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Synthesis and optimization of novel and selective muscarinic M_3 receptor antagonists☆

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Abstract—A series of constrained piperidine analogues were synthesized as novel muscarinic M_3 receptor antagonists. Evaluation of these compounds in binding assays revealed that they not only have high affinity for the M_3 receptor but also have high selectivity over the M_2 receptor.

Overactive bladder (OAB) arises from the uncontrolled spontaneous activity of the detrusor muscle during bladder filling leading to the symptoms of urinary urgency and increased frequency of micturition with or without incontinence. Urinary bladder contraction is predominantly under the control of the parasympathetic system, where the primary neuronal input is via cholinergic muscarinic receptors. Five distinct muscarinic subtypes are known to exist (M_1–M_5) and at least two of them are involved in normal and disturbed bladder contraction. In patients with an overactive bladder, blocking the muscarinic receptors in the detrusor muscle will cause less frequent and less forceful bladder contractions allowing improved bladder filling and reduced urge incontinence. However, since the muscarinic receptors are widely distributed throughout the body, an antimuscarinic action at organ systems other than the urinary bladder can lead to several adverse effects including dry mouth, constipation, blurred vision, headache, somnolence and tachycardia. It is believed that achieving subtype selectivity may lead to organ selectivity. The human urinary bladder smooth muscle contains a mixed population of muscarinic M_2 and M_3 receptor subtypes. Although the M_2 receptors predominate in number, the M_3 receptors are mainly responsible for normal micturition contraction because they mediate direct contraction of the detrusor muscle.[1] The M_2 receptor contributes by reversing the relaxation produced by β-adrenoceptor stimulation. However, blockade of the M_2 receptor may lead to adverse cardiac effects, owing to the presence of functional M_2 receptors in the heart. Thus, a dual M_3/M_2 receptor antagonist would produce the required relaxation of the bladder but might also induce cardiac toxicity. Indeed, the importance of selectivity is reflected in the recently launched drugs darifenacin and solefenacin, which have been reported[2] to have M_3/M_2 selectivity of 59- and 12-fold, respectively. There are few reports of compounds having high M_3 receptor selectivity,[3] and the present study highlights the high selectivity seen in a novel class of azabicyclohexanes.

Recently,[4] we disclosed compounds of general structures **1** and **2** as having high affinity for the M_3 receptor but moderate selectivity over the M_2 receptor. With a quest to achieve higher affinity and selectivity, we designed a new series of compounds **3** based on series **2** in which the acetylenic group has been replaced by the more rigid bicyclic moiety, (1α,5α,6α)-6 amino-3-azabicyclo[3.1.0]hexane. As with other M_3 receptor antagonists,

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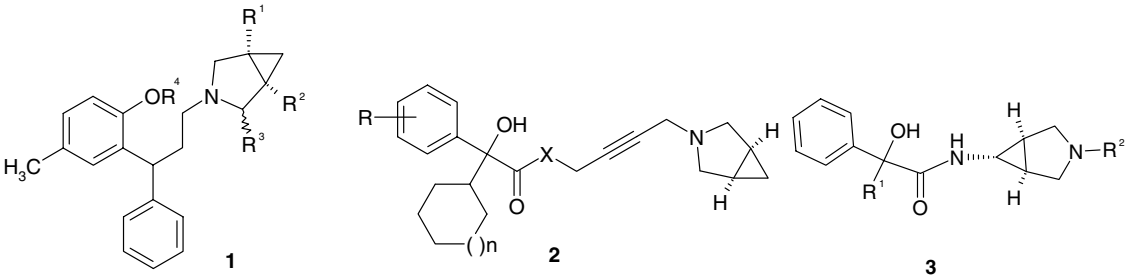
many of the key pharmacophoric features such as a bulky hydrophobic group, a hydroxyl group, a basic nitrogen atom and a set distance between the carbonyl group and the basic nitrogen were maintained. This design is also supported by reports[5,6] that piperidine and piperazine may be used as the required amine portion.

