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Muscarinic acetylcholine receptor antagonists: SAR and optimization of tyrosine ureas

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ABSTRACT

SAR exploration of multiple regions of a tyrosine urea template led to the identification of very potent muscarinic acetylcholine receptor antagonists such as **10b** with good subtype selectivity for M_3 over M_1. The structure–activity relationships (SAR) and optimization of the tyrosine urea series are described.

Five muscarinic acetylcholine receptor (mAChR) subtypes, M_1–M_5, are known to date.[1–3] These seven-transmembrane (7TM) receptors share a common orthosteric ligand-binding site with an extremely high sequence homology, which explains why it has been difficult historically to identify subtype-selective ligands.[3] M_1–M_5 mAChRs are widely distributed in mammalian organs and the central and peripheral nerve system where they mediate important neuronal and autocrine functions.[4,5] In the mammalian lung, only M_1, M_2, and M_3 mAChRs have been recognized as playing important and functional roles.[6] M_3 is predominantly expressed on airway smooth muscle and mediates smooth muscle contraction.[7] M_2 is primarily found on postganglionic nerve termini and functions to limit acetylcholine release from parasympathetic nerves.[8] M_1 is found in parasympathetic ganglia and facilitates neurotransmission through ganglia thus enhancing cholinergic reflexes.[9]

In chronic obstructive pulmonary disease (COPD) and asthma, inflammatory conditions lead to loss of neuronal inhibitory activity mediated by M_2 on parasympathetic nerves, causing excess acetylcholine reflexes[10] which result in airway hyperreactivity and hyperresponsiveness mediated by increased acetylcholine release and thus excess stimulation of M_3. Therefore, potent mAChR antagonists, particularly directed toward the M_3 subtype, would be useful as therapeutics in these mAChRs-mediated disease states. Inhaled delivery could potentially reduce side effects mediated by peripheral and/or central M_1, M_2, or M_3 antagonism[5] by avoiding substantial systemic exposure. We previously reported a novel muscarinic acetylcholine receptor antagonist series exemplified by **2a** with high potency, outstanding selectivity, and excellent in vivo efficacy and long duration of action in the bronchoconstriction model in mice via inhaled delivery (Fig. 1).[11] Herein, we describe SAR and optimization of this tyrosine urea series from high-throughput screen (HTS) hit **1a** to the identification of lead compounds such as **10b** and **2a**.

The HTS hit **1a**,[11] a mixture of two diastereoisomers, was an antagonist in a M_3 fluorometric imaging plate reader (FLIPR) assay[12] with a pIC_{50} of 7.7 and was about 10-fold selective for M_3 over M_2 and 100-fold selective for M_3 over M_1 (Fig. 1).[13,14] On the basis of its good potency and subtype selectivity, **1a** was considered an acceptable starting point for our lead optimization program aimed at improving potency via SAR exploration.

A robust solid-phase synthesis was developed to efficiently explore multiple regions of the series in parallel (Scheme 1).

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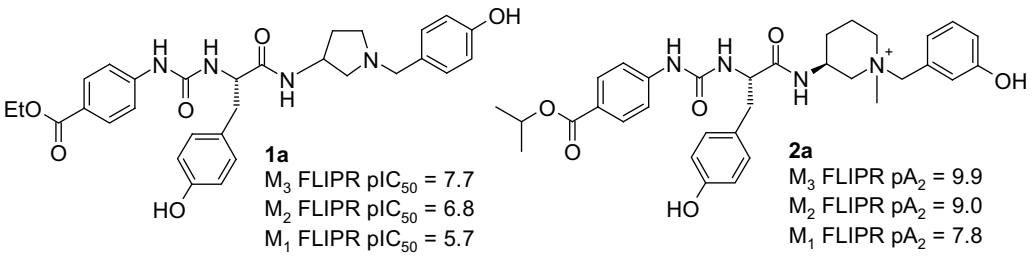
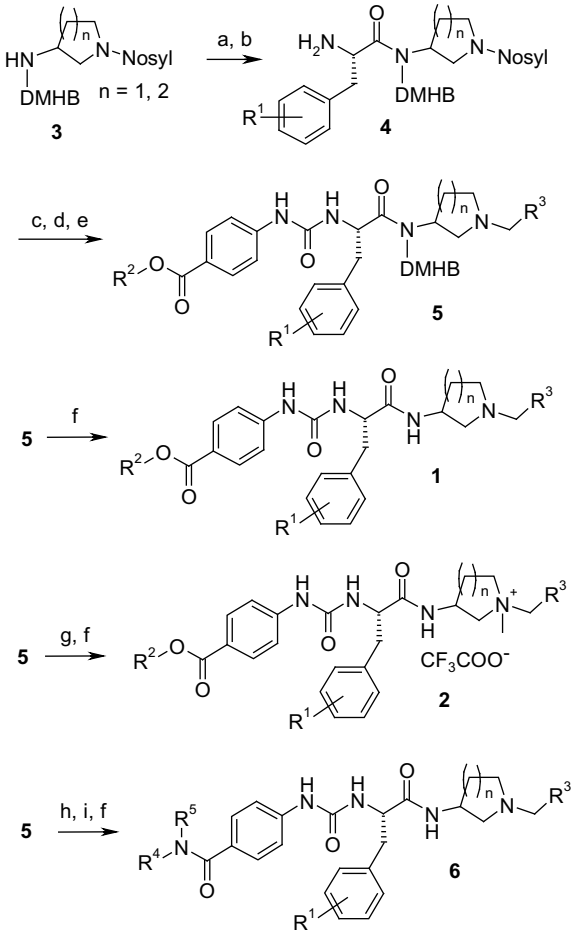


