Improving the Nicotinic Pharmacophore with a Series of (Isoxazole)methylene-1-azacyclic Compounds: Synthesis, Structure−Activity Relationship, and Molecular Modeling

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A series of (isoxazole)methylene-1-azacyclic compounds was prepared. The compounds were tested for affinity to central nicotinic acetylcholine receptors (nAChRs) and central muscarinic receptors. The compounds covered a broad range of affinities for the nAChRs (IC_{50} = 0.32 to >1000 nM), with selectivities for the nAChRs over the muscarinic receptors in the range of 3−183. The high-affinity compound (*Z*)-**26** (3-(4-methyl-5-isoxazolyl)methylene-1-azabicyclo-[2.2.2]octane, IC_{50} = 3.2 nM) having only one energy minimum was used as the reference structure in a computational study. This ligand has enabled definition of an important distance parameter, and the existence of this parameter was supported by showing that other potent nicotinic ligands (for example, nicotine and epibatidine) fit the model.

Introduction

Research in the nicotinic area has over the years demonstrated a variety of diseases or disease states in which nicotinic therapy would have beneficial effect. Among these are Alzheimer's disease, Parkinson's disease, Tourette's syndrome, anxiety, pain, and depression.[1−4] Several compounds with high affinity for the central nicotinic acetylcholine receptors (nAChRs) have been described, e.g., epibatidine,[4,5] ABT 418,[6,7] and recently a series of pyridyl ethers which include the very potent compound A85380.[8]

The structures of epibatidine and ABT 418 suggested that combining the steric volume close to the sp^3 hybridized nitrogen, as in epibatidine, with the isoxazole from ABT 418 could lead to new structures possessing affinity for the nAChRs. This led to some novel (isoxazole)methylene-1-azabicyclic compounds, including (*Z*)-**14**, with nanomolar affinities for the central nAChRs and possessing agonist properties.[9] In the present work, the series was continued by varying the nature of the azacycle and the isoxazole substitution pattern, in order to examine the borders of the nicotinic pharmacophore.

The nicotinic pharmacophore has been developing since 1970, when Beers and Reich were the first to suggest two elements essential for nicotinic agonist activity.[10] They proposed that the specific binding of nicotinic agonists to the nAChRs is mediated by two elements: (1) a Coulombic interaction involving the cationic center and (2) a hydrogen bond between the receptor and an acceptor group in the ligand which is formed approximately 5.9 Å from the center of the positive charge.

In 1986 Sheridan and co-workers proposed a triangle as a model of the nicotinic pharmacophore.[11] They suggested that the essential groups in the pharmacophore are (A) a cationic center (e.g., a protonated sp^3 nitrogen), (B) an electronegative atom capable of forming a hydrogen bond, and (C) a dummy point or an atom, which defines a line along which the hydrogen bond may form. For (*S*)-nicotine this latter point is considered to be a point toward the centroid of the pyridine ring. The optimal distances between the three points were estimated to (A−B) 4.8 ± 0.3 Å; (A−C) 4.0 ± 0.3 Å; (B−C) 1.2 Å.

Since then the interest has primarily been focusing on the internitrogen (N−N) distance of the ligand's supposed binding conformation (Table 1). The main question underlying the dispute about the correct N−N distance is: Which low-energy conformation of epibatidine is responsible for the affinity for the nAChRs?

As epibatidine only contains one rotatable bond, its flexibility is very limited. With the exception of one report,[12] the conformational studies of epibatidine published till now[5,8,13−15] have all found approximately the same two low-energy conformers. The actual conformers found vary a little depending on whether the compounds under study are protonated or not. The two conformers are almost equal energetically, they only vary 180° in rotation of the pyridine ring. Thus, depending on the epibatidine conformer chosen as reference structure, the study comes out with an N−N distance close to either 4.6 or 5.5 Å.

In this report the synthesis and in vitro binding data for a large number of compounds in the 3-(isoxazole)-methylene-1-azacyclic series are presented. Furthermore, the structure−activity relationships based on molecular modeling of the compounds are thoroughly discussed. We also report the definition of an important distance parameter, which predicts affinity for the nAChRs better than the N−N distance does.

Chemistry

Isoxazoles (**1**−**3**) were synthesized following a modified literature procedure.[16] Aldehydes were reacted with

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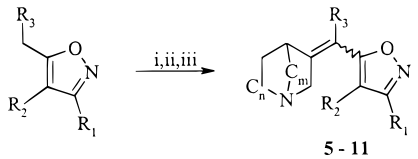
Table 1. Various N−N Distances Proposed over the Years

year	compounds studied	N−N distance (Å)
1986[a]	nicotine, cytisine, ferruginine methiodide, muscarone	4.8
1994[b]	nicotine, epibatidine	5.5
1996[c]	A85380, epibatidine	4.6, 5.6 or > 6
1998[d]	A85380 analogues, epibatidine	4.5

[a] Reference 11. [b] Reference 5. [c] Reference 8. [d] Reference 15.

Scheme 1[a]



[a] For R_1, R_2, and R_3, see Tables 2 and 3. Reagents and conditions: (i) *n*-BuLi or LDA in THF, −78 °C, N_2. (ii) $m = 2$, $n = 1$: 1-azabicyclo[2.2.2]octane-3-one, −78 °C, N_2. $m = 1$, $n = 2$: 1-azabicyclo[3.2.1]octane-5-one, −78 °C, N_2. (iii) NEt_3, EtOH, $SOCl_2$, −78° → 20 °C, N_2.

hydroxylamine hydrochloride to form the corresponding oximes. Subsequently the oximes were α-brominated with *N*-bromosuccinimide and then treated with triethylamine causing elimination of HBr to give the corresponding nitrile oxides. A 1,3-dipolar cycloaddition reaction between the nitrile oxides and isopropenyl acetate produced the 4,5-dihydroisoxazoles, which upon treatment with hydrochloric acid finally eliminated acetic acid to give the desired isoxazoles (3-isopropyl-5-methylisoxazole (**1**), 3-ethyl-5-methylisoxazole (**2**), and 3-propyl-5-methylisoxazole (**3**)).

3-Methoxymethyl-5-methylisoxazole (**4**) was prepared from 3-hydroxymethyl-5-methylisoxazole by deprotonation with sodium hydride followed by alkylation with methyl iodide.

