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QUINUCLIDIN-2-ENE - BASED MUSCARINIC ANTAGONISTS

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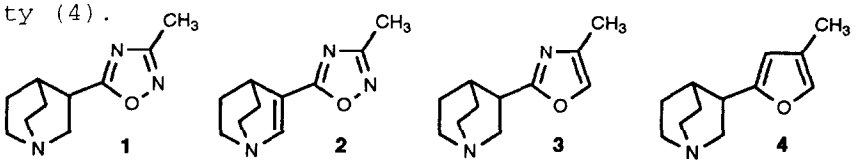
Summary

A series of achiral 3-heteroaryl substituted quinuclidin-2-ene derivatives and related compounds have been synthesized by facile methods. The compounds were evaluated for muscarinic and antimuscarinic properties in receptor binding studies using (-)-[^{3}H]-QNB as the radioligand and in a functional assay using isolated guinea pig urinary bladder. 3-(2-Benzofuranyl)-quinuclidin-2-ene (**15**) displayed the highest M_1-receptor affinity in the present series (K_i = 9.6 nM).

Key Words: muscarinic antagonists, quinuclidin-2-ene derivatives

Muscarinic receptors have been divided into M_1, M_2, M_3 and M_4 subtypes on the basis of pharmacological studies and molecular biological approaches have led to the identification of five genetically distinct muscarinic receptor subtypes, m1-m5 (1-3). The m1, m2, m3 and m4 receptors have been shown to correlate pharmacologically to the M_1, M_2, M_3, and M_4 receptors, respectively (3).

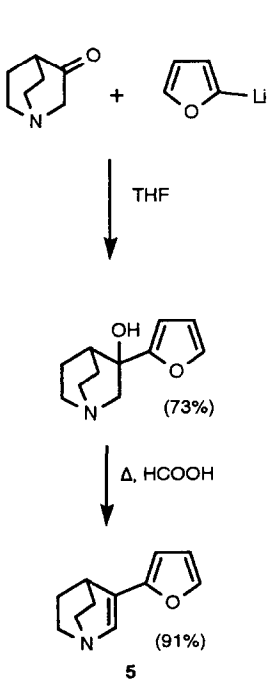
In an ongoing project we have attempted to generate subtype-selective muscarinic antagonists by structural modifications of 3-heteroaryl-quinuclidines, previously identified as potent muscarinic agonists. The 1,2,4-oxadiazole derivative **1** was among the most potent and efficacious in this series of agonists (4). However, binding experiments indicated that introduction of 2,3 unsaturation in the quinuclidine moiety of **1**, affording **2**, was detrimental to efficacy and also resulted in reduced affinity (4). It was also shown that removal of the heteroatoms of **1**, producing **3** and **4**, resulted in diminished muscarinic efficacy and affinity (4).



In the present study, we have attempted to combine the structural features which reduce efficacy without producing a corresponding loss in affinity. A 2,3 positioned double bond was introduced and a 3-substituted heteroaryl ring, containing only one heteroatom was appended to the C3-position of the quinuclidin-2-ene moiety. The results are noteworthy in that the derivatives lack efficacy and because some compounds are relatively potent. An additional advantage is that, in contrast to the corresponding chiral quinuclidine derivatives, the quinuclidin-2-ene derivatives are achiral. However, in general, the new compounds exhibit low subtype selectivity.

Chemistry

Most of the 3-heteroaryl quinuclidin-2-ene derivatives were prepared in a two-step sequence: addition of the appropriate heteroaryllithium compound to quinuclidin-3-one produced an alcohol which was readily dehydrated by heating in concentrated formic acid (Scheme 1). This strategy was, however, not applicable to the 3-benzofuranyl derivative because 3-lithiobenzofuran undergoes a competitive ring-opening (5). Instead, we choose to reverse the reactivity of the two reaction components. 3-Lithio-quinuclidin-2-ene, prepared from quinuclidin-3-one by a Shapiro reaction (6) was added to 2,3-dihydrobenzofuran-3-one and the resulting alcohol was readily dehydrated under the standard conditions (Scheme 2).



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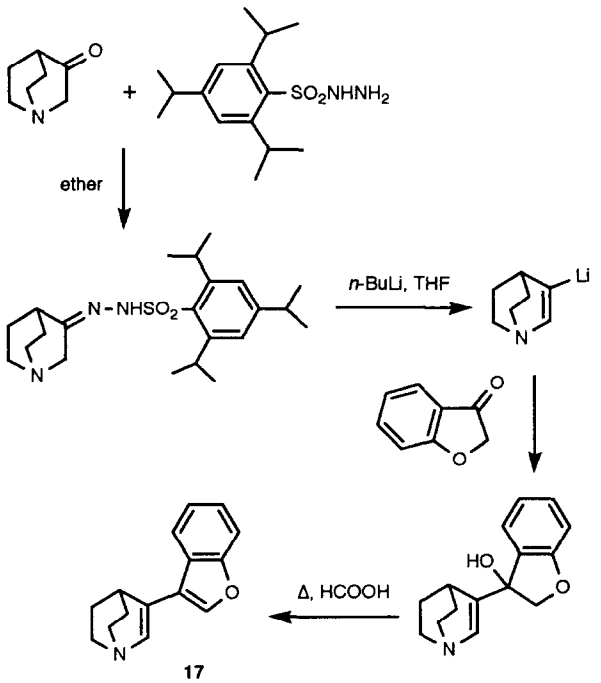
Scheme 1