Figure 1. In vitro profile of HTS hit **1a** and structure of lead compound **2a**.

Resin-bound nosyl-protected diamines **3**[11] were coupled with Fmoc-protected L-amino acids, followed by Fmoc removal, to produce resin-bound amines **4**. Urea formation from intermediates **4**, nosyl-group removal and subsequent reductive amination afforded resin-bound intermediates **5**, which were then cleaved from resin to produce the targeted tertiary amines **1** in good overall yields.[15] Alkylation of tertiary amines **5**, followed by resin cleavage afforded the desired quaternary ammonium salts **2**. Intermediates **5** were also converted to amides **6** in good yields via hydrolysis of the ester, amide formation from the resulting acid, and resin cleavage. Using this robust solid-phase synthesis, the right-hand side (RHS) N-capping group, quaternary ammonium salt, central diamine and amino acid, and left-hand side (LHS) ester and amide moieties were explored in parallel.

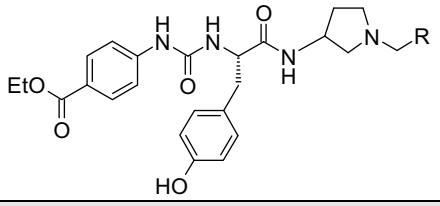


Scheme 1. Reagents and conditions: (a) Fmoc amino acids, DIC, HOAt, NMP, rt; (b) 20% of piperidine in NMP, rt; (c) 4-isocyanatobenzoates, DCE, rt; or 4-aminobenzoates, 1,1′-(oxomethanediyl)bis-1*H*-pyrrole, DIEA, DCE, rt; (d) K_2CO_3, PhSH, NMP, rt; (e) R^3CHO, $Na(OAc)_3BH$, 10% of HOAc in NMP, rt; (f) 50% of TFA in DCE, rt; (g) MeI, CH_3CN, rt; (h) potassium trimethylsilanolate, THF, rt; (i) R^4R^5NH, PyBOP, NMP, rt.

Modifications to the RHS N-capping group were well tolerated (Table 1). Substitution at the *para*- (**1a** and **1c**), *meta*- (**1b** and **1d**), or *ortho*-position (**1e**) of the benzyl group gave similar M_3 FLIPR potency. 3,4-Disubstituted compounds such as **1f** had similar potency as mono-substituted benzyl analogs. A wide range of substituents on the benzyl ring was tolerated—from electron-donating groups such as hydroxyl (**1b**) and methoxy (**1d**) to hydrogen (**1j**) and electron-withdrawing groups such as chloro (**1h**), cyano (**1g**), and trifluoromethyl (**1i**). While a small alkyl group such as ethyl (**1k**) was significantly less potent, a larger alkyl group such as cyclopropylmethyl (**1m**) was about equipotent compared to benzyl analogs. Modifications to this region did not have substantial impact on sub-type selectivity—the compounds were 10-fold selective for M_3 over M_2 and 100-fold selective for M_3 over M_1 in general.

