give methyl ester **5** (30 mg, 96%) as a colorless oil: $R_f = 0.63$ (5% MeOH–$CHCl_3$); IR (neat) 2935, 2865, 1656, 1447, 1360, 1300, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44–1.48 (m, 1H), 1.70–1.83 (m, 3H), 2.18 (br s, 1H), 2.76 (d, $J = 13.4$ Hz, 1H), 3.01–3.05 (m, 1H), 3.08 (d, $J = 13.4$ Hz, 1H), 3.33 (d, $J = 16.7$ Hz, 1H), 3.52 (dd, $J = 7.2$, 9.3 Hz, 1H), 3.66 (dd, $J = 6.6$, 9.3 Hz, 1H), 3.77 (s, 3H), 3.91 (d, $J = 16.7$ Hz, 1H), 4.53 (d, $J = 12.3$ Hz, 1H), 4.60 (d, $J = 12.3$ Hz, 1H), 7.26–7.35 (m, 5H), 11.80 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 19.4, 22.7, 31.7, 46.3, 51.3, 52.0, 60.4, 70.7, 73.2, 98.6, 127.6, 127.7 (2C), 128.4 (2C), 138.3, 171.1, 171.9 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 318 $[M+1]^+$; M_r (+CI) 318.17084 $[M+1]^+$ (calcd for $C_{18}H_{24}NO_4$, 318.17053). Anal. Calcd for $C_{18}H_{23}NO_4 \cdot 0.1H_2O$: C, 67.74; H, 7.29; N, 4.39. Found C, 67.81; H, 7.58; N, 4.23.

7.1.2. Synthesis of *exo*-8-benzyloxymethyl-3-allyloxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (6). With the same procedure employed for the preparation of **5**, **1** (43 mg, 0.13 mmol), allyl alcohol (177 μL, 2.60 mmol), and 4-(dimethylamino) pyridine (16 mg, 0.13 mmol) gave **6** (18 mg, 40%) as an oil after PTLC purification (2.5% MeOH–$CHCl_3$): $R_f = 0.69$ (5% MeOH–$CHCl_3$); IR (neat) 2934, 2865, 1728, 1654, 1452, 1367, 1292, 1207 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.49 (m, 1H), 1.70–1.79 (m, 3H), 2.19 (br s, 1H), 2.77 (d, $J = 13.8$ Hz, 1H), 2.99–3.03 (m, 1H), 3.08 (d, $J = 13.8$ Hz, 1H), 3.37 (d, $J = 17.0$ Hz, 1H), 3.53 (dd, $J = 7.5$, 9.3 Hz, 1H), 3.66 (dd, $J = 6.6$, 9.3 Hz, 1H), 3.94 (d, $J = 17.0$ Hz, 1H), 4.54 (d, $J = 12.5$ Hz, 1H), 4.60 (d, $J = 12.5$ Hz, 1H), 4.68 (d, $J = 5.4$ Hz, 2H), 5.26 (dd, $J = 2.2$, 10.5 Hz, 1H), 5.34 (dd, $J = 2.2$, 17.4 Hz, 1H), 5.91–6.00 (m, 1H), 7.26–7.36 (m, 5H), 11.78 (s, 1H); ^{13}C

NMR ($CDCl_3$, 75 MHz) 19.5, 22.8, 31.7, 46.3, 52.1, 60.4, 64.7, 70.8, 73.2, 98.8, 118.0, 127.6, 127.7 (2C), 128.4 (2C), 132.0, 138.4, 170.5, 172.4 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 344 $[M+1]^+$; M_r (+CI) 344.18650 $[M+1]^+$ (calcd for $C_{20}H_{26}NO_4$, 344.18618). Anal. Calcd for $C_{20}H_{25}NO_4 \cdot 0.3H_2O \cdot 0.3\ C_6H_5CH_3$: C, 70.51; H, 7.50; N, 3.72. Found C, 70.26, H, 7.89, N, 3.45.

7.1.3. Synthesis of *exo*-8-benzyloxymethyl-3-propargyloxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (7). With the same procedure employed for the preparation of **5**, methyl ester **5** (53 mg, 0.17 mmol) and propargyl alcohol (194 mg, 3.34 mmol) gave **7** (17 mg, 30%) following PTLC purification (EtOAc/hexanes = 3/1): $R_f = 0.46$ (EtOAc/hexanes = 3/1); IR (neat) 2926, 2859, 1659, 1622, 1453, 1370, 1290, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25–1.40 (m, 1H), 1.71–1.79 (m, 3H), 2.22 (br s, 1H), 2.50–2.52 (m, 1H), 2.78 (d, $J = 13.8$ Hz, 1H), 3.01–3.05 (m, 1H), 3.11 (d, $J = 13.8$ Hz, 1H), 3.38 (d, $J = 16.7$ Hz, 1H), 3.56 (dd, $J = 7.5$, 9.0 Hz, 1H), 3.67 (dd, $J = 6.6$, 9.0 Hz, 1H), 4.54 (d, $J = 12.5$ Hz, 1H), 4.60 (d, $J = 12.5$ Hz, 1H), 4.77–4.79 (m, 2H), 7.27–7.37 (m, 5H), 11.62 (br s, 1H); MS (+CI) 342 $[M+1]^+$; M_r (+CI) 342.16989 $[M+1]^+$ (calcd for $C_{20}H_{24}NO_4$, 342.17053).

