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Communications to the Editor

Synthesis and Modeling Studies of a Potent Conformationally Rigid Muscarinic Agonist: 1-Azabicyclo[2.2.1]heptanespirofuranone

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Introduction. The prediction by the cholinergic hypothesis[1] that enhancement of cholinergic transmission would alleviate the short memory impairment associated with Alzheimer's disease (AD) has been supported by the successful clinical use of cholinesterase inhibitors such as tetrahydroaminoacridine (tacrine),[2a] 2,3-dihydro-5,6-dimethoxy-2-[{1-(phenylmethyl)-4-piperidinyl}methyl]-1*H*-inden-1-one hydrochloride (donepezil or Aricept), and (*S*)-ethylmethylcarbamic acid 3-[1-(dimethylamino)ethyl]phenyl ester (rivastimine or Exelon)[2c] in some patients suffering AD. However, the discovery of an M1-selective centrally acting muscarinic agonist for the treatment of AD remains elusive.[3] Acetylcholine (Ach) is a small but very flexible molecule with a number of rotatable bonds. Although the preferred conformation as determined by X-ray[4a] and aqueous solution NMR[4b] is shown to be gauche, the biological relevance of this conformation at the receptor level awaits to be explored. Earlier work with semirigid muscarinic agonists has yielded various proposals, such as Schulman's pharmacophore model,[4c] on the active conformation of Ach or an agonist that would afford better interaction with mAChR (muscarinic acetylcholine receptors).[4c−f] Recently, molecular models of the transmembrane domains of mAChR have been constructed to explain the three-dimensional interaction of mAChR with its ligands.[5] Conformationally rigid, potent agonists are important tools to ascertain the nature of the interaction of a muscarinic agonist ligand. The information gathered from the molecular modeling study will be useful for the interpretation of the structure−activity relationship (SAR) and for the design and development of a new generation of a muscarinic receptor subtype-selective agonist. To date, this type of information is still lacking despite intensive efforts that have been made in the past[6] in synthesizing potent muscarinic agonists with conformational rigidity. In this communication we would like to report the first example of a potent conformationally rigid muscarinic agonist.

Synthesis. Following the preparation of the corresponding 3-quinuclidyl spirofuranone **7**,[7a] dihydro-4′-methylspiro[1-azabicyclo[2.2.1]heptane-3,5′(4′*H*)-furan]-3′-one (**1**) was synthesized starting from the ketone **2**[8a] (Scheme 1). The Horner−Emmons reaction of the ketone **2** with triethyl phosphonoacetate gave the conjugated ester **3** in 85% yield. The ketoester **4** was prepared from the Michael addition of the oxyanion of ethyl (*S*)-(−)-lactate, which was generated from NaH in dimethylformamide (DMF), to the ester **3** followed by the Dieckmann cyclization. The resulting ketoester **4** was then hydrolyzed and decarboxylated in refluxing 1 N HCl to **1** as a mixture of the syn (the methyl group is cis to the nitrogen) and anti isomers in 61% yield (purified).[9]

The X-ray study of the fumarate of the syn racemate **1a** (vide infra) indicated that the furan ring oxygen is in the exo position and that the methyl group is syn to the bridgehead nitrogen, as depicted in Scheme 1. The exo direction of the ring oxygen is somewhat expected because of the exo attack by the oxyanion being less sterically hindered than the endo one.

Similarly, **5** and **6** were prepared and separated by column chromatography. The NOE (nuclear Overhauser effect) between the 4′-methine proton of the furan ring and the 4-methine proton of the 1-azabicyclo-[2.2.1]heptane ring which was only observed for the syn

