Discovery of Novel 1-Azoniabicyclo[2.2.2]octane Muscarinic Acetylcholine Receptor Antagonists

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Received December 18, 2008

A novel 4-hydroxyl(diphenyl)methyl substituted quinuclidine series was discovered as a very promising class of muscarinic antagonists. The structure−activity relationships of the connectivity of the diphenyl moiety to the quinuclidine core and around the ring nitrogen side chain are described. Computational docking studies using an homology model of the M_3 receptor readily explained the observed structure−activity relationship of the various compounds. Compound **14o** was identified as a very potent, slowly reversible M_3 antagonist with a very long in vivo duration of bronchoprotection.

Introduction

Muscarinic acetylcholine receptors (mAChRs[a]) belong to the superfamily of G-protein coupled 7-transmembrane (TM) receptors. Five subtypes of mAChRs, termed M_1-M_5, have been identified to-date.[1,2] The mAChRs are widely distributed in mammalian organs where they mediate many of the vital functions.[1−3] In the lungs, mAChRs have been localized to smooth muscle in the trachea and bronchi, the submucosal glands, and the parasympathetic ganglia.[4] The three subtypes of mAChRs, which are known to exert their physiological effect in the lungs through the action of the native ligand, acetylcholine, are the M_1, M_2, and M_3 receptors.[4] The M_3 mAChRs are located on the airway smooth muscle and also on the pulmonary submucosal glands where they mediate muscle contraction and mucus secretion respectively.[5,6] The M_2 mAChRs, which make up the majority of the cholinergic receptor population on airway smooth muscle, are also located on postganglionic parasympathetic nerves,[7] where their role is autoinhibitory, to provide tightly regulated control of acetylcholine release. M_1 mAChRs are found in the pulmonary parasympathetic ganglia where they function to facilitate neurotransmission.[8]

mAChR dysfunction in the lungs has been noted in a variety of different pathophysiological states.[9] In particular, in asthma and chronic obstructive pulmonary disease (COPD), inflammatory conditions lead to loss of inhibitory M_2 mAChR autoreceptor function on parasympathetic nerves supplying the pulmonary smooth muscle, causing increased acetylcholine release following vagal nerve stimulation.[10] This mAChR dysfunction results in airway hyperreactivity and hyperresponsiveness mediated by increased stimulation of M_3 mAChRs, which in turn lead to the constriction of the airways. Thus mAChR antagonists, particularly of the M_3 subtype, are useful therapeutics in mAChR-mediated disease states and particularly COPD. Inhaled anticholinergic agents approved for the treatment of COPD include ipratropium bromide, oxitropium bromide, and more recently tiotropium bromide.[11] Ipratropium and oxitropium have relatively short durations of bronchodilation (4−8 h), whereas tiotropium has a duration of action of over 24 h, making it suitable for once-daily treatment.[12] As part of a general strategy to develop new respiratory products, our goal was to discover novel long-acting muscarinic antagonists.

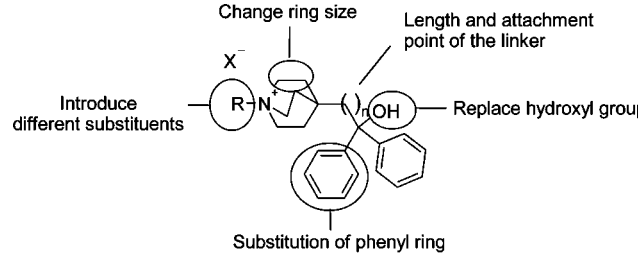

Figure 1. General Strategy.

In previous communications, our research team disclosed several series of mAChR antagonists, which act as potent and highly efficacious muscarinic antagonists.[13,14] To provide some structural diversity in our investigation, we turned our attention to compounds possessing a [2.2.2] bicyclic core (Figure 1). Such molecules would possess the key pharmacophoric features for muscarinic activity, i.e., a positive center and two aromatic groups, connected together via an alkyl linker. The [2.2.2] quinuclidine ring is a known muscarinic pharmacophore,[15,16] providing a certain degree of confidence that this approach could succeed. As outlined in Figure 1, our general strategy was primarily based on the exploration of the length and connectivity point of the linker as well as the chemical space around the aromatic moieties and the N substituents. Other areas of investigation were the ring size and the replacement of the hydroxyl group. In the course of our study, the azabicyclic derivatives were evaluated for their muscarinic affinity using recombinant muscarinic M_1, M_2, and M_3 receptors. Some selected compounds were also further investigated in a methacholine-induced bronchoconstriction model in the mouse. In this paper, we report the synthesis, structure−activity relationship (SAR) at the M_3 receptor, and pharmacological evaluation of this new series of muscarinic antagonists.

Chemistry

The preparation of the 3-substituted azabicyclic derivatives is depicted in Scheme 1. The commercially available 3-quinu-

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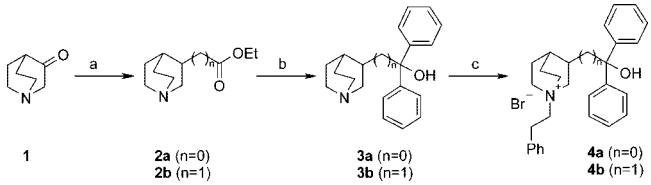
[a] Abbreviations: mAChR, muscarinic acetycholine receptor; TM, transmembrane; COPD, chronic obstructive pulmonary disease; SAR, structure−activity relationship; ACh, acetylcholine; CHO, Chinese hamster ovary; FLIPR, fluorometric imaging plate reader; SPA, scintillation proximity assay; Penh, enhanced pause; i.n., intranasal; GPCR, G-protein coupled receptor; ECL, extracellular loop; MLR, multiple linear regression, NQ, not quantifiable; ESI, electrospray ionization; HRMS, high resolution mass spectrum; SD, steepest descent; ABNR, adopted basis Newton−Raphson; LC, liquid chromatography.

10.1021/jm801601v CCC: $40.75
Published on Web 03/24/2009

Scheme 1. Preparation of the C-3 Substituted Derivatives[a]



[a] Reagents and conditions: (a) See ref 15; (b) PhMgBr (4−5 equiv), THF; (c) $PhCH_2CH_2Br$, CH_3CN, $CHCl_3$.

clidinone **1** was transformed into the ethyl ester **2b** ($n = 1$) in four steps following procedures previously described in the literature.[17] Further condensation of **2b** with phenylmagnesium bromide afforded the tertiary alcohol **3b**. N-Alklyation of **3b** with phenethyl bromide gave the corresponding quinuclidium quaternary salt **4b**. In a similar fashion to **2b**, the commercially available ester **2a** ($n = 0$) was processed to the corresponding tertiary amine **3a** and quaternary ammonium **4a**.

As shown in Scheme 2, the 4-subsitututed azabicyclic derivatives were obtained from the commercially available ethyl 4-piperidinecarboxylate precursor **5** in four synthetic steps. Compound **5** was reacted sequentially with 1-bromo-2-chloroethane in the presence of potassium carbonate and then with LDA in THF to give the quinuclidine intermediate **8**. Subsequent condensation of **8** with an organometallic reagent resulted in the formation of the tertiary alcohol **10**, which could be further processed to the nitrile derivative **12** by treatment with TMSCN and $AlCl_3$. N-Alkylation of **10** and **12** with 2-bromoethyl phenylmethyl ether resulted in the formation of the corresponding quaternary salts **14o** and **16**. A similar synthetic route was also used for the preparation of the [2.2.1] derivatives **13** and **15** from the ester derivative **7**, which could be prepared from the spiro derivative **6** by following procedures previously described in the literature.[18] Compounds from Tables 2−4 were prepared in an analogous manner to **14o** but replacing 2-bromoethyl phenylmethyl ether with the appropriate alkylating agents (**14a**−**n**, **14p**−**r**, Table 2 and **17a**−**o**, Table 3) or PhMgBr with the appropriate organometallic reagent (**18a**−**e**, Table 4).

Pharmacology

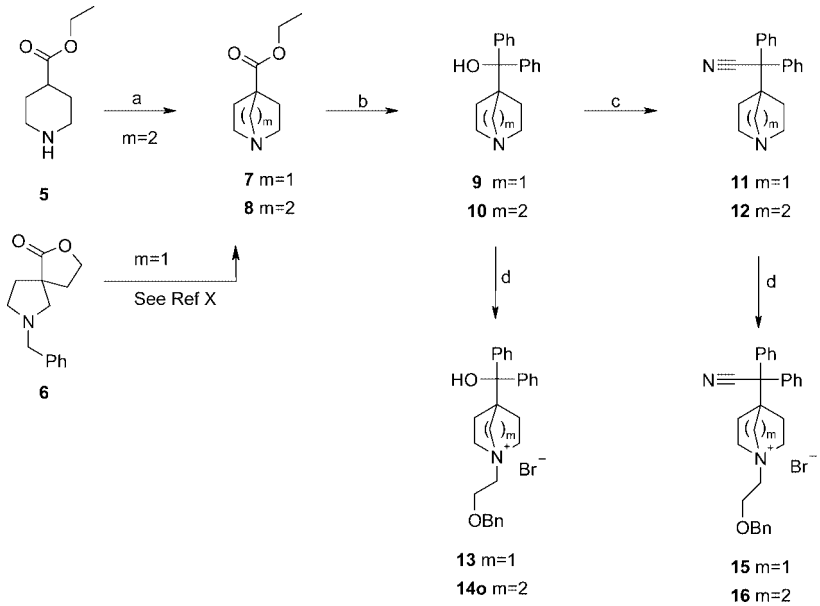
The functional activities and potencies of the synthesized compounds were evaluated by calcium mobilization assays to determine inhibition of acetylcholine(Ach)-induced receptor-activation at cloned human M_3 receptors expressed in Chinese hamster ovary (CHO) cells. The inhibitory potency of a compound was evaluated at a single ACh concentration to determine its IC_{50}. When a compound IC_{50} was lower than 10 nM, antagonist potency was further quantified with a pA_2 by measuring the ratio of the M_3 EC_{50} in the presence and absence of the compound.[19] The functional reversibility of antagonist blockade of the receptor was evaluated by a similar methodology following 90 min incubation with vehicle, 1.0, 10, or 100 nM compound, and washout of vehicle or compound for 180 min using 18 buffer changes. Following washout, cells were stimulated with ACh and calcium mobilization was measured by fluorometric imaging plate reader (FLIPR). Antagonist binding was also assessed in radioligand binding assays conducted using cell membrane preparations from CHO cells expressing human M_1, M_2, or M_3 receptors in a scintillation proximity assay (SPA) with 0.5 nM [^{3}H]-*N*-methyl scopolamine as the ligand. The binding potency was determined as a K_i. Functional activity of antagonists was determined in human bronchial tissues from primary, secondary, or tertiary airways obtained at autopsy, cut into strips, and then connected to force-displacement transducers suspended in standard organ baths. Tissue strips were preincubated with the test compound for 2 h and then incubated with increasing concentrations of carbachol. The mechanical responses were recorded isometrically, and the potencies of the compounds were determined as pA_2 values. To assess reversibility and to calculate the off-rate, the antagonists were allowed to equilibrate with the bronchial strips until they reached maximum inhibition of carbachol-induced contraction. At this point, antagonists were removed from the perfusate and tissues were superfused with buffer containing carbachol with tension recovery measured over time. The results are expressed as the time in minutes for 50% reversal of antagonist blockade of carbachol-induced contraction (off t_{50}). The in vivo efficacy of the compounds was determined by their airway responsiveness to methacholine in a mouse plethysmography model. Barometric plethysmography was used to measure enhanced pause (Penh), a unitless measure that has been shown to correlate with the changes in airway resistance that occur during bronchial challenge with methacholine.[20] Mice were pretreated intranasally (i.n.) with a solution of the compound in 50 μL of vehicle (10% DMSO) and were then placed in the plethysmography chamber a given amount of time following drug administration. For potency determination, a dose response to a given compound was performed, and all Penh measurements were taken 15 min following i.n. drug administration. For duration of action determination, Penh measurements were taken anywhere from 15 min to 96 h following i.n. drug administration.