7.1.4. Synthesis of *exo*-8-benzyloxymethyl-3-hexyloxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (8). An anhydrous toluene solution (2.1 mL) of **1** (43 mg, 0.13 mmol), hexyl alcohol (0.49 mL, 3.90 mmol), and 1-hydroxy-3-chlorotetrabutyldistannoxane (7 mg, 0.013 mmol) was heated to reflux (48 h). The solution was concentrated in vacuo and the residue was purified by PTLC (2.5% MeOH–$CHCl_3$) to give hexyl ester **8** (18 mg, 35%) as a brown oil: $R_f = 0.77$ (5% MeOH–$CHCl_3$); IR (neat) 2931, 1652, 1292, 1207 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, $J = 6.6$ Hz, 3H), 1.26–1.47 (m, 7H), 1.62–1.82 (m, 5H), 2.18 (br s, 1H), 2.76 (d, $J = 13.5$ Hz, 1H), 3.01–3.05 (m, 1H), 3.07 (d, $J = 13.5$ Hz, 1H), 3.33 (d, $J = 16.8$ Hz, 1H), 3.52 (dd, $J = 7.5$, 9.3 Hz, 1H), 3.66 (dd, $J = 6.9$, 9.3 Hz, 1H), 3.91 (d, $J = 16.8$ Hz, 1H), 4.16 (t, $J = 6.6$ Hz, 2H), 4.54 (d, $J = 12.3$ Hz, 1H), 4.61 (d, $J = 12.3$ Hz, 1H), 7.26–7.36 (m, 5H), 11.86 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 19.7, 22.7, 23.0, 25.8, 28.8, 31.6, 32.0, 46.6, 52.4, 60.6, 64.6, 71.1, 73.4, 99.3, 127.8, 127.9 (2C), 128.6 (2C), 138.7, 171.2, 172.1 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 388 $[M+1]^+$; M_r (+CI) 388.24907 $[M+1]^+$ (calcd for $C_{23}H_{34}NO_4$, 388.24878). Anal. Calcd for $C_{23}H_{33}NO_4$: C, 71.29; H, 8.58; N, 3.61. Found C, 71.39; H, 8.76; N, 3.45.

7.1.5. Synthesis of *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-methoxy-1-azabicyclo[3.3.1]non-3-ene (9). Compound **1** (47 mg, 0.14 mmol), tri-*n*-butylphosphine (53 μL, 0.21 mmol) and MeOH (9 μL, 0.21 mmol) were dissolved in dry benzene (4.7 mL), and then 1,1′-azobis(*N*,*N*-dimethylformamide) (37 mg, 0.21 mmol) was added. The resulting mixture was stirred at room temperature (20 h) and then concentrated in vacuo. The residue was purified by PTLC (EtOAc/hexanes = 3/1) to give **9** (21 mg, 49%) as an oil: $R_f = 0.36$ (5% MeOH–$CHCl_3$); IR (neat) 2934, 1637, 1454, 1275, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.29 (t, $J = 7.2$ Hz, 3H), 1.40–1.49 (m, 1H), 1.61–1.82 (m, 3H), 2.41 (br s, 1H), 2.74 (d, $J = 13.7$ Hz, 1H), 3.02–3.04 (m, 1H), 3.10 (d, $J = 13.7$ Hz, 1H), 3.44 (d, $J = 17.7$ Hz, 1H), 3.52 (dd, $J = 7.4$, 9.5 Hz, 1H), 3.65 (dd, $J = 6.5$, 9.5 Hz, 1H), 3.75 (s, 3H), 3.95 (d, $J = 17.7$ Hz, 1H), 4.19 (q, $J = 7.2$ Hz, 2H), 4.53 (d, $J = 12.3$ Hz, 1H), 4.60 (d, $J = 12.3$ Hz, 1H), 7.27–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.3, 19.0, 22.6, 28.3, 46.9, 54.7, 55.9, 59.8, 60.0, 70.7, 73.1, 108.2, 127.5, 127.6 (2C), 128.3 (2C), 138.4, 163.3, 165.8 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 346 $[M+1]^+$; M_r (+CI) 346.20180 $[M+1]^+$ (calcd for $C_{20}H_{28}NO_4$, 346.20183). Anal. Calcd for $C_{20}H_{27}NO_4 \cdot 0.2H_2O \cdot 0.2C_6H_6$: C, 69.83; H, 7.91; N, 3.83. Found C, 69.78; H, 8.25; N, 3.50.

7.1.6. Synthesis of *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-propargyloxy-1-azabicyclo[3.3.1]non-3-ene (10). With the same procedure employed for the preparation of **9**, **1** (51 mg, 0.15 mmol) and propargyl alcohol (10 μL, 0.17 mmol) gave **10** (6 mg, 11%) following PTLC purification (EtOAc/hexanes = 3/1): $R_f = 0.39$ (EtOAc/hexanes = 3/1); IR (neat) 2926, 1641, 1456 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, $J = 7.2$ Hz, 3H), 1.38–1.46 (m, 1H), 1.66–1.76 (m, 3H), 2.40 (br s, 1H), 2.51 (t, $J = 2.3$ Hz, 1H), 2.75 (d, $J = 13.2$ Hz, 1H), 3.01–3.05 (m, 1H), 3.10 (d, $J = 13.2$ Hz, 1H), 3.46 (d, $J = 17.7$ Hz, 1H), 3.53 (dd, $J = 7.2$, 9.3 Hz, 1H), 3.66 (dd, $J = 6.9$, 9.3 Hz, 1H), 3.98 (d, $J = 17.7$ Hz, 1H), 4.21 (q, $J = 7.2$ Hz, 2H), 4.54 (d, $J = 12.3$ Hz, 1H), 4.59–4.69 (m, 3H), 7.26–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.3, 19.2, 22.5, 29.8, 46.8, 54.8, 57.2, 60.1, 60.2, 70.8, 73.2, 75.6, 78.9, 112.5, 127.6, 127.7 (2C), 128.4 (2C), 138.4, 161.5, 165.5 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 370 $[M+1]^+$; M_r (+CI) 370.20215 $[M+1]^+$ (calcd for $C_{22}H_{28}NO_4$, 370.21083). Anal. Calcd for $C_{22}H_{27}NO_4 \cdot 0.2H_2O$: C, 70.83; H, 7.40; N, 3.76. Found C, 70.98; H, 7.80; N, 3.68.

