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The discovery of new spirocyclic muscarinic M_3 antagonists

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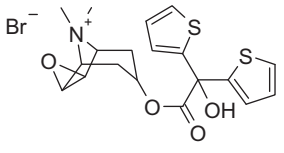
ABSTRACT

The optimisation of a new series of high potency muscarinic M3 antagonists, derived from high throughput screening library hit is described.

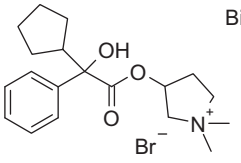
Muscarinic acetylcholine receptors belong to the G-protein-coupled receptors family and currently five different receptor subtypes (M_1–M_5) have been identified.[1] The M_3 receptor subtype is coupled to G_q and mediates an increase in phosphatidyl inositol hydrolysis and calcium release from internal stores. Muscarinic receptors are widely distributed, both in the central nervous system and in the periphery. The prime function of M_3 antagonists acting in the lung is to antagonise acetyl choline induced smooth muscle contraction, leading to bronchodilation, and as a consequence one of the principle therapeutic applications of M_3 antagonists lies in chronic obstructive pulmonary disease (COPD).[2]

Tiotropium bromide is, at present, considered the bronchodilator of choice[3] and is generally well tolerated with dry-mouth being the main observed side-effect. Tiotropium bromide directly targets the lung through inhaled formulations (in combination with a β_2-agonist) and this method of administration has been shown to reduce the adverse events seen through antagonism of cardiac M_2 receptors (leading to tachycardia), as the metabolically unstable pan muscarinic antagonist becomes rapidly metabolised upon systemic exposure. Glycopyrrolate bromide (Phase 2b—Novartis)[4] and aclidininium bromide (Phase 3—Almirall-Forest)[5] are a selection of newer muscarinic antagonists in late stage clinical



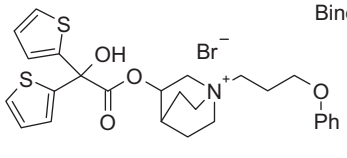
Binding	pIC_{50}
M_3	10.3
M_1	10.5
M_2	10.5
M_4	10.4
M_5	10.0

Tiotropium Bromide



Binding	pIC_{50}
M_3	9.6
M_1	9.9
M_2	9.3
M_4	9.8
M_5	9.7

Glycopyrrolate Bromide



Binding	pIC_{50}
M_3	9.9
M_1	10.0
M_2	9.9
M_4	9.7
M_5	9.8

Aclidinium Bromide

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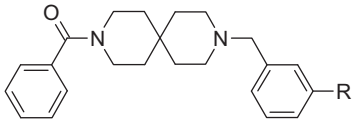
development for COPD, and once more these compounds are reported to be non-selective muscarinic M_{1-5} antagonists. The fixed charge present in Tiotropium, glycopyrrolate and aclidinium limits absorption of the compound into the systemic circulation and once absorbed the compounds are rapidly metabolised thus limiting systemic exposure.

The selectivity profile of M_3 antagonists has been debated widely in the literature[6] however, there is still a need for safer M_3 antagonists for the treatment of COPD[7] and our program set out with the aim of looking for new muscarinic antagonists with an improved muscarinic M_3 safety profile for the treatment of COPD.

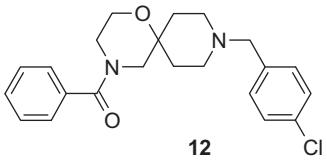
Compounds **1** and **2** were identified from a targeted medium throughput screen and possessed promising potency as well as attractive structural features amenable to optimisation by rapid parallel synthesis. The affinity (pIC_{50}) of compound binding was determined by competition binding of [^{3}H]*N*-methyl scopolamine to CHO-K1 cell membranes expressing the human muscarinic acetylcholine M_3 or M_2 receptor in a SPA format.[8]



Binding	**1** R=Cl pIC_{50}	**2** R=Me pIC_{50}
M_3	7.0	7.0
M_2	8.4	8.6

Further exploration of the 3,9-diaza-spiro[5,5]undecanes **1** and **2** proved unsuccessful (Table 1) as although good potency was achieved, the compounds were shown to possess a high degree of M_2 selectivity, which was contrary to the project aim.