To avoid substantial systemic exposure thus reduce potential side effects mediated by peripheral and/or central M_1, M_2, or M_3 antagonism via minimizing cell membrane permeability, we attempted to convert tertiary amines to quaternary ammonium salts and were pleased to find that quaternary ammonium salts such as **2b** were slightly more potent and had similar subtype selectivity compared to the corresponding tertiary amine **1n** (Fig. 2).[16] Introducing the quaternary ammonium moiety indeed reduced membrane permeability and thereby oral bioavailability significantly. For example, compound **2a** had extremely low artificial membrane permeability (less than 3 nm/s) and no oral bioavailability.[11]

Table 1
SAR of the RHS N-capping group



Compound	R	FLIPR pIC_{50}[a]		
		M_3	M_2	M_1
1a	4-Hydroxyphenyl	7.7	6.8	5.7
1b	3-Hydroxyphenyl	7.7	6.8	6.1
1c	4-Methoxyphenyl	7.7	6.6	6.3
1d	3-Methoxyphenyl	7.6	6.5	5.6
1e	2-Methoxyphenyl	7.3	6.8	6.0
1f	3,4-Methylenedioxyphenyl	7.5	6.7	6.0
1g	3-Cyanophenyl	8.2	7.0	6.6
1h	3-Chlorophenyl	8.1	6.9	5.9
1i	3-Trifluoromethylphenyl	7.8	6.2	<5.5
1j	Phenyl	7.8	6.7	5.7
1k	Methyl	6.1	5.6	<5.5
1m	Cyclopropyl	7.6	7.0	6.2

[a] Means of at least two determinations with standard deviation of <±0.3.

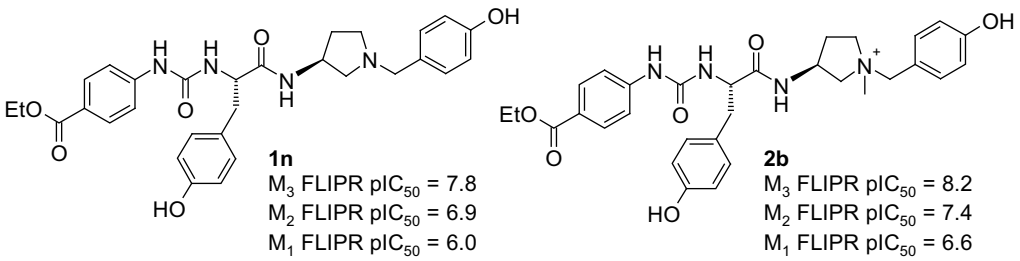
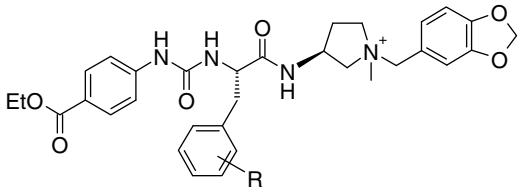


Figure 2. Potency comparison of tertiary amine **1n** with quaternary ammonium salt **2b**.

Table 2
SAR of the central tyrosine moiety



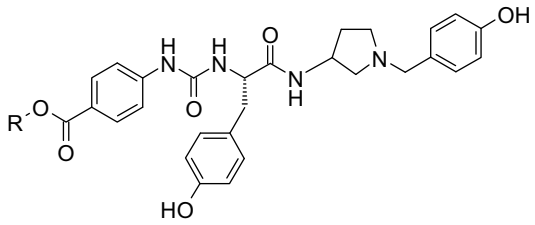
Compound	R	FLIPR pIC_{50}[a]		
		M_3	M_2	M_1
2c	4-Hydroxy	8.2	7.4	6.8
2d	4-Methoxy	7.4	8.3	7.1
2e	4-Amino	7.8	7.8	5.9
2f	4-Methyl	7.8	8.0	7.0
2g	4-Fluoro	7.8	8.0	7.0
2h	4-Chloro	8.0	8.2	7.4
2i	4-Bromo	7.9	8.4	7.5
2j	4-Cyano	7.3	7.9	6.6
2k	4-Trifluoromethyl	7.5	8.0	7.3
2l	4-Phenyl	7.8	8.3	7.9
2m	4-Phenylcarbonyl	7.9	8.6	8.0
2n	3-Chloro	8.1	8.4	7.6
2o	2-Chloro	6.9	7.0	5.7
2p	3,4-Dichloro	7.6	7.9	7.4
2q	3,4-Dimethoxy	5.9	7.3	6.2

[a] Means of at least two determinations with standard deviation of <±0.3.