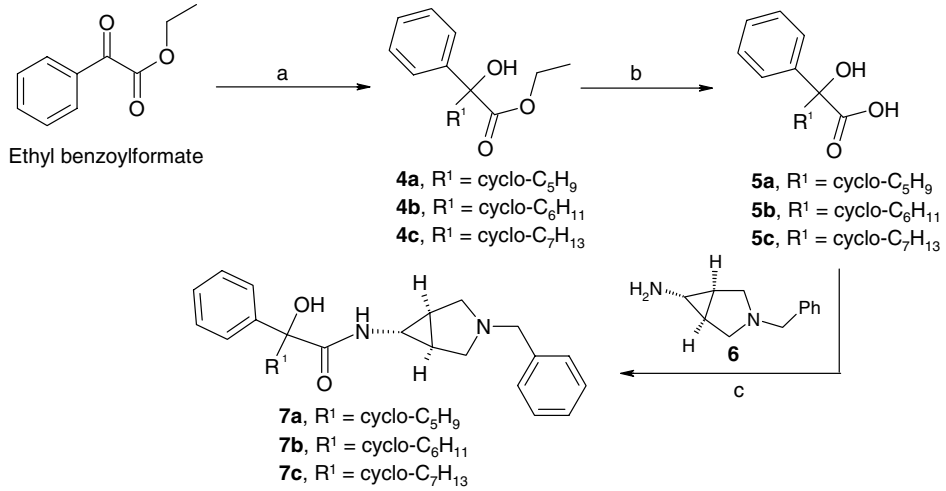


A number of compounds were synthesized with the general structure **3**, and the present work discusses the optimization of the groups R^1 and R^2.

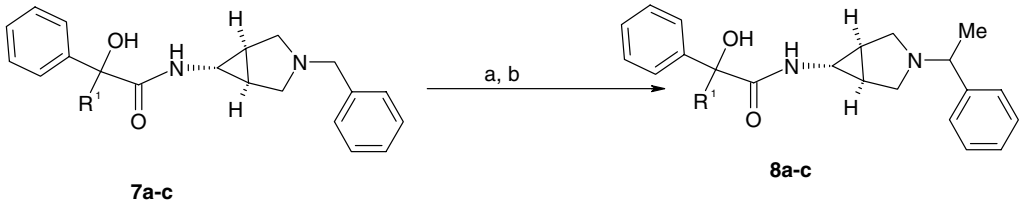
rac-2-Cycloalkyl-2-phenyl-2-hydroxy acetic acids **5a–c** were prepared by the addition of cycloalkyl magnesium bromides to ethyl benzoylformate followed by basic hydrolysis using literature precedent.[7] The amidic condensation of amine **6**[8] with the acids (**5a–c**) in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBT) and *N*-methylmorpholine (NMM) gave **7a–c** (Scheme 1). Compound **7d** was obtained by coupling *rac*-mandelic acid with amine **6**.

Compounds **7a–c** were debenzylated in quantitative yields under hydrogenation conditions using Pd/C as catalyst in methanol and the crude secondary amines converted to compounds **8a–c** by reductive amination with acetophenone (**8a**) or alkylation with 1-bromo-ethylbenzene (**8b–c**) (Scheme 2). The products were diastereomeric mixtures.

Compounds **8d–m** in which R^1 was fixed as cyclopentyl were obtained from compound **7a** by hydrogenation followed by reductive amination reactions with 2-thiophene carboxaldehyde (**8d**), 4-methylacetophenone (**8e**) and 3-phenylpropanal (**8l**). Compounds **8f–k** and **8m** were obtained by hydrogenation of **7a** followed by alkylation with various bromides under alkaline conditions



Scheme 1. Reagent and conditions: (a) R^1MgBr, THF, 0 °C to rt, 20–60%; (b) KOH, MeOH, rt, 70–95%; (c) **6**, EDC, HOBT, NMM, DMF, 0 °C to rt, 70–80%.