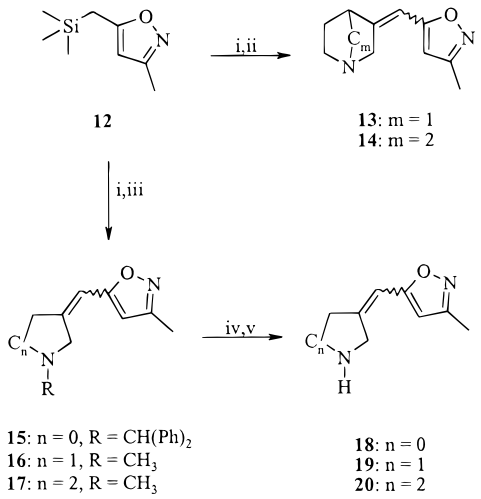
Compounds **5**−**11** were synthesized in a one-pot reaction by direct coupling of the azacyclic ketone and the isoxazole (Scheme 1). The substituted 5-methylisoxazoles were selectively deprotonated with LDA or *n*-BuLi and reacted with an azacyclic ketone to give the desired C−C bond formation. The formed alcohol was treated directly with thionyl chloride, triethylamine, and in some cases ethanol. The chloro intermediate spontaneously eliminated HCl to give the desired products **5**−**11** as a mixture of the (*E*)- and (*Z*)-isomers in a total yield of around 70%. The isomers were separated by column chromatography and/or fractional crystallization, giving the compounds **5**−**11** in 4.4−29% yield. A complete separation of the (*Z*)- and (*E*)-isomers was not possible in all cases. The compounds containing a chiral center ((*Z*)-**11** and (*E*)-**11**) were prepared as racemates.

Compounds **13**−**17** were prepared by a direct coupling between 3-methyl-5-trimethylsilanylmethylisoxazole (**12**)[17] and the appropiate azacyclic ketone (Scheme 2).

Treatment of **12** with LDA or *n*-BuLi generated the α-silyl carbanion, which then reacted with a ketone, again with a mixture (approximately 1:1) of the (*E*)- and (*Z*)-isomers in about 60−80% yield as the result. The isomers were separated using column chromatography and/or fractional crystallization to give **13**−**17** in 1.1−13% yield.

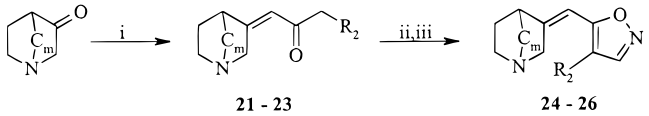
Compounds **15**−**17** were subsequently dealkylated to **18**−**20** by treatment with 1-chloroethyl chloroformate in toluene followed by reflux in methanol.

Scheme 2[a]



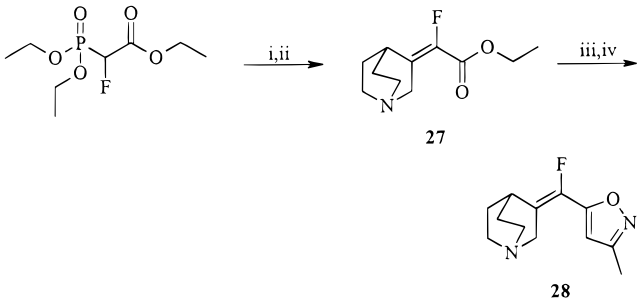
[a] Reagents and conditions: (i) LDA or *n*-BuLi in THF, −78 °C, N_2. (ii) Azabicyclic ketone, −78 → 20 °C, N_2. (iii) Azacyclic ketone, −78 → 20 °C, N_2. (iv) 1-Chloroethyl chloroformate in toluene, reflux. (v) Reflux in MeOH.

Scheme 3[a]



[a] For R_2, see Tables 2 and 3. Reagents and conditions: (i) Dimethyl 2-oxoalkylphosphonate and KOH in water, 0 °C. (ii) DMF-DMA, 90 °C, N_2. (iii) CH_3CO_2H and $NH_2OH{\cdot}HCl$ in water, 90 °C.

Scheme 4[a]

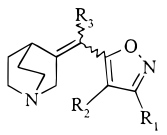


[a] Reagents and conditions: (i) *t*-BuOK, DMF, 0 °C, N_2. (ii) 1-Azabicyclo[2.2.2]octan-3-one, 0 → 20 °C. (iii) Acetone oxime, *n*-BuLi, N_2, 0 °C. (iv) Concd. H_2SO_4, 20 °C.

The α,β-unsaturated ketones **21**−**23** were formed as a mixture of their (*E*)- and (*Z*)-isomers by a Horner−Wadsworth−Emmons reaction between the appropriate azacyclic ketones and oxoalkyl phosphonates (Scheme 3). After separation of the (*Z*)- and (*E*)-enones, the (*Z*)-isomers (**21**−**23**) were reacted with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) in a modified Mannich reaction. The intermediate formed was treated with glacial acetic acid and hydroxylamine to give the desired (*Z*)-isoxazoles **24**−**26** in 18−37% yield.

The α,β-unsaturated fluoro ester **27** was synthesized by a similar reaction between triethyl-2-fluoro-2-phosphonoacetate and 1-azabicyclo[2.2.2]octan-3-one (Scheme 4). Following a modified literature procedure,[18] **27** was then treated with deprotonated acetone oxime to induce the cyclization, whereupon stirring with concentrated sul-

Table 2. In Vitro Binding Data for the 1-Azabicyclo[2.2.2]octan-isoxazoles[a]

Compound	 R_1	R_2	R_3	Isomer Purity[b] (%)	[^{3}H]MCC cortex IC_{50} (nM)	[^{3}H]-Oxo-M cortex IC_{50} (nM)	Selectivity
(Z)-5	Et	H	H	87	100 ± 45	566 ± 335	5.6
(E)-5	Et	H	H	93	> 1000	867 ± 286	-
(Z)-6	MOM	H	H	88	218 ± 38	1470 ± 260	6.7
(E)-6	MOM	H	H	> 99	> 1000	4000 ± 1070	-
(Z)-7	n-Pr	H	H	> 99	167 ± 27	852#	4.9
(E)-7	n-Pr	H	H	95	> 1000	1930 ± 429	-
(Z)-8	i-Pr	H	H	> 99	107 ± 57	911 ± 84*	8.5
(E)-8	i-Pr	H	H	99	> 1000	2280 ± 115	-
(Z)-9	Me	H	Me	> 99	715 ± 257*	2263#	3.2
(E)-10	Me	Me	H	> 99	> 1000*	373 ± 162	-
(Z)-14	Me	H	H	81	3.9 ± 1.2	658 ± 230	169
(E)-14	Me	H	H	97	504 ± 59	1810 ± 446	3.6
(Z)-25	H	H	H	87	1.65 ± 0.35*	312 ± 105	183
(Z)-26	H	Me	H	> 99	3.2 ± 2.0	418 ± 120	131
28	Me	H	F	78	255 ± 85	1450 ± 227	5.0
ABT 418	-	-	-	-	56 ± 3.1	47000 ± 3000*	840
(S)-Nicotine	-	-	-	-	6.6 ± 3.1	32000#	4850
(±)-Epibatidine	-	-	-	-	0.30 ± 0.07	> 1*	>3

