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Discovery of (3-*endo*)-3-(2-cyano-2,2-diphenylethyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide as an efficacious inhaled muscarinic acetylcholine receptor antagonist for the treatment of COPD

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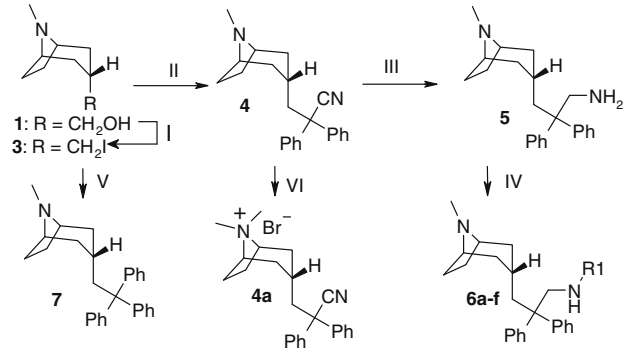
ABSTRACT

Design and syntheses of a novel series of muscarinic antagonists are reported. These efforts have culminated in the discovery of (3-*endo*)-3-(2-cyano-2,2-diphenylethyl)-8,8-dimethyl-8-azoniabicyclo-[3.2.1]octane bromide (**4a**) as a potent and pan-active muscarinic antagonist as well as a functionally active compound in a murine model of bronchoconstriction. The compound has also displayed pharmacokinetic characteristics suitable for inhaled delivery.

Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder that causes significant deterioration of lung function and chronic breathlessness.[1] Six hundred million people worldwide already live with COPD, but its prevalence is predicted to rise to become the world's third leading cause of death by 2020.[2] Rather than a single pathologic condition, COPD is an umbrella term encompassing chronic bronchitis, emphysema and small airways disease.[3] Bronchodilator drugs form a preferred choice of COPD maintenance therapy with two principle classes of bronchodilation agent widely prescriped,[4,5] β_2-adrenoceptor agonists such as Serevent® (salmeterol xinafoate) and muscarinic acetylcholine receptor (mAChR) antagonists such as Spiriva® (tiotropium bromide).[6,7] There are five sub-types (M_1–M_5) mAChRs playing a number of different pharmacological roles both centrally and peripherally.[8–10] As such, it would be beneficial to deliver an anti-cholinergic to the lung that was not systemically active. Both M_2 and M_3 mAChRs are located in smooth muscle and mucosal glands and have been considered as a therapeutical targets for COPD treatment.[11–14] Herein, we disclose the design and syntheses of a novel series of long-acting mAChR antagonists as exemplified by (3-*endo*)-3-(2-cyano-2,2-diphenylethyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide (**4a**).

Compounds described in this paper were synthesized from two known intermediates (Schemes 1 and 2): 3-hydroxymethyltropane (**1**) and 1,1-diphenyl-2-(3-tropanyl)ethanol (**2**).[15,16] Starting from **1**, iodination followed by alkylation of the resultant 3-iodomethyltropane (**3**) with diphenylacetonitrile in the presence of NaH provided nitrile **4**. Reduction of **4** with borane furnished the primary amine **5**, which was utilized to prepare a small array of compound



Scheme 1. Preparation of muscarinic antagonists. Reagents and conditions: (I) I_2, PPh_3, 77%; (II) Ph_2CHCN, NaH, 93%; (III) BH_3·THF, 63%; (IV) (**6a**) $ClSO_2NCO$, 38%; (**6b**) $PhCH_2NCO$ 19%; (**6c**) CH_3CH_2NCO, 45%; (**6d**) Ac_2O, 29%; (**6e**) $PhSO_2Cl$, 54%; (**6f**) $MeSO_2Cl$, 28%; (V) Ph_3CH, *n*-Buli, DMF, 17%; (VI) CH_3Br.

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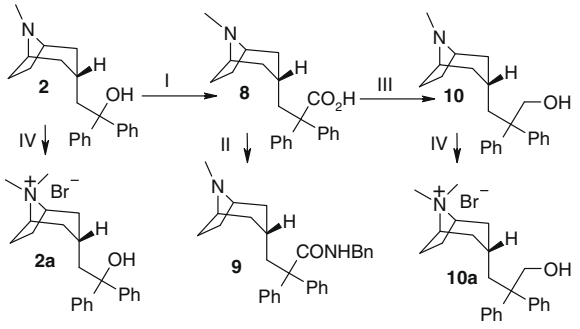


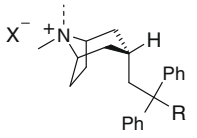
Scheme 2. Preparation of muscarinic antagonists. Reagents and conditions: (I) HCO_2H, H_2SO_4, 48%; (II) $PhCH_2NH_2$, EDC, HOBt, TEA, 30%; (III) LAH, THF, microwave, 100 °C, 71%; (IV) CH_3Br.

including ureas **6a–c**; amide **6d**; and sulfonamides **6e–f**. Alkylation of **3** with triphenyl methane was studied under various conditions furnishing compound **7** with a modest yield. This is very likely due to the steric hindrance exhibited by the three phenyl groups. Acid **8** was prepared by mixing **2** with formic acid and concentrated sulfuric acid in a freezer (−20 °C) for one week.[17] Coupling with benzyl amine afforded amide **9**. Microwave assisted reduction of **8** with $LiAlH_4$ furnished the primary alcohol **10**. Quaternization of the tertiary amine **2**, **4**, or **10** with CH_3Br then gave rise to the corresponding quaternary salt **2a**, **4a** and **10a**.