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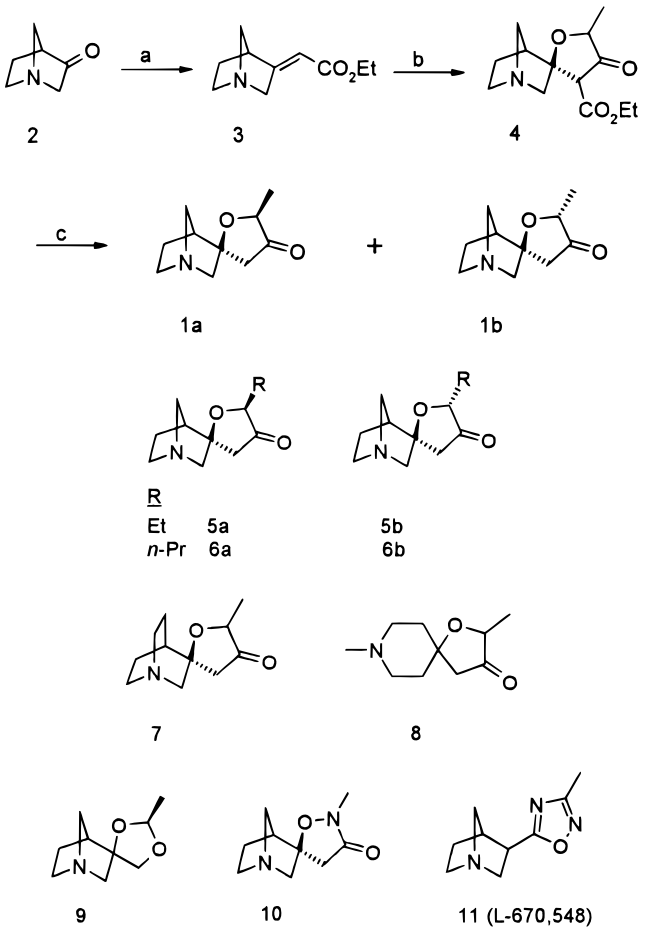
Scheme 1[a]



[a] (a) $(EtO)_2P(O)CH_2COOEt$/Na/EtOH; 85%; (b) (*S*)-(−)-MeCH(OH)COOEt/NaH/DMF; (c) 1 N HCl.

Table 1. Biological Activities[7a,c] of Azabicyclo[2.2.1]-spirofurans

compd	[^{3}H]Oxo-M, K_i (μM)[a] ± SD	intrinsic activity (% max),[b] PI stimulation, M1[c] ± SD
1a	0.0008 ± 0.0001	81 ± 9
5a	0.0004 ± 0	47 ± 7
5b	0.0037 ± 0.0004	39 ± 6
6a	0.002 ± 0.0003	0
6b	0.002 ± 0.0003	0
7[7a]	0.030 ± 0.002	9 ± 9
8[7a]	0.0067 ± 0.0002	51 ± 4.6
9[d]	0.014[9c]	75[d]
10[18]	0.004 ± 0.0005	22.6 ± 2.4
11[8]	0.0002 ± 0	106 ± 21
carbachol	0.009 ± 0.0014	100

[a] [^{3}H]Oxotremorine-M affinity.[7a] [b] Intrinsic activity is expressed as a percentage of the response to the full agonist carbachol. [c] Stimulation of the PI hydrolysis in rat hippocampal slices. [d] The mixture of the syn and anti isomers of compound **8** was reported to have a $pD_2 = 6.5$ and 75% efficacy in the ganglion preparation.[6b]

isomers allows us to assign the syn and anti configurations of **5** and **6** unambiguously.

Results and Discussion. In contrast to the inactivity of the quinuclidine analogue **7**,[7a] **1a** was found to exhibit high affinity in the [^{3}H]Oxo-M binding ($K_i = 0.8$ nM) and 81% efficacy ($EC_{50} = 0.32$ μM) in stimulation of phosphatidyl inositol (PI) hydrolysis as compared to that of carbachol (Table 1). This compound **1a** is much better than the [4.5]decanespirofuranone **8**[7a−c] in terms of affinity and efficacy (M1), although neither is subtype-selective. Both compounds exhibited 100% inhibition on cAMP formation in guinea pig heart, an M2 functional assay.[7c] The size limit for agonism for the quinuclidine ring vs 1-azabicyclo[2.2.1]heptane is very striking and quite different from that of the oxadiazole series reported by Saunders et al.[8] However, similar observations have been reported in the literature when there is an increase in size of a muscarinic agonist.[10] Whereas increase in the size of the alkyl group on the 4′-position of the furan ring (see **5a**,**b** and **6a**,**b** in Table 1) did not affect the Oxo-M affinity, the intrinsic activity was reduced drastically, showing the typical SAR trend for muscarinic agonists.[10] The affinity of four stereoisomers of the rigid dioxolane analogue **9** was reported to be in the range of 0.014−0.55 μM in the Oxo-M assay,[6] which shows that they are much less active than **1a**, albeit they are also rigid analogues.

Figure 1. X-ray structure of **1a**. Compound **1a**, $C_{12}H_7NO_6$, crystallizes in the monoclinic space group $P2_1/c$ (systematic absences $0k0$, k = odd; and $h0l$, l = odd) with $a = 13.837(2)$, $b = 8.280(2)$, $c = 11.244(2)$ Å; $\beta = 92.69(1)°$, $Z = 4$, $d_{calc} = 1.400$ g/cm^3.