[a] Values represent mean ± SEM. $n \geq 3$, except *$n = 2$, and # $n = 1$. [b] Measured by GC.

furic acid caused elimination of water to selectively produce the (*E*)-isomer of **28**.

The relative configuration of the (*Z*)- and (*E*)-compounds was determined on the basis of ^{1}H NMR experiments. After the individual protons were assigned from COSY experiments, NOE effects were used for determination of the spatial arrangement around the double bond.

Biology

The oxalate or hydrochloride salts of the compounds were tested in a binding assay, using the displacement of [^{3}H]methylcarbamylcholine ([^{3}H]MCC)[19,20] from native cholinergic binding sites in rat brain cortex as a measure of their affinity for nAChRs. As the ligands for the nAChRs receptors often display some affinity for the muscarinic acetylcholine receptors, the affinity for these was measured using [^{3}H]oxotremorine-M (Oxo-M)[21] as radioligand.

Computational Studies

As the conformational freedom in the compounds containing an azabicycle and an aromatic heterocycle is very limited, a conformational search was performed by rotation around the single bond connecting the two heterocycles. The more flexible compounds were investigated using the molecular dynamics option in Sybyl 6.5.[22]

For the further studies, only low-energy conformers with a $\Delta E < 3.0$ kcal/mol were used.

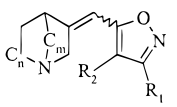
As all of the compounds in this series that have been tested in a functional assay proved to be nicotinic agonists,[9] it is assumed that they interact with the nAChRs in the same way as nicotine and epibatidine.

Results and Discussion

Structure−Activity Relationships. In the present collection of compounds, explorations were concentrated on alkyl substituents in the isoxazole ring, substituents of different character on the methylene bridge, and the nature of the nonaromatic heterocycle.

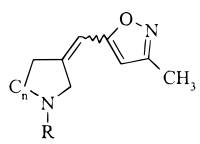
The 1-Azabicyclo[2.2.2]octane Series. Generally, the (*Z*)-isomers are more potent than the (*E*)-isomers (Table 2). Hence, (*E*)-**14**, with an affinity of 504 nM for the nAChRs, was the most potent of the (*E*)-compounds.

Table 3. In Vitro Binding Data for the 1-Azabicyclo[3.2.1]octan- and [2.2.1]heptan-isoxazoles[a]

Compound	 m	n	R_1	R_2	Isomer Purity[b] (%)	[^{3}H]MCC cortex IC_{50} (nM)	[^{3}H]-Oxo-M cortex IC_{50} (nM)	Selectivity
(Z)-13	1	1	Me	H	> 99	29 ± 6	276 ± 57	9.5
(E)-13	1	1	Me	H	> 99	23 ± 4	1020 ± 192	44
(Z)-24	1	1	H	Me	> 99	0.32* ± 0.13	19#	59
(Z)-11	1	2	Me	H	> 99	130 ± 31	898 ± 66	6.9
(E)-11	1	2	Me	H	> 99	53 ± 22	1570 ± 185*	30

[a] Values represent mean ± SEM $n \geq 3$, except *$n = 2$, and # $n = 1$. [b] Measured by GC.

Table 4. In Vitro Binding Data for the 1-Azacyclo-isoxazoles[a]

Compound	 n	R	Isomer Purity[b] (%)	[^{3}H]MCC cortex IC_{50} (nM)	[^{3}H]-Oxo-M cortex IC_{50} (nM)	Selectivity
(Z)-16	1	Me	> 99	783 ± 81	4420 ± 893	5.6
(E)-16	1	Me	> 99	> 1000	15700 ± 6020	-
(Z)-17	2	Me	> 99	> 1000	5760 ± 248	-
(E)-17	2	Me	98	> 1000	9540 ± 1160	-
18	0	H	-	> 1000	19300 ± 8590	-
(Z)-19	1	H	> 99	> 1000	3290 ± 1960	-
(Z)-20	2	H	91	87 ± 13	7220 ± 2480	89
(E)-20	2	H	93	> 1000*	6950 ± 1350	-

[a] Values represent mean ± SEM $n \geq 3$, except *$n = 2$. [b] Measured by GC.

In the 1-azabicyclo[2.2.2]octane series different substitutions within the isoxazole ring show that the optimum R_1 substituent has to be small. Thus, (*Z*)-**14** displayed an affinity of 3.9 nM and (*Z*)-**25** an affinity of 1.7 nM compared to 6.6 nM for (*S*)-nicotine. There was a substantial decrease in activity in going from methyl ((*Z*)-**14**) to larger R_1 substituents. Changing methyl to ethyl ((*Z*)-**5**) led to a 26-fold decrease in affinity and a further change to propyl ((*Z*)-**7**) and isopropyl ((*Z*)-**8**) lowered the affinity 45 and 27 times, respectively. It is noteworthy that (*Z*)-**6** with the electronically differing R_1 substituent CH_2OCH_3 had almost the same nicotinic affinity as (*Z*)-**7**.

Interchanging the R_1 and R_2 substituents in (*Z*)-**14** gave the equipotent (*Z*)-**26** (3.2 nM). On the methylene bridge, methyl ((*Z*)-**9**) and fluorine (**28**) were introduced instead of hydrogen, but with a lowering of affinity as the result.

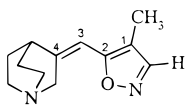
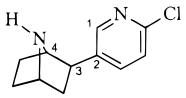
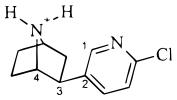
All compounds displayed selectivity toward the nicotinic receptor when compared to the muscarinic receptors, the best compounds showing a selectivity around 150.