Results and Discussion

To determine the most prominent SAR features (attachment point and length of the linker, N substitution) around the quinuclidine ring, we first proceeded with a rapid examination of the series of analogues shown in Table 1. The hydroxyl-(diphenyl)methyl quinuclidine derivatives **3a**, **10**, and **19** exhibited $IC_{50} > 1000$ nM at the M_3 receptor. In contrast, the homoanalogue **3b**, which incorporated an extra methylene spacer between the diaryl moiety and the quinuclidine ring, was 7 fold more potent than **3a**. A homology model of the M_3 muscarinic receptor was constructed using the X-ray structure of bovine rhodopsin as a template[21] and will be used throughout this paper to provide a posterior rationalization of our results. Although several more recent GPCR structures have been solved by crystallography, no improvement has been observed with these over the use of rhodopsin as a template. In particular, it seems unlikely that the helical segment found in the second extracellular loop of the β-2 receptor structure will be common to other family members. Docking of the three compounds **3a**, **10**, and **19** suggested that a common mode of binding existed for the hydroxyl(diphenyl)methyl group, which mainly consisted of a strong hydrogen bond between the hydroxyl hydrogen and $ser^{7:46}$. The phenyl rings of this group sit in a hydrophobic pocket lined by $met^{2:58}$, $ile^{2:53}$, $phe^{6:44}$, and $tyr^{7:43}$ (Figure 2a).[22]

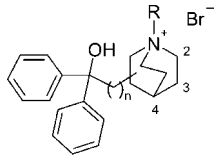
With an N-unsubstituted quinuclidine, the protonated nitrogen tends to form highly directional hydrogen bonds or salt bridges. This is reflected in a localized positive electrostatic potential as shown in Figure 3, left. The expected binding site, which is the conserved (in aminergic G-protein coupled receptors (GPCRs)) $asp^{3:32}$ residue in TM3, already has three hydrogen bonds from $ser^{2:57}$, $tyr^{7:39}$, and $tyr^{7:43}$. In addition to breaking these hydrogen bonds upon formation of a salt bridge with the quinuclidine nitrogen, the three compounds cannot readily adopt a conformation where the NH is orientated toward the aspartate without

Scheme 2. Preparation of the C-4 Substituted Derivatives[a]



[a] Reagents and conditions: (a) 1-bromo-2-chloroethane, K_2CO_3, acetone; then LDA, THF; (b) PhLi (2.5 eq), THF; (c) $AlCl_3$, TMSCN, DCE, 85 °C; (d) $PhCH_2CH_2Br$, CH_3CN, $CHCl_3$.

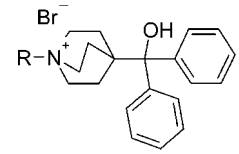
Table 1. Structure and M_3 Activity of Initial Leads



no.	attachment point	*n*	R	M_3 potency IC_{50}[a] (nM)
19	2	0		6599
3a	3	0		1104
10	4	0		1068
3b	3	1		145
4a	3	0	2-phenylethyl	2824
4b	3	1	2-phenylethyl	122
14a	4	0	2-phenylethyl	87

[a] Values are the mean of two or more independent assays.

Table 2. M_3 Activity of Quinuclidinium Salts **14a−s**



		M_3 activity	
no.	R	IC_{50}[a] (nM)	pA_2
14a	$-(CH_2)_2Ph$	87	
14b	−Me	935	
14c	$-CH_2$-^cPr	1956	
14d	−Et	104	
14e	−Pr	208	
14f	−Bu	135	
14g	$-(CH_2)_2OMe$	30	
14h	$-(CH_2)_2OPh$	<10	9.1
14i	$-(CH_2)_2NH_2$	850	
14j	$-(CH_2)_2OH$	486	
14k	$-(CH_2)_3OPh$	<10	9.4
14l	$-(CH_2)_4OPh$	9.5	8.0
14m	$-(CH_2)_3OH$	29509	
14n	$-(CH_2)_3OMe$	829	
14o	$-(CH_2)_2OCH_2Ph$	<10	10.5
14p	$-(CH_2)_4Ph$	<10	9.3
14q	$-(CH_2)_5OPh$	306	

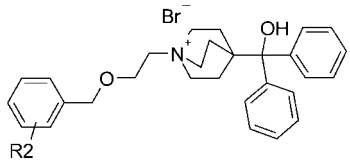
[a] Values are the mean of two or more independent assays.

disrupting the binding of the hydroxyl(diphenyl)methyl group. Compound **3b**, however, can dock reasonably close to the aspartate although tyr$^{7:39}$ and tyr$^{7:43}$ must rotate out of the way to accommodate the quinuclidine ring. The increased binding energy of the salt bridge in **3b** is reflected in its increased potency as shown in Table 1. Formation of a quaternary center causes the positive charge to diffuse around the hydrogens of the methylene groups adjacent to the nitrogen (Figure 3, right).

Quaternary compounds therefore favor nondirectional electrostatic interactions, especially of the $\pi-$cation type. In compound **14a**, the attachment of a phenethyl chain onto the N atom of the quinuclidine ring led to a significant increase in potency compared to the N-unsubstituted compound **10**. In contrast, the *N*-phenethyl derivatives **4a** and **4b** were in the same potency range as their respective parent compounds **3a** and **3b**. Docking of **14a** into the M_3 receptor model showed good $\pi-$cation interactions with trp$^{6:48}$, tyr$^{6:51}$, and tyr$^{7:39}$. The quaternary center also formed nonspecific electrostatic interactions with asp$^{3:32}$ and ser$^{3:36}$ (Figure 2b). The hydroxyl(diphenyl)methyl group docked in the same pocket as before with the hydrogen bond to ser$^{7:46}$. What was particularly interesting was that the terminal phenethyl chain sat in a hydrophobic pocket bounded by leu$^{3:29}$, val$^{6:55}$, tyr$^{3:33}$, and a phenylalanine, phe$^{4:79}$, in the extracellular loop 2 (ECL2) domain. There was a particularly good $\pi-\pi$ stacking interaction with tyr$^{3:33}$. These results suggested that further exploration of the SAR around the pending chain of 4-hydroxy(diphenyl)methyl substituted quinuclidium salts had the potential to lead to further potency increase. Such C_3 symmetric 4-substitued quinuclidines would also possess the added advantages to be achiral entities and more readily accessible synthetically. Consequently, we focused our attention primarily on this series of molecules.

Table 2 shows the SAR of a series of analogues in which various pending chains were attached to the quinuclidine nitrogen. Compounds with small alkyl chains (**14b−f**) were less potent than the phenethyl analog **14a**. This is in agreement with

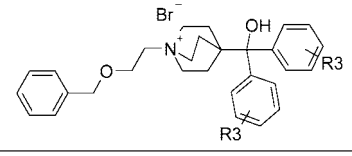
Table 3. Effect of Substitution of the Benzyl Moiety on M_3 Activity



		M_3 activity	
no.	R2	IC_{50} (nM)[a]	pA_2
14o	H	<10	10.5
17a	3-OMe	<10	8.7
17b	4-^{t}Bu	39	
17c	4-F	<10	10.1
17d	4-Cl	<10	10.2
17e	4-Br	<10	10.4
17f	4-CN	<10	9.4
17g	3-F	<10	10.6
17h	2-CN	5403	
17i	3-Br	<10	9.9
17k	3-Me	<10	10.0
17l	2-Br	22	8.5
17m	4-Me	<10	10.4
17n	2-Me	13	8.4
17o	2-F	<10	10.4

[a] Values are the mean of two or more independent assays.

Table 4. Modification to the Diphenyl Portion of **14o**



		M_3 activity	
no.	R3	IC_{50} (nM)[a]	pA_2
14o	H	<10	10.5
18a	4-F	<10	10.2
18b	3-OMe	17	
18c	3-F	<10	9.6
18d	4-OMe	36	
18e	4-Me	12	

[a] Values are the mean of two or more independent assays.

the docking studies, which showed that these compounds would not have the $\pi-\pi$ stacking interaction with tyr$^{3:33}$. However, the ether derivative, **14g**, was found to be 3-fold more active than **14a**, suggesting than the presence of an oxygen atom on the ring could be beneficial to activity, probably through the acceptance of a hydrogen bond from a nearby residue. The most likely candidates for this were tyr$^{6:51}$ and ser$^{3:36}$. Replacement of the terminal methyl of **14g** with a phenyl ring as in compound **14h** further increases the potency, whereas both the amine **14i** and the alcohol **14j** were found to lose significant activity. On the basis of these initial results, we then examined the sequential introduction of methylene spacers onto the side chain. This would maintain the hydrogen bond to the ether oxygen but would also allow the phenyl ring to pick up additional $\pi-\pi$ interactions with other aromatic residues such as trp$^{7:35}$ or the ECL2 phe$^{4:79}$ higher up in the binding pocket. We found that the maximum chain length between the quinuclidine ring and the phenyl ring was four atoms as exemplified by compounds **14k**, **14o**, and **14p**. In particular, we observed that the benzyloxy derivative **14o** was an order of magnitude more potent at the receptor than **14k** and **14p**, suggesting that the γ-positioning of the oxygen on the chain is optimum. The docking of **14k** and **14o** is shown in parts a and b of Figure 4, respectively. Compound **14k** has hydrogen bonds to the phenoxy oxygen from both tyr$^{6:51}$ and ser$^{3:36}$. This however, has the effect of pulling the terminal phenyl group down from the hydrophobic pocket so that it forms far from ideal $\pi-\pi$ interactions with tyr$^{3:33}$ and phe$^{4:79}$. Also this lowering of the ligand in the pocket results in less favorable $\pi-$cation and other electrostatic interactions around the quaternary center. Compound **14o**, however, shows much better interactions overall. There is only one hydrogen bond between the benzyloxy oxygen and tyr$^{6:51}$,but ser$^{3:36}$ now show a good electrostatic interaction with the quaternary center as does asp$^{3:32}$. The quaternary center also shows $\pi-$cation interactions with trp$^{6:48}$ and tyr$^{7:39}$. Removal of the phenyl ring as in **14m** and **14n** resulted as expected in significant loss of activity, in agreement with what was observed for **14i** and **14j**. The modeling is therefore in excellent agreement with the observed activities.

Next we investigated the effect of substituting the side chain aromatic ring by preparing a series of analogues of **14o** (Table 3). Generally, we found that introducing any group at the ortho position was detrimental to activity (compounds **17h**, **17n**, and **17n**). In fact, with the exception of the 3-fluoro analogue (**17g**), all compounds had a lower pA_2 value than the unsubstituted compound. This would suggest that the effect of substitution was largely steric. A multiple linear regression study (MLR) was performed using a variety of steric parameters (substituent volume, Verloop L and B1 values, ellipsoid volume, etc.), but no satisfactory MLR equations were obtained. Docking studies however did shed some light on these substituent effects. The least potent analogue was the 2-cyano compound (**17h**). Upon docking, this was shown to have a large detrimental effect on the electrostatic interactions around the quinuclidine quaternary center. In particular, asp$^{3:32}$ was forced to rotate away from the quinuclidine's adjacent methylene group and thus form a hydrogen bond with tyr$^{7:43}$. Tyr$^{7:39}$ was also displaced, resulting in a weakening of its $\pi-$cation interaction (Figure 5a). Whereas the loss of potency with compound **17h** was mostly electrostatic, the 4-*tert*-butyl analogue (**17b**) was found to displace the aromatic side chains surrounding the terminal phenyl ring of the ligand (Figure 5b). Thus the *tert*-butyl group itself formed a hydrophobic interaction with trp$^{7:35}$, but the other π-stacking residues, trp$^{3:28}$, tyr$^{7:39}$, phe$^{4:79}$, and tyr$^{3:33}$, were further from the terminal phenyl ring than in the unsubstituted compound **14o**.