7.1.7. Synthesis of *exo*-8-benzyloxymethyl-1-azabicyclo-[3.3.1]nonan-4-one (11). To a toluene solution (18 mL) of **1** (149 mg, 0.45 mmol) was added 4-(dimethylamino) pyridine (58 mg, 0.47 mmol), and then the reaction mixture was heated to reflux (3 days). The solution was concentrated in vacuo and the residue was purified by column chromatography (2.5% MeOH–$CHCl_3$) to give **11** (67 mg, 58%) as a colorless oil: $R_f = 0.54$ (5% MeOH–$CHCl_3$); IR (neat) 2937, 2872, 1693, 1645, 1457, 1354, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.58–1.83 (m, 3H), 1.93–2.03 (m, 1H), 2.38 (br s, 1H), 2.39–2.45 (m, 1H), 2.57–2.69 (m, 1H), 2.84 (d, $J = 14.1$ Hz, 1H), 3.14–3.18 (m, 1H), 3.24–3.40 (m, 3H), 3.51 (dd, $J = 7.2$, 9.5 Hz, 1H), 3.64 (dd, $J = 6.6$, 9.5 Hz, 1H), 4.55 (d, $J = 12.3$ Hz, 1H), 4.61 (d, $J = 12.3$ Hz, 1H), 7.25–7.38 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) 21.3, 23.6, 39.8, 44.5, 48.3, 53.5, 58.1, 71.9, 73.1, 127.5 (3C), 128.3

(2C), 138.2, 212.3 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 260 $[M+1]^+$; M_r (+CI) 260.16547 $[M+1]^+$ (calcd for $C_{16}H_{22}NO_2$, 260.16505). Anal. Calcd for $C_{16}H_{21}NO_2 \cdot 0.6H_2O$: C, 71.14; H, 8.28; N, 5.18. Found C, 71.52; H, 8.31; N, 4.85.

7.1.8. Synthesis of (*E*)-*exo*-8-benzyloxymethyl-1-azabicyclo[3.3.1]nonan-4-one, *O*-methyloxime (12) and (*Z*)-*exo*-8-benzyloxymethyl-1-azabicyclo[3.3.1]nonan-4-one, *O*-methyloxime (13). Compound **11** (54 mg, 0.21 mmol) and *O*-methoxylamine hydrochloride (18 mg, 0.21 mmol) were dissolved in MeOH (2.5 mL), and then triethylamine (30 μL, 0.21 mmol) was added and the reaction was maintained at room temperature (24 h). The reaction solution was evaporated in vacuo and the residue was purified by PTLC (4% MeOH–$CHCl_3$) to give *E*-isomer **12** (29 mg, 48%) and *Z*-isomer **13** (15 mg, 25%).

Compound 12: yellow oil: $R_f = 0.45$ (5% MeOH–$CHCl_3$); IR (neat) 2941, 1642, 1458, 1275 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.56–1.66 (m, 2H), 1.77–1.85 (m, 1H), 1.96–2.02 (m, 1H), 2.35 (br s, 1H), 2.37–2.41 (m, 1H), 2.78 (d, $J = 17.0$ Hz, 1H), 2.99 (dd, $J = 5.6$, 17.0 Hz, 1H), 3.13–3.21 (m, 4H), 3.50 (dd, $J = 7.4$, 9.3 Hz, 1H), 3.62 (dd, $J = 6.6$, 9.3 Hz, 1H), 3.83 (s, 3H), 4.53 (d, $J = 12.5$ Hz, 1H), 4.60 (d, $J = 12.5$ Hz, 1H), 7.28–7.35 (m, 5H), the ^1H NMR assignments were consistent with the COSY and the NOESY spectra; ^{13}C NMR ($CDCl_3$, 125 MHz) 22.2, 24.1, 26.8, 32.9, 48.8, 52.5, 58.0, 61.2, 71.7, 73.2, 127.6, 127.7 (2C), 128.4 (2C), 138.4, 160.3 ppm, the assignments were consistent with the DEPT and the HMQC spectra; MS (+CI) 289 $[M+1]^+$; M_r (+CI) 289.191 33 $[M+1]^+$ (calcd for $C_{17}H_{25}N_2O_2$, 289.191 60). Anal. Calcd for $C_{17}H_{24}N_2O_2 \cdot 0.18CH_3OH$: C, 70.15; H, 8.41; N, 9.52. Found C, 70.45; H, 8.49; N, 9.14.

Compound 13: yellow oil: $R_f = 0.36$ (5% MeOH–$CHCl_3$); IR (neat) 2935, 2866, 1626, 1458, 1285 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.56–1.61 (m, 1H), 1.68–1.71 (m, 1H), 1.78–1.86 (m, 1H), 1.92–1.99 (m, 1H), 2.31 (dd, $J = 5.5$, 16.0 Hz, 1H), 2.59–2.63 (m, 1H), 2.65 (d, $J = 16.0$ Hz, 1H), 3.07–3.28 (m, 4H), 3.22 (br s, 1H), 3.49 (dd, $J = 7.3$, 9.3 Hz, 1H), 3.60 (dd, $J = 6.5$, 9.3 Hz, 1H), 3.80 (s, 3H), 4.53 (d, $J = 12.3$ Hz, 1H), 4.59 (d, $J = 12.3$ Hz, 1H), 7.27–7.36 (m, 5H), the ^1H NMR assignments were consistent with the COSY and the NOESY spectra; ^{13}C NMR ($CDCl_3$, 125 MHz) 21.7, 24.1, 27.3, 28.6, 48.2, 54.0, 57.7, 61.1, 72.2, 73.2, 127.6, 127.7 (2C), 128.4 (2C), 138.4, 161.0 ppm, the assignments were consistent with the DEPT and the HMQC spectra; MS (+CI) 289 $[M+1]^+$; M_r (+CI) 289.19129 $[M+1]^+$ (calcd for $C_{17}H_{25}N_2O_2$, 289.19160). Anal. Calcd for $C_{17}H_{24}N_2O_2 \cdot 0.15\ CH_3OH$: C, 70.26; H, 8.40; N, 9.55. Found C, 70.49; H, 8.55; N, 9.23.