Other Azacycles. Interchanging the R_1 and R_2 substituents from (*Z*)-**13** to (*Z*)-**24**, both having the (*R*/*S*)-1-azabicyclo[2.2.1]heptane moiety, enhances the nicotinic affinity by a factor 91, as (*Z*)-**24** becomes equipotent with (±)-epibatidine (Table 3). Analogues containing (*R*/*S*)-1-azabicyclo[3.2.1]octane have lower affinities. Interestingly, the (*E*)-isomers (*E*)-**11** and (*E*)-**13** are relatively potent (53 and 23 nM).

Some azamonocycles (piperidine; **17**, **20**, pyrrolidine; **16**, **19**, and azetidine; **18**) were also tested (Table 4), but except for (*Z*)-**20** all the compounds had a low affinity for the nAChR.

Computational Studies. For the study, 16 compounds from the 3-(isoxazole)methylene-1-azacyclic series were chosen. (*R*/*S*)-Epibatidine, (*S*)-nicotine, ABT 418, an Anatoxin-a analogue (**29**)[1], Epiboxidine,[23] a

Table 5. Results from the Molecular Modeling[a]

Compound	Torsion τ (°)	ΔE (kcal/mol)	RMS[b]	N-N⁺ distance (Å)	N⁺-b distance (Å)	a-b distance (Å)
 (*Z*)-**26**	180	0	0	5.6	7.3	7.2
 (R)-Epibatidine	7 -173 80 -99	0 0.1 1.4 1.4	0.571 0.373 0.845 0.403	4.7 5.5 4.8 5.5	6.4	7.7
 (S)-Epibatidine	-7 173 -80 99	0 0.1 1.4 1.4	0.571 0.373 0.845 0.403	4.7 5.5 4.8 5.5	6.4	7.7

[a] See Supporting Information for the full results from the conformational search. [b] Superpositioning with (*Z*)-**26**_a using the sp^3 nitrogen (N^+), the anionic site point (a), and the hydrogen bond donor site point (b).

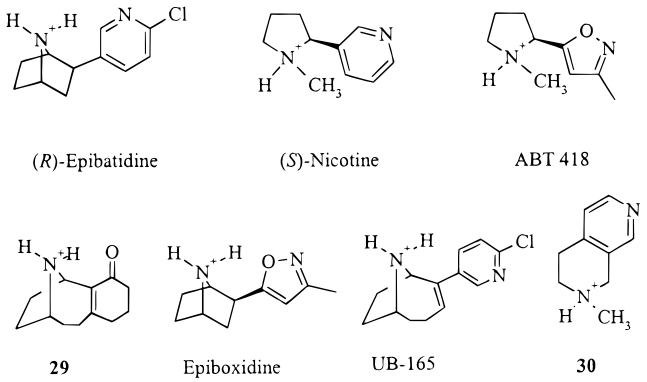


Figure 1. Reference compounds used in the computational studies. The numbered atoms are the ones employed in measuring the torsional angle (*τ*).

mixed Anatoxin-a/epibatidine analogue named UB-165,[24] and a rigid nicotine analogue[25] (**30**) were used as reference compounds (Figure 1).

As the sp^3 hybridized nitrogen atom probably will be protonated under physiological conditions, the protonated form of the compounds was chosen for the study.

Conformational Search. The conformational search of (*S*)-nicotine gave rise to 20 different low-energy conformers with a $\Delta E < 3.0$ kcal/mol. The 20 conformers occurred in pairs of almost equal energy, differing by approximately 180° in the rotation of the pyridine ring. For (*R*)- and (*S*)-epibatidine, four low-energy conformers were found (Table 5). These conformers also occurred in pairs as described for (*S*)-nicotine.

In the (*Z*)-series the individual compounds have differing numbers of low-energy structures, but the global energy minimum is in every instance the one which possesses a torsion (*τ*) of 180° or the pair of conformers that have a *τ* close to 180°. In the low-energy conformer the R_2 group is directed away from the azacycle, thereby avoiding its hydrogen atoms (Table 5, (*Z*)-**26**, R_2 = Me).

In the (*E*)-series the compounds have four ((*E*)-**14**) or two ((*R*/*S*)-(*E*)-**11** and (*R*/*S*)-(*E*)-**13**) low-energy conformations with a $\Delta E < 3.0$ kcal/mol. The global minima all have a *τ* close to 180°.

Compound **29** is quite rigid due to the integrated double bond, consequently the conformational search revealed only three low-energy conformers. UB-165 displays the same symmetry as (*S*)-nicotine and (*R*/*S*)-epibatidine, thus the low-energy conformers occur in pairs differing 180° in rotation of the pyridine ring. The low-energy conformations of **30** are symmetrical around 0°, reflecting the position of the *N*-methyl group (axial/equatorial).

Superpositioning. For several years it has been accepted that the presence of a positively charged nitrogen atom and a hydrogen bond acceptor in the ligand was important for activity at the nAChRs.[10,11] In the present study it is assumed that the sp^2 hybridized nitrogen lone pair interacts with a hydrogen bond donor in the receptor, and even though the presence of an anionic site within the nicotinic receptor has been the matter of some discussion,[26−29] it is also assumed, that such a site is present and interacts with the protonated nitrogen atom. Thus, the compounds were superpositioned using the sp^3 hybridized nitrogen atom (N^+), its complementary anionic site point (a), and the hydrogen bond donor site point (b) complementary to the sp^2 nitrogen atom (Figure 2). The site points were placed 2.9 Å away from the nitrogen atoms. With the

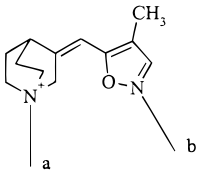


Figure 2. The position of the elements (N^+, a, b) used for superpositioning the compounds.

points chosen for superpositioning, all the chiral compounds display symmetrical results for their enantiomers, so from now on only the (*R*)-enantiomers are mentioned. As the high-affinity compound (*Z*)-**26** has only one energy minimum, this conformer ((*Z*)-**26**_a) was used as the reference structure in the superpositioning.

Superpositioning (*Z*)-**26**_a on the four low-energy conformers of (*R*)-epibatidine gave an excellent fit with the conformer possessing the global energy minimum ($\tau = 7°$). The carbon skeleton of (*R*)-epibatidine follows the skeleton of (*Z*)-**26**_a very closely; the aromatic rings are coplanar and very close to one another. In addition, the steric bulk around the sp^3 nitrogen atom occupies the same space (Figure 3).