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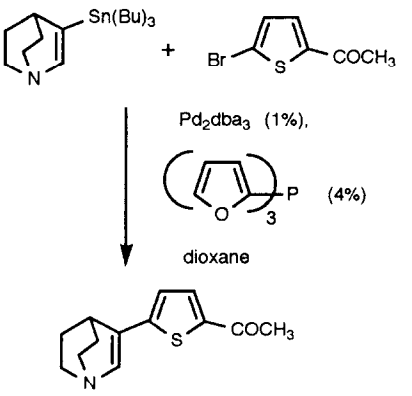
Scheme 2

The 3-lithio derivative was a quite useful synthetic intermediate as it could also be converted into a tin derivative by treatment with trialkyltin chloride. This opened the possibility of synthesizing derivatives by palladium catalyzed reactions between the tin derivative of quinuclidin-2-ene and appropriate (hetero)arylhalides (Scheme 3).



Scheme 3

Pharmacology

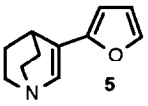
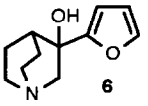
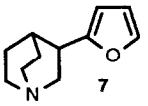
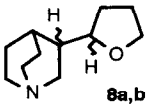
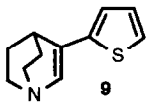
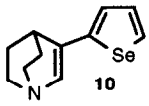
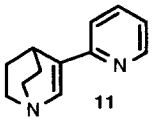
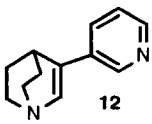
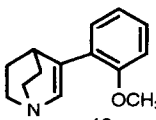
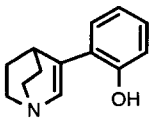
Below are given short descriptions of the pharmacological methods. Detailed accounts of the methods have been reported previously (7-9).

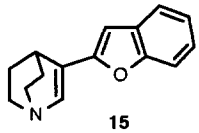
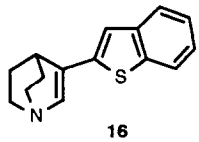
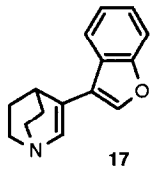
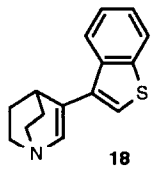
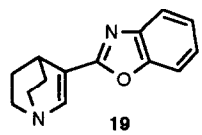
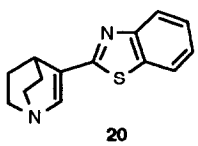
Receptor binding assays: The receptor affinities of the new compounds were determined by in vitro receptor binding assays. The muscarinic receptors in various tissue preparations from guinea pigs were labeled with the muscarinic antagonist (-)-[^{3}H]QNB. The inhibition constants (K_i-values) of the compounds were determined for the muscarinic receptors in the cerebral cortex (M_1), heart (M_2), and parotid gland (M_3) to obtain an estimate of the selectivity. The use of tissue from different regions was necessary because the radioligand used, (-)-[^{3}H]QNB, binds with high affinity (about 50 - 20 pM) to all muscarinic receptor subtypes.

Functional studies on isolated guinea pig bladder : In order to detect possible intrinsic activity of the compounds we performed functional studies on the isolated guinea pig bladder. Therefore, a range of concentrations of the target compound were added to a bath containing a bladder preparation. Antimuscarinic potencies (K_B values) were evaluated by first adding the target compound and then carbachol. In the presence of an antagonist, the concentration response curves to carbachol shifted in parallel towards higher concentrations and the maximal response remained unaffected. EC_{50} values for carbachol in the absence (control) and presence of antagonist were graphically derived and dose ratios (r) were calculated. Dissociation constants (K_B values) for the antagonists were then calculated by $K_B = [A]/(r-1)$ (A is the concentration of test compound). There was a good correlation between receptor binding data (K_i values) and functional data in the bladder (K_B values; data not shown) since the same rank order of potency was observed for all receptor subtypes. None of the compounds in the present study exhibited muscarinic agonist activity when tested in concentrations of 10 - 1000 μM.

Results. The approach in which small heteroaryl substituents were appended to the quinuclidine-2-ene nucleus was successful. 3-(2-furanyl)quinuclidin-2-ene **5**, the first compound in this

Table 1. Quinuclidine-Based Muscarinic Antagonists: Affinites (K_i) for Muscarinic Receptors.