Molecular Modeling. Examination of the calculated electrostatic and GRID[11] maps around compound **1a,** which was constructed in the protonated form based on the X-ray crystallographic data[12] (Figure 1), afforded information as to the optimal position of potential interaction sites between the ligand and its receptor. Consequently, the requisite groups were orientated in space to achieve maximal interaction with compound **1a** and thus provided a pseudoreceptor structure. Thus, a deprotonated carboxylic acid group was placed in the GRID defining area adjacent to the protonated ring nitrogen of compound **1a**. Similarly, aliphatic hydroxyl groups were constructed to maximally interact with both the ring ether and carbonyl oxygen atoms. The resultant pseudosite groups were individually anchored by a terminal methyl group, and the complex structure was geometry-optimized. The distances between the protonated nitrogen and the ether oxygen of the furan and the carbonyl carbon are 3.418 and 5.652 Å, respectively, and the torsional angles of NCCO and C2C3C2′C-(=O) are 119.4° and −94.7°, respectively (Table 2). A diverse subset of muscarinic agonists was "docked" into the pseudoreceptor site model defined by these studies, and the resultant alignment was employed in 3D QSAR studies (Figure 2). A comparative molecular field analysis (CoMFA)[13] was performed on 80 ligands modeled in the pseudoreceptor site using an sp^3 carbon probe bearing a unit positive charge, with a grid spacing of 2 Å. The ligands included in the model encompassed

Table 2. Atomic Distances and Torsional Angles

	atomic distances (Å)		torsional angles (deg)
compd	N^+-O (sp^3)	N^+-O of C=O	NCCO
1a	3.418	5.652	119.4
Ach	3.826	6.030	171.9
11	4.757	5.738[a]	122.8

[a] The distance for this compound is the protonated nitrogen and nitrogen of the imidate.

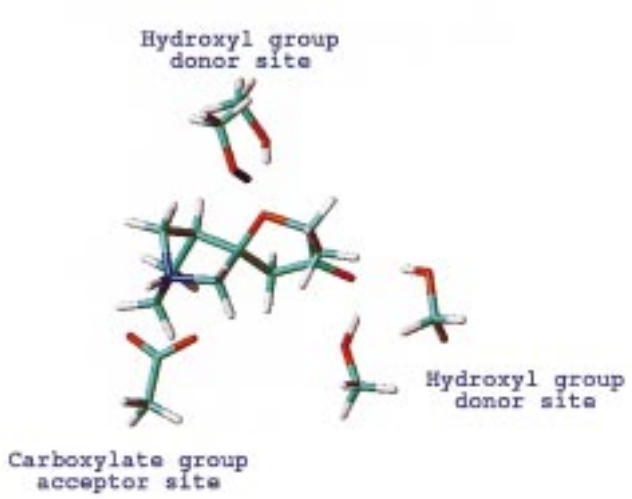


Figure 2. Compound **1a** docked into the pseudosite with acid acceptor group aligned to protonated basic nitrogen and hydroxyl donor groups aligned to carbonyl and ring ether oxygens.

those described within this report together with an extensive sample of acylhydrazones, hydrazides, and oximes.[14] The model obtained had a cross-validated r^2 of 0.556, employing six components, with a final r^2 of 0.900, standard error of 0.278. This model was further refined by modifications in individual molecule orientation within the pseudosite, the use of alternative probe atoms of differing charge, the use of smaller grid spacing, and the incorporation of external hydropathic fields calculated by HINT.[15] The final model was employed in the design and affinity prediction of a number of potential muscarinic ligands.

A number of site-directed mutagenesis studies of muscarinic receptors have implicated certain residues that may be involved in ligand binding.[16] Conserved residues involved in receptor function are most likely to be situated on the inside of the transmembrane bundle. Residues identified in the muscarinic M1 receptor that are believed to influence the affinity or efficacy of the ligands include Asp105, Thr192, and Tyr381.[17] The pseudosite model is consistent with these observations in that the carboxylic acid acceptor group could match Asp105, and some of the hydroxyl donor groups may match either Thr192 or Tyr381. Further validation of the pseudosite model comes from its ability to accommodate a series of muscarinic agonists, including both the natural ligand acetylcholine and a super agonist **11**, 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo-[2.2.1]heptane (L 670,548)[8a] (Figure 3). The corresponding functional groups in acetylcholine interact with the receptor site model in a fashion similar to that in compound **1a**; that is, the basic amine interacts with the carboxylic acid moiety and the ester oxygens interact with the hydroxyl groups. The Merck super agonist (L 670,548) shows a similar interaction profile, with the oxadiazole moiety functioning as an ester bioisostere and interacting with the hydroxyl groups.