Of the 20 low-energy conformers found for (*S*)-nicotine, three overlapped well with (*Z*)-**26**_a (Figure 3). These had conformational energies 1.5, 1.7, and 2.4 kcal/mol above the minimum. As the three energy minima represent different conformations, it is difficult to interpret the relation between conformation and nicotinic activity for (*S*)-nicotine.

In the case of ABT 418, the overlapping conformation had an energy 1.5 kcal/mol above the global minimum. All other reference compounds (**29**, Epiboxidine, UB-165, and **30**) overlapped well with (*Z*)-**26**_a in their global minimum conformation. Thus the RMS values were low, and the carbon skeletons of the compounds followed that of (*Z*)-**26**_a very well.

(*Z*)-**8** was included as an example of the compounds with large R_1 substituents. As expected, the R_1 substituent affected neither the number nor the geometry of the low-energy conformations found compared to, e.g., (*Z*)-**14**. Thus the drop in affinity (a factor of 27 compared to (*Z*)-**14**) must be ascribed to unfavorable interactions between the R_1 substituent and the receptor.

The R_3 position was examined with **28** and (*Z*)-**9**. Having fluorine in the R_3 position of (**28**) had no effect on the low-energy conformation; instead the electrostatic potential surface was changed. Hence in **28** there is a slightly more negative area around the fluorine atom, which must be poorly tolerated by the receptor (IC_{50} = 755 nM). Having methyl in the R_3 position ((*Z*)-**9**) led to major conformational changes. The global energy minimum has a $\tau = \pm 141°$, and the energy gap to the desirable conformation with $\tau = 180°$ is as large as 4.5 kcal/mol.

The low-energy conformers of the two piperidine compounds (*Z*)-**20** and (*Z*)-**17** are very much alike when viewed as models, and at first it is difficult to explain the marked difference in affinity. Yet, a comparison with (*S*)-nicotine and ABT 418 shows that the *N*-methyl of (*Z*)-**17** points in the opposite direction to the *N*-methyl groups of ABT 418 and the three conformers of (*S*)-nicotine, which superimpose (*Z*)-**26**_a (Figure 4). The drastic effect that N-methylation in some cases has on the affinity has previously been ascribed to the possibility that the various amine substituents experience different receptor interactions, due to the varying directions in which they are oriented.[13]

The relatively low affinity of (*Z*)-**20** (87 nM), compared to (*Z*)-**26**, is probably due to the changes in electrostatic potential in the aliphatic area around the sp^3 hybridized nitrogen atom. These changes are apparent when compared to both (*Z*)-**26** (monoprotonated amine) and epibatidine (diprotonated amine).

(*R*)- and (*S*)-(*Z*)-**11** also have some extra volume in the aliphatic area close to the *N*-methyl group of (*Z*)-**17**, but the extra volume must be placed in a more tolerable area of the receptor, as the affinity of (*Z*)-**11** (130 nM) is not as low as the affinity of (*Z*)-**17** (>1000 nM).

The pyrrolidine and azetidine compounds (*Z*)-**16** and **18** cannot achieve a conformation that superimposes (*Z*)-**26**_a. This explains their low affinities (783 nM and >1000 nM).

As mentioned above, (*E*)-**11** and (*E*)-**13** are active (53 and 23 nM). Attempts to fit these compounds into the derived model fail if the points mentioned above (N^+, a, b) are used. However, if the site point complementary to the isoxazole oxygen (2.9 Å away) is used instead of the sp^2 nitrogen site point, the overlap is much better (Figure 5). For all the (*E*)-compounds, local minimum conformers are used for the superposition, not the global minima, as these cannot superimpose (*Z*)-**26**_a to any reasonable extent. The local minima are 1.2−2.6 kcal/mol above their global minimum. Still the superposi-

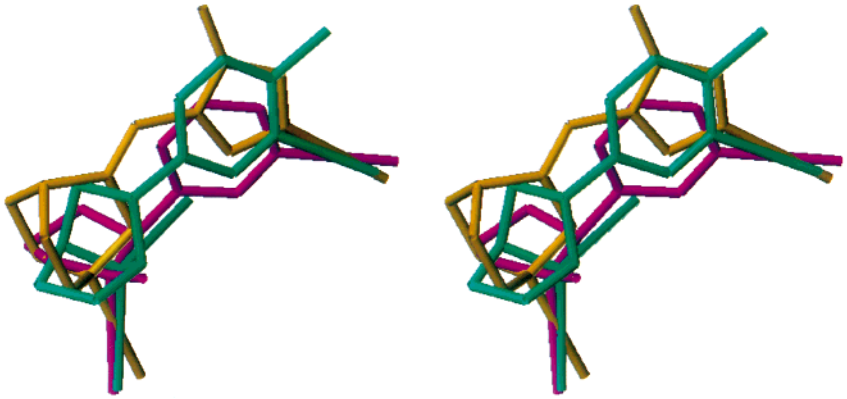


Figure 3. Stereoview of (*Z*)-**26**_a (orange) superimposed with (*R*)-epibatidine (magenta) and one of the three overlapping conformations of (*S*)-nicotine ($\tau = -67°$, dark green).

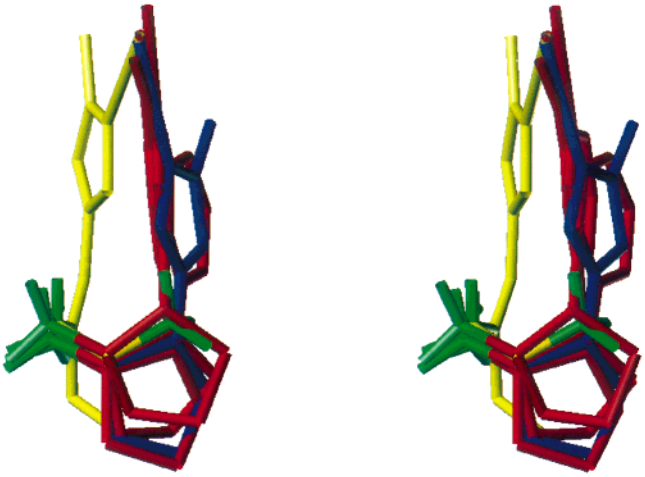


Figure 4. Stereoview of (*Z*)-**17** (lime) superimposed with ABT 418 (blue) and the three overlapping conformations of (*S*)-nicotine (red), showing the orientation of the *N*-methyl groups (green). All hydrogen atoms, except for those present in the *N*-methyl groups, are deleted.

tioning of the (*E*)-compounds is worse than for the (*Z*)-compounds. A possible explanation of the high affinity possessed by (*E*)-**13** may be the larger energy gain associated with forming a hydrogen bond to the oxygen atom instead of to the nitrogen atom.