A number of substituents on the central phenyl ring was explored (Table 2). In general, substituents on the phenyl ring did not have substantial impact on M_3 potency. Electron-donating groups such as hydroxyl (**2c**), methoxy (**2d**), amino (**2e**), and methyl (**2f**); halo groups such as fluoro (**2g**), chloro (**2h**), and bromo (**2i**); and electron-withdrawing groups such as cyano (**2j**) and trifluoromethyl (**2k**) all had good potency in the M_3 FLIPR assay. While moving the chloro group from the *para*-position (**2h**) to the *meta*-position (**2n**) maintained M_3 potency, the *ortho*-chloro group (**2o**) resulted in about 10-fold potency loss. 3,4-Disubstituted compounds such as **2p** and **2q** were less potent compared to the corresponding mono-substituted analogs. In contrast to the modest impact on M_3 potency, the substituents had a significant impact on mAChR subtype selectivity. Except for the 4-hydroxy (**2c**), other substituents (**2d**–**q**) resulted in equi- or more potent compounds against M_2 compared to M_3. In most cases, subtype selectivity for M_3 over M_1 was also not as good as that of compound **2c**. On the basis of both M_3 potency and subtype selectivity, 4-hydroxy (**2c**) was optimal.

For the LHS ester moiety (Table 3), isopropyl (**1r**) was optimal among the six groups examined. Compound **1r** had a pIC_{50} of 8.3 in the M_3 FLIPR assay, 4-fold more potent than the HTS hit **1a**, and good subtype selectivity—greater than 10-fold selective for M_3 over M_2 and 100-fold selective for M_3 over M_1. Amides, bioisosteres of the esters, were also investigated (Table 4). Similar to the

Table 3
SAR of the LHS ester moiety

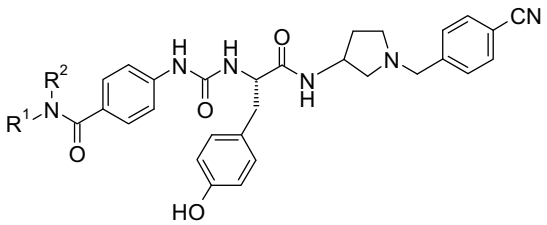


Compound	R	FLIPR pIC_{50}[a]		
		M_3	M_2	M_1
1p	Methyl	7.6	6.5	6.0
1a	Ethyl	7.7	6.8	5.7
1q	Propyl	8.0	7.0	6.0
1r	Isopropyl	8.3	7.1	6.0
1s	Isobutyl	6.6	5.9	<5.5
1t	Cyclopropylmethyl	7.0	6.4	6.0

[a] Means of at least two determinations with standard deviation of <±0.3.

SAR of esters, *N*-isopropyl (**6d**) was the most potent in the M_3 FLIPR assay. Small alkyl groups such as *N*-methyl (**6a**) and large alkyl groups such as *N*-phenyl (**6f**) and *N*-benzyl (**6g**) were significantly less potent. A tertiary amide was tolerated but did not provide any potency enhancement (**6h** vs **6b**). The morpholine analog (**6i**) was also less potent. The amides exemplified by **6d** had similar subtype

Table 4
SAR of the LHS amide moiety



Compound	NR^1R^2	FLIPR pIC_{50}[a]		
		M_3	M_2	M_1
6a	*N*-Methylamino	5.6	<5.5	<5.5
6b	*N*-Ethylamino	6.5	<5.5	<5.5
6c	*N*-Propylamino	6.3	<5.5	<5.5
6d	*N*-Isopropylamino	7.0	5.8	<5.5
6e	*N*-Cyclopropylamino	6.8	5.8	<5.5
6f	*N*-Phenylamino	6.3	<5.5	<5.5
6g	*N*-Benzylamino	5.8	<5.5	<5.5
6h	*N*,*N*-Diethylamino	6.4	<5.5	<5.5
6i	4-Morpholino	6.0	<5.5	<5.5

[a] Means of at least two determinations with standard deviation of <±0.3.