7.1.9. Synthesis of *exo*-8-hydroxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (14). A solution of **1** (320 mg, 0.97 mmol) in $CHCl_3$ (29 mL) and MeOH (3 mL) was hydrogenated over 10% Pd–C (96 mg) under 20 atm of H_2 (20 h). The mixture was filtered, and the filtrate was evaporated in vacuo. The residue was purified by column chromatography (10% MeOH–$CHCl_3$) to give **14** (186 mg, 80%) as a semisolid: $R_f = 0.29$ (5% MeOH–$CHCl_3$); IR (neat) 2936, 2873, 1656, 1365, 1299, 1209 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.12–1.17 (m, 1H), 1.32 (t, $J = 7.2$ Hz, 3H), 1.73–1.89 (m, 3H), 2.23 (br s, 1H), 2.71 (d, $J = 13.4$ Hz, 1H), 2.90–2.97 (m, 1H), 3.03 (d, $J = 13.4$ Hz, 1H), 3.21–3.32 (m, 1H), 3.31 (d, $J = 16.5$ Hz, 1H), 3.73 (d, $J = 10.5$ Hz, 1H), 3.92 (d, $J = 16.5$ Hz, 1H), 4.24 (q, $J = 7.2$ Hz, 2H), 11.90 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 19.2, 22.2, 31.5, 44.7, 51.6, 60.0, 60.8, 62.7, 96.9, 170.1, 170.8 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 242 $[M+1]^+$; M_r (+CI) 242.13882 $[M+1]^+$ (calcd for $C_{12}H_{20}NO_4$, 242.13923). Anal. Calcd for $C_{12}H_{19}NO_4 \cdot 0.9\ H_2O$: C, 55.97; H, 8.14; N, 5.44. Found C, 56.19; H, 7.79; N, 5.28.

7.1.10. Synthesis of *exo*-8-acetoxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (15). To a mixture of **14** (30 mg, 0.13 mmol) in CH_2Cl_2 (3 mL) was successively added triethylamine (19 μL, 0.13 mmol) and acetyl chloride (9 μL, 0.13 mmol). The solution was stirred at room temperature (3 h), and then concentrated to dryness. The residue was purified by PTLC (2.5% MeOH–$CHCl_3$) to give **15** (22 mg, 62%) as a colorless oil: $R_f = 0.64$ (5% MeOH–$CHCl_3$); IR (neat) 2938, 1737, 1655, 1370, 1294, 1211 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.30–1.37 (m, 1H), 1.32 (t, $J = 7.2$ Hz, 3H), 1.76–1.86 (m, 3H), 2.09 (s, 3H), 2.22 (br s, 1H), 2.78 (d, $J = 13.7$ Hz, 1H), 3.03–3.07 (m, 1H), 3.12 (d, $J = 13.7$ Hz, 1H), 3.32 (d, $J = 16.5$ Hz, 1H), 3.89 (d, $J = 16.5$ Hz, 1H), 4.12–4.33 (m, 4H), 11.88 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 19.5, 21.0, 22.7, 31.6, 46.1, 52.1, 59.4, 60.3, 64.5, 99.0, 170.9, 171.0, 171.8 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 284 $[M+1]^+$; M_r (+CI) 284.14991 $[M+1]^+$ (calcd for $C_{14}H_{22}NO_5$, 284.14980). Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94. Found C, 59.70; H, 7.78; N, 4.80.

7.1.11. Synthesis of *exo*-8-(2′,2′-diphenyl)propionoxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]-non-3-ene (16). To a mixture of **14** (30 mg, 0.13 mmol) in CH_2Cl_2 (3 mL) was successively added triethylamine (19 μL, 0.13 mmol) and a solution of 2,2-diphenylpropionyl chloride (32 mg, 0.13 mmol) in CH_2Cl_2 (0.2 mL). The solution was stirred at room temperature (6 h), and then concentrated to dryness. The residue was purified by PTLC (2.5% MeOH–$CHCl_3$) to give **16** (31 mg, 54%) as a colorless oil: $R_f = 0.77$ (5% MeOH–$CHCl_3$); IR (neat) 2936, 1728, 1655, 1453, 1369, 1294, 1210 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.11–1.23 (m, 1H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.59–1.73 (m, 3H), 1.93 (s, 3H), 2.14 (br s, 1H), 2.68 (d, $J = 13.7$ Hz, 1H), 2.95–2.98 (m, 1H), 3.02 (d, $J = 13.7$ Hz, 1H), 3.25 (d, $J = 16.8$ Hz, 1H), 3.81 (d, $J = 16.8$ Hz, 1H), 4.18 (dd, $J = 7.8$, 10.5 Hz, 1H), 4.22 (q, $J = 7.2$ Hz, 2H), 4.43 (dd, $J = 6.8$ Hz,

10.5 Hz, 1H), 7.21-7.32 (m, 10 H), 11.85 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 19.0, 22.5, 27.1, 31.5, 46.1, 52.0, 56.6, 59.1, 60.2, 65.3, 98.8, 126.7 (2C), 127.9 (2C), 128.0 (2C), 128.1 (2C), 128.2 (2C), 144.3, 144.4, 170.8, 171.9, 174.8 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 450 $[M+1]^+$; M_r (+CI) 450.22854 $[M+1]^+$ (calcd for $C_{27}H_{32}NO_5$, 450.22805). Anal. Calcd for $C_{27}H_{31}NO_5 \cdot 0.3H_2O$: C, 71.28; H, 7.00; N, 3.08. Found C, 71.48; H, 7.26, N, 2.76.