In support of the model presented in this paper, the effects of various alkyl substitutions on nicotine itself[30] are exactly as would be expected from our model, since it places the 5- and 6-positions of the pyridine ring in nicotine at the 4- and 3-positions in the isoxazole ring in (*Z*)-**26** (Figure 3).

The Internitrogen Distance. Surprisingly (*Z*)-**26**_a, which has an N−N^+ distance of 5.6 Å, overlaid the low-energy conformer of (*R*)-epibatidine, which has an N−N^+ distance of 4.7 Å. This, combined with the inconsistency in the reports of the N−N^+ distance led to the idea that the essential parameter might not be the distance between the nitrogens, but the interaction sites between the ligands and the receptor.

It is assumed that a hydrogen bond donor is involved in binding. Thus, if the center of charge is important,[10] one would expect the distance between the hydrogen bond donor site point (b) and the cationic center (N^+) to be essential (Figure 2). Similarly if an anionic binding site is involved in ligand binding, the distance between the hydrogen bond donor site point (b) and the assumed anionic binding site (a) would be essential. Thus the N−N^+ distance, the (N^+−b) distance, and the (a−b) distance were measured and plotted (Figure 6) for the molecules in which substituent effects could be ruled out. The molecules were represented by their supposed binding conformations and grouped according to their activity level. It turns out that neither the N−N^+ distance nor the (N^+−b) distance have optimum values, which are restricted to the high-affinity compounds (IC_{50} < 10 nM). Furthermore all high-affinity compounds from the new series presented in this report have N−N^+ distances that exceed the 4.8 ± 0.3 Å proposed by Sheridan et al.[11]

Interestingly, all binding conformations of the high-affinity compounds have (a−b) distances between 7.0 and 8.0 Å, and only two of the other compounds ((*Z*)-**16** and (*Z*)-**13**) have an (a−b) distance in that range. On this basis, it is proposed that the nicotinic agonists interact with their receptors through one hydrogen bond and one Coulombic interaction as proposed by Beers and Reich.[10] As the high-affinity compounds all have approximately the same (a−b) distance, it is very likely that there is an anionic binding site within the receptor. The optimum conformation of this anionic site is then recognized by the compounds possessing an (a−b) distance of 7.0−8.0 Å.

Conclusion

The series of structures presented in this report include compounds with very high affinity for nicotinic acetylcholine receptors and minimal affinity for muscarinic receptors. Computational studies of a large number of these compounds have clarified that future modeling of nicotinic agonists should be performed using the global energy minimum of epibatidine (in which τ = 7°) as reference structure and that it is important to use site points when superimposing different molecules. In addition, when designing novel nicotinic agonists, attention should be paid to the following features of the molecule: (a) the (a−b) distance, since there is a strong indication that the optimum (a−b) distance is between 7 and 8.0 Å, and (b) the aliphatic area around the sp^3 nitrogen atom, as the receptor seems to be sensitive to changes in steric bulk and electrostatic potential.

Experimental Section

^{1}H NMR spectra were recorded at 300 MHz on a Bruker AC-300 MHz FT-NMR instrument, and COESY, NOESY, and ROESY spectra were recorded at 400 MHz on a Bruker AC-400 MHz FT-NMR instrument. Mass spectra were recorded on a Finnigan 5100 mass spectrometer, and melting points (uncorrected) were determined on a Buchi capillary melting point apparatus. Column chromatography was performed on silica gel 60 (70−230 mesh, ASTM, Merck). Elemental analyses

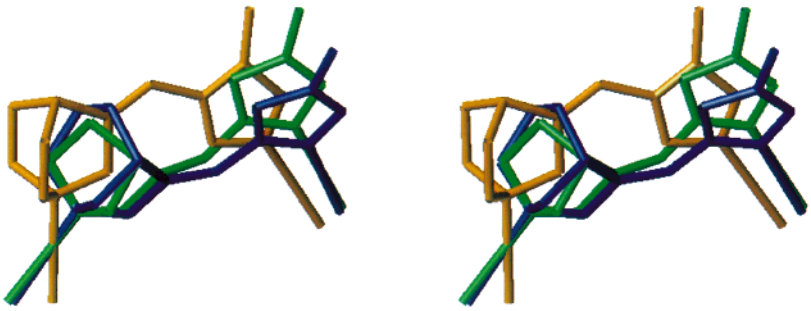


Figure 5. Stereoview of (*E*)-**11** (blue) and (*E*)-**13** (green) superimposed with (*Z*)-**26** (orange) using the site point complementary to the isoxazole oxygen atoms. All hydrogen atoms are deleted.

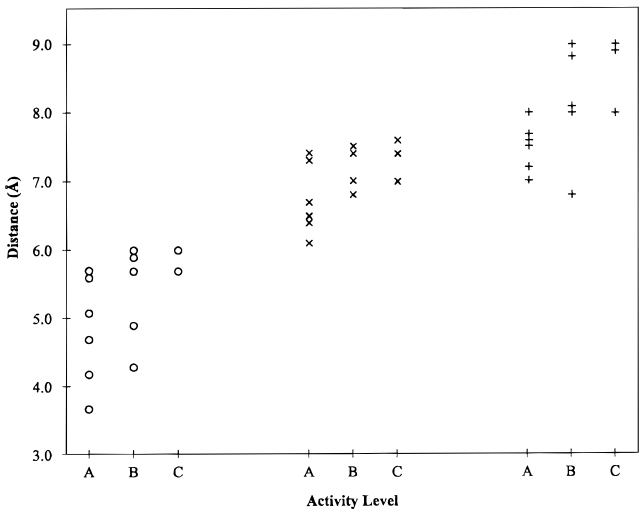


Figure 6. Interatomic distances measured in the compound's supposed binding conformation. ○: N−N$^+$, ×: N$^+$−b, +: a−b. Activity level A: IC_{50} < 10 nM; (*Z*)-**26**, (*Z*)-**14**, (*R*)-(*Z*)-**24**, (*Z*)-**25**, (*R*)-epibatidine, **29**, epiboxidine, and UB-165. Activity level B: IC_{50} = 10−100 nM; (*R*)-(*Z*)-**13**, (*R*)-(*E*)-**13**, (*R*)-(*E*)-**11**, ABT 418, and **30**. Activity level C: IC_{50} = 100−1000 nM; (*Z*)-**16**, **18**, and (*E*)-**14**.

were performed by Novo Nordisk Microanalytical Laboratory, Denmark, and were within ±0.4% of the calculated values.

