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Discovery of novel 8-azoniabicyclo[3.2.1]octane carbamates as muscarinic acetylcholine receptor antagonists

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Abstract—In the course of our research program to develop novel muscarinic receptor antagonists for the treatment of COPD, new tropane carbamate derivatives were identified as potent anti-muscarinic agents. The synthesis, structure–activity relationships and pharmacological evaluation that led to the identification of compound **5o**, are described.

Muscarinic acetylcholine receptors (mAChRs) belong to the superfamily of G-protein coupled 7-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 respiratory system, 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 airways have been identified as M_1, M_2, and M_3.[4] The M_3 mAChRs are located on the airway smooth muscles and also on the pulmonary submucosal glands where they stimulate 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 muscles, are located on postganglionic parasympathetic nerves.[7] Their role is to provide a tight control of acetylcholine release. M_1 mAChRs are found in the pulmonary parasympathetic ganglia where they function to enhance neurotransmission.[8]

Muscarinic acetylcholine receptor 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 muscarinic acetylcholine 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. Thus the identification of potent mAChR antagonists, particularly of the M_3 subtype, would be useful as therapeutics in these mAChR-mediated disease states.

In the course of our research program to develop novel muscarinic receptor antagonists for the treatment of COPD, we identified new tropane carbamate derivatives as potent anti-muscarinic agents. These compounds were prepared as both their tertiary and quaternary derivatives and evaluated for their muscarinic affinity using recombinant muscarinic M_1, M_2, and M_3 receptors. Some selected compounds were also investigated in a methacholine-induced bronchoconstriction model in mice. In this communication, we report the synthesis, structure–activity relationships, and pharmacological evaluation of this new series of muscarinic antagonists.

The synthetic route to the tropane carbamate derivatives is outlined in Scheme 1. The Curtius reaction of an aromatic carboxylic acid **1** with ethanol using diphenylphosphoryl azide (DPPA) gives the corresponding ethyl carbamate **2**.[11] Alkylation of compound **2** with an alkyl bromide using sodium hydride in DMF produces the branched alkyl carbamate **3**. These intermediates can be further elaborated to the disubstituted carbamate compounds **4** by displacement of the alkoxy

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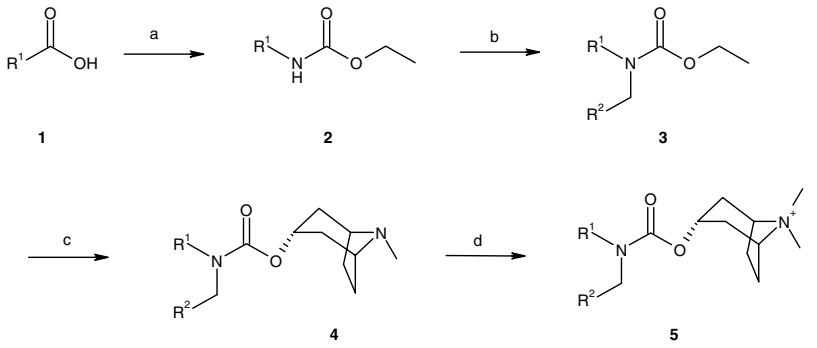
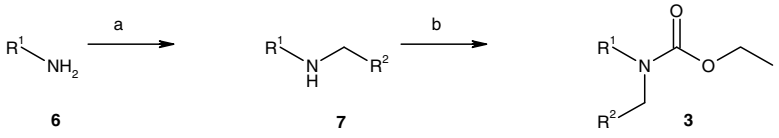


Scheme 1. Reagents and conditions: (a) DPPA, EtOH; (b) R^2CH_2Br, NaH, DMF; (c) tropinol, NaH, toluene; (d) MeBr, CH_2Cl_2, CH_3CN.

moiety with tropanol using sodium hydride in toluene.[12] Subsequent reaction of **4** with methyl bromide affords the related dimethyl quaternary ammonium salt **5**.