7.1.12. Synthesis of *exo*-8-ethylaminocarbonyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo [3.3.1]non-3-ene (17). To a mixture of **14** (34 mg, 0.14 mmol) in CH_2Cl_2 (3.4 mL) was added ethyl isocyanate (56 μL, 0.71 mmol). The solution was stirred at room temperature (13 h), and then the solution was concentrated in vacuo. The residue was purified by PTLC (5% MeOH–$CHCl_3$) to give **17** (38 mg, 88%) as a colorless oil: $R_f = 0.52$ (5% MeOH–$CHCl_3$); IR (neat) 2943, 1650, 1531, 1294, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.14 (t, $J = 7.2$ Hz, 3H), 1.21–1.26 (m, 1H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.76–1.84 (m, 3H), 2.22 (br s, 1H), 2.78 (d, $J = 13.5$ Hz, 1H), 3.02–3.04 (m, 1H), 3.13–3.27 (m, 3H), 3.32 (d, $J = 17.0$ Hz, 1H), 3.90 (d, $J = 17.0$ Hz, 1H), 4.09–4.19 (m, 2H), 4.23 (q, $J = 7.2$ Hz, 2H), 4.83 (br s, 1H), 11.88 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 15.2, 19.4, 22.7, 31.6, 35.9, 45.9, 52.1, 59.8, 60.2, 64.5, 99.0, 156.4, 170.9, 171.8 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 313 $[M+1]^+$; M_r (+CI) 313.17609 $[M+1]^+$ (calcd for $C_{15}H_{25}N_2O_5$, 313.17635). Anal. Calcd for $C_{15}H_{24}N_2O_5 \cdot 0.3H_2O$: C, 56.70; H, 7.80; N, 8.82. Found C, 56.86; H, 7.88; N, 8.56.

7.1.13. Synthesis of *exo*-8-phenylaminocarbonyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo [3.3.1]non-3-ene (18). With the same procedure employed for the preparation of **17**, **14** (32 mg, 0.13 mmol) and phenyl isocyanate (72 μL, 0.66 mmol) gave **18** (18 mg, 37%) as a semisolid after PTLC purification (EtOAc/hexanes = 3/1): $R_f = 0.37$ (EtOAc/hexanes = 3/1); IR (neat) 2935, 1725, 1655, 1610, 1540, 1448, 1299, 1215 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, $J = 7.2$ Hz, 3H), 1.36–1.39 (m, 1H), 1.83–1.95 (m, 3H), 2.24 (br s, 1H), 2.80 (d, $J = 13.5$ Hz, 1H), 3.03–3.12 (m, 1H), 3.18 (d, $J = 13.5$ Hz, 1H), 3.34 (d, $J = 16.8$ Hz, 1H), 3.92 (d, $J = 16.8$ Hz, 1H), 4.23 (q, $J = 7.2$ Hz, 2H), 4.20–4.27 (m, 1H), 4.38 (dd, $J = 8.7$ Hz, 1H), 6.86 (s, 1H), 7.06 (t, $J = 7.2$ Hz, 1H), 7.27–7.39 (m, 4 H), 11.89 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 19.5, 22.8, 31.6, 45.7, 52.0, 59.7, 60.3, 64.7, 99.0, 118.6, 123.4 (2C), 129.0 (2C), 137.8, 153.4, 170.8, 171.7 ppm; MS (+CI) 361 $[M + 1]^+$; M_r (+CI) 361.17646 $[M+1]^+$ (calcd for $C_{19}H_{25}N_2O_5$, 361.17635). Anal. Calcd for $C_{19}H_{24}N_2O_5 \cdot 1H_2O$: C, 60.31; H, 6.93; N, 7.40. Found C, 60.45; H, 6.71; N, 7.13.

7.1.14. Synthesis of *exo*-8-chloromethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo [3.3.1]non-3-ene (19). To a mixture of **14** (25 mg, 0.10 mmol) in CH_2Cl_2 (2.5 mL) was added Et_3N (17 μL, 0.12 mmol) and methanesulfonyl chloride (17 μL, 0.22 mmol). The reaction solution was stirred at room temperature (24 h), and then concentrated in vacuo. The residue was purified by PTLC (5% MeOH–$CHCl_3$) to give **19** (20 mg, 74%) as an oil: $R_f = 0.65$ (EtOAc/hexanes = 3/1); IR (neat) 2939, 1652, 1293, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32 (t, $J = 7.2$ Hz, 3H), 1.58–1.67 (m, 2H), 1.78–1.86 (m, 2H), 2.22 (br s, 1H), 2.78 (d, $J = 13.7$ Hz, 1H), 2.98–3.02 (m, 1H), 3.02 (d, $J = 13.7$ Hz, 1H), 3.33 (d, $J = 16.8$ Hz, 1H), 3.60 (dd, $J = 8.1$, 11.0 Hz, 1H), 3.73 (dd, $J = 6.9$, 11.0 Hz, 1H), 3.89 (d, $J = 16.8$ Hz, 1H), 4.23 (q, $J = 7.2$ Hz, 2H), 11.89 (s, 1H); MS (+CI) 260 $[M+1]^+$; M_r (+CI) 260.10522 $[M+1]^+$ (calcd for $C_{12}H_{19}ClNO_3$, 260.10535).