As shown in Table 4, we also explored briefly the substitution of the diphenylmethyl portion. The most active compounds of this study were the fluoro substituted derivatives **18a** and **18c**, which were slightly less potent at M_3 than **14o** but significantly more potent than the corresponding methoxy derivatives **18b** and **18d**. These results suggested that substitution of these two aromatic rings was unlikely to lead to a greater potency increase and consequently was not investigated any further. These results are comparable to some previous observations made in a carbamate series developed by our team.[23]

We also explored the effect of substituting the hydroxyl group with a nitrile moiety. As shown in Table 5, this transformation resulted in a marked potency decrease for compound **16**. Finally, the slight reduction of the carbocycle size of **14o** to a [2.2.1] system was a change that was tolerated but also led to a decrease in potency (compounds **13** and **15**).

On the basis of their high potencies at M_3, the pharmacological profiles compounds **14o** and **14k** were further investigated. As shown in Table 6, radioligand binding studies demonstrated that both compounds potently competed with [^{3}H]-*N*-methyl scopolamine binding to any of the three muscarinic receptors

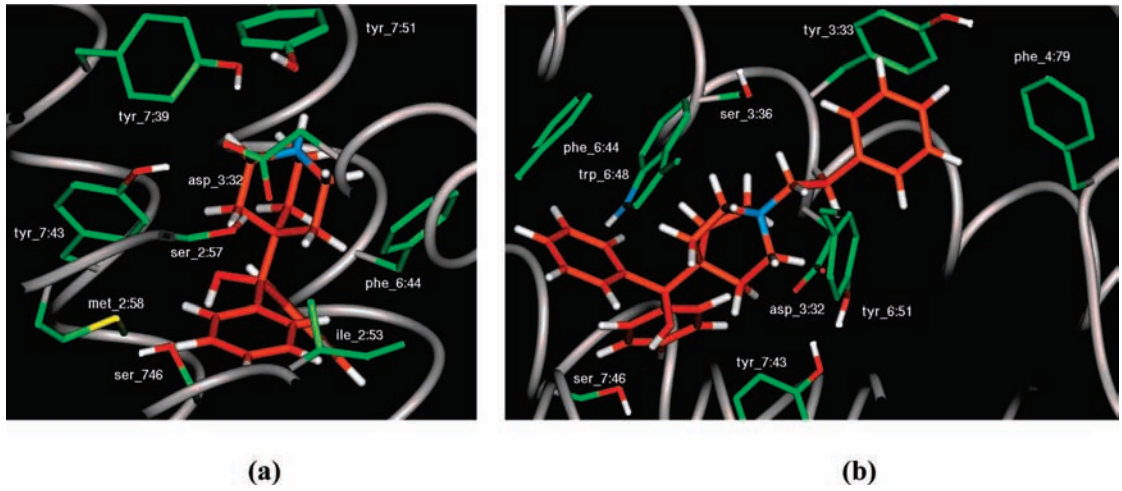


Figure 2. (a) Compound **10** docked in the M_3 receptor model showing the hydrogen bond of the ligand hydroxyl to ser$^{7:46}$ and the hydrophobic interactions between the two phenyl rings and tyr$^{7:43}$. met$^{2:58}$, ile$^{2:53}$, and phe$^{6:44}$. Tyr$^{7:39}$, tyr$^{7:43}$, and ser$^{2:57}$ form hydrogen bonds with asp$^{3:32}$, which as can be seen, cannot approach the quinuclidine NH. Instead, it forms a hydrogen bond with tyr$^{7:51}$. (b) Compound **14a** docking where the hydroxydiphenylmethyl group has the same interactions as compound **10**. The methylene groups adjacent to the nitrogen of the quaternary center are now surrounded by asp$^{3:32}$, ser$^{3:36}$, trp$^{6:48}$, tyr$^{6:51}$, and tyr$^{7:39}$ (not shown). The pendant phenethyl group lies between tyr$^{3:33}$ and phe$^{4:79}$.

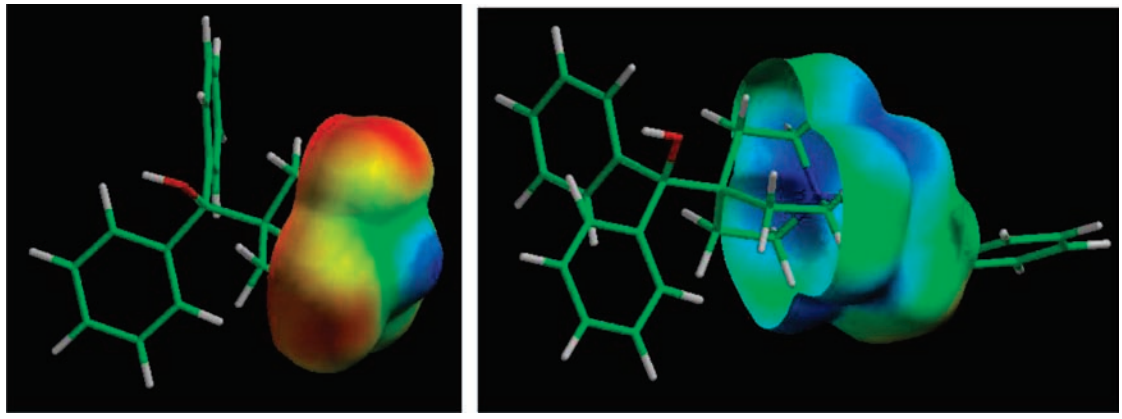


Figure 3. (left) A partial electron density surface around the protonated nitrogen of the quinuclidine in compound **10**. The electrostatic potential plotted on this surface shows a highly localized positive (blue) region around the NH group. (right) In compound **14a**, the same surface surrounding the adjacent methylenes of the phenethyl quaternary salt shows a diffused positive charge surrounding the quinuclidine nitrogen.

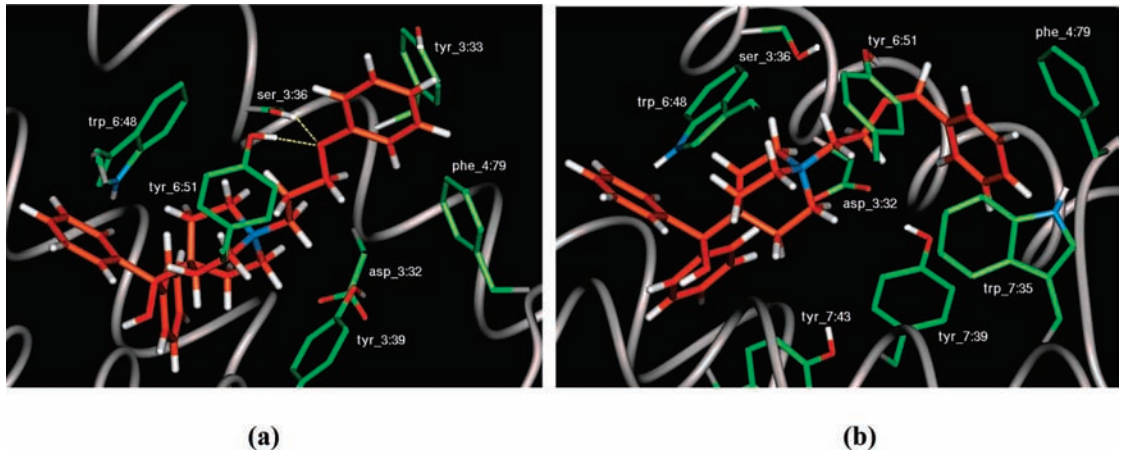


Figure 4. (a) Compound **14k** docked in the M_3 model. There are strong hydrogen bonds between the phenoxy oxygen and ser$^{3:36}$ and tyr$^{6:51}$. Trp$^{6:48}$ is not especially close to the quaternary center, and phe$^{4:79}$ and tyr$^{3:33}$ are not making good interactions with the terminal phenyl ring. (b) Compound **14o** in the M_3 model showing much better interactions with the quaternary center. The benzyloxy oxygen has only one hydrogen bond with tyr$^{6:51}$. There are good $\pi-\pi$ interactions between the terminal phenyl ring and phe$^{4:79}$, trp$^{3:28}$ (not shown), and trp$^{7:35}$.

subtypes (M_1-M_3). At the M_3 receptor, **14o** had a higher affinity (K_i: 0.06 nM) than **14k** (K_i: 0.14 nM). This potency difference is in agreement with the pA_2 values previously discussed. The binding assays also showed that both compounds were pan active muscarinic antagonists with strong affinities with the M_1 and M_2 receptors.

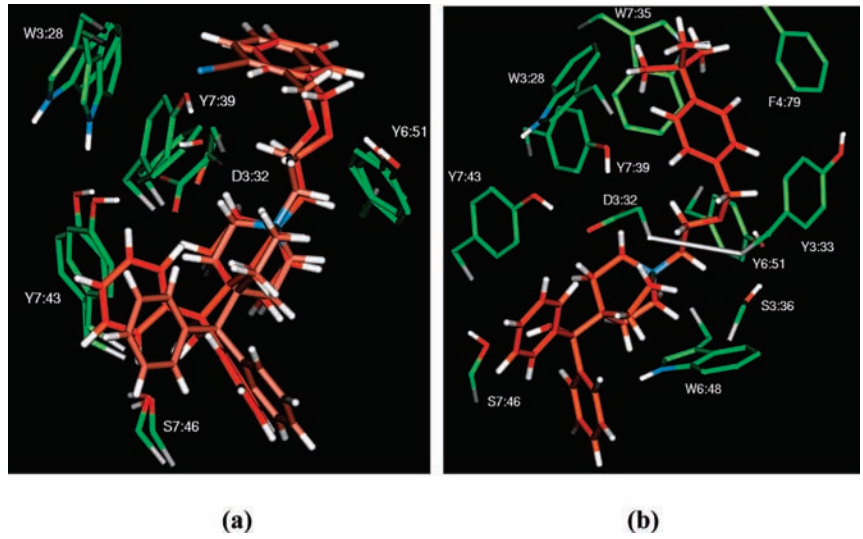


Figure 5. The backbone ribbons have been removed for clarity. (a) the docking of the 2-cyano analogue, **17h** (pink), overlaid with the docking of the unsubstituted compound, **14o** (orange). There is considerable displacement of the residues around the quaternary methylenes, especially $tyr^{7:39}$ and $asp^{3:32}$. The latter in fact is now oriented away from the quinuclidine and forms a strong hydrogen bond with $tyr^{7:43}$. $Trp^{3:28}$ is displaced away from its orthogonal $\pi-\pi$ interaction with the terminal phenyl ring. (b) In the docking of compound **17b**, there is a general weakening of the $\pi-\pi$ interactions around the terminal phenyl ring. The *tert*-butyl group forms a hydrophobic interaction with $trp^{7:35}$ (top middle), while the other π-stacking residues, $trp^{3:28}$, $tyr^{7:39}$, $phe^{4:79}$, and $tyr^{3:33}$, are all displaced further away from the phenyl ring of the ligand.

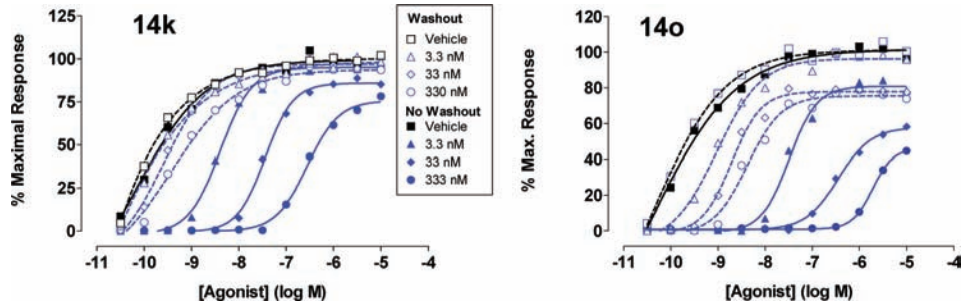


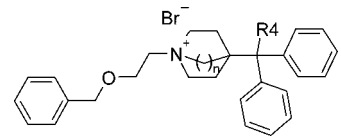
Figure 6. Concentration-dependent shifts in actylcholine concentration response curve after 90 min incubation with **14o** and **14k** and reversibility following a 180 min wash-out at the human M_3 receptor.