Structure	K_i (nM) M_1	M_2	M_3
 **5**	300	390	1100
 **6**	30000	—	—
 **7**	540	1500	3100
 **8a,b**	8100 2200	>20000 5000	24000 13000
 **9**	290	620	1200
 **10**	410	990	1100
 **11**	1200	1900	4000
 **12**	2200	2800	5900
 **13**	220	410	740
 **14**	360	330	1400

Structure	K_i (nM) M_1	M_2	M_3
 **15**	9.6	31	59
 **16**	81	270	420
 **17**	34	99	160
 **18**	37	96	110
 **19**	100	400	720
 **20**	170	600	1100

series to be synthesized, showed an inhibition constant of 300, 390 and 1100 nM for the M_1, M_2, and M_3 receptors, respectively (Table 1). However, in general, the novel compounds presented herein displayed less than seven-fold selectivity for any of the muscarinic receptor subtypes. Reduction of the double bond in the nitrogen-containing ring system in **5**, giving **7**, slightly decreased affinity for all three receptor subtypes whereas the additional reduction also of the furanyl substituent afforded two diastereomers (**8a,b**) with considerably lower affinity. Similarly, introduction of a 3-hydroxyl substituent in **5** to give **6** considerably decreased the affinity for the muscarinic receptors. In contrast, replacement of the furanyl oxygen of **5** with a sulfur (**9**) or a selenium (**10**) atom only slightly affected affinity, demonstrating that the absence of a strict requirement for an oxygen atom in this position. Substitution with the electron poor 2- or 3-pyridyl substituents (**11** and **12**) decreased affinity as compared to **5**. However, affinity was retained when the 2-furanyl substituent was replaced by 2-methoxyphenyl- (**13**) or 2-hydroxyphenyl (**14**) substituents.

Interestingly, the affinity of **5** could be markedly improved by fusion of the furanyl substituent with a benzene ring, affording the potent 2-benzofuranyl derivative **15**. The 3-substituted regioisomer (**17**) of the 2-benzofuranyl derivative was about three fold less potent in terms of affinity. However, whereas replacement of the oxygen in **5** with a sulfur atom did not affect affinity, the benzothiophene analog of **15** (**16**) was less potent. Similarly, the affinity of a benzoxazole analog (**19**) was much lower than that of **15**.

Discussion

In order to try to deduce the bioactive conformations of the antimuscarinic quinuclidin-2-ene derivatives, we performed some semiempirical calculations (Fig. 1).

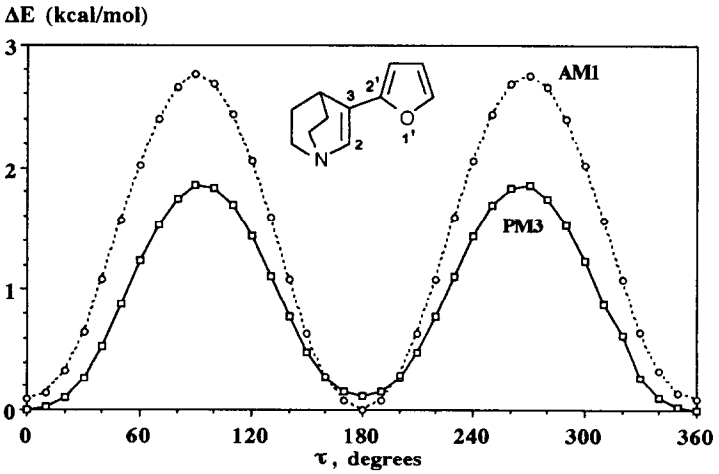


Fig. 1
Conformational energy curves for rotation about τ(2,3,2',1') in compound **5** obtained using the AM1 and PM3 programs.

It was apparent that these derivatives preferentially adopt conformations in which the heteroaryl substituent is coplanar with the double bond of the nitrogen-containing ring system and that such conformers are likely to correspond to those interacting with the muscarinic receptors. The conformational behavior of the corresponding quinuclidine derivatives is different but conformations which overlap fairly well with the quinuclidin-2-ene derivatives are still energetically accessible.

The most potent antagonist in the present series, compound **15**, was well accomodated within a previously defined model (10) of the m1-receptor: docking experiments indicate that the quinuclidin-2-ene ring is located in an area of the receptor defined by Val102, Ala160 and Val385, that is, in the same location as the quinuclidine ring of potent agonists such as L-670,548 (11). The aromatic part of the molecule is directed away from the agonist binding site with the indole ring of Trp400 (TM7) forming an edge to face interaction with the benzenoid part of the benzofuranyl substituent. This favourable interaction is not possible with the smaller and less potent furanyl derivative **5**. The relevance of these docking experiments was supported by a good correlation between the magnitude of the electrostatic potential in the benzene nucleus and the M_1-receptor affinity of the benzofuranyl (**15**), benzothienyl (**16**), benzoxazolyl (**19**) and benzothiazolyl (**20**) derivatives.

Acknowledgment

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