The following compounds were prepared according to literature procedures: 3,4,5-trimethylisoxazole,[31,32] *N*-benzhydrylazetidin-3-one,[33] 1-methylpiperidin-3-one,[34] dimethyl-2-oxobutanephosphonate,[35] 1-azabicyclo[3.2.1]octan-3-one,[36] 5-ethyl-3-methylisoxazole,[37] ABT 418,[6] and (±)-epibatidine.[38]

The *Z*/*E*-purity of the compounds subjected to biological testing was measured by gas chromatography (Chrompack CP9001).

Method A. 3-Isopropyl-5-methylisoxazole (1). To a solution of isobutyraldehyde (4.5 mL, 0.050 mol) in methanol (150 mL) was slowly added a mixture of $NaHCO_3$ (8.4 g, 0.10 mol) and hydroxylamine hydrochloride (7.0 g, 0.10 mol) under N_2. The mixture was refluxed for 45 min and then stirred at 20 °C for 30 min, whereupon TLC (dichloromethane/methanol 25:1) showed that all starting material had reacted.

Distillation at 34 °C/0.5 mmHg gave 2.4 g (81%) of isopropyloxime. The compound was used without further purification.

A solution of isopropyloxime (2.2 g, 0.25 mol) in dry DMF (30 mL) was stirred under a stream of N_2, and *N*-bromosuccinimide (4.6 g, 0.026 mol) was slowly added. After the mixture was stirred for 90 min at 20 °C, TLC (ethyl acetate) showed that no oxime was left. The mixture was cooled to 0 °C, isopropenyl acetate (18 mL, 0.25 mol) was added, followed by dropwise addition of triethylamine (7.0 mL, 0.050 mol) dissolved in dry DMF (15 mL). The reaction mixture was stirred overnight at 20 °C, whereupon 7 N HCl (10 mL, 0.070 mol) was added, and stirring continued overnight at 20 °C. TLC showed that the reaction had reached completion. Addition of water (50 mL), extraction with diethyl ether (3 × 50 mL), washing of the combined extracts with a saturated solution of $NaHCO_3$ (2 × 25 mL) and water (25 mL), drying, and evaporation gave 2.5 g of an orange oil. Distillation at reduced pressure (0.05 mmHg, 25 °C) gave 1.5 g (48%) of 3-isopropyl-5-methylisoxazole (**1**) as a colorless oil.

The overall yield was 39% from isobutyraldehyde. The product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.85 (s, 1H, Ar-H), 3.01 (heptet, 1H, J = 7 Hz, CH-isopropyl), 2.38 (s, 3H, Ar-CH_3), 1.26 (d, 6H, J = 7 Hz, CH_3).

Compounds **2** and **3** were prepared following method A.

3-Methoxymethyl-5-methylisoxazol (4). A 55% suspension of sodium hydride in mineral oil (1.00 g, 0.020 mol) was added to a solution of 3-hydroxymethyl-5-methylisoxazole (2.30 g, 0.020 mol) in dry THF (40 mL). The sodium salt precipitated when the reaction mixture was stirred for a few minutes under N_2 at 20 °C. Upon cooling to 0 °C methyl iodide (2.50 g, 0.020 mol) was slowly added. When the addition was complete, the temperature was raised to 20 °C and stirring continued for 1 h. The reaction was quenched with ethanol (20 mL), whereupon water (50 mL) and diethyl ether (150 mL) were added. The phases were separated, and the organic phase was washed with 1 N HCl (75 mL) and 1 N NaOH (75 mL). Drying ($MgSO_4$) and evaporation gave 2.40 g (94%) of 3-methoxymethyl-5-methylisoxazol (**4**) which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.01 (s, 1H, Ar-H), 4.48 (s, 2H, Ar-CH_2O), 3.40 (s, 3H, OCH_3), 2.44 (s, 3H, Ar-CH_3)

Method B. 3-(3-Ethyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.2]octane (5). A solution of 3-ethyl-5-methylisoxazole (**2**) (0.43 g, 3.9 mmol) in THF (25 mL) was cooled to −78 °C, and *n*-butyllithium (2.5 M in hexanes, 5.5 mmol, 2.2 mL) was added dropwise under N_2. After the mixture was stirred for 15 min at −78 °C, a solution of 1-azabicyclo[2.2.2]octan-3-one (0.41 g, 3.3 mmol) in THF (8 mL) was slowly added. Stirring was continued at −78 °C for 90 min, whereupon triethylamine (0.92 mL, 6.6 mmol), ethanol (0.1 mL), and thionyl chloride (1.2 mL, 17 mmol) were added. After being stirred for 1 h at −78 °C, the reaction mixture was allowed to warm to 20 °C and stirred for 2 h. Addition of diethyl ether (100 mL) and extraction with 1 N HCl (3 × 50 mL) gave an aqueous layer, which upon basification (K_2CO_3) was extracted with dichloromethane (4 × 50 mL). The organic extracts were dried ($MgSO_4$) and concentrated in vacuo to give 0.65 g of a brown oil. Column chromatography (ethyl acetate/methanol/ammonium hydroxide, 25% in water: 72/25/3) gave 316 mg (44%) of (*E*)-**5** ((*E*)-3-(3-ethyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.2]-octane) in the first fractions and 63 mg (8.7%) of (*Z*)-**5** ((*Z*)-3-(3-ethyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.2]octane) in the later fractions as colorless oils. None of the compounds were absolutely free of the other isomer, but crystallization with HCl in ethyl acetate and subsequent recrystallizations from acetone gave the almost pure compounds as their hydrochlorides.

(*Z*)-5. Mp: 142−144 °C. MS: *m*/*z* 218 (M$^+$). GC: 87% *Z*. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.25−6.15 (m, 1H, CH=), 5.87 (s, 1H, Ar-H), 3.80 (s, 2H, NCH_2C=), 3.22−3.03 (m, 4H, NCH_2), 2.69 (quartet, 2H, J = 8 Hz, Ar-CH_2), 2.59−2.49 (m, 1H, CH), 1.93−1.68 (m, 4H, CH_2), 1.27 (t, 3H, J = 8 Hz, CH_3). Anal. ($C_{13}H_{18}N_2O$, HCl, $^1/_4H_2O$) C, H, N.