Studies to evaluate the reversibility of the compounds at the M_3 receptor were conducted in the FLIPR assay using a 90 min incubation followed by a 180 min washout period. As shown in Figure 6, following the washout, acetylcholine concentration−response curves did not fully reverse to levels obtained in the absence of drug at any concentration of **14o** tested. These data indicate that antagonist blockade of the receptor remained after washout, suggesting that **14o** is slowly reversible at the M_3 receptor. In contrast, compound **14k** was found to be fully reversible under the same conditions.

The compounds were also evaluated against the endogenous receptors in isolated human bronchial tissues strips. In these studies, **14o** and **14k** were potent inhibitors at the endogenous human M_3 receptors of carbachol-induced contractions, with pA_2's of 9.5 and 8.9, respectively. In an additional study, the off-rates from endogenous muscarinic receptors were determined on human tissues superfused with buffer containing carbachol in the presence of antagonist. At 10 nM, **14o** had a off t_{50} of 145 min compared to 61 min for **14k**. Taken together, these in vitro data suggest that the longer off rate of **14o** may be a function of its lower reversibility and higher potency at the M_3 receptor.

As reported in Table 6, the pharmacokinetic profiles of both compounds in the rat were characterized by low oral bioavailability and high plasma clearance. These properties are preferred to optimize the topical efficacy of these agents in the lungs and

Table 5. [2.2.1] and Nitriles



			M_3 activity	
no.	*n*	R4	IC_{50} (nM)[a]	pA_2
14o	2	OH	<10	10.5
16	2	CN	<10	8.5
13	1	OH	<10	9.4
15	1	CN	<10	9.3

[a] Values are the mean of two or more independent assays.

to minimize potential systemic-mediated side effects.[24,25] Considering these characteristics, the compounds were examined in our mouse in vivo model. Following intranasal administration, **14k** and **14o** demonstrated potent inhibition of metacholine-induced bronchoconstriction with respective ED_{50} of 0.08 and 0.02 μg. When dosed at the ED_{80}, the compounds exhibited very long duration of action. Greater than 50% bronchoprotection was observed at 34 h for **14k** and at 48 h for **14o**.

Conclusion

In conclusion, this work has led to the identification of a 4-substituted quinuclidine series as a very promising class of

Table 6. Additional Data on Selected Compound

	binding affinity (K_i)[a]			in vitro tissue studies		mouse in vivo studies		PK properties		
no.	M_1	M_2	M_3	pA_2[b]	off t_{50}[c] (min)	ED_{50}[d] (μg/mouse)	duration[e] (h)	species	Cl[f] (ml/min/kg)	F[f,g] (%)
14k	0.64	0.94	0.13	8.9	57	0.08	34 (0.5 ug)	rat	37	NQ[h]
14o	0.16	0.15	0.06	9.5	145	0.02	>48 (0.05 ug)	rat	92	NQ[h]

[a] Radioligand binding assays were conducted using CHO cell membrane preparations in SPA format vs 0.5 nM [^{3}H]-*N*-methyl scopolamine. Values are the mean of three independent assays. [b] Potency against carbachol-induced contraction in human bronchus. [c] Reversal time for 50% of carbachol-induced contraction to return in human bronchus after incubation to maximum relaxation at [10 nM] antagonist. [d] Potency in conscious mice against MCh-induced bronchoconstriction. [e] Duration of bronchoprotection in conscious mice against MCh-induced bronchoconstriction. Time to 50% loss of maximum protection (dose) [f] Cl: systemic plasma clearance. iv doses: 3.5 mg/kg (**14k**), 0.5 mg/kg (**14o**). [g] F: percent bioavailability. po doses: 7.1 mg/kg (**14k**), 2 mg/kg (**14o**). [h] NQ = no quantifiable plasma levels following oral administration.

muscarinic antagonists. Through optimizing the connectivity of the diphenyl moiety to the quinuclidine core as well as the nature and substitution of the ring nitrogen side chain, compounds **14k** and **14o** were identified as very potent M_3 antagonists. Computational docking studies using a homology model of the M_3 receptor readily explained the observed SAR of the various compounds. Kinetic washout studies at the human M_3 cloned receptor and in human bronchus showed that **14o** had a slower reversibility profile than **14k**, correlating with its higher potency at the receptor. Compound **14o** also displayed longer in vivo activity in a mouse bronchoconstriction model than **14k**. Taken together, these results suggest that **14o** has a better likelihood than **14k** of achieving long duration of bronchodilation in humans. Further pharmacological studies of these compounds will be reported elsewhere.

Experimental Section

Chemistry. All materials and reagents were used as is unless otherwise indicated. Air- or moisture-sensitive reactions were carried out under a nitrogen atmosphere. Flash chromatography was performed using silica gel (EM Science, 230−400 mesh) under standard techniques or using silica gel cartridges (RediSep normal phase disposable flash columns) on an Isco CombiFlash. Preparative and analytical HPLC were carried out on a Gilson 306 HPLC system. Unless otherwise stated, the conditions for the preparative reverse phase HPLC purifications used YMC Combiscreen ODS-A 75 mm × 30 mm (30 mL/min; gradient, (A) acetonitrile, (B) water; 10−80% A during 10 min) with UV detection at 214 nm. The ^{1}H NMR spectra were recorded at 400 MHz using a Bruker Avance 400 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield of tetramethylsilane (δ scale). Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Coupling constants (*J*) are reported in hertz and refer to apparent multiplicities and not true coupling constants. Mass spectra were recorded on an Applied Biosystems MDS Sciex API 150EX single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. High-resolution mass spectral (HRMS) data were determined on a Bruker Daltonics 7T FTICR-MS equipped with an Apollo ESI interface. The purity of the tested compounds not subjected to combustion analysis was determined by liquid chromatography (LC) on a Schimadzu LC system. Unless stated otherwise, the conditions for the LC used an Aquasil C18 40 mm × 1 mm column (flow rate: 300 μL/min, 10% solution A, 90% solution B; A: water with 0.02% TFA; B: acetonitrile with 0.018% TFA, duration 4.2 min) with UV detection at 214 nm. All reported compounds, with the exception of **17b**, possess a purity of at least 95%.

The syntheses and characterizations of intermediates **2b**, **6**, **7**, and test compound **19** have already been reported.[17,18,31]

1-Azabicyclo[2.2.2]oct-3-yl(diphenyl)methanol (3a). A solution of phenylmagnesium bromide (3.0 M in diethyl ether, 1.1 mL, 3.3 mmol) was chilled down to 0 °C under argon. Methyl 1-azabicyclo-[2.2.2]octane-3-carboxylate (**2a**) (0.125 g, 0.74 mmol) in THF (5 mL) was slowly added to the reaction mixture at 0 °C over 30 min. The reaction was allowed to warm up to room temperature, then heated to 60 °C for three and a half-hours and then cooled to room temperature overnight. The reaction was cooled to 0 °C and quenched with saturated NH_4Cl (aqueous) and then evaporated to dryness under vacuum. H_2O and EtOAc were added, and the aqueous phase was further extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by preparatory HPLC to give the TFA salt of the title compound, which was subsequently converted to its free base utilizing saturated K_2CO_3 to give the desired compound **3a** (0.0633 g, 29.3%). LC/MS (ES) *m/z* 294 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.51 (d, J = 7.33 Hz, 2 H), 7.46 (d, J = 7.07 Hz, 2 H), 7.29 (q, J = 8.17 Hz, 4 H), 7.20−7.10 (m, 2 H), 5.76 (s, 1 H), 3.01−2.82 (m, 5 H), 2.80−2.73 (m, 1 H), 2.42−2.31 (m, 1 H), 1.67−1.60 (m, 1 H), 1.53−1.46 (m, 2 H), 1.15−1.26 (m, 1 H). HRMS calcd for ($C_{20}H_{24}NO$) 294.1852, found 294.18499.

2-(1-Azabicyclo[2.2.2]oct-3-yl)-1,1-diphenylethanol (3b). A solution of phenylmagnesium bromide (3.0 M in diethyl ether, 8.5 mL, 25.5 mmol) was chilled down to 0 °C under argon. Ethyl 1-azabicyclo[2.2.2]oct-3-ylacetate (**2b**) (1.0296 g, 5.22 mmol) in THF (10 mL) was slowly added to the reaction mixture at 0 °C over 50 min. The reaction was allowed to warm up to room temperature and then heated to 55 °C overnight. The reaction was cooled to 0 °C, quenched with saturated NH_4Cl (aqueous), and then evaporated to dryness under vacuum. H_2O and EtOAc were added, and the aqueous phase was further extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by preparatory HPLC to give the title compound (0.560 g, 35%). LC/MS (ES) *m/z* 308 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.47−7.41 (m, 4H), 7.30−7.24 (m, 4H), 7.19−7.13 (m, 2H), 5.47 (s, 1H), 2.72−2.50 (m, 5H), 2.48−2.43 (m, 1H), 2.37−2.32 (m, 2H), 1.85−1.65 (m, 2H), 1.52−1.45 (m, 2H), 1.38−1.30 (m, 2H). HRMS calcd for ($C_{21}H_{26}NO$) 308.2009, found 308.2008.

3-[Hydroxy(diphenyl)methyl]-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane Bromide (4a). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-3-yl(diphenyl)-methanol (**3a**) (0.040 g, 0.136 mmol) and (2-bromoethyl)benzene (0.028 mL, 0.204 mmol) in $2CH_3CN/3CHCl_3$ (4.5 mL) were reacted to give the desired product (0.0284 g, 43.5%). LC/MS (ES) *m/z* 398 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.49 (t, J = 6.40 Hz, 4 H), 7.40−7.17 (m, 11 H), 5.73 (s, 1 H), 3.54−3.42 (m, 1 H), 3.40−3.23 (m, 4 H), 3.18 (d, J = 5.02 Hz, 1 H), 3.16−3.06 (m, 1 H), 2.98−2.82 (m, 2 H), 2.63−2.56 (m, 1 H), 2.17−2.01 (m, 2 H), 1.92−1.63 (m, 4 H). HRMS calcd for ($C_{28}H_{32}NO$) 398.2478, found 398.2477.

3-(2-Hydroxy-2,2-diphenylethyl)-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane Bromide (4b). Following the general procedure outlined in **14h**, 2-(1-azabicyclo[2.2.2]oct-3-yl)-1,1-diphenylethanol hydrochloride (**3b**) (0.100 g, 0.291 mmol) (Note: Triethylamine (0.41 mL, 0.291 mmol) was added to the reaction to basify **3b**) and (2-bromoethyl)benzene (0.060 mL, 0.436 mmol) in $2CH_3CN/3CHCl_3$ (1.5 mL) were reacted to give the desired product (0.0558 g, 39.0%). LC/MS (ES) *m/z* 412 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.58−7.45 (m, 4 H), 7.39−7.17 (m, 11 H), 6.19 (s, 1 H), 3.57−3.36 (m, 9 H), 3.08−2.83 (m, 3 H),

2.57−2.52 (m, 2 H), 2.08−1.94 (m, 1 H), 1.88−1.76 (m, 1 H), 1.73 (br s, 1 H), 1.69−1.54 (m, 1 H). HRMS calcd for ($C_{29}H_{34}NO$) 412.2635, found 412.2630.

Ethyl 1-Azabicyclo[2.2.2]octane-4-carboxylate (8). To a solution of ethyl nipecotate (**5**) (20.0 mL, 130 mmol) in acetone (180 mL) was added 1-bromo-2-chloroethane (21.6 mL, 260 mmol) followed by anhydrous K_2CO_3 (27.12 g, 196 mmol). The reaction mixture was stirred for 24 h and then concentrated under vacuum. The resulting residue was treated with H_2O (75 mL) and extracted with Et_2O. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under vacuum. Purification of the crude residue by flash chromatography (50% Et_2O/50% hexane) on silica gel gave ethyl 1-(2-chloroethyl)-4-piperidinecarboxylate (10.99 g, 38.6%). LC/MS (ES) *m*/*z* 220 $(M + H)^+$. A solution of ethyl 1-(2-chloroethyl)-4-piperidinecarboxylate (20.42 g, 92.9 mmol) in THF (600 mL) was cooled to −50 °C under Ar. LDA (2.0 M in heptane/THF/ethyl benzene, 70 mL, 140 mmol) was slowly added to the solution at −50 °C over 25 min. The reaction was warmed up to room temperature over 16 h. The reaction was quenched with K_2CO_3 (saturated aqueous) (500 mL) and extracted with Et_2O (3 × 500 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting orange oil was coevaporated three times with DCM to remove excess ethyl benzene to give the title compound (16.29 g, 95.7%). LC/MS (ES) *m*/*z* 184 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.05 (q, J = 7.07 Hz, 2 H), 2.74 (t, J = 7.58 Hz, 6 H), 1.58 (t, J = 7.58 Hz, 6 H), 1.17 (t, J = 7.07 Hz, 3 H).