As a consequence, effort was focussed into exploring other spirocyclic ring systems and a range of compounds were prepared from which compound **12** proved interesting as a new lead, due to the higher degree of chemical novelty and improved profile for M_3 versus M_2.



12

Binding	pIC_{50}
M_3	6.6
M_2	6.9

Table 1
Selected binding affinities for the 3,9-diaza-spiro[5,5]undecanes series

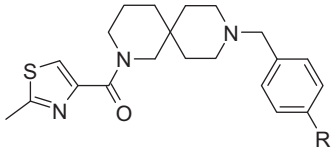
Entry	R^1	R^2	M_3 pIC_{50}[a]	M_2 pIC_{50}[a]
3	Phenyl	Phenyl	6.9	8.2
4	3-Pyridyl	Phenyl	<6.0	7.1
5	2-Furyl	Phenyl	<6.0	7.1
6	3-(2,5-Dimethylthiazole)	Phenyl	i.a.	6.5
7	3-Thienyl	Phenyl	6.5	7.8
8	3-(2,4-Dimethylisoxazole)	Phenyl	i.a.	6.1
9	2-Isoxazolyl	Phenyl	i.a.	6.1
10	Pyrimidinyl	Phenyl	i.a.	6.6
11	2-Ethoxyphenyl	Phenyl	6.1	7.1

i.a. <50% inhibition at 1 μM.
[a] Affinities measured ±0.2 ($n \geqslant 2$).

Parallel synthesis was employed to explore the series and a summary of key results are shown (Table 2).

Analysis of the results showed that the substituted thiazole amides (e.g., **25**–**33**) were the most interesting series to explore as they were generally more potent than the corresponding phenyl **12** and other heterocyclic compounds (e.g., **13**–**24**). Interestingly, the parent 4-thiazole **24** has reduced affinity compared to the 2-methyl substituted thiazole **25**. Further exploration of the thiazole 2-substituent showed an improvement for the 2-ethyl compound **28** and an even better profile was obtained with the 2-*iso*-propyl compound **31** through reduction of the M2 activity, whilst maintaining potent M3 activity. Interestingly, the 2,5-dimethyl substituted thiazole **20** showed a decrease in affinity and the incorporation of a 2-*tert*-butyl group **29** showed a dramatic loss in potency at both the M_3 and M_2 receptors. Exploration of the substitution pattern on the piperidine *N*-benzyl ring demonstrated that 3-chloro substitution **26** was favoured over the corresponding 2-chloro and 4-chloro isomers **27** and **25**.

Surprisingly, conversion of the 1-oxa-4,9-diazaspiro[5,5]-undec-9-ane spirocyclic ring system to the corresponding 2, 9-diaza-spiro[5,5]undecanes **35** and **36** (synthesised from the corresponding commercially available intermediates) afforded compounds with much reduced M_3 and M_2 activity, thus demonstrating the importance of the morpholine ring in driving muscarinic activity within this series.



Binding	**35** R=Cl pIC_{50}	**36** R=H pIC_{50}
M_3	<6.0	<6.0
M_2	<6.0	<6.0

The observation that compounds **35** and **36** have much reduced activity cannot readily be explained. However, pharmacophore modelling of the overlay of tiotropium and glycopyrrolate[9] shows four distinct pharmacophore points (Fig. 1), which are also present in **25**.

Overlaying the 4-point pharmacophore analysis onto compound **35** shows that the key acceptor 1 is missing (Fig. 2) and this may well explain the dramatic loss of activity shown over the replacement of the morpholine **25** with piperidine in **35**.

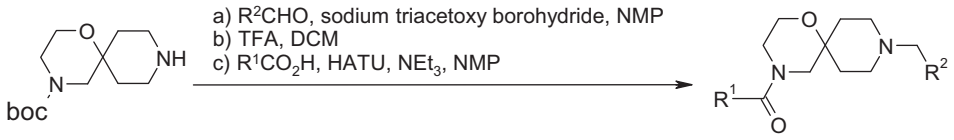
The boc-protected spirocyclic piperidine morpholine was prepared as shown in Scheme 1.