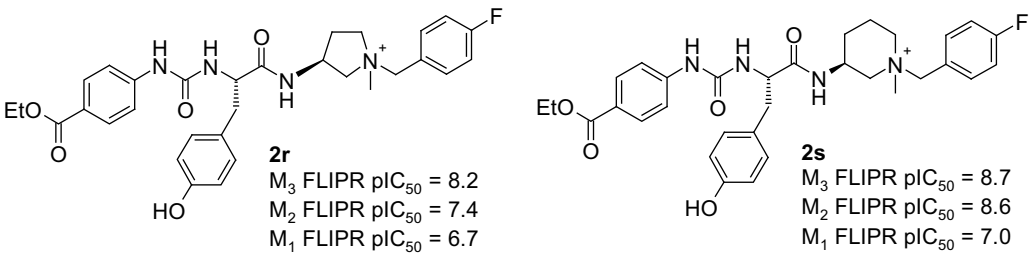
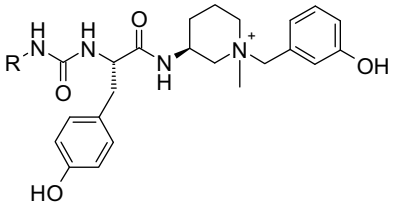
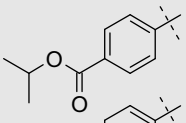
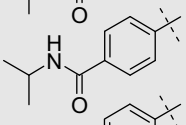
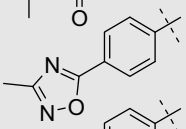
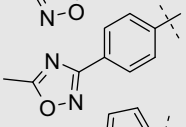
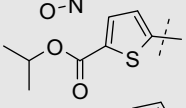
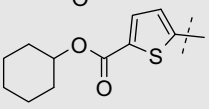


Figure 3. Potency comparison of pyrrolidinium salt **2r** with piperidinium salt **2s**.

Table 5
Further optimization of the LHS moiety

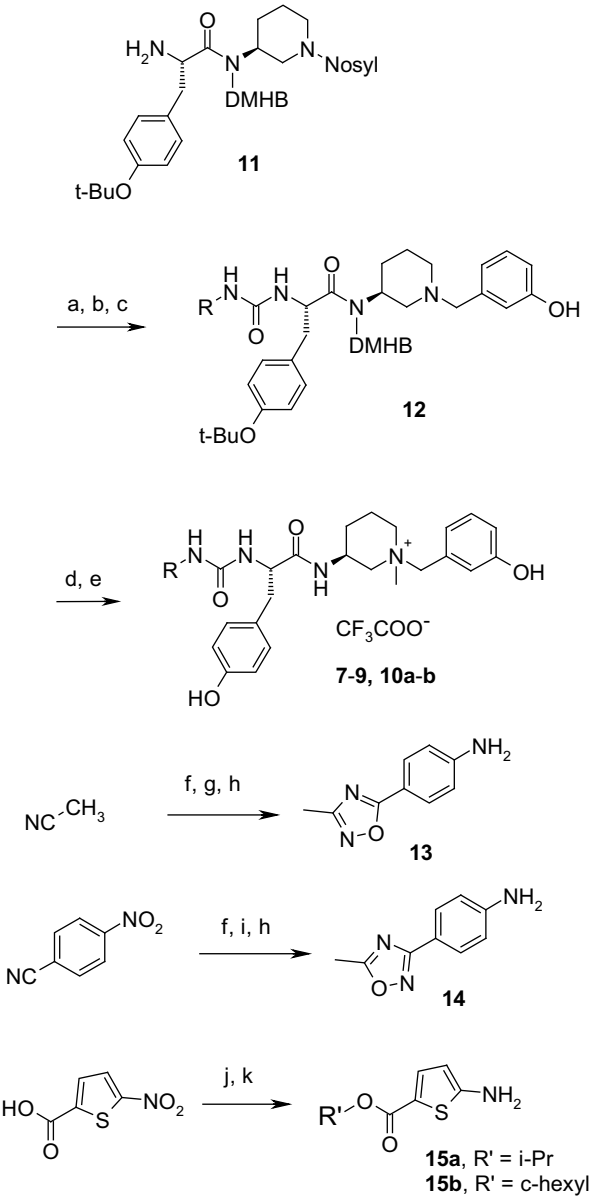


Compound	R	FLIPR pA_2[a]		
		M_3	M_2	M_1
2a		9.9	9.0	7.8
7		9.2	8.4	7.1
8		8.7	8.2	6.8
9		8.7	8.2	6.9
10a		10.0	9.7	7.5
10b		10.7	10.3	8.6

[a] Means of at least two determinations with standard deviation of <±0.3.