1-Azabicyclo[2.2.1]hept-4-yl(diphenyl)methanol (9). A solution of ethyl 1-azabicyclo[2.2.1]heptane-4-carboxylate (**7**) (4 g, 23 mmol) in THF (60 mL) was added dropwise to a solution of phenyllithium (1.5 M in 70% cyclohexane/30% diethyl ether, 63.1 mL, 94.6 mmol) precooled to −30 °C under Ar. The reaction was warmed to RT and stirred for 16 h. The reaction was treated with water and extracted with ethyl acetate. The combined extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. Trituration (diethyl ether) and then filtration afforded the title compound (3.595 g, 54%). LC/MS (ES) *m*/*z* 280 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.53−7.49 (m, 4 H), 7.34−7.22 (m, 6 H), 2.89−2.86 (m, 2 H), 2.76−2.73 (m, 4 H), 1.91−1.86 (m, 2 H), 1.73−1.64 (m, 2 H).

1-Azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (10). A solution of phenyllithium (1.5−1.7 M in 70 cyclohexane/30 ether, 20.0 mL, 32 mmol) was chilled down to −30 °C under Argon. Ethyl 1-azabicyclo[2.2.2]octane-4-carboxylate (**8**) (1.51 g, 8.23 mmol) in THF (20 mL) was slowly added to the reaction mixture at −30 °C over 25 min. The reaction was allowed to warm up to room temperature over 16 h. The reaction was quenched with H_2O and then evaporated to dryness under vacuum. H_2O and EtOAc were added, causing a white solid to crash out. This solid was filtered off to give the title compound (0.79 g). The aqueous phase was further extracted with EtOAc, the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was treated with EtOAc and hexane and filtered to yield more of the title compound (0.67 g). Total yield (1.46 g, 60.7%). LC/MS (ES) *m*/*z* 294 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.53 (d, J = 7.58 Hz, 4 H), 7.27 (t, J = 7.58 Hz, 4 H), 7.21−7.13 (m, 2 H), 5.43 (s, 1 H), 2.73 (d, J = 7.58 Hz, 6 H), 1.65 (d, J = 7.58 Hz, 6 H). Anal. ($C_{20}H_{23}NO$) C, H, N. HRMS calcd for ($C_{20}H_{24}NO$) 294.1852, found 294.1852.

1-Azabicyclo[2.2.1]hept-4-yl(diphenyl)acetonitrile (11). To a suspension of 1-azabicyclo[2.2.1]hept-4-yl(diphenyl)methanol (**9**) (0.906 g, 3.24 mmol) in 1,2-dichloroethane (48 mL) was added $AlCl_3$ (2.028 g, 15.3 mmol). The reaction was allowed to stir for 10 min, and then TMSCN (2.04 mL, 15.3 mmol) was added. The reaction was sealed and heated to 85 °C overnight. The reaction mixture was poured into a separatory funnel containing K_2CO_3 (aq satd) (200 mL) and EtOAc (200 mL). Extraction with EtOAc (3 × 200 mL) was performed. The combined organics were dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was dissolved in DMSO and purified by preparatory HPLC (w/0.1% TFA). The combined fractions were concentrated down under vacuum to remove the CH_3CN. The resulting water layer was basified to pH = 12 with 5 N NaOH and then extracted with EtOAc (3 × 150 mL). The combined fractions were dried over $MgSO_4$, filtered, and concentrated under vacuum to give the title compound (0.53 g, 56.7%). LC/MS (ES) *m*/*z* 289 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.48−7.40 (m, 8 H), 7.40−7.32 (m, 2 H), 2.83−2.76 (m, 2 H), 2.68−2.61 (m, 2 H), 2.57 (br s, 2 H), 1.68−1.61 (m, 4 H).

1-Azabicyclo[2.2.2]oct-4-yl(diphenyl)acetonitrile (12). Following the general procedure outlined in **11**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.3055 g, 1.04 mmol), $AlCl_3$ (0.6675 g, 5.04 mmol), and TMSCN (0.68 mL, 5.10 mmol) in 1,2-dichloroethane (17 mL) were reacted to give the desired product (0.185 g, 59.7%). LC/MS (ES) *m*/*z* 303 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.48 (d, J = 7.81 Hz, 4 H), 7.40 (t, J = 7.55 Hz, 4 H), 7.34 (d, J = 7.30 Hz, 2 H), 2.80 (t, J = 7.55 Hz, 6 H), 1.75 (t, J = 7.55 Hz, 6 H).

4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.1]heptane Bromide (13). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.1]hept-4-yl(diphenyl)methanol (**9**) (50 mg, 0.18 mmol) and 2-bromoethyl phenylmethyl ether (0.04 mL, 0.25 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (32 mg, 36%). LC/MS (ES) *m*/*z* 414 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.44 (d, J = 7.58 Hz, 4 H), 7.42−7.25 (m, 11 H), 6.41 (s, 1 H), 4.53 (s, 2 H), 3.86−3.79 (m, 2 H), 3.72−3.65 (m, 4 H), 3.61−3.54 (m, 4 H), 2.20−2.12 (m, 2 H), 2.04−1.97 (m, 2 H). HRMS calcd for ($C_{28}H_{32}NO_2$) 414.2428, found 414.2429.

4-[Hydroxy(diphenyl)methyl]-1-(2-phenylethyl)-1-azoniabicyclo[2.2.2]octane Bromide (14a). To a solution of 1-azabicyclo-[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0775 g, 0.264 mmol) in CH_3CN/DCM/MeOH (2 mL/2 mL/1 mL) was added (2-bromoethyl)benzene (0.38 mL, 2.78 mmol). The solution was allowed to stir at room temperature for 4 days and then concentrated under vacuum to give a white solid. This residue was dissolved in DMSO and purified by preparatory HPLC to give the title compound (0.0612 g, 48.6%). LC/MS (ES) *m*/*z* 398 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.56 (d, J = 7.51 Hz, 4 H), 7.36−7.23 (m, 11 H), 5.96 (s, 1 H), 3.47 (t, J = 7.49 Hz, 6 H), 3.32−3.28 (m, 2 H), 2.99−2.95 (m, 2 H), 2.03 (t, J = 7.49 Hz, 6 H). HRMS calcd for ($C_{28}H_{32}NO$) 398.2478, found 398.2478.

4-[Hydroxy(diphenyl)methyl]-1-methyl-1-azoniabicyclo[2.2.2]-octane Bromide (14b). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0638 g, 0.217 mmol) and bromomethane (2.0 M in t-butylmethyl ether, 0.250 mL, 0.500 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0739 g, 88.0%). LC/MS (ES) *m*/*z* 308$(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.58 Hz, 4 H), 7.33 (t, J = 7.58 Hz, 4 H), 7.27−7.22 (m, 2 H), 5.96 (s, 1 H), 3.40 (t, J = 7.58 Hz, 6 H), 2.87 (s, 3 H), 2.04−1.93 (m, 6 H). HRMS calcd for ($C_{21}H_{26}NO$) 308.2009, found 308.2008.

1-(Cyclopropylmethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (14c). To a solution of 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0552 g, 0.188 mmol) in $2CH_3CN/3CHCl_3$ (2.5 mL) was added (bromomethyl)-cyclopropane (0.025 mL, 0.257 mmol). The solution was heated at 60 °C for 16 h, cooled down to room temperature, and the solvents were evaporated under vacuum. The residue was taken up in 2.5 mL of DMSO and purified by preparatory HPLC (without TFA) to give the title compound (0.031 9 g, 39.9%). LC/MS (ES) *m*/*z* 348 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.57 (d, J = 8.06 Hz, 4 H), 7.34 (t, J = 7.60 Hz, 4 H), 7.57 (t, J = 7.2 Hz, 2 H), 5.97 (s, 1 H), 3.46 (t, J = 7.20 Hz, 6 H), 3.02 (d, J = 6.80 Hz, 2 H), 2.03 (t, J = 7.20 Hz, 6 H), 1.15−1.04 (m, 1 H), 0.72−0.62 (m, 2 H), 0.48−0.32 (m, 2 H). HRMS calcd for ($C_{24}H_{30}NO$) 348.2322, found 348.2321.

1-Ethyl-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]-octane Bromide (14d). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0581 g, 0.198 mmol) and bromoethane (0.030 mL, 0.402 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product

(0.0434 g, 54.9%). LC/MS (ES) *m/z* 322 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.33 Hz, 4 H), 7.38−7.19 (m, 8 H), 6.98−6.88 (m, 3 H), 5.95 (s, 1 H), 3.99 (t, J = 5.68 Hz, 2 H), 3.38 (t, J = 7.45 Hz, 6 H), 3.12 (br s, 2 H), 2.01 (t, J = 6.95 Hz, 6 H), 1.84−1.65 (m, 4 H). HRMS calcd for ($C_{22}H_{28}NO$) 322.2165, found 322.2164.

4-[Hydroxy(diphenyl)methyl]-1-propyl-1-azoniabicyclo[2.2.2]-octane Bromide (14e). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0518 g, 0.176 mmol) and 1-bromopropane (0.030 mL, 0.330 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0548 g, 75.1%). LC/MS (ES) *m/z* 336 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.55 Hz, 4 H), 7.32 (t, J = 7.68 Hz, 4 H) 7.27−7.21 (m, 2 H), 5.95 (s, 1 H), 3.39−3.32 (m, 6 H), 3.00 (dd, J = 12.21, 4.66 Hz, 2 H), 1.99 (t, J = 7.43 Hz, 6 H), 1.65−1.57 (m, 2 H), 0.87 (t, J = 7.30 Hz, 3 H). HRMS calcd for ($C_{23}H_{30}NO$) 336.2322, found 336.2321.

1-Butyl-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]-octane Bromide (14f). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0496 g, 0.169 mmol) and 1-bromobutane (0.030 mL, 0.279 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0509 g, 70.7%). LC/MS (ES) *m/z* 350 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.55 Hz, 4 H), 7.32 (t, J = 7.68 Hz, 4 H), 7.27−7.20 (m, 2 H), 5.95 (s, 1 H), 3.36 (t, J = 7.43 Hz, 6 H), 3.10−3.01 (m, 2 H), 1.99 (t, J = 7.30 Hz, 6 H), 1.63−1.52 (m, 2 H), 1.33−1.21 (m, 2 H), 0.90 (t, J = 7.30 Hz, 3 H). HRMS calcd for ($C_{24}H_{32}NO$) 350.2478, found 350.2477.

4-[Hydroxy(diphenyl)methyl]-1-[2-(methyloxy)ethyl]-1-azoniabicyclo[2.2.2]octane Bromide (14g). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (0.0597 g, 0.203 mmol) and 2-bromoethyl methyl ether (0.030 mL, 0.319 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0372 g, 42.8%). LC/MS (ES) *m/z* 352 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.33 Hz, 4 H), 7.32 (t, J = 7.58 Hz, 4 H), 7.28−7.22 (m, 2 H), 5.95 (s, 1 H), 3.71−3.65 (m, 2 H), 3.44 (t, J = 7.58 Hz, 6 H), 3.32−3.28 (2 H, m), 3.26 (s, 3 H), 2.00 (t, J = 7.33 Hz, 6 H). HRMS calcd for ($C_{23}H_{30}NO_2$) 352.2271, found 352.2271.