The known epoxide **37**[10] was reacted with ammonia in methanol to give exclusively the primary amine **38**, which was in turn reacted with chloroacetyl chloride to afford **39** in a high yielding 2-step process. Reduction followed by non-acidic work up afforded the mono-protected 1-oxa-4,9-diazaspiro[5,5]undec-9-ane **40** that served as the template in the resulting parallel synthesis strategy.

A new and potent muscarinic antagonist series has been disclosed. Brief exploration of the SAR has demonstrated that an improved selectivity profile for M_3 over M_2 can be achieved by substitution of the 2-position of the thiazole ring and the 2-*iso*-propyl substituted thiazole amide **31** demonstrates a compound with an acceptable muscarinic M_3 profile. Brief exploration of the piperidine *N*-benzyl substituent showed substitution in the 3-position was favoured in a series of chlorine-substituted isomers.

Compound **25** was shown to have reasonable in vitro pharmaco-kinetic (human microsomal clearance 11 and rat hepatocyte clearance 47 μL/min/mg) and physic-chemical properties (human PPB 57% free, Log *D* 2.2, MWt 405) and work is continuing in our laboratories to further optimize the chemical series.

Table 2
Selected binding affinities for spirocyclic piperidine morpholine series



Entry	R^1	R^2	M_3 pIC_{50}[a]	M_2 pIC_{50}[a]
13	5-Benzofuryl	4-Chlorophenyl	7.5	7.5
14	2-(5-Methylfuryl)	4-Chlorophenyl	7.3	7.3
15	2-Furyl	4-Chlorophenyl	7.2	7.6
16	2-Thienyl	4-Chlorophenyl	7.2	7.7
17	2-(5-Hydroxymethylfuryl)	4-Chlorophenyl	7.0	6.9
18	3-Furyl	4-Chlorophenyl	6.9	7.2
19	4-Thiadiazolyl	4-Chlorophenyl	6.7	7.4
20	3-(2,5-Dimethylthiazolyl)	4-Chlorophenyl	6.7	7.6
21	2-Pyrimidinyl	4-Chlorophenyl	6.4	6.9
22	3-Isoxazolyl	4-Chlorophenyl	6.4	7.1
23	3-Pyridyl	4-Chlorophenyl	<6	ND
24	4-Thiazolyl	4-Chlorophenyl	6.8	7.3
25	4-(2-Methylthiazoly)	4-Chlorophenyl	8.0	8.5
26	4-(2-Methylthiazoly)	3-Chlorophenyl	8.9	8.9
27	4-(2-Methylthiazolyl)	2-Chlorophenyl	8.1	8.5
28	4-(2-Ethylthiazolyl)	4-Chlorophenyl	7.8	7.9
29	4-(2-*tert*-Butylthiazolyl)	4-Chlorophenyl	<6	<6
30	4-(2-*iso*-Propylthiazolyl)	4-Chlorophenyl	7.8	7.5
31	4-(2-*iso*-Propylthiazolyl)	Phenyl	8.4	7.8
32	4-(2-*iso*-Propylthiazolyl)	2-Chlorophenyl	7.7	7.6
33	4-(2-*iso*-Propylthiazolyl)	3-Chlorophenyl	8.3	8.0
34	4-(2-Methylthiazolyl)	Methyl	6.4	6.1

ND not determined.
[a] Affinities measured ±0.2 ($n \geqslant 2$).

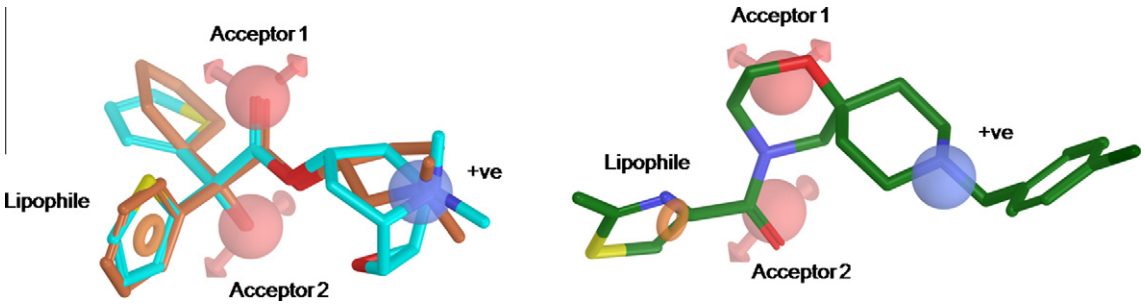


Figure 1. 4-Point pharmacophore mapping of the overlays of tiotropium and glycopyrrolate (left) and compound **25** (right). The +ve term indicates either a protonated or quarternised amine.