Compound **7** provided useful information for QSAR models developed from the pseudosite alignment to predict binding affinity to the muscarinic M1 receptor, in terms of the steric requirements around the basic nitrogen. The lower affinity of compound **9** compared to **1a** can be explained by a weaker interaction with the hydroxyl site donating to the corresponding carbonyl oxygen of **1a**. However, the M1 efficacy of compound **10**[18] is much less than that of compound **1a**, although they have similar affinities in the Oxo-M assay. The oxazolidinone has been shown to be a somewhat planar ring[19] as compared to the somewhat puckered furanone ring. As a result, the N-Me of **10** is aligned in the same plane of the oxazolidinone ring, while the 4′-methyl group of the furanone is either above or below the plane of the furanone ring as it is attached to an sp^3 carbon. It is surprising to observe such a big difference in the M1 intrinsic activity, due to such a minor difference in geometric configuration, but not in the M2 functional activity.[18] In contrast to the basic nitrogen, the M1 receptor at the recognition site is less sensitive to the size increase at the 4′-position of the furan ring (compounds **5** and **6**), albeit their functional activity starts to fall. Therefore, QSAR models derived from this alignment were not predictive of functional activity, suggesting the possibility of separate binding and efficacy sites within the transmembrane helical domain. In addition, these results plus the previous observations on piperidinespirofurans[7a] suggest that there is a small pocket or cavity in M1 receptors which is located above and below the furanone plane and that a methyl group provides the best fit to these sites. The cavity is more sensitive to change in ligand size in the putative efficacy sites affecting function, than in recognition sites. However, this pocket appears only important for M1, but not M2, receptors.

Attempts to search for a new pharmacophore or a meaningful convergence from the geometric parameters (Table 2) of these three potent muscarinic agonists reveal that the distance of N^+-O of the carbonyl group lies in the range of 5.652−6.030 Å, while that of N^+-O (sp^3) varies from one to the other. To interact with this site model, Ach achieves a trans or extended configuration as evidenced by a dihedral angle of 171.9° for the NCCO backbone. This result is quite different from those obtained from the X-ray and NMR studies where Ach exists in a gauche arrangement.[4a,b] This plus the synclinal trans conformations of **1a** (119.4°) and **11** (122.8°) is in full agreement with the previous report that the trans arrangement of NCCO such as in trans ACTM (2-acetoxycyclopropyltrimethylammonium iodide)[20] and (+)-(2*S*,3*R*,5*S*)-muscarine[21] is generally preferred for muscarinic activity.[22]

Schulman et al.[4c] have developed a pharmacophore model for the active conformation of muscarinic agonists. Two interaction sites, P (carboxylate oxygen for the cationic head)and Q (hydrogen-bonding site), which are located 3.0 and 1.2 Å from the nitrogen and the ether oxygen, respectively, were defined in this model. Two geometric parameters, a characteristic distance PQ

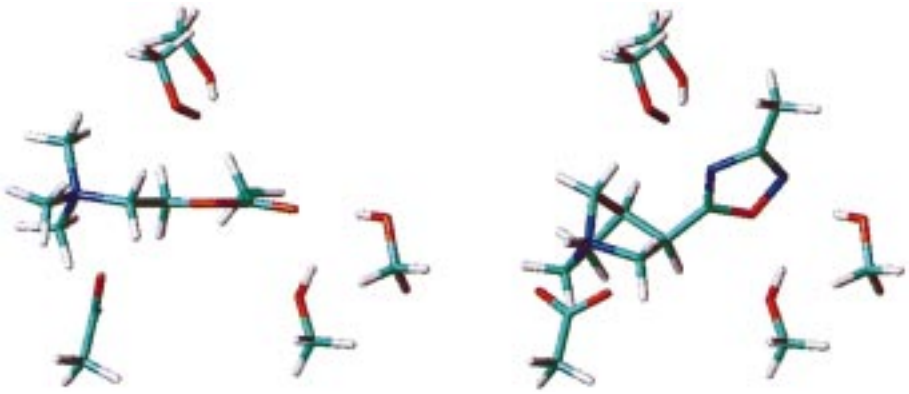


Figure 3. Acetylcholine (left) and **11**, L 670,548 (right), bound into the pseudosite model derived from studies on the rigid muscarinic agonist compound **1a**.