4-[Hydroxy(diphenyl)methyl]-1-[2-(phenyloxy)ethyl]-1-azoniabicyclo[2.2.2]octane Bromide (14h). To a solution of 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.055 0 g, 0.187 mmol) in $2CH_3CN/3CHCl_3$ (2.5 mL) was added 2-bromoethyl phenyl ether (0.06 0 g, 0.29 mmol). The solution was stirred at 60 °C for 16 h. The reaction was cooled down to room temperature and then diluted with ethyl acetate and hexane, causing a solid to crash out of solution. This solid was filtered off and washed with hexane to give the title compound (0.063 g, 67.6%). LC/MS (ES) *m/z* 414 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.58 Hz, 4 H), 7.36−7.28 (m, 6 H), 7.27−7.21 (m, 2 H), 7.03−6.95 (m, 3 H), 5.95 (s, 1 H), 4.40−4.36 (m, 2 H), 3.59−3.52 (m, 8 H), 2.03 (t, J = 7.33 Hz, 6 H). HRMS calcd for ($C_{28}H_{32}NO_2$) 414.2428, found 414.2427.

[1-(2-Aminoethyl)-1-azoniabicyclo[2.2.2]oct-4-yl](diphenyl)-methanolate Trifluoroacetate (14i). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (0.0916 g, 0.312 mmol) and 2-(2-bromoethyl)-1*H*-isoindole-1,3(2*H*)-dione (0.130 g, 0.512 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give 1-[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane bromide (0.0881 g, 51.8%). LC/MS (ES) *m/z* 467$(M)^+$. To a solution of 1-[2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane bromide (0.078 g, 0.142 mmol) in EtOH (4.0 mL) was added hydrazine (0.25 mL, 7.96 mmol). The solution was stirred at room temperature for 16 h and then filtered. The filtrate was concentrated and taken up in 2.5 mL of DMSO and purified by preparatory HPLC (with TFA) to give the title compound (0.0200 g, 31.2%). LC/MS (ES) *m/z* 338 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.16 (br s, 3 H), 7.55 (d, J = 7.33 Hz, 4 H), 7.33 (t, J = 7.58 Hz, 4 H), 7.28−7.22 (m, 2 H), 5.99 (br s, 1 H), 3.45 (t, J = 7.33 Hz, 6 H), 3.33−3.27 (m, 2 H), 3.27−3.18 (m, 2 H), 2.02 (t, J = 7.33 Hz, 6 H). HRMS calcd for ($C_{22}H_{29}N_2O$) 337.2274, found 337.2273.

4-[Hydroxy(diphenyl)methyl]-1-(2-hydroxyethyl)-1-azoniabicyclo[2.2.2]octane Bromide (14j). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (0.0638 g, 0.217 mmol) and 2-bromoethanol (0.035 mL, 0.494 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0541 g, 60.1%). LC/MS (ES) *m/z* 338 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.58 Hz, 4 H), 7.32 (t, J = 7.58 Hz, 4 H), 7.27−7.21 (m, 2 H), 5.95 (s, 1 H), 5.21 (t, J = 4.80 Hz, 1 H), 3.77 (q, J = 4.88 Hz, 2 H), 3.47 (t, J = 7.45 Hz, 6 H), 3.21−3.15 (m, 2 H), 2.00 (t, J = 7.33 Hz, 6 H). HRMS calcd for ($C_{22}H_{28}NO_2$) 338.21146, found 338.21114. Anal. ($C_{22}H_{28}NO_2Br \cdot 0.26H_2O$) C, H, N, Br.

4-[Hydroxy(diphenyl)methyl]-1-[3-(phenyloxy)propyl]-1-azoniabicyclo[2.2.2]octane Bromide (14k). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (0.045 g, 0.153 mmol) and 3-bromopropyl phenyl ether (0.035 mL, 0.222 mmol) in $2CH_3CN/3CHCl_3$ (3.0 mL) were reacted to give the desired product (0.0662 g, 86.0%). LC/MS (ES) *m/z* 428 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.56 (d, J = 7.33 Hz, 4 H), 7.36−7.22 (m, 8 H), 6.98−6.90 (m, 3 H), 5.96 (s, 1 H), 4.02 (t, J = 5.81 Hz, 2 H), 3.43 (t, J = 7.45 Hz, 6 H), 3.30−3.23 (m, 2 H), 2.15−2.07 (m, 2 H), 2.05−1.98 (m, 6 H). HRMS calcd for ($C_{29}H_{34}NO_2$) 428.2584, found 428.2583.

4-[Hydroxy(diphenyl)methyl]-1-[4-(phenyloxy)butyl]-1-azoniabicyclo[2.2.2]octane Bromide (14l). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (0.0604 g, 0.206 mmol) and 4-bromobutyl phenyl ether (0.106 g, 0.463 mmol) in $2CH_3CN/3CHCl_3$ (5.0 mL) were reacted to give the desired product (0.0649 g, 64.9%). LC/MS (ES) *m/z* 442 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.33 Hz, 4 H), 7.38−7.19 (m, 8 H), 6.98−6.88 (m, 3 H), 5.95 (s, 1 H), 3.99 (t, J = 5.68 Hz, 2 H), 3.38 (t, J = 7.45 Hz, 6 H), 3.12 (br s, 2 H), 2.01 (t, J = 6.95 Hz, 6 H), 1.84−1.65 (m, 4 H). HRMS calcd for ($C_{30}H_{36}NO_2$) 442.2741, found 442.2735.

4-[Hydroxy(diphenyl)methyl]-1-(3-hydroxypropyl)-1-azoniabicyclo[2.2.2]octane Bromide (14m). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (0.0628 g, 0.214 mmol) and 3-bromo-1-propanol (0.030 mL, 0.343 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0661 g, 71.8%). LC/MS (ES) *m/z* 352 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.58 Hz, 4 H), 7.33 (t, J = 7.58 Hz, 4 H), 7.27−7.21 (m, 2 H), 5.96 (s, 1 H), 4.77 (t, J = 4.93 Hz, 1 H), 3.44 (q, J = 5.31 Hz, 2 H), 3.38 (t, J = 7.45 Hz, 6 H), 3.17−3.10 (m, 2 H), 2.04−1.96 (m, 3 H), 1.82−1.71 (m, 2 H). HRMS calcd for ($C_{23}H_{30}NO_2$) 352.2271, found 352.2270.

4-[Hydroxy(diphenyl)methyl]-1-[3-(methyloxy)propyl]-1-azoniabicyclo[2.2.2]octane Bromide (14n). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (0.0632 g, 0.215 mmol) and 3-bromopropyl methyl ether (0.0461 g, 0.301 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0826 g, 86.0%). LC/MS (ES) *m/z* 366 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.07 Hz, 4 H), 7.33 (t, J = 6.69 Hz, 4 H), 7.28−7.21 (m, 2 H), 5.95 (s, 1 H), 3.43−3.31 (m, 8 H), 3.23 (s, 3 H), 3.17−3.08 (m, 2 H), 2.04−1.96 (m, 6 H), 1.90−1.83 (m, 2 H). HRMS calcd for ($C_{24}H_{32}NO_2$) 366.2428, found 366.2427.

4-[Hydroxy(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.2]octane Bromide (14o). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (3.30 g, 11.2 mmol) and 2-bromoethyl phenylmethyl ether (2.31 mL, 14.6 mmol) in $2CH_3CN/3CHCl_3$ (200 mL) were reacted to give the desired product (2.47 g, 43.3%). LC/MS (ES) *m/z* 428 $(M)^+$. ^{1}H NMR (DMSO-d_6) δ 7.56 (d, 4H, J = 1.2), 7.28 (m, 11H), 5.95 (s, 1H), 4.50 (s, 2H), 3.81 (d, 2H, J = 4.0), 3.48 (t, 6H, J = 7.2), 3.38 (d, 2H, J = 4.0), 2.01 (t, 6H, J = 7.2). Anal. ($C_{29}H_{34}NO_2Br$) C, H, N.

4-[Hydroxy(diphenyl)methyl]-1-(4-phenylbutyl)-1-azoniabicyclo[2.2.2]octane Bromide (14p). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (79 mg, 0.269 mmol) and (4-bromobutyl)benzene (73 mg, 0.343 mmol) in $2CH_3CN/3CHCl_3$ (5.0 mL) were reacted to give the desired product (0.0998 g, 66.6%). LC/MS (ES) *m/z* 426 $(M)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.54 (d, J = 7.28 Hz, 4 H), 7.36−7.16 (m, 11 H), 5.94 (s, 1 H), 3.39−3.34 (m, 6 H), 3.33−3.29 (m, 2 H), 3.12 −3.06 (m, 2 H), 2.60 (t, J = 7.28 Hz, 2 H), 2.04−1.94 (m, 6 H), 1.68−1.50 (m, 4 H). HRMS calcd for ($C_{30}H_{36}NO$) 426.2791, found 426.2787.

4-[Hydroxy(diphenyl)methyl]-1-[5-(phenyloxy)pentyl]-1-azoniabicyclo[2.2.2]octane Bromide (14q). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-methanol (**10**) (52.1 mg, 0.178 mmol) and 5-bromopentyl phenyl ether (52 mg, 0.214 mmol) in $2CH_3CN/3CHCl_3$ (5.0 mL) were reacted to give the desired product (0.0725 g, 62.5%). LC/MS (ES) *m/z* 456 $(M)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.53 Hz, 4 H), 7.23−7.36 (m, 8 H), 6.94−6.88 (m, 3 H), 5.95 (s, 1 H), 3.96 (t, J = 6.27 Hz, 2 H), 3.38 (t, J = 6.78 Hz, 6 H), 3.06 (br. s., 1 H), 3.09 (d, J = 7.78 Hz, 1 H), 2.00 (t, J = 6.90 Hz, 6 H), 1.73 (d, J = 7.78 Hz, 2 H), 1.76 (br s, 2 H), 1.40 (br s, 2 H). HRMS calcd for ($C_{31}H_{38}NO_2$) 456.2897, found 456.2893.

4-[Cyano(diphenyl)methyl]-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.1]heptane Bromide (15). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.1]hept-4-yl(diphenyl)acetonitrile (**11**) (0.044 g, 0.152 mmol) and 2-bromoethyl phenylmethyl ether (0.040 mL, 0.253 mmol) in $2CH_3CN/3CHCl_3$ (3.5 mL) were reacted to give the desired product (0.0281 g, 37.0%). LC/MS (ES) *m/z* 423 $(M)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.50−7.43 (m, 6 H), 7.40−7.32 (m, 4 H), 4.55 (s, 2 H), 3.89−3.84 (m, 2 H), 3.79−3.72 (m, 8 H), 2.30−2.23 (m, 2 H), 2.19−2.12 (m, 2 H). HRMS calcd for ($C_{29}H_{31}N_2O$) 423.2431, found 423.2431.

4-[Cyano(diphenyl)methyl]-1-{3-[(phenylmethyl)oxy]propyl}-1-azoniabicyclo[2.2.2]octane Bromide (16). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)-acetonitrile (**12**) (0.0495 g, 0.164 mmol) and 3-bromopropyl phenylmethyl ether (0.050 mL, 0.283 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0579 g, 66.6%). LC/MS (ES) *m/z* 451 $(M)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.55−7.49 (m, 4 H), 7.45 (t, J = 7.45 Hz, 4 H), 7.42−7.27 (m, 7 H), 4.47 (s, 2 H), 3.54−3.47 (m, 8 H), 3.25−3.18 (m, 2 H), 2.13 (t, J = 7.07 Hz, 6 H), 1.97−1.89 (m, 2 H). HRMS calcd for ($C_{31}H_{35}N_2O$) 451.2744, found 451.2741.