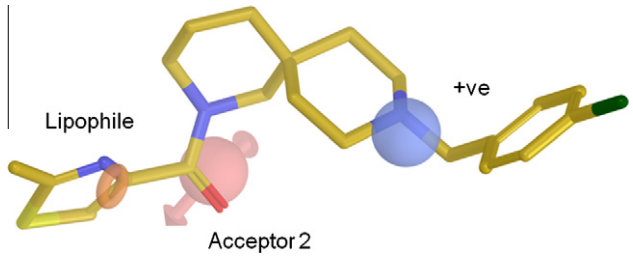
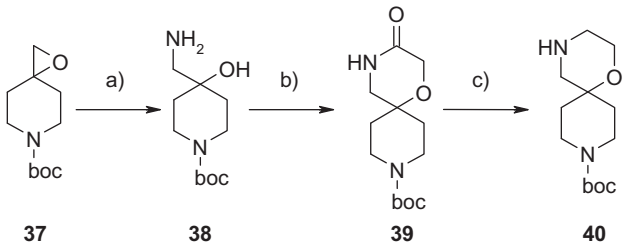


Figure 2. Overlay of the 4-point pharmacophore mapping with **35**, highlighting the loss of the key acceptor 1.



Scheme 1. Reagents and conditions: (a) 7 N ammonia in methanol (30 equiv), rt, 3 days (100%); (b) (i) chloroacetyl chloride (1.25 equiv), K_2CO_3 (2.5 equiv), H_2O/EtOAc; (ii) KO^{t}Bu, (1.25 equiv), ^{t}BuOH, 90 °C (67%); (c) (i) BH_3/THF (4 equiv), THF, 55 °C, 2 h; (ii) MeOH, $Me_2NCH_2CH_2NMe_2$, reflux, 6 h (95%).

Acknowledgements

The authors thank Dr. Simon Teague and Dr. Tim Luker for helpful discussions.

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8. The affinity (pIC_{50}) of compounds binding to the M_3 receptor was determined by competition binding of [^{3}H]*N*-methyl scopolamine (NMS) to CHO-K1 (Chinese Hamster Ovary) cell membranes expressing the human muscarinic acetylcholine M_3 or M_2 receptor (M_3-ACh) in a scintillation proximity assay (SPA) format. SPA beads were pre-coated with membranes and then incubated at 2 mg of beads per well with serial dilutions of compounds, [^{3}H]NMS at half K_D (experimentally determined dissociation constant) and assay buffer (20 mM HEPES pH 7.4 containing 5 mM $MgCl_2$). The assay was conducted in a final volume of 200 μL, in the presence of 1% (v/v) dimethyl sulphoxide (DMSO). Total binding of [^{3}H]NMS was determined in the absence of competing compound and non-specific binding of [^{3}H]NMS was determined in the presence of 1 μM atropine. The plates were incubated for 16 h at room temperature and then read on a Wallac Microbeta™ using a normalised ^{3}H protocol. pIC_{50}, values were determined as the negative logarithm of the molar concentration of compound required for 50% reduction in specific [^{3}H]-NMS binding.
9. Pharmacophore modelling was performed using the Schrödinger software suite within Maestro (Maestro, version 9.0, Schrödinger, LLC, New York, NY, 2009). 3D conformations of the molecules were generated and ionisation states at pH 7.0 ± 1.0 were assigned by the small molecule preparation utility LigPrep (LigPrep, version 2.3, Schrödinger, LLC, New York, NY, 2009). Phase (Phase, version 3.1, Schrödinger, LLC, New York, NY, 2009) was used to identify a 4-point pharmacophore model that encompassed all three active ligands, using default parameters for both the identification of potential hypotheses and subsequent scoring of the candidates.
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