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# Design, synthesis and structure–activity relationship of N-substituted tropane muscarinic acetylcholine receptor antagonists

Dramane I. Lainé [†], Hongxing Yan, Haibo Xie, Roderick S. Davis, Jeremy Dufour, Katherine L. Widdowson, Michael R. Palovich, Zehong Wan, James J. Foley, Dulcie B. Schmidt, Gerald E. Hunsberger, Miriam Burman, Alicia M. Bacon, Edward F. Webb, Mark A. Luttmann, Michael Salmon [‡], Henry M. Sarau, Sandra T. Umbrecht, Philip S. Landis, Brian J. Peck, Jakob Busch-Petersen *

*GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA*

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ABSTRACT

A novel series of N-substituted tropane derivatives was characterized as potent muscarinic acetylcholine receptor antagonists (mAChRs). Kinetic washout studies showed that the N-endosubstituted analog **24** displayed much slower reversibility at mAChRs than the methyl-substituted parent molecule darotropium. In addition, it was shown that this characteristic appeared to translate into enhanced which duration of action in a mouse model of bronchonstriction.



Muscarinic acetylcholine receptors (mAChRs) 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 (Ach), 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. Thus, mAChR antagonists 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 action (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. The investigation of several series of muscarinic antagonists developed within our laboratory has previously been disclosed.[13–18] Darotropium bromide **1** (Fig. 1), in particular, has been reported as a very potent mAChR antagonist with long duration of action in a mice.[13,14] However, further work

* Corresponding author. Tel.: +1 610 270 7831; fax: +1 610 270 4451.
*E-mail address:* jakob.2.busch-petersen@gsk.com (J. Busch-Petersen).
† Present address: Hoffmann-La Roche Inc. 340 Kingsland Street, Nutley, NJ 07110, USA.
‡ Present address: Merck Research Laboratories, 33 Avenue Louis Pasteur, Boston, MA 021115, USA.

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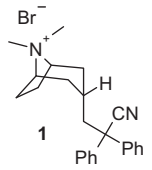
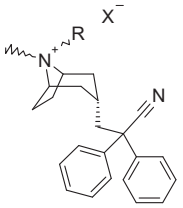



**Figure 1.** Darotropium bromide.

was carried out in order to enhance the understanding of the interplay between molecular structure, potency, receptor kinetics and duration of action. In this letter we report the structural–activity relationships (SAR) at the $M_3$ receptor of a *N*-substituted series of analogs of **1**.

Two complementary methods of preparation of the differentially substituted quaternary salts are shown in Scheme 1. The previously described nitrile **2**[13] was treated with an alkyl halide (other than a methylating agent) in the presence of a base to give quaternary salts **3**–**15** as a mixture of two isomers. One of the compounds formed possessed the pending chain on the opposite side as the C-3 substituent (referred to as *N-Exo*) whereas the other isomer had the chain on the same side of the C-3 substituent (referred to as *N-Endo*). Although synthetically convenient, this approach did not prove to be very selective and variable ratio of both isomers were obtained. Subsequent SAR studies, however, showed that the *N-Endo* derivatives were the more potent of the two isomers. Consequently, a more appropriate route to the preparation to these isomers was designed. Treatment of the known alcohol **16** with iodine,[19] followed by reaction with diphenylacetonitrile and a base afforded the nitrile derivative **5**. Removal of the benzyl group either by hydrogenation or via a quaternization/dealkylation

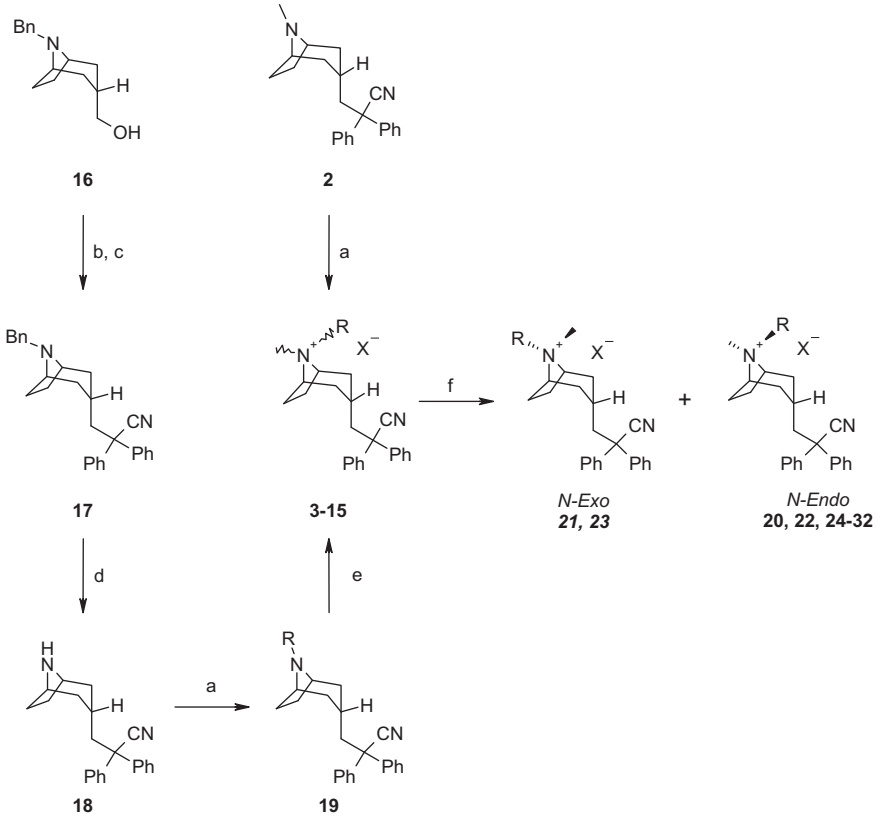
**Table 1**
Tropane N-substitution-initial SAR