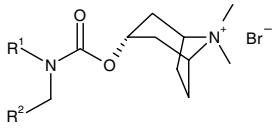
Alternatively, a second method can be utilized to prepare the carbamate derivatives **3** as shown in Scheme 2. The secondary amine **7** can be prepared via a reductive amination reaction between a primary amine **6** and an aldehyde using solid supported cyanoborohydride resin.[13] Subsequently, **7** can be reacted with the commercially available ethyl chloroformate under basic conditions to give compound **3**. Full experimental details and characterization data for key compounds have been described elsewhere.[14]

The functional activities and potencies of the synthesized compounds were evaluated by monitoring calcium mobilization of receptor-activated CHO-cells expressing



Scheme 2. Reagents and conditions: (a) R^2CHO, polymer-supported cyanoborohydride; (b) ethylchloroformate.

Table 1. *N*,*N*-disubstituted carbamate tropane salts



Compound	R^{1c}	R^{2c}	IC_{50}[a] (nM)			Selectivity[b]	
			M_1	M_2	M_3	M_1/M_3	M_2/M_3
5a	Ph	Ph	4.5	48.0	4.6	1.0	10.4
5b	Ph	2-Th	2.4	12.5	2.3	1.0	5.4
5c	Ph	3-Th	2.6	30.2	2.8	0.9	10.8
5d	Ph	4Br–Ph	33.4	399.8	56.6	0.6	7.1
5e	Ph	4OMe–Ph	86.0	687.5	154.4	0.6	4.4
5f	Ph	3Br–Ph	210.3	519.8	342.1	0.6	1.5
5g	Ph	2Br–Ph	420.0	666.4	407.2	1.0	1.6
5h	Ph	4CN–Ph	257.9	1490.1	713.9	0.4	2.1
5i	Ph	3OMe	611.8	1000.3	971.7	0.6	1.0
5j	Ph	5Me–2-Th	15.8	60.8	21.2	0.8	2.9
5k	Ph	3Me–2-Th	3.8	34.7	6.4	0.6	5.4
5l	Ph	3-Furan	12.2	53.7	16.0	0.8	3.4
5m	Ph	3Me–2-Furan	15.2	228.5	42.3	0.4	5.4
5n	2-Th	Ph	2.7	13.8	3.0	0.2	5.7
5o	3-Th	Ph	2.1	10.9	2.0	1.0	5.5
5p	3-Th	3-Th	1.6	7.2	1.0	1.6	7.2
5q	3-Th	*c*-Hex	5.8	55.9	11.3	0.5	5.0
5r	3-Th	3CN–Ph	55.6	638.5	137.4	0.4	4.7

[a] Values are means of two or more independent assays.
[b] The selectivity is calculated as the ratio between the IC_{50} values at M_3 and M_1 or M_2 muscarinic receptors.
[c] Th, thiophene.

cloned M_1–M_3 receptors and the selectivity for M_3 over the other receptor subtypes was examined.

Table 1 summarizes the results obtained for the *N*,*N*-dimethyl carbamate quaternary salts. Generally, it was observed that the introduction of bulky substituents on the phenyl ring, especially at the meta position, appears to be detrimental to the activity of the molecules at the M_3 receptor. Thus the 3-bromo, the 4-cyano, and 3-methoxy derivatives **5f**, **5h**, and **5i** are all significantly less potent than the unsubstituted compound **5a**. This suggests that the decrease in activity is linked to the size of the group and not to the variations on the electronic effect on the aromatic ring. Replacement of either phenyl ring with the isostericaly equivalent thiophene moiety leads to a slight increase of activity at the M_3 receptor (**5b**, **5c**, and **5n**–**p**). However, the furan derivatives, **5l** and **5m**, display less activity than compound **5a**. This series of compounds was found to have similar potencies at the M_1 and M_3 receptors. Activities at the M_2 receptor were markedly reduced compared to the other subtypes. This was illustrated by the 10-fold selectivity for M_3 over M_2 displayed by the derivatives **5a** and **5c**. Interestingly, the *N*-methyl carbamate derivatives display very similar structure activity relationships at R^1 and R^2 to their quaternary salt analogues but with a lesser level of activity (Table 2). For example, the M_3 activity of the most potent thiophene derivative **4p** was found to be several folds lower than that of the corresponding salt **5p**. The decrease in potency might be due to the stronger interaction of the positive tropane nitrogen with the aspartate residue identified in the receptor binding site.[15] No general selectivity trend for one subtype over another was observed for the *N*-methyl derivatives.