4-[Hydroxy(diphenyl)methyl]-1-[2-({[3-(methyloxy)phenyl]-methyl}oxy)ethyl]-1-azoniabicyclo[2.2.2]octane Bromide (17a). 1-Azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (30 mg, 0.102 mmol) was added to a solution of 1-{[(2-bromoethyl)oxy]methyl}-3-(methyloxy)benzene (35 mg, 0.143 mmol) in $2CH_3CN/3CHCl_3$ (3 mL). The reaction was heated at 60 °C for 96 h. The reaction was concentrated, and the crude product was washed with EtOAc (3 × 1 mL) and then MeOH (1 × 1 mL). The product was dried under high vacuum to give the title compound (7.7 mg, 14%). LC/MS (ES) *m/z* 458 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 7.58 (d, J = 7.6 Hz, 4 H), 7.32 (t, J = 7.6 Hz, 4 H), 7.28−7.23 (comp, 3 H), 6.95−6.90 (m, 2 H), 6.86 (dd, J = 7.5, 1.89 Hz, 1 H), 3.86−3.82 (m, 2 H), 4.52 (s, 3 H), 3.77 (s, 3 H), 3.53−3.48 (m, 6 H), 3.42−3.36 (m, 2 H), 2.22−2.16 (m, 6 H).

1-[2-({[4-(1,1-Dimethylethyl)phenyl]methyl}oxy)ethyl]-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17b). Following the general procedure outlined in **17a**, 1-azabicyclo-[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (30 mg, 0.102 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-4-(1,1-dimethylethyl)benzene (25 mg, 0.143 mmol) in $2CH_3CN/3CHCl_3$ (3.0 mL) were reacted to give the desired product (9 mg, 16%). LC/MS (ES) *m/z* 484 $(M)^+$. Purity by LC: 89%. ^1H NMR (400 MHz, MeOD) δ ppm 7.58 (d, J = 7.8 Hz, 4 H), 7.39 (d, J = 8.3 Hz, 2 H), 7.34−7.24 (comp, 8 H), 4.50 (s, 2 H), 3.85−3.79 (m, 2 H), 3.52−3.46 (m, 6 H), 3.40−3.35 (m, 2 H), 2.21−2.15 (m, 6 H), 1.31 (s, 9 H).

1-(2-{[(4-Fluorophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17c). Following the general procedure outlined in **17a**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (30 mg, 0.102 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-4-fluorobenzene (33 mg, 0.143 mmol) in $2CH_3CN/3CHCl_3$ (3.0 mL) were reacted to give the desired product (9 mg, 16%). LC/MS (ES) *m/z* 446 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 7.60−7.55 (m, 4 H), 7.39−7.36 (m, 2 H), 7.34−7.30 (m, 4 H), 7.26−7.22 (m, 2 H), 7.07 (ddd, J = 8.9, 6.7, 2.0 Hz, 2 H), 4.53 (s, 2 H), 3.89−3.83 (m, 2 H), 3.56−3.50 (m, 6 H), 3.44−3.39 (m, 2 H), 2.22−2.16 (m, 6 H). Anal. ($C_{29}H_{33}FNO_2Br$) C, H, N, Br. HRMS calcd for ($C_{29}H_{33}FNO_2$) 446.2490, found 446.2489.

1-(2-{[(4-Chlorophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17d). Following the general procedure outlined in **17a**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (30 mg, 0.102 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-4-chlorobenzene (36 mg, 0.143 mmol) in $2CH_3CN/3CHCl_3$ (3.0 mL) were reacted to give the desired product (17.4 mg, 32%). LC/MS (ES) *m/z* 462 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 7.59−7.57 (s, 4 H), 7.35−7.29 (comp, 8 H), 7.26−7.22 (m, 2 H), 4.53 (s, 2 H), 3.90−3.84 (m, 2 H), 3.56−3.50 (m, 6 H), 3.45−3.39 (m, 2 H), 2.23−2.17 (m, 6 H). Anal. ($C_{29}H_{33}ClNO_2Br$) C, H, N. HRMS calcd for ($C_{29}H_{33}ClNO_2$) 462.2194, found 462.2198.

1-(2-{[(4-Bromophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17e). Following the general procedure outlined in **17a**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (30 mg, 0.102 mmol) and 1-bromo-4-{[(2-bromoethyl)oxy]methyl}benzene (42 mg, 0.143 mmol) in $2CH_3CN/3CHCl_3$ (3.0 mL) were reacted to give the desired product (19.4 mg, 32%). LC/MS (ES) *m/z* 506 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 7.58 (d, J = 7.6 Hz, 4 H), 7.52−7.48 (m, 2 H), 7.34−7.22 (comp, 8 H), 4.51 (s, 2 H), 3.89−3.83 (m, 2 H), 3.56−3.49 (m, 6 H), 3.44−3.38 (m, 2 H), 2.23−2.16 (m, 6 H). HRMS calcd for ($C_{29}H_{33}BrNO_2$) 506.1689, found 506.1690.

1-(2-{[(4-Cyanophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17f). Following the general procedure outlined in **17a**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (30 mg, 0.102 mmol) and 4-{[(2-bromoethyl)oxy]methyl}benzonitrile (34 mg, 0.143 mmol) in $2CH_3CN/3CHCl_3$ (3.0 mL) were reacted to give the desired product (40 mg, 74%). LC/MS (ES) *m/z* 453 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 7.71 (d, J = 8.1 Hz, 2 H), 7.60−7.53 (m, 4 H), 7.32 (t, J = 7.6 Hz, 4 H), 7.24 (t, J = 7.3 Hz, 2 H), 4.65 (s, 2 H), 3.94−3.92 (m, 2 H), 3.60−3.53 (m, 6 H), 3.48−3.46 (m, 2 H), 2.24−2.18 (m, 6 H). HRMS calcd for ($C_{30}H_{33}N_2O_2$) 453.2537, found 453.2539.

1-(2-{[(3-Fluorophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17g). Following the general procedure outlined in **17a**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (94 mg, 0.321 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-3-fluorobenzene (75 mg, 0.321 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (50 mg, 30%). LC/MS (ES) *m/z* 446 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 2.24−2.17 (m, 6 H) 3.47−3.41 (m, 2 H) 3.58−3.51 (m, 6 H) 3.92−3.86 (m, 2 H) 4.57 (s, 2 H) 7.02 (td, J = 8.5, 2.4 Hz, 1 H) 7.11 (d, J = 9.6 Hz, 1 H) 7.16 (d, J = 7.6 Hz, 1 H) 7.24 (t, J = 7.3 Hz, 2 H) 7.37−7.29 (m, 5 H) 7.58 (d, J = 7.3 Hz, 4 H). HRMS calcd for ($C_{29}H_{33}FNO_2$) 446.2490, found 446.2493.

1-(2-{[(2-Cyanophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17h). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (35.4 mg, 0.121 mmol) and 2-{[(2-bromoethyl)oxy]methyl}benzonitrile (29 mg, 0.121 mmol) in $2CH_3CN/3CHCl_3$ (1.5 mL) were reacted to give the desired product (40 mg, 61.5%). LC/MS (ES) *m/z* 453 $(M)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.88 (d, J = 7.03 Hz, 1 H), 7.74 (t, J = 7.65 Hz, 1 H), 7.62 (d, J = 7.78 Hz, 1 H), 7.55 (d, J = 7.53 Hz, 5 H), 7.32 (t, J = 7.65 Hz, 4 H), 7.25 (d, J = 7.03 Hz, 2 H), 5.96 (s, 1 H), 4.68 (s, 2 H), 3.92 (br s, 2 H), 3.49 (t, J = 7.15 Hz, 6 H), 3.40

(br s, 2 H), 2.01 (t, J = 7.03 Hz, 6 H). HRMS calcd for ($C_{30}H_{33}N_2O_2$) 453.2537, found 453.2532.

1-(2-{[(3-Bromophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17i). Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (61.9 mg, 0.211 mmol) and 2-bromoethyl (3-bromophenyl)methyl ether (62 mg, 0.211 mmol) in $2CH_3CN$/$3CHCl_3$ (1.5 mL) were reacted to give the desired product (17 mg, 13.72%). LC/MS (ES) m/z 506 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.58−7.49 (m, 6 H), 7.36−7.29 (m, 6 H), 7.27−7.22 (m, 2 H), 5.99 (br s, 1 H), 4.50 (s, 2 H), 3.82 (br s, 2 H), 3.52−3.43 (m, 6 H), 3.42−3.31 (m, 2 H), 2.08−1.90 (m, 6 H). HRMS calcd for ($C_{29}H_{33}BrNO_2$) 506.1689, found 506.1683.

1-(2-{[(2-Fluorophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17j). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (177 mg, 0.602 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-2-fluorobenzene (169 mg, 0.602 mmol) in $2CH_3CN$/$3CHCl_3$ (5.0 mL) were reacted to give the desired product (36 mg, 10.8%). LC/MS (ES) m/z 446 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.53 Hz, 4 H), 7.45 (t, J = 7.53 Hz,1 H), 7.39 (d, J = 7.53 Hz, 1 H), 7.33 (t, J = 7.65 Hz, 4 H), 7.27−7.20 (m, 4 H), 5.95 (s, 1 H), 4.56 (s, 2 H), 3.85 (d, J = 4.02 Hz, 2 H), 3.46 (t, J = 7.40 Hz, 6 H), 3.40−3.34 (m, 2 H), 2.00 (t, J = 7.28 Hz, 6 H). HRMS calcd for ($C_{29}H_{33}FNO_2$) 446.2490, found 446.2485.

4-[Hydroxy(diphenyl)methyl]-1-(2-{[(3-methylphenyl)methyl]oxy}ethyl)-1-azoniabicyclo[2.2.2]octane Bromide (17k). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (119 mg, 0.407 mmol) and 2-bromoethyl (3-methylphenyl)methyl ether (153 mg, 0.407 mmol) in $2CH_3CN$/$3CHCl_3$ (5.0 mL) were reacted to give the desired product (33 mg, 14.7%). LC/MS (ES) m/z 442 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.28 Hz, 4 H), 7.33 (t, J = 7.53 Hz, 4 H), 7.24 (t, J = 7.15 Hz, 3 H), 7.14−7.10 (m, 3 H), 5.95 (s, 1 H), 4.46 (s, 2 H), 3.82−3.78 (m, 2 H), 3.46 (t, J = 7.53 Hz, 6 H), 3.40−3.29 (m, 2 H), 2.29 (s, 3 H), 2.00 (t, J = 7.40 Hz, 6 H). Anal. ($C_{30}H_{36}NO_2 \cdot 0.5H_2O$) C, H, N, Br. HRMS calcd for ($C_{30}H_{36}NO_2$) 442.2741, found 442.2736.

1-(2-{[(2-Bromophenyl)methyl]oxy}ethyl)-4-[hydroxy(diphenyl)methyl]-1-azoniabicyclo[2.2.2]octane Bromide (17l). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (136 mg, 0.464 mmol) and 2-bromoethyl (2-bromophenyl)methyl ether (192 mg, 0.464 mmol) in $2CH_3CN$/$3CHCl_3$ (5.0 mL) were reacted to give the desired product (48 mg, 16.75%). LC/MS (ES) m/z 506 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.62 (d, J = 1.00 Hz, 1 H), 7.55 (d, J = 7.53 Hz, 4 H), 7.51−7.46 (m, 1 H), 7.41 (t, J = 6.90 Hz, 1 H), 7.35−7.29 (m, 4 H), 7.25 (q, J = 7.11 Hz, 3 H), 5.95 (s, 1 H), 4.55 (s, 2 H), 3.94−3.87 (m, 2 H), 3.49 (t, J = 7.40 Hz, 6 H), 3.43−3.38 (m, 2 H), 2.00 (t, J = 7.40 Hz, 6 H). Anal. ($C_{29}H_{33}NO_2Br_2 \cdot 1.5H_2O$) C, H, N, Br. HRMS calcd for ($C_{29}H_{33}BrNO_2$) 506.1689, found 506.1683.