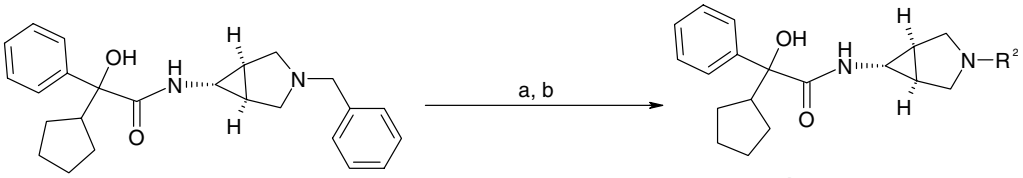
Scheme 2. Reagent and conditions: (a) H_2, Pd/C, MeOH, rt, 80–90%; (b) PhCOMe, $Na(OAc)_3BH$, HOAc, THF, rt, 16% (**8a**) or PhCH(Br)Me, K_2CO_3, KI, CH_3CN, reflux, 83% (**8b**) and 30% (**8c**).

(Scheme 3). The *R* and *S* isomers of compound **8m** and the *R* isomer of **8i** were prepared from the enantiomers of **5a**.[9]

The muscarinic M_3 receptor binding affinity of compounds **7a–d** and **8a–c** indicates a preference for bulkier groups at R^1 (Table 1). When R^2 is benzyl, M_3/M_2 selectivity increases with increasing bulk at R^1, but interestingly no such an effect is observed when R^2 is α-methylbenzyl.

A comparison of compounds in which the R^1 group was initially maintained as a cyclopentyl group (Table 2) reveals that the thiophenyl compound **8d** has lower muscarinic M_3 receptor affinity than the phenyl analogue **7a**, indicating in this case that thiophene does not behave as bioisostere for benzene. Comparing compound **7a** with compounds **8k** and **8l** shows that optimum M_3 receptor affinity and selectivity arises when the phenyl ring is one atom or preferably two atoms away from the tertiary nitrogen atom. Branching of the methylene linker produces a small increase in M_3 receptor affinity and M_3/M_2 receptor selectivity (compare compound **7a** with compounds **8a** and **8e**), but this small increase is accompanied with more complexity in the molecule i.e. an additional chiral centre.

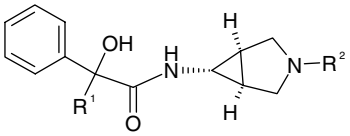
Keeping in mind the preference for R^2 to be an arylethyl group, we decided to study the effect of substitution on the phenyl ring of the R^2 group (compounds **8f–i**). Unfortunately, in all cases, substitution by electron-donating and electron-withdrawing or alkyl groups results in decreased M_3 receptor affinity and decreased M_3/M_2 receptor selectivity. When the R^2

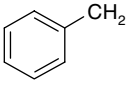
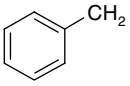
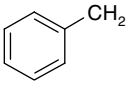
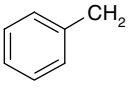
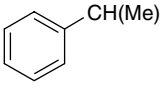
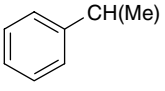
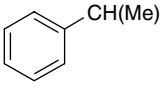


7a **8d-m**

Scheme 3. Reagent and conditions: (a) H_2, Pd/C, MeOH, rt, 87%; (b) aldehyde or ketone, $Na(OAc)_3BH$, HOAc, THF, rt, 15–20% or alkyl bromide, K_2CO_3, KI, CH_3CN, reflux, 30–70%.