7.1.15. Synthesis of *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-methyl-1-azabicyclo[3.3.1]non-3-ene iodide (20). To a diethyl ether solution (1.6 mL) of **1** (33 mg, 0.1 mmol) was added dropwise iodomethane (19 μL, 0.3 mmol). The reaction mixture was stirred at room temperature (30 h) and then the precipitate was filtered, and washed with diethyl ether. The solid was dried to give **20** (30 mg, 63%) as a yellow solid: mp 143–146 °C; $R_f = 0.15$ (10% MeOH–$CHCl_3$); IR (neat) 1641, 1267, 1229 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.26 (t, $J = 7.2$ Hz, 3 H), 1.60–1.63 (m, 1H), 1.87–2.13 (m, 3H), 2.86 (br s, 1H), 3.23 (s, 3 H), 3.30–3.42 (m, 1H), 3.52 (d, $J = 12.6$ Hz, 1H), 3.77–4.34 (m, 7 H), 4.55–4.62 (m, 2H), 7.32–7.41 (m, 5H), 11.64 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) 14.0, 19.8, 21.1, 32.4, 52.6, 55.8, 60.9, 61.3, 68.5, 70.0, 72.2, 94.0, 127.5, 127.6, 127.7, 128.3 (2C), 137.4, 167.5, 168.0 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 346 $[M-I]^+$; MS (–CI) 345 $[(M-I)-1]^-$; M_r (+CI) 346.20275 $[M-I]^+$ (calcd for $C_{20}H_{28}NO_4$, 346.20183). Anal. Calcd for $C_{20}H_{28}INO_4$: C, 50.75; H, 5.96; N, 2.96. Found C, 50.45; N, 6.05; N, 2.84.

7.1.16. Synthesis of *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-ethyl-1-azabicyclo[3.3.1]non-3-ene trifluoromethanesulfonate (21). To a dry $CHCl_3$ solution (1.5 mL) of **1** (33 mg, 0.10 mmol) was added ethyl trifluoromethanesulfonate (16 μL, 0.12 mmol) and the reaction mixture was stirred at room temperature (6 h). The solution was evaporated in vacuo, and the residue was triturated with diethyl ether (10 mL). The precipitate was filtered and dried to give **21** (39 mg, 77%) as a hygroscopic solid: $R_f = 0.19$ (10% MeOH–$CHCl_3$); IR (neat) 2989, 1728, 1677, 1643, 1460, 1369, 1263 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32 (t, $J = 7.2$ Hz, 3H), 1.41 (t, $J = 6.6$ Hz, 3H), 1.77–1.80 (m, 1H), 1.93–2.21 (m, 3H), 2.80 (br s, 1 H), 3.21–3.38 (m, 1H), 3.47–3.76 (m, 4H), 3.87–4.29 (m, 6H), 4.54 (d, $J = 11.7$ Hz, 1H), 4.59 (d, $J = 11.7$ Hz, 1H), 7.27–7.35 (m, 5H), 11.99 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 7.5, 13.9, 20.5, 22.4, 33.1, 57.2, 58.7, 61.7, 61.8, 67.4, 69.2, 73.7, 93.8, 128.1, 128.3, 128.5, 128.6 (2C), 136.4, 168.2, 168.8 ppm, the assignments were consistent with the DEPT spectrum; MS (+CI) 360 $[M–OSO_2CF_3]^+$; MS (–CI) 509 $[M]^-$; M_r (–CI) 509.16823 $[M]^-$ (calcd for $C_{22}H_{30}F_3NO_7S$,

509.16951). Anal. Calcd for $C_{22}H_{30}F_3NO_7S{\cdot}0.5H_2O$: C, 50.96; H, 6.02; N, 2.70. Found C, 50.84; H, 6.01; N, 2.64.

7.1.17. Synthesis of *exo*-8-benzyloxymethyl-3-allyloxycarbonyl-4-methoxy-1-azabicyclo[3.3.1]non-3-ene (22). Using the same procedure employed for the preparation of **9** and using **6** (52 mg, 0.15 mmol) and MeOH (31 μL, 0.76 mmol) gave **22** (6 mg, 11%) following PTLC purification (EtOAc/hexanes = 1/1): $R_f = 0.37$ (5% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.55–1.66 (m, 2H), 1.85–1.89 (m, 2H), 2.53 (br s, 1H), 2.83 (d, $J = 12.3$ Hz, 1H), 3.10–3.11 (m, 1H), 3.22 (d, $J = 12.3$ Hz, 1 H), 3.52 (d, $J = 17.9$ Hz, 1H), 3.60–3.70 (m, 2H), 3.78 (s, 3H), 4.07 (d, $J = 17.9$ Hz, 1H), 4.55 (d, $J = 12.2$ Hz, 1H), 4.60 (d, $J = 12.2$ Hz, 1H), 4.65 (d, $J = 5.4$ Hz, 2H), 5.23 (dd, $J = 1.8$, 10.5 Hz, 1H), 5.35 (dd, $J = 1.8$, 17.0 Hz, 1H), 5.90–6.07 (m, 1H), 7.25-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) 18.9, 22.4, 28.1, 46.7, 54.2, 55.8, 60.3, 64.6, 70.4, 73.2, 90.8, 117.7, 127.6, 127.7 (2C), 128.4 (2C), 132.5, 138.1, 161.1, 163.8 ppm; MS (+CI) 358 $[M+1]^+$; M_r (+CI) 358.20181 (calcd for $C_{21}H_{28}NO_4$ 358.20134).

7.1.18. Synthesis of *endo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-methoxy-1-azabicyclo[3.3.1]non-3-ene (23). With the same procedure employed for the preparation of **9**, **2** (66 mg, 0.2 mmol) and MeOH (16 μL, 0.4 mmol) gave **23** (15 mg, 22%) following PTLC purification (3% MeOH–$CHCl_3$): $R_f = 0.33$ (5% MeOH–$CHCl_3$); IR (neat) 2934, 2862, 1701, 1632, 1454, 1369, 1268, 1207 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, $J = 7.2$ Hz, 3H), 1.38–1.41 (m, 2H), 1.80–1.85 (m, 2H), 2.48 (br s, 1H), 3.04–3.14 (m, 3H), 3.35 (dd, $J = 5.4$, 9.6 Hz, 1H), 3.48 (d, $J = 18.0$ Hz, 1H), 3.51 (s, 3H), 3.52 (dd, $J = 7.5$, 9.6 Hz, 1H), 3.62 (d, $J = 18.0$ Hz, 1H), 4.18 (q, $J = 7.2$ Hz, 2H), 4.56 (s, 2H), 7.27–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.3, 21.3, 27.6, 28.4, 45.1, 53.8, 55.7, 59.7, 61.5, 71.8, 73.2, 108.3, 127.6, 127.7 (2C), 128.3 (2C), 138.2, 163.7, 165.7 ppm; MS (+CI) 346 $[M+1]^+$; M_r (+CI) 346.20170 $[M+1]^+$ (calcd for $C_{20}H_{28}NO_4$, 346.20183). Anal. Calcd for $C_{20}H_{27}NO_4{\cdot}0.3H_2O$: C, 68.47; H, 7.93; N, 3.99. Found C, 68.48; H, 8.25; N, 4.21.