| Entry | Stereochemistry | R | X | $M_3$ $IC_{50}$[a] | $M_3$ $pA_2$[b] |
|---|---|---|---|---|---|
| 1 | | –Me | Br | <10 | 10.7 |
| 3 | Mixed | $-CH_2-{}^cPr$ | Br | <10 | 10.3 |
| 4 | Mixed | –Et | Br | <10 | 10.3 |
| 5 | Mixed | –Pr | Br | <10 | 10.3 |
| 6 | Mixed | $-(CH_2)_2OH$ | Br | <10 | 9.8 |
| 7 | Mixed | $-CH_2CH{=}CH_2$ | I | <10 | 8.9 |
| 8 | Mixed | $-(CH_2)_2OMe$ | Br | <10 | 9.3 |
| 9 | Mixed | $-(CH_2)_3CN$ | Br | <10 | 9.8 |
| 10 | Mixed | $-CH_2-{}^cHex$ | Br | 89.6 | |
| 11 | Mixed | $-CH_2-Ph$ | Br | 318 | |
| 12 | Mixed | $-(CH_2)_2{}^cHex$ | Br | 109 | |
| 13 | Mixed | $-(CH_2)_4CH{=}CH_2$ | Br | 85.2 | |
| 14 | Mixed | $-(CH_2)_2O(CH_2)_2OMe$ | Br | <10 | 9.2 |
| 15 | Mixed | $-(CH_2)_{11}CH_3$ | Br | 64 | |
| 20 | *N-Endo* | $-CH_2-{}^cPr$ | | <10 | 10.6 |
| 21 | *N-Exo* | $-CH_2-{}^cPr$ | | 26.3 | |
| 22 | *N-Endo* | $-(CH_2)_4CH{=}CH_2$ | | <10 | >11 |
| 23 | *N-Exo* | $-(CH_2)_4CH{=}CH_2$ | | 32 | |

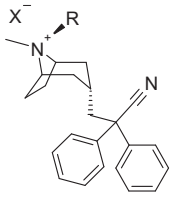
[a] Functional assays were performed on CHO cells expressing cloned human $M_3$ receptors. Cells were stimulated with ACh. Calcium mobilization was measured using the standard FLIPR protocol.[22] Values are in nM and are a mean of two or more independent assays.

[b] The $pA_2$ value was determined by measuring the ratio of the ACh $EC_{50}$ in the presence and absence of compound.[21]




**Scheme 1.** Preparation of mixed and stereospecific quaternary ammonium salts. Reagents and conditions: (a) RX, $K_2CO_3$; (b) $I_2$, PS-$PPh_3$, DCM; (c) PhCH(CN)Ph, NaH, DMF; (d) $ClCO_2CHClCH_3$, mw, 140 °C or $H_2$, 10% $Pd(OH)_2$; (e) MeX, $K_2CO_3$; (f) separation by chromatography.