4-[Hydroxy(diphenyl)methyl]-1-(2-{[(4-methylphenyl)methyl]oxy}ethyl)-1-azoniabicyclo[2.2.2]octane Bromide (17m). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (171 mg, 0.583 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-4-methylbenzene (167 mg, 0.583 mmol) in $2CH_3CN$/$3CHCl_3$ (5.0 mL) were reacted to give the desired product (72.7 mg, 22.67%). LC/MS (ES) m/z 442 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.53 Hz, 4 H), 7.33 (t, J = 7.65 Hz, 4 H), 7.28−7.20 (m, 4 H), 7.19−7.14 (m, 2 H), 5.95 (s, 1 H), 4.44 (s, 2 H), 3.82−3.75 (m, 2 H), 3.46 (t, J = 7.40 Hz, 6 H), 3.38−3.34 (m, 2 H), 2.29 (s, 3 H), 2.00 (t, J = 7.40 Hz, 6 H). Anal. ($C_{30}H_{36}NO_2Br \cdot 1.1H_2O$) C, H, N, Br. HRMS calcd for ($C_{30}H_{36}NO_2$) 442.2741, found 442.2736.

4-[Hydroxy(diphenyl)methyl]-1-(2-{[(2-methylphenyl)methyl]oxy}ethyl)-1-azoniabicyclo[2.2.2]octane (17n). Following the general procedure outlined in **14c**, 1-azabicyclo[2.2.2]oct-4-yl(diphenyl)methanol (**10**) (174 mg, 0.593 mmol) and 1-{[(2-bromoethyl)oxy]methyl}-2-methylbenzene (194 mg, 0.593 mmol) in $2CH_3CN$/$3CHCl_3$ (5.0 mL) were reacted to give the desired product (40.6 mg, 13.1%). LC/MS (ES) m/z 442 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.55 (d, J = 7.53 Hz, 4 H), 7.35−7.28 (m, 5 H), 7.25 (d, J = 7.28 Hz, 2 H), 7.22−7.17 (m, 3 H), 5.95 (s, 1 H), 4.50 (s, 2 H), 3.87−3.80 (m, 2 H), 3.46 (t, J = 7.40 Hz, 6 H), 3.39−3.35 (m, 2 H), 2.26 (s, 3 H), 2.00 (t, J = 7.28 Hz, 6 H). Anal. ($C_{30}H_{36}NO_2Br \cdot 1.2H_2O$) C, H, N, Br. HRMS calcd for ($C_{30}H_{36}NO_2$) 442.2741, found 442.2737.

4-[Bis(4-fluorophenyl)(hydroxy)methyl]-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.2]octane Bromide (18a). A solution of 4-fluorophenylmagnesiumbromide (1.0 M in THF, 4.4 mL, 4.4 mmol) was chilled down to 0 °C under Ar. Ethyl 1-azabicyclo-[2.2.2]octane-4-carboxylate (**8**) (0.1973 g, 1.08 mmol) in THF (4 mL) was slowly added to the reaction mixture at 0 °C over 20 min. The reaction was allowed to warm up to room temperature and then heated at 60 °C for 16 h. The reaction was chilled in an ice bath, quenched with saturated NH_4Cl, and concentrated under vacuum. The resulting residue was treated with H_2O and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under vacuum to yield 1-azabicyclo[2.2.2]oct-4-yl[bis(4-fluorophenyl)]methanol (0.3152 g, 88.9%). LC/MS (ES) m/z 330 $(M + H)^+$. Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl[bis(4-fluorophenyl)]methanol (0.0489 g, 0.148 mmol) and 2-bromoethyl phenylmethyl ether (0.0352 mL, 0.222 mmol) in $2CH_3CN$/$3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0534 g, 66.1%). LC/MS (ES) m/z 464 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.56 (dd, J = 8.84, 5.56 Hz, 4 H), 7.40−7.28 (m, 5 H), 7.19−7.12 (m, 2 H), 7.15 (t, J = 8.84 Hz, 2 H), 6.09 (s, 1 H), 4.50 (s, 2 H), 3.85−3.785 (m, 2 H), 3.47 (t, J = 7.33 Hz, 6 H), 3.40−3.35 (m, 2 H), 1.97 (t, J = 7.20 Hz, 6 H). HRMS calcd for ($C_{29}H_{32}F_2NO_2$) 464.2396, found 464.2398.

4-(Hydroxy{bis[3-(methyloxy)phenyl]}methyl)-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.2]octane Bromide (18b). Following the general procedure outlined in **18a**, ethyl 1-azabicyclo-[2.2.2]octane-4-carboxylate (**8**) (0.1608 g, 0.877 mmol) and 3-(methyloxy)phenylmagnesiumbromide (1.0 M in THF, 3.3 mL, 3.3 mmol) in THF (4.0 mL) were reacted to give 1-azabicyclo[2.2.2]oct-4-yl{bis[3-(methyloxy)phenyl]}methanol (0.2881 g, 92.9%). LC/MS (ES) m/z 354 $(M + H)^+$. Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl{bis[3-(methyloxy)-phenyl]}methanol (0.0538 g, 0.152 mmol) and 2-bromoethyl phenylmethyl ether (0.0361 mL, 0.228 mmol) in $2CH_3CN$/$3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0292 g, 33.8%). LC/MS (ES) m/z 488 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.37−7.30 (m, 5 H), 7.25 (t, J = 8.08 Hz, 2 H), 7.14 (d, J = 8.59 Hz, 2 H), 7.08 (t, J = 2.02 Hz, 2 H), 6.84 (dd, J = 7.96, 2.40 Hz, 2 H), 5.96 (s, 1 H), 4.50 (s, 2 H), 3.84−3.79 (m, 2 H), 3.73 (s, 6 H), 3.50−3.42 (m, 6 H), 3.39−3.33 (m, 2 H), 2.03 − 1.96 (m, 6 H). HRMS calcd for ($C_{31}H_{38}NO_4$) 488.2795, found 488.2796.

4-[Bis(3-fluorophenyl)(hydroxy)methyl]-1-{2-[(phenylmethyl)-oxy]ethyl}-1-azoniabicyclo[2.2.2]octane Bromide (18c). Following the general procedure outlined in **18a**, ethyl 1-azabicyclo-[2.2.2]octane-4-carboxylate (**8**) (0.1756 g, 0.958 mmol) and 3-fluorophenylmagnesiumbromide (1.0 M in THF, 3.3 mL, 3.3 mmol) in THF (4.0 mL) were reacted to give 1-azabicyclo[2.2.2]oct-4-yl[bis(3-fluorophenyl)]methanol (0.242 g, 76.7%). LC/MS (ES) m/z 330 $(M + H)^+$. Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl[bis(3-fluorophenyl)]methanol (0.0507 g, 0.154 mmol) and 2-bromoethyl phenylmethyl ether (0.0365 mL, 0.230 mmol) in $2CH_3CN$/$3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0362 g, 43.2%). LC/MS (ES) m/z 464 $(M)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.43−7.30 (m, 11 H), 7.17−7.11 (m, 2 H), 6.27 (s, 1 H), 4.50 (s, 2 H), 3.85−3.79 (m, 2 H), 3.48 (t, J = 7.45 Hz, 6 H), 3.40−3.35 (m, 2 H), 1.98 (t, J = 7.20 Hz, 6 H). HRMS calcd for ($C_{29}H_{32}F_2NO_2$) 464.2396, found 464.2396.

4-(Hydroxy{bis[4-(methyloxy)phenyl]}methyl)-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.2]octane Bromide (18d). Following the general procedure outlined in **18a**, ethyl 1-azabicyclo-[2.2.2]octane-4-carboxylate (**8**) (0.1587 g, 0.866 mmol) and 4-(me-

thyloxy)phenylmagnesiumbromide (0.5 M in THF, 6.5 mL, 3.25 mmol) in THF (4.0 mL) were reacted to give 1-azabicyclo[2.2.2]oct-4-yl{bis[4-(methyloxy)phenyl]}methanol (0.273 g, 89.0%). LC/MS (ES) *m*/*z* 354 $(M + H)^+$. Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl{bis[4-(methyloxy)phenyl]}-methanol (0.0498 g, 0.141 mmol) and 2-bromoethyl phenylmethyl ether (0.0334 mL, 0.211 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0374 g, 46.7%). LC/MS (ES) *m*/*z* 488 $(M)^+$. ^1H NMR (400 MHz, MeOD) δ ppm 7.48 (t, $J =$ 6.8 Hz, 4 H), 7.37−7.30 (m, 5 H), 6.87 (d, $J = 6.8$ Hz, 4 H), 4.56 (s, 2 H), 3.87−3.85 (m, 2 H), 3.78 (s, 6 H), 3.51 (t, $J = 6.4$ Hz, 6 H), 3.42−3.40 (m, 2 H), 2.15 (t, $J = 6.4$ Hz, 6 H). HRMS calcd for ($C_{31}H_{38}NO_4$) 488.2795, found 488.2796.

4-{Hydroxy[bis(4-methylphenyl)]methyl}-1-{2-[(phenylmethyl)oxy]ethyl}-1-azoniabicyclo[2.2.2]octane Bromide (18e). Following the general procedure outlined in **18a**, ethyl 1-azabicyclo-[2.2.2]octane-4-carboxylate (**8**) (0.1509 g, 0.823 mmol) and 4-methylphenylmagnesiumbromide (1.0 M in THF, 3.3 mL, 3.3 mmol) in THF (4.0 mL) were reacted to give 1-azabicyclo[2.2.2]oct-4-yl[bis(4-methylphenyl)]methanol (0.2291 g, 86.6%). LC/MS (ES) *m*/*z* 322 $(M + H)^+$. Following the general procedure outlined in **14h**, 1-azabicyclo[2.2.2]oct-4-yl[bis(4-methylphenyl)]methanol (0.0525 g, 0.163 mmol) and 2-bromoethyl phenylmethyl ether (0.0387 mL, 0.245 mmol) in $2CH_3CN/3CHCl_3$ (4.0 mL) were reacted to give the desired product (0.0465 g, 53.1%). LC/MS (ES) *m*/*z* 456$(M)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.40−7.30 (m, 9 H), 7.21 (t, $J = 7.6$ Hz, 2 H), 7.06 (d, $J = 6.8$ Hz, 2 H), 5.86 (s, 1 H), 4.51 (s, 2 H), 3.82 (br. S, 2 H), 3.46 (br s, 6 H), 3.36 (br. S, 2 H), 2.30 (s, 6H), 2.00 (br s, 6 H). HRMS calcd for ($C_{31}H_{38}NO_2$) 456.2897, found 456.2900.

Computational Modeling. The model of the M_3 receptor transmembrane helix bundle was built using standard homology techniques, with the bovine rhodopsin X-ray structure[21] as a template (PDB code 2F88). Extracellular loops were added to this using a procedure developed "in house", which makes use of a combined distance geometry sampling and molecular dynamics simulation.[26] The side chains of this model were then refined using the Karplus standard rotamer library.[27] The final model was optimized fully (500 steps of steepest descent (SD) followed by 5000 steps of adopted basis Newton−Raphson (ABNR)) with the CHARMm force field[28] using helical distance constraints between the *i*th and $I +$ fourth residues (except proline), within a range of 1.8−2.4 Å, to maintain the backbone hydrogen bonds of the helix bundle. Ligand docking was performed manually using multiple low energy conformations of the compounds and adjustment of the protein side chains, again using the Karplus rotamer library. Each pose was minimized as above with CHARMm.. Visualizations and pictures were generated with the Quanta modeling package.[29] The ligands themselves were built with the Spartan program[30] with initial minimization at the AM1 semiempirical level. 3-21G* natural atomic orbital charges were then calculated and used for the optimizations with CHARMm. The electrostatic potential surfaces depicted in Figure 2 were also calculated in Spartan at the 3-21G* level.

Acknowledgment. We thank Sandra Umbrecht and Giovanni Vitulli for providing the PK data. We also thank Dulcie Schmidt and Michael Hafey for excellent technical assistance with the FLIPR assays and Henry M. Sarau for his continuous support of the program.

Supporting Information Available: The general method of preparation of the noncommercially available alkylating agents to prepare the compounds **17a**−**17o** (Table 3). The elemental analyses data for compounds **10**, **14j**, **14o**, **17c**, **17d**, **17k**, **17l**, **17m**, and **17n**. The general methods for the in vitro and in vivo assays (Tables 1−6, Figure 6). The pA_2 data at the M_1 and M_2 receptors for compounds **14k** and **14o**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM801601V