7.1.19. Pharmacological analyses. hM_1–hM_5 Expression in COS-7 cells and membrane preparation. COS-7 cells were subcultured in 150 mm dishes to a density of 20,000 cells/cm^2. The cells were transfected with Fugene 6 transfection reagent (according to Roche Molecular Biochemical's specifications) and pcDNA3.1 DNA containing sequence coding for the hM_1, hM_2, hM_3, hM_4, or hM_5 receptor (approximately 20 μg DNA/150 mm dish). Cell lysates were harvested after 24 h by scraping the cells and sonicating in buffer (5 mM Tris (pH 7.5), 1 mM $MgCl_2$, and protease inhibitors). Membranes were isolated by differential centrifugation and resuspended in freezing buffer (20 mM HEPES (pH 8.0), 250 mM sucrose, 0.1 mM EDTA, and protease inhibitors). Binding assays were carried out as described below.

7.1.20. hM_1–hM_5 Binding assay. Competition binding assays were performed essentially as described[28] in a volume of 2 mL using 10 μL membrane (50–100 μg of protein), 200 pM [^{3}H]QNB (approx. 20,000 cpm/assay), and various concentrations of competing agents in binding buffer (20 mM HEPES (pH 7.5), 150 mM NaCl, 3 mM $MgCl_2$, and 1 mM EDTA). Incubations were for 90 min at 30 °C. The assays were terminated by addition of 4 mL of wash buffer (20 mM Tris (pH 7.5), 150 mM NaCl, and 2 mM $MgCl_2$), quick filtration over Whatman GF/A filters, and two washes with 4 mL of wash buffer. The filters were placed into scintillation vials with 5 mL of scintillation fluid and radioactivity quantitated.

7.1.21. Inositol phosphate accumulation assay. COS-7 cells were subcultured in 12-well culture dishes to a density of 20,000 cells/cm^2. The cells were transfected with Fugene 6 transfection reagent (according to manufacturer's specifications) and pcDNA3.1 DNA containing sequence coding for hM_1 receptor (approximately 20 μg DNA/150 mm dish). Approximately 24 h after the addition of DNA and transfection agent, the inositol lipid pool of cells was radiolabeled by incubating the cells overnight in inositol-free DMEM medium containing 1 μCi of *myo*-[^{3}H]inositol per well. Approximately 12 h post-labeling, [^{3}H]inositol accumulation was initiated by addition of LiCl (final concentration of 10 mM) and either vehicle or the indicated drug concentration. After 30 min, the assay was terminated by aspirating the medium and adding 750 μL of ice-cold 50 mM formic acid. The samples were neutralized 30 min later with 250 μL of 150 mM NH_4OH. [^{3}H]Inositol phosphates were isolated by ion exchange chromatography on Dowex AG 1-X8 columns and quantified by liquid scintillation counting.

7.1.22. Statistical analysis. Nonlinear regression and paired *t*-test were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

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References and notes

1. (a) Sternbach, L. H.; Kaiser, S. *J. Am. Chem. Soc.* **1952**, *74*, 2215; (b) Sternbach, L. H.; Kaiser, S. *J. Am. Chem. Soc.* **1952**, *74*, 2219.
2. Orlek, B. S.; Blaney, F. E.; Brown, F.; Clark, M. S. G.; Hadley, M. S.; Hatcher, J.; Riley, G. J.; Rosenberg, H. W.;