Screening of the GSK collection of compounds based on the topography of the binding site of the muscarinic receptors M_1–M_3, we uncovered the potent and pan-active antagonist **2** (Table 1). This compound also shows high binding affinity for the three receptors. It contains a bulky lipophilic unit (e.g., diphenyl methylene) linked with a tertiary amine (e.g., tropane), two key structural features in well-documented muscarinic antagonists.[18] The lipophilic part has been considered to drive interactions with the receptors, while quaternization of the tertiary amine was utilized to improve anti-muscarinic activities as well as to reduce side effects related to CNS penetration.[19] We therefore started by searching for appropriate replacements of the tertiary –OH that is directly attached to the lipophilic unit in the lead with the purpose of improving its potency against M_3 receptors via modulating lipophilicity. Topological polar surface area (TPSA),[20] a calculated property that has been widely used to assess molecular interactions with transport membranes, was later found to exhibit better correlation with the lipophilicity of this chemical series than log *D* or log *P*. For examples, insertion of an additional methylene group does not change TPSA and the resultant primary alcohol **10** is as potent as **2**. Replacement with a tertiary nitrile (e.g., **4**) slightly increases TPSA value, but the resultant compound is ten fold more potent as demonstrated later in the binding assays. Dramatic modifications of the polar surface area have been detrimental to potency as evidenced by ureas **6a–c**, amide **6d**, sulfonamides **6e–f**, triphenylmethane **7**, acid **8**, and amide **9**.

The compound inhibitory potency, IC_{50} of an EC_{80} concentration of ACh, as well as a determination of any direct stimulation of the receptors by the compounds was first determined using a standard FLIPR protocol.[22,23] Binding affinities of analogs with IC_{50} less than 10 nM in the FLIPR assays were measured in radioligand binding assays. Compounds **2**, **2a**, **4**, **4a**, **10** and **10a** all display high affinities (K_i) with the three receptors. All compounds were devoid of any agonist activities (data not shown). The antagonist potency of all high potency compounds (IC_{50} <10 nM) was also further quantified on FLIPR (pA_2 determination) by measuring the ratio of the ACh EC_{50} in presence and absence of a single concentration

Table 1
Evaluation of tropane series of muscarinic antagonists in vitro and in vivo



Compd	R	TPSA[a]	X[b]	FLIPR (IC_{50}, nM)[c]			Binding affinity (pK_i)[d]			FLIPR kinetics (pA2)[e]			In vivo
				M_1	M_2	M_3	M_1	M_2	M_3	M_1	M_2	M_3	Inh.[f]
2	–OH	23.5	—	<10	<10	<10	9.3	8.2	8.7	9.1	8.4	8.6	<5%[g]
2a	–OH	—	Br	<10	<10	<10	8.8	8.4	8.5	9.6	9.3	9.3	34%
4	–CN	27.0	—	<10	<10	<10	10.0	9.3	9.8	10.8	9.5	10.2	11%
4a	–CN	—	Br	<10	<10	<10	10.3	9.8	10.2	11.4	10.3	10.7	>95%
6a	$–CH_2NHC(O)NH_2$	58.4	—	3829	5345	6399	—	—	—	—	—	—	—
6b	$–CH_2NHC(O)NHBn$	44.4	—	1017	1273	1338	—	—	—	—	—	—	—
6c	$–CH_2NHC(O)NHEt$	44.4	—	1276	1553	1939	—	—	—	—	—	—	—
6d	$–CH_2NHC(O)CH_3$	32.3	—	2198	3478	2895	—	—	—	—	—	—	—
6e	$–CH_2NHS(O_2)Ph$	49.4	—	770	458	1059	—	—	—	—	—	—	—
6f	$–CH_2NHS(O_2)CH_3$	49.4	—	1597	2119	2732	—	—	—	—	—	—	—
7	–Ph	3.24	—	105	1195	206	—	—	—	—	—	—	—
8	$–CO_2H$	40.5	—	277	240	687	—	—	—	—	—	—	—
9	–C(O)NHBn	32.4	—	596	545	839	—	—	—	—	—	—	—
10	$–CH_2OH$	23.5	—	<10	<10	<10	9.0	7.9	8.6	8.9	8.2	8.4	15%
10a	$–CH_2OH$	—	Br	<10	<10	<10	8.9	8.4	8.6	8.8	8.3	8.5	23%

[a] TPSA values of tertiary amines were calculated with a GSK in-house program according to Ref. 20.
[b] Compound is a tertiary amine unless the counter ion (X) is specified.
[c] Functional assays were performed on CHO cells expressing cloned human M_1–M_3 receptors. Cells were stimulated with Ach. Calcium mobilization was measured using a standard FLIPR protocol. Values are the mean of two or more independent assays.
[d] Radioligand binding assays were conducted using CHO cell membrane preparations in SPA format versus 0.5 nM [^{3}H]-*N*-methyl scopolamine. Values are the mean of two or more independent assays.
[e] The pA2 was determined by measuring the ratio of the ACh EC_{50} in presence and absence of compound.[21]
[f] Screening was done with aerosolized methacholin induced bronchoconstriction in conscious mice. Compound was given by inhalation 24 h prior to the challenging at a dose of 5 μg per mouse.
[g] Compound was administered at a dose of 50 μg per mouse.