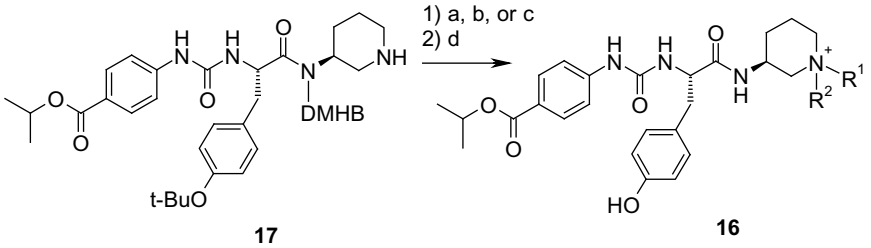
selectivity as esters. In addition, the central diamine moiety was briefly examined. As shown in Figure 3, piperidinium salt **2s** was slightly more potent than pyrrolidinium salt **2r** in the M_3 FLIPR assay. **2s** had similar subtype selectivity for M_3 over M_1 but was less subtype selective for M_3 over M_2 compared to **2r**.

Having identified the preferred structural motifs in each of the five regions examined, we then combined the best SAR elements into single molecules and further optimized two of these regions. As expected, combining the isopropyl ester, 3(*S*)-amino piperidine and quaternary ammonium salt into a single molecule (**2a**) resulted in excellent M_3 potency ($pA_2 = 9.9$) (Table 5).[17] **2a** also had good subtype selectivity—about 10-fold selective for M_3 over M_2 and 100-fold selective for M_3 over M_1. Compared to ester **2a**, isopropyl amide **7** was less potent, but still possessed high M_3 potency with a pA_2 of 9.2 and good subtype selectivity for M_3 over M_2 and M_1. Other ester bioisosteres such as oxadiazole were explored. Oxadiazoles **8** and **9** had good M_3 potency and were



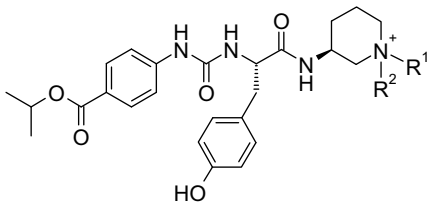
Scheme 2. Reagents and conditions: (a) anilines, CDI, DIEA, DCE, rt; (b) K_2CO_3, PhSH, NMP, rt; (c) 3-hydroxybenzaldehyde, $Na(OAc)_3BH$, 10% of HOAc in NMP, rt; (d) MeI, CH_3CN, rt; (e) 20% of TFA in DCE, rt; (f) NH_2OH, EtOH, reflux; (g) *p*-NO_2–PhCOCl, pyridine, heating; (h) Na_2S, dioxane/H_2O (1:1), heating; (i) MeCOCl, pyridine, heating; (j) $(COCl)_2$, DCM, DMAP, TEA, isopropanol or cyclohexanol, rt; (k) Pd/C, H_2 (15 psi), EtOH, rt.

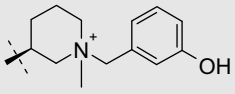
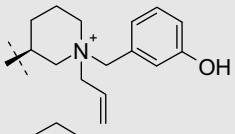
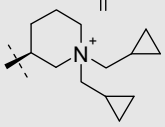
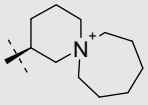
subtype-selective for M_3 over M_2 and M_1, but were significantly less potent than ester **2a**. The LHS phenyl group could be replaced by a thiophene group. Isopropyl thiophenecarboxylate **10a** had



Scheme 3. Reagents and conditions: (a) i—allyl bromide, CH_3CN, rt; ii—3-hydroxybenzaldehyde, $Na(OAc)_3BH$, 10% of HOAc in NMP, rt; (b) i—cyclopropylcarboxyaldehyde, $Na(OAc)_3BH$, 10% of HOAc in NMP, rt; ii—cyclopropylmethylbromide, CH_3CN, 75 °C; (c) 1,6-hexyldibromide, CH_3CN, 75 °C; (d) 50% of TFA in DCE, rt.