**Table 2**
$M_3$ activities of *N-Endo* derivatives



| Entry | R | X | $M_3$ $IC_{50}$ [a] | $M_3$ $pA_2$ [b] |
|---|---|---|---|---|
| 20 | $-CH_2-{}^cPr$ | Br | <10 | 10.6 |
| 24 | $-(CH_2)_4CH{=}CH_2$ | Br | <10 | >11 |
| 25 | $-(CH_2)_2OCH_2Ph$ | I | <10 | >11 |
| 26 | $-(CH_2)_3Ph$ | Br | <10 | >11 |
| 27 | $-(CH_2)_3OPh$ | Br | <10 | >11 |
| 28 | $-(CH_2)_3CH_3$ | Br | <10 | >11 |
| 29 | $-(CH_2)_5CH_3$ | Br | <10 | >11 |
| 30 | $-(CH_2)_2OPh$ | Br | <10 | >11 |
| 31 | $-(CH_2)_3OCH_2Ph$ | Br | <10 | 10.4 |
| 32 | $-(CH_2)_7CH_3$ | Br | <10 | 10.1 |

[a] Functional assays were performed on CHO cells expressing cloned human M3 receptors. Cells were stimulated with ACh. Calcium mobilization was measured using the standard FLIPR protocol.[22] Values are in nM and are a mean of two or more independent assays.

[b] The $pA_2$ value was determined by measuring the ratio of the ACh $EC_{50}$ in the presence and absence of compound.[21]

two-steps process, led to the secondary amine **17**.[20] The larger substituent was attached onto **17** by treatment with the appropriate alkyl halide to give the tertiary amine **18**. Subsequent methylation of **18** delivered a mixture of the two diastereoisomeric salts which could be separated by chromatography to give the *N-Endo* derivatives (**24**–**32**) as the major products and the *N-Exo* derivatives as the minor products. Using this approach, we found that the selectivity was generally high for the desired *N-Endo* derivative (80–90%). This selectivity probably originated from the preferential approach of the electrophile from the least hindered face.

The compound inhibitory potency ($IC_{50}$ of an $EC_{80}$ concentration of Ach) at cloned human $M_3$ receptors was first determined by calcium mobilization assays. When a compound $IC_{50}$ was lower than 10 nM, antagonist potency was further quantified by a $pA_2$.[21]

In order to probe the initial SAR for potency around the N-substitution, a series of derivatives where one of the methyl groups of **1** was replaced with a variety of substituents was quickly synthesized. At that point, and chiefly for practical reasons, the compounds were tested as mixtures of diasterosiomers of unknown composition (Table 1). Generally, it was observed that the introduction of relatively small groups, such as ethyl, propyl or cyclopropylmethyl gave compounds with slightly decreased potency compared to **1** (**3**–**5**). Elongation of the chain by one of two atoms further decreased the compound potencies by about half a log unit (**6**–**9**). The activity of compound containing cyclohexyl or phenyl rings at the terminal chain position was also markedly reduced (**10**–**12**). Finally, we found out that the introduction of chains containing 6 or more carbon atoms did not increase compound activity either (**13**–**15**). Although, this first round of SAR was somewhat disappointing, we decided to proceed with the separation of the isomeric mixture of the most potent compound, **3**, in the hope that one of the diasteroisomers could be differentiated with regards to potency. Indeed this proved to be the case as the isomer **20** with the larger group pointing towards the same side as the biphenylcyano moiety (*N-Endo*) was found to be almost equipotent to **1**. Positioning the substituent in the opposite direction (*N-Exo*), however, led to a sharp lost of potency (**21**). Similar results were obtained for the alkene derivative **13**. In that case, the *N-Endo* derivative, **22**, was found to be much more potent than **13**, suggesting that this isomer was only present as a minor component of the original mixture. As with the cyclopropyl derivatives, the *N-Exo* isomer **23** was markedly less potent than the *N-Endo* isomer. These results clearly indicated the importance of the stereochemistry at the tropane nitrogen and suggested that the testing of the compounds as diasterosiomeric mixtures was not the most appropriate way to proceed.

The next step of our investigation was to further explore the SAR of the *N-Endo* derivatives. The compounds were prepared using the chemical route via the secondary amine **18**, as previously described. As shown in Table 2, a wide variety of groups were tolerated at that position. It appears that the receptor pocket accommodating these substituents can tolerate linear alkyl chains of up to 7 carbon atoms. In fact, the majority of the compounds were so potent that their $pA_2$'s could not be accurately determined using this assay and they are simply listed as >11.

To further assess the pharmacological profile of the series the *N-Endo* derivatives, one exemplar, **24** was chosen for additional evaluation and comparison with the previously reported profile

**Table 3**
Comparison **24** versus **1**

| Properties | | **1** | **24** |
|---|---|---|---|
| Binding affinities[a] | $M_1$ (nM) | 0.07 ± 0.01 | 0.12 ± 0.02 |
| | $M_2$ (nM) | 0.17 ± 0.01 | 0.10 ± 0.02 |
| | $M_3$ (nM) | 0.06 ± 0.02 | 0.16 ± 0.02 |
| In vitro tissue studies | $pA_2$[b] | 10.0 ± 0.2 | NC[c] |
| | Off $t_{50}$ (min)[d] | 85 ± 5 | >600 |
| Mouse in vivo studies | $ED_{50}$ (μg/mouse)[e] | 0.01 | 0.02 |
| | Hours duration (dose (μg/mouse)) [f] | ~48 (0.5) | >96 (0.05) |
| Rat PK properties | Cl (ml/min/kg)[g] | 74 ± 21 | 125 ± 14 |
| | F (%)[h] | NQ[i] | NQ |

[a] 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 three independent assays.