Table 3. Dihedral Angle τPNOQ[a] and Other Distance Parameters (Å) Used in Schulman's Model[4c]

compd	P−N	O−Q	P−Q	P−C	τPNOQ[b] (deg)
1a	2.406	1.841	5.872	6.194	−78.3
Ach	3.44	3.332	5.299 (6.7)	7.41 (8.5)	−117.9 (117)
11	2.40	2.501[c]	5.865[c]	8.333	−66.1[c]

[a] P is the carboxylate oxgen of Asp; Q is the hydrogen atom of the hydrogen-bonding hydroxyl group; C is the methyl carbon. The data in parentheses are taken from the report by Schulman et al.[4c] [b] The negative number is due to the difference in the alignments used in the model. [c] The nitrogen of the imidate was used for measurement instead of the sp^3 oxygen due to the alignment of the nitrogen being more similar to that of the oxygen of **1a** and Ach.

(6.6−6.8 Å) and a dihedral angle τPNOQ (100−117°) were found to be more suitable to describe the muscarinic pharmacophore. To further validate our model, the carboxylate oxygen of Asp and the hydroxyl hydrogen were used as P and Q, respectively, and the certain geometric parameters were measured for comparison (Table 3). Among the parameters obtained, the distances of |PQ| for the three potent agonists shown here appear relatively constant (5.299−5.872 Å) which are shorter than the literature value of 6.7 Å for Ach.[4c] The range of the dihedral angles τPNOQ (−66.1° to −117.9°) obtained from this model is wider and that of the |PC| distance is shorter than the Schulman's model, although the angle for Ach (−117.9°) is the same as the reported one.[4c] It is not known if the difference between our model and theirs is due to the constraints of their model using fixed distances of |PN| and |OQ|. This comparison suggests that |PQ| is an important geometric parameter for muscarinic agonists.

In summary, the rigid analogue **1a** has been demonstrated to be a full and nonselective muscarinic agonist. The pseudosite model derived from compound **1a** can accommodate a number of muscarinic agonists such as acetylcholine and L 670,548 (**11**). The model appears to serve well for muscarinic agonists at the recognition site and was employed in 3D QSAR studies. A predictive CoMFA model has been developed that serves to validate the pseudoreceptor site as being biologically relevant. The model will be useful to serve as a template for designing a second generation of selective muscarinic agonists. The results from this study further suggest that both the dihedral angle of NCCO in the synclinal trans arrangement and the |PQ| distance are important for muscarinic agonists.

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(9) The overall yield, starting from the ketone **2**, of the fumarate salt of pure **1a** (the syn isomer) which was isolated and purified from a mixture of syn and anti isomers of **1** was 6%.

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(11) Compound **1a** was constructed in the protonated form, from the X-ray crystallographic data[12] (Figure 1), using the Sybyl suite of programs, version 6.3, Tripos Associates, St. Louis, MO. Geometry optimization was performed initially with the Tripos force field, followed by the 3-21G* Hamiltonian as implemented within Spartan, Wavefunction, Irvine, Ca. Atomic charges were calculated via natural population analysis and fitted to the electrostatic potential over the surface density. Potential energy isosurfaces were contoured to identify the maximal regions of electrostatic attraction and repulsion from the proton probe. The attractive areas were concentrated around the carbonyl and ring ether oxygens, along the vectors of their respective lone pairs. The molecular environment was subsequently sampled by an assortment of probe atoms and groups using GRID, Molecular Diversity Limited, Oxford, England, to identify potential interaction sites. Primary interaction domains were examined with carboxylic acid and aliphatic and aromatic hydroxyl probe groups. The resulting interaction maps obtained by GRID were consistent with the electrostatic potential maps and provided the pertinent information for construction of a pseudoreceptor site model.

(12) The cell constants were determined from a least-squares fit of the setting angles for 25 accurately centered reflections. X-ray intensity data were collected on an Enraf-Nonius CAD4 diffractometer employing graphite-monochromated Cu Kα radiation ($\lambda = 1.541\ 84$ Å) and using the $\omega-2\theta$ scan technique. A total of 2485 reflections were measured over the ranges: $4° \le 2\theta \le 130°$, $-16 \le h \le 16$, $0 \le k \le 9$, $0 \le l \le 13$. Three standard reflections measured every 3500 s of X-ray exposure showed no intensity decay over the course of data collection. The intensity data were corrected for Lorentz and polarization effects but not for absorption. Of the reflections measured, a total of 1715 unique reflections with $F^2 > 3\sigma(F^2)$ were used during subsequent structure refinement. The structure was solved by direct methods (SIR88). Refinement was by full-matrix least squares technique based on F to minimize the quantity $\sum w(|F_o| - |F_c|)^2$ with $\omega = 1/\sigma^2(F)$. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were refined isotropically. Refinement converged to $R_1 = 0.052$ and $R_2 = 0.075$.

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