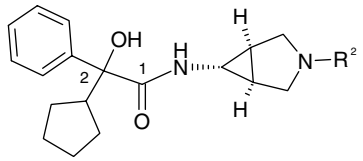
Table 1. Variation at R^1 and R^2

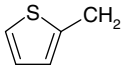
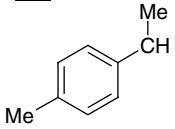
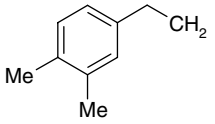
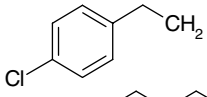
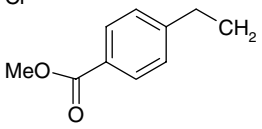
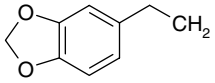
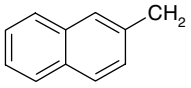
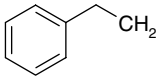
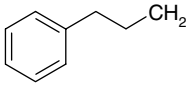
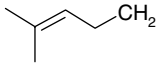
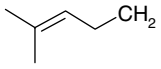
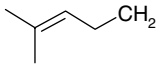
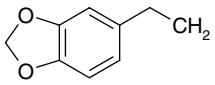


Compound	R^1	R^2	n[a]	$K_i \pm$ SEM (nM)[10]		M_3/M_2
				M_3	M_2	
7a	Cyclo-C_5H_9		3	96 ± 20.7	1422.3 ± 127.3	15
7b	Cyclo-C_6H_{11}		3	31.7 ± 8.4	943.3 ± 149.2	30
7c	Cyclo-C_7H_{13}		3	6.8 ± 0.5	349 ± 65.6	51
7d	H		1	>1000	>1000	NA
8a	Cyclo-C_5H_9		3	25.1 ± 7.6	630 ± 59	25
8b	Cyclo-C_6H_{11}		3	5.9 ± 1.4	154 ± 12	26
8c	Cyclo-C_7H_{13}		3	1.8 ± 0.2	39 ± 6.5	22

[a] *n* is the number of experiments.

Table 2. Variation at R^2



Compound	R^2	n[a]	$K_i \pm$ SEM (nM)[10]		M_3/M_2
			M_3	M_2	
8d		1	524	3981	8
8e		3	58.9 ± 16	1230 ± 148	21
8f		2	43.6 ± 13	812 ± 27	19
8g		3	31.3 ± 4.4	298.3 ± 59.5	10
8h		2	43.6 ± 7	537 ± 38	12
8i		3	20 ± 3.8	277 ± 60	14
8j		3	42 ± 2	778.3 ± 140.8	19
8k		3	9.3 ± 0.2	335.3 ± 14.4	36
8l		1	776	2630	3
8m		3	33 ± 11.7	1370 ± 277	41
(2*R*)-**8m**		4	12.4 ± 2.5	564 ± 110	46
(2*S*)-**8m**		1	71	1139	16
(2*R*)-**8i**		4	12.3 ± 2.9	646 ± 113	52
Darifenacin	—	5	2.5 ± 0.45	63 ± 4.4	25
Solefenacin	—	5	40.5 ± 16.5	273 ± 50	7

[a] n is number of experiments.

group is an arylmethyl group, it is found that the naphthylmethyl analogue **8j** has higher M_3 receptor affinity and higher M_3/M_2 receptor selectivity than the phenylmethyl compound **7a**, indicating a lack of steric hindrance at the receptor close to the basic nitrogen atom.

We have shown previously when R^1 is a cycloalkyl group, that the R enantiomer is favoured.[4] For example, when R^1 is cyclopentyl and R^2 is 2-methyl-2-pentenyl, (2*R*)-**8m** has a higher M_3 receptor affinity than the racemate. This trend is followed in the current (1α, 5α, 6α)-6 amino-3-azabicyclo[3.1.0]hexane series: the 2*R* isomer of the benzodioxol **8i** has not only a higher M_3 receptor affinity but also a higher M_3/M_2 receptor selectivity than the racemate.

In conclusion, we have discovered a novel class of compounds with high affinity for the M_3 receptor and high selectivity over the M_2 receptor. Whilst we have described the SAR of compounds in which the R^1 group is cyclopentyl, compounds having improved in vitro profiles might be produced when the R^1 group is the more bulky cycloheptyl. In addition, highly potent antagonists might result from the separation of the compounds into their homochiral forms and such compounds might be developed as selective M_3 muscarinic receptor antagonists for the treatment of OAB.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.081.

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