[b] Potency against carbachol-induced contraction in human bronchus.

[c] NC = non competitive.

[d] Reversal time for 50% of carbachol-induced contraction to return to maximum relaxation in human bronchus after incubation at [10 nM] antagonist (The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents).

[e] Potency in conscious mice against MCh-induced bronchoconstriction ($n$ = 1).

[f] Duration of bronchoprotection in conscious mice against methacholine-induced bronchoconstriction. Time to 50% loss of maximum protection.

[g] Cl – systemic plasma clearance. iv doses: 1 mg/kg.

[h] F – percent bioavailability. po doses: 2 mg/kg.

[i] NQ = no quantifiable plasma levels following oral administration. (All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.).

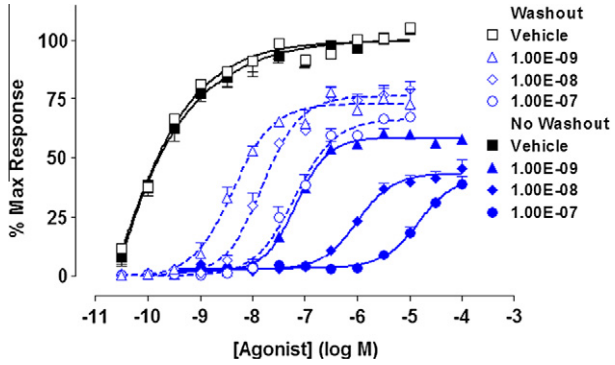



**Figure 2.** Concentration-dependent shifts in ACh concentration response curve after 90 min incubation with **24** and reversibility following a 180 min wash-out at the human $M_3$ receptor.

of **1** (Table 3).[14] The selection was made on the basis of the ability of **24** to form a stable crystalline form with a high melting point (mp >200 °C), which is considered a desirable property for a dry powder inhaled drug as it may help prevent polymorphic transformation or chemical degradation during manufacturing processes such as micronisation or spray-drying.[23] In radioligand binding studies, **24** was found to be comparably potent to **1** and competed with [$^3$H]-*N*-methylscopolamine binding to $M_3$ mAChR ($K_i$ of 0.10 nM). No clear selectivity for $M_3$ over the two other receptor subtypes was observed for both molecules. Studies to evaluate the reversibility of **24** at the $M_3$ receptor were also conducted in the FLIPR assay using a 30 min incubation interval followed by a 180 min washout period. As shown in Figure 2, following the washout, acetylcholine concentration–response curves did not fully reverse to levels obtained in the absence of drug at any concentration of the compound tested. Similar findings were obtained at endogenously expressed $M_3$ mAChRs. Thus, in human bronchus kinetic studies, **24** was found to have a very long off rate (off $t_{50}$ >600 min), which was considerably longer than **1** (85 min). Taken together, these data indicate that antagonist blockade remained after washout, suggesting that **24** is very slowly reversible at the $M_3$ receptor. To examine the impact of the compound kinetics on in vivo duration of action, **24** was further characterized in a mouse plethysmography model. Both compounds exhibited similar potency when dosed intranasally with $ED_{50}$'s of 0.02 and 0.01 μg per animal for **24** and **1**, respectively. However, when dosed at the $ED_{80}$ (0.05 μg/mouse), **24** displayed considerable longer duration of bronchoprotection (>96 h) than **1** (~48 h) even though the latter was given at a dose which exceeded the $ED_{90}$ (0.5 μg/mouse). This is most likely due to the slower target off-rate of **24**. From a pharmacokinetic viewpoint, both compounds are characterized in the art as having very high systemic clearance and low bioavailability; two properties considered desirable for a drug delivered by inhalation.

In conclusion, a novel series of *N-Endo* tropane derivatives was characterized as potent $M_3$ mAChR antagonists. Kinetic washout studies at the human $M_3$ cloned receptor and in human bronchus showed that analog **24** displayed slower reversibility at the mAChRs than the parent molecule **1**. This profile translated in a much longer in vivo duration of action for **24** compared to **1**. Taken together, these results suggest that the introduction of alkyl chains on the tropane nitrogen of **1** has created a novel class of mAChR antagonists with a better likelihood to achieve prolonged duration of bronchodilation in humans. Further studies of these compounds will be reported elsewhere.

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