Wadsworth, H. J.; Wayman, P. *J. Med. Chem.* **1991**, *34*, 2726.
3. Mitch, C. H.; Brown, T. J.; Bymaster, F. P.; Calligaro, D. O.; Dieckman, D.; Merrit, L.; Peters, S. C.; Quimby, S. J.; Shannon, H. E.; Shipley, L. A.; Ward, J. S.; Hansen, K.; Olesen, P. H.; Sauerberg, P.; Sheardown, M. J.; Swedberg, M. D. B.; Suzdak, P.; Greenwood, B. *J. Med. Chem.* **1997**, *40*, 538.
4. Tecle, H.; Barrett, S. D.; Lauffer, D. J.; Augelli-Szafran, C.; Brann, M. R.; Callahan, M. J.; Caprathe, B. W.; Davis, R. E.; Doyle, P. D.; Eubanks, D.; Lipinski, W.; Mirzadegan, T.; Moos, W. H.; Moreland, D. W.; Nelson, C. B.; Pavia, M. R.; Raby, C.; Schwarz, R. D.; Spencer, C. J.; Thomas, A. J.; Jaen, J. C. *J. Med. Chem.* **1998**, *41*, 2524.
5. Greenlee, W.; Clader, J.; Asberom, T.; McCombie, S.; Ford, J.; Guzik, H.; Kozlowski, J.; Li, S.; Liu, C.; Lowe, D.; Vice, S.; Zhao, H.; Zhou, G.; Billard, W.; Binch, H.; Crosby, R.; Duffy, R.; Lachowicz, J.; Coffin, V.; Watkins, R.; Ruperto, V.; Strader, C.; Taylor, L.; Cox, K. *Il Farmaco* **2001**, *56*, 247.
6. Kim, M. G.; Bodor, E. T.; Wang, C.; Harden, T. K.; Kohn, H. *J. Med. Chem.* **2003**, *46*, 2216.
7. (a) Brown, J. H.; Taylor, P. A. In *The Pharmacological Basis of Therapeutics*; Hardman, J. G., Limbird, L. E., Eds.; 9th ed.; McGraw-Hill: New York, 1996; pp 141–160, Chapter 7; (b) Cannon, J. G. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; 5th ed.; John Wiley and Sons: New York, 1996; pp 3–58, Chapter 25; (c) Sastry, V. R. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; 5th ed.; John Wiley and Sons: New York, 1996; pp 59–117, Chapter 26; (d) Latin, D. L. In *Principles of Medicinal Chemistry*; Foye, W. O., Lemke, T. L., Williams, D. A., Eds.; 4th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 1995; pp 321–344. Chapter 17.
8. Felder, C. C.; Bymaster, F. P.; Ward, J.; DeLapp, N. *J. Med. Chem.* **2000**, *43*, 4333.
9. (a) Bräuner-Osborne, H.; Ebert, G.; Brann, M. R.; Falch, E.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1995**, *38*, 2188; (b) Krogsgaard-Larsen, P.; Falch, E.; Sauerberg, P.; Freedman, S. B.; Lembøl, H. L.; Meier, E. *Trends Pharmacol. Sci.* **1988**, *Suppl*, 69–74.
10. Moser, U.; Lambrecht, G.; Wagner, M.; Wess, J.; Mutschler, E. *Br. J. Pharmacol.* **1989**, *96*, 319.
11. Messer, W. S., Jr.; Abuh, Y. F.; Ryan, K.; Shepherd, M. A.; Schroeder, M., et al. *Drug Dev. Res.* **1997**, *40*, 171.
12. Augelli-Szafran, C. E.; Blankley, C. J.; Jaen, J. C.; Moreland, D. W.; Nelson, C. B.; Penvose-Yi, J. R.; Schwarz, R. D.; Thomas, A. J. *J. Med. Chem.* **1999**, *42*, 356.
13. Bromridge, S. M.; Brown, F.; Cassidy, F.; Clark, M. S. G.; Dabbs, S.; Hadley, M. S.; Hawkins, J.; Loudon, J. M.; Naylor, C. B.; Orlek, B. S.; Riley, G. J. *J. Med. Chem.* **1997**, *40*, 4265.
14. Ringdahl, B.; Ehler, F. J.; Jenden, D. *J. Mol. Pharmacol.* **1982**, *21*, 594.
15. Leader, H.; Wolfe, A. D.; Chiang, P. K.; Gordon, R. K. *J. Med. Chem.* **2002**, *45*, 902.
16. Sabb, A. L.; Stein, R. P.; Vogel, R. L.; Tasse, R.; Amburn, S.; Fairman, D. K.; Malhorta, D.; Boast, C. A.; Bartolomeo, A.; Morris, H.; Sailer, T.; Moyer, J. A.; Abou-Gharbia, M.; Ho, D. M. *Drug Dev. Res.* **1997**, *40*, 185.
17. Tumiatti, V.; Angeli, P.; Andrisano, V.; Bolognesi, M. L.; Cavalli, A.; Marucci, G.; Minarini, A.; Recanatini, M.; Rosini, M.; Melchiorre, C. *Bioorg. Med. Chem.* **2000**, *8*, 681.
18. Gilbert, J. C.; Kelly, T. A. *J. Org. Chem.* **1988**, *53*, 449.
19. (a) Otera, J.; Danoh, N.; Nozaki, H. *J. Org. Chem.* **1991**, *56*, 5307; (b) Otera, J. *Chem. Rev.* **1993**, *93*, 1449.
20. Tsunoda, T.; Yamamiya, Y.; Ito, S. *Tetrahedron Lett.* **1993**, *34*, 1639.
21. Hawkes, G. E.; Herwig, K.; Roberts, J. D. *J. Org. Chem.* **1974**, *39*, 1017.
22. Biemann, K. In *Tables of Spectral Data for Structural Determination of Organic Compounds*; Fresenius, W., Huber, J. F. K., Pungor, E., Rechnitz, G. A., Simon, W., West, Th. S., Eds.; 2nd ed.; Springer: Berlin, 1989; p H75.
23. Ref. 21, pp C90–C97.
24. Birdsall, N. J. M. Muscarinic Acetylcholine Receptors. In *The IUPHAR Compendium of Receptor Characterization and Classification*; Girdlestone, D., Ed.; IUPHAR Media Ltd: London, 2000; pp 54–63.
25. Bolden, C.; Cusack, B.; Richelson, E. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 576.
26. Eglen, R. M.; Choppin, A.; Dillon, M. P.; Hedge, S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 426.
27. (a) Wang, Y.; Chackalamannil, S.; Hu, Z.; Greenlee, W.; Clader, J.; Boyle, C.; Kaminski, J.; Billard, W.; Binch, H., III; Croslby, G.; Ruperto, V.; Duffy, R.; Cohen-Williams, M.; Coffin, V.; Cox, K.; Grotz, D.; Lachowicz, J. *J. Med. Chem.* **2002**, *45*, 5415; (b) Kozlowski, J. A.; Zhou, G.; Tagat, J. R.; Lin, S.; McCombie, S. W.; Ruperto, V.; Duffy, R.; McQuade, R. A.; Crosby, G., Jr.; Taylor, L. A.; Billard, W.; Binch, H., III; Lachowicz, J. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 791; (c) Wang, Y.; Chackalamannil, S.; Hu, Z.; McKittrick, B.; Ruperto, V.; Duffy, R. A.; Lachowicz, J. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1087, and references therein.
28. Evans, T.; Smith, M. M.; Tanner, L. I.; Harden, T. K. *Mol. Pharmacol.* **1984**, *26*, 395.