Table 6
Further optimization of the quaternary ammonium salt moiety



Compound		FLIPR pA_2[a]		
		M_3	M_2	M_1
2a		9.9	9.0	7.8
16a		10.0	8.8	7.8
16b		9.2	8.3	7.4
16c		8.5	8.3	7.1

[a] Means of at least two determinations with standard deviation of <±0.3.

similar M_3 potency to isopropyl benzoate **2a**. Further optimization of the ester group resulted in cyclohexyl ester **10b**, which was the most potent M_3 antagonist to date with a pA_2 of 10.7. Compared to **2a**, compounds **10a** and **10b** were slightly less selective for M_3 over M_2, but maintained good subtype selectivity for M_3 over M_1. Synthesis of compounds **7, 8, 9**, **10a**, and **10b** is outlined in Scheme 2. Urea formation from resin-bound primary amine **11**, prepared according to Scheme 1, and commercially available 4-amino-*N*-isopropyl benzamide and anilines **13, 14**, **15a**, and **15b** (vide infra), followed by nosyl removal and reductive amination, afforded resin-bound intermediates **12**. Alkylation of tertiary amines **12**, followed by resin cleavage and simultaneous removal of the *tert*-butyl protecting group, produced the desired quaternary ammonium salts **7, 8, 9, 10a**, and **10b**. Anilines **13** and **14** were synthesized in a 3-step sequence—formation of amidoximes from nitriles and hydroxylamine,[18] cyclization of amidoximes with acid chlorides to form oxadiazoles,[19] and reduction of the nitro group.[20] 2-Amino-5-thiophenecarboxylates **15a** and **15b** were prepared from commercially available 2-nitro-5-thiophenecarboxylic acid under standard ester formation and hydrogenation conditions.

We then further explored the quaternary ammonium salt moiety. In addition to *N*-methyl quaternary ammonium salt **2a**, other quaternary ammonium salts exemplified by *N*-allyl ammonium salt **16a** had excellent M_3 potency (pA_2 = 10.0) and good subtype selectivity (greater than 10-fold selective for M_3 over M_2 and 100-fold selective for M_3 over M_1) (Table 6). Symmetrical quaternary ammonium salts such as *N*,*N*-dicyclopropylmethyl compound **16b** also possessed high M_3 potency and good subtype selectivity for M_3 over M_2 and M_1, but were less potent compared to **2a** and **16a**. In addition, compound **16c**, which possesses a spiro quaternary ammonium center, was less potent and less selective for M_3 over M_2 and M_1 compared to **16b**, but still showed good M_3 potency with a pA_2 of 8.5. Symmetrical quaternary ammonium salts exemplified by **16b** and **16c** eliminated the chiral center at the quaternary ammonium nitrogen. Synthesis of compounds **16a, 16b**, and **16c** is outlined in Scheme 3. Resin-bound piperidine **17** was prepared according to Scheme 1. Reductive amination of intermediate **17**, alkylation of the resulting tertiary amines and subsequent resin cleavage and simultaneous removal of the *tert*-butyl protecting group afforded the desired compounds **16a** and **16b**. Compound **16c** was prepared via alkylation of **17** with 1,6-hexyldibromide, resin cleavage and protecting group removal.

In summary, SAR exploration of multiple regions of the HTS hit **1a** led to the identification of key structural motifs necessary for achieving high M_3 potency and good subtype selectivity. Further optimization of this series resulted in highly potent M_3 antagonists such as **2a** and **10b** with greater than 100-fold subtype selectivity for M_3 over M_1.

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References and notes

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12. Measuring inhibition of acetylcholine-induced $[Ca^{2+}]_i$-mobilization in Chinese Hamster Ovary (CHO) cells stably expressing human recombinant M_3 receptor.

13. The biological assay results in the paper are a mean of at least two determinations with standard deviation of <±0.3 unless otherwise noted.
14. For M_3, M_2, and M_1 FLIPR assay details, see Supporting information in Ref. 11.
15. For representative experimental procedures, see Supporting information in Ref. 11.
16. The preferred stereochemistry was elucidated previously and the (3*S*,3′*S*) diastereoisomer was the most preferred, see Ref. 11 for details.
17. The pIC_{50} limit of the M_3 FLIPR assay was about 9.0. pA_2 was determined and used to compare potency for compounds with pIC_{50} reaching the limit. See Ref. 11 for assay details of pA_2 determination.
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