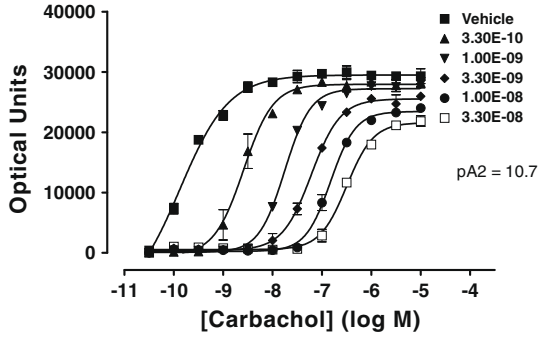


Figure 1. Functional antagonism of **4a** in Ach-induced calcium mobilization (FLIPR) employing CHO-M_3.

of compound.[21] All six compounds effectively antagonized the agonist-induced responses resulting in a rightward shift of the acetylcholine concentration response curve in a manner similar to **4a** at the M_3 receptor as shown in Figure 1. PA_2 values are in good agreement with the corresponding binding affinities (e.g., pK_i). Schild analysis[21,24] of **4a** against M_3, Figure 1, would suggest competitive antagonism.

Quaternary salts (e.g., **2a**, **4a** and **10a)** were prepared to investigate its impact on potency. In general, their in vitro potency is comparable to the amine. All quaternary salts, however, demonstrate much greater magnitude inhibition than the corresponding tertiary amines in response to aerosolized methacholine induced bronchoconstriction in conscious mice when dosed by inhalation 24 h prior to the challenging at the dose of 5 μg per mouse. Of the compounds screened, **4a** exhibited the greatest inhibition and was thus selected for more of detailed studies in the same murine model. This inhibition was found to be dose dependent with ED_{50} of 0.01 μg/mouse (Fig. 2) and the bronchoprotection was extended up to 48 h when given by inhalation at two different doses of 0.5 μg per mouse and 5.0 μg per mouse (Fig. 3).

The pharmacokinetics of **4a** in the rat are characterized by low drug absorption, high plasma clearance, high-to-moderate volume of distribution at steady state, a short terminal half-life and low oral bioavailability. These characteristics are considered desirable for an inhaled drug candidate. These results, as well as detailed SAR discussions of this novel series of antagonists, will be reported in due course.

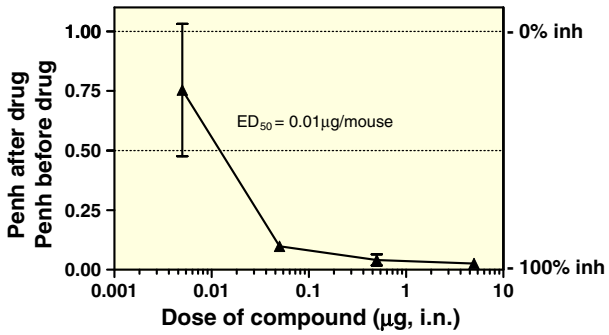


Figure 2. Dose–response of bronchoprotection for **4a**.[25]

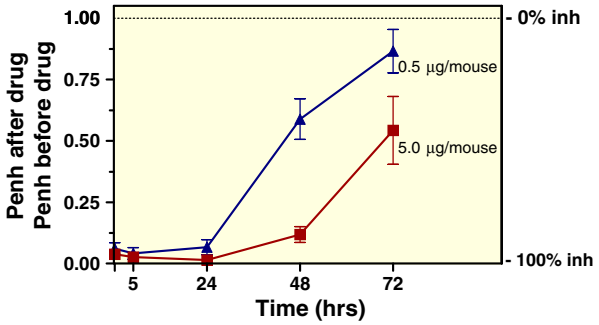


Figure 3. Duration of bronchoprotection for **4a**.[26]

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25. Balb/C mice ($n = 4$) were treated with the compound intra-nasally (in) and then challenged after 5 h with 30 mg/ml methacholine (aerosolized, 2 min). The magnitude of bronchoconstriction was measured as Penh over the next 5 min using a standard Buxco plethysmography system. The data is expressed as a ratio of the Penh achieved with methacholine after drug was given to that achieved before drug was given. A ratio of 1.0 (dotted line) indicates that there was no change in the Penh in response to aerosolized methacholine (i.e., 0% inhibition), and a ratio of 0.0 means that there was no bronchoconstriction in response to methacholine challenge (i.e., 100% inh.).
26. Same as Ref. 25 except the mice were treated with the compound for 0.25–48 h before the challenging.