In order to evaluate their in vivo efficacy, selected compounds (**5a**–**c**, **n**–**p**) with high affinities were examined in a metacholine-induced bronchoconstriction model in the mouse as shown in Figure 1. In these experiments, greater than 85% bronchoprotection was observed at 15 min postdosing (5 μg/mouse) for all the compounds. At 5 h, the two thiophene derivatives **5n** and **5o** still exhibited over 80% of bronchoprotection, and at 24 h a remaining level of bronchoprotection of 25% was observed for compound **5o** thus demonstrating the prolonged in vivo efficacy of this compound.

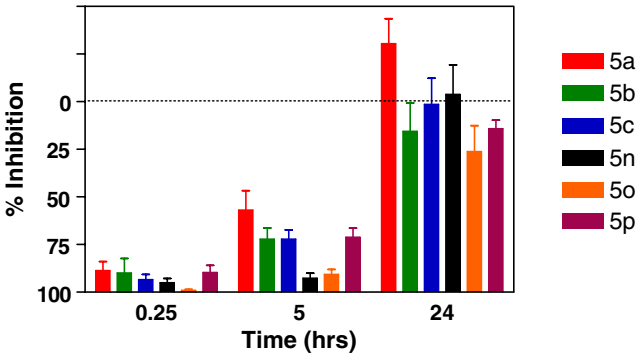
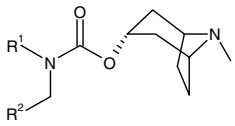


Figure 1. Duration of action in methacholine-induced bronchoconstriction in mice.

Table 2. Carbamate tropane



Compound	R^{1c}	R^{2c}	IC_{50}[a] (nM)			Selectivity[b]	
			M_1	M_2	M_3	M_1/M_3	M_2/M_3
4a	Ph	Ph	161.3	253.8	74.5	2.2	3.4
4b	Ph	2-Th	48.3	38.2	20.3	2.4	1.9
4c	Ph	3-Th	73.8	90.7	27.5	2.7	3.3
4d	Ph	4Br–Ph	812.8	1423.4	720.7	1.1	2.0
4e	Ph	4OMe–Ph	734.4	1364.1	776.3	0.9	1.8
4f	Ph	3Br–Ph	1964.3	2157.2	2310.7	0.9	0.9
4g	Ph	2Br–Ph	1394	1404.7	1143.6	1.2	1.2
4h	Ph	4CN–Ph	847.4	1348.8	1161.3	0.7	1.1
4i	Ph	3OMe	1206.8	1170.8	2135.4	0.6	0.5
4j	Ph	5Me–2-Th	518.1	409.8	261.6	2.0	0.4
4k	Ph	3Me–2-Th	117.3	190.5	108	1.1	1.8
4l	Ph	3-Furan	84.5	82.5	57.3	1.5	1.4
4m	Ph	3Me–2-Furan	349.2	467.7	428.3	0.8	1.1
4n	2-Th	Ph	45.1	26.5	22.9	2.0	1.2
4o	3-Th	Ph	19.1	39.1	10.9	1.7	3.6
4p	3-Th	3-Th	13.8	19.6	7.2	1.9	2.7
4q	3-Th	*c*-Hex	125.5	271.2	109.0	1.1	2.5
4r	3-Th	4CN–Ph	75.8	362.7	136.9	0.6	2.6

[a] Values are means of two or more independent assays.
[b] The selectivity is calculated as the ratio between the IC_{50} values at M_3 and M_1 or M_2 muscarinic receptors.
[c] Th, thiophene.

In conclusion, a new series of *N*,*N*-dimethyl tropane quaternary salts was synthesized and evaluated for muscarinic activities. A number of derivatives showed high affinities for the M_3 and M_1 receptors and moderate selectivity over the M_2 receptor subtype. Further in vivo evaluation of selected compounds demonstrated that this series of molecules have efficacy in an anti-bronchoconstriction model via blockade of the M_3 receptors located on the airways smooth muscles. The inhibition of the M_1 receptors, found in the pulmonary parasympathetic ganglia, might also be contributing to that effect via inhibition of pulmonary neurotransmission.

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