(*E*)-5. Mp: 194−197 °C. MS: *m*/*z* 218 (M$^+$). GC: 93% *E*. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.10−6.00 (m, 1H, CH=), 5.93 (s, 1H, Ar-H), 3.59 (s, 2H, NCH_2C=), 3.52−3.42 (m, 1H, CH), 3.06−2.82 (m, 4H, NCH_2), 2.68 (quartet, 2H, J = 8 Hz, Ar-CH_2), 1.90−1.64 (m, 4H, CH_2), 1.27 (t, 3H, J = 8 Hz, CH_3). Anal. ($C_{13}H_{18}N_2O$, HCl, $^1/_4H_2O$) C, H, N.

Compounds **6**, **7**, **8**, **9**, **10**, and **11** were prepared according to method B.

Method C. (*R*/*S*)-3-(3-Methyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.1]heptane (13). To a solution of 3-methyl-5-trimethylsilanylmethylisoxazole[39] (**12**) (3.38 g, 20.0 mmol) in THF (100 mL) under N_2 was added *n*-butyllithium (2.5 M in hexanes, 8.0 mL, 20 mmol) dropwise at −78 °C. After the mixture was stirred for 60 min at −78 °C, a solution of (*R*/*S*)-1-azabicyclo[2.2.1]heptan-3-one[9] in THF (20 mL) was added dropwise and stirring continued at −78 °C for 60 min, whereupon concentrated HCl (10 mL) was added, and the reaction was allowed to warm to 20 °C. The reaction mixture was poured onto ice and washed with diethyl ether (30 mL). Basification of the aqueous phase (K_2CO_3), extraction with diethyl ether (5 × 20 mL) and dichloromethane (2 × 20 mL), drying ($MgSO_4$), and evaporation gave 3.1 g (86%) of a yellow oil. Column chromatography (ethyl acetate/methanol/ammonium hydroxide, 25% in water: 65:33.2) gave 170 mg (4.5%) of (*R*/*S*)-(*Z*)-3-(3-methyl-5-isoxazolyl)methylene-1-azabicyclo-[2.2.1]heptane ((*Z*)-**13**), 170 mg (4.5%) of (*R*/*S*)-(*E*)-3-(3-methyl-5-isoxazolyl)methylene-1-azabicyclo[2.2.1]heptane ((*E*)-**13**), and 2.56 g (67%) of a mixture of the two products. The products

were crystallized with oxalic acid in acetone (1.2 equiv) in acetone to give the oxalate salts.

(*Z*)-13. Mp: 174−176 °C. MS: *m*/*z* 190 (M^+). GC: >99% *Z*. ^{1}H NMR (300 MHz, DMSO): δ 6.62 (s, 1 H, CH═), 6.32 (s, 1 H, Ar-H), 4.26 (d, 1 H, J = 17 Hz, NCH_2C═), 4.16 (d, 1 H, J = 17 Hz, NCH_2C═), 3.55 (d, 1 H, J = 5 Hz, CH), 3.44−3.33 (m, 1 H, NCH_2), 3.31−3.19 (m, 3 H, NCH_2), 2.27−2.18 (m, 1 H, CH_2), 2.22 (s, 3 H, Ar-CH_3),1.69−1.62 (m, 1 H, CH_2). Anal. ($C_{11}H_{14}N_2O$, $C_2H_2O_4$, $^1/_4H_2O$) C, H, N.

(*E*)-13. Mp: 168−170 °C. MS: *m*/*z* 190 (M^+). GC: >99% *E*. ^{1}H NMR (300 MHz, DMSO): δ 6.42 (s, 1 H, Ar-H), 6.37 (s, 1 H, CH═), 4.09 (d, 1 H, J = 17 Hz, NCH_2C═), 3.96 (d, 1 H, J = 5 Hz, CH), 3.90 (d, 1 H, J = 17 Hz, NCH_2C═), 3.41 (t, 1 H, J = 15 Hz, NCH_2), 3.22 (m, 3 H, NCH_2), 2.31−2.23 (m, 1 H, CH_2), 2.23 (s, 3 H, Ar-CH_3), 1.65−1.58 (m, 1 H, CH_2). Anal. ($C_{11}H_{14}N_2O$, $C_2H_2O_4$) C, H, N.

Compounds **14**, **15**, and **17** were prepared according to method C.

1-Methyl-3-(3-methyl-5-isoxazolyl)methylenepyrrolidine (16). A solution of diisopropylamine (0.83 mL, 6.3 mmol) in THF (15 mL) was cooled to 0 °C, and *n*-butyllithium (2.36 M in hexanes, 6.3 mmol) was added under N_2. After the mixture was stirred for 60 min at 0 °C, 3-methyl-5-trimethylsilanylmethylisoxazole[39] (**12**) (0.98 g, 5.8 mmol) was added dropwise. Stirring was continued for 15 min at 0 °C, followed by 15 min at −78 °C, during which a dark orange color appeared. 1-Methylpyrrolidinone[40] (0.53 g, 5.3 mmol) was dissolved in THF (2 mL) and added dropwise to the anion solution at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, upon which it was allowed to warm to 20 °C and stirred at that temperature for 2 h. HCl (1 N, 50 mL) and ethyl acetate (50 mL) were added, and the layers separated. Further extraction with 1 N HCl (2 × 50 mL), basification (K_2CO_3) of the aqueous layers, and extraction of these with dichloromethane (4 × 50 mL) gave a solution, which upon drying and evaporation yielded 0.63 g of a brown oil. Column chromatography (eluent: ethyl acetate/methanol/ammonium hydroxide, 25% in water: 75/25/0.5) followed by crystallization with oxalic acid in acetone gave 16 mg (1.1%) of (*Z*)-1-methyl-3-(3-methyl-5-isoxazolyl)methylenepyrrolidine ((*Z*)-**16**) oxalate and 57 mg (4.0%) of (*E*)-1-methyl-3-(3-methyl-5-isoxazolyl)-methylenepyrrolidine ((*E*)-**16**) oxalate.

(*Z*)-16. Mp: 177−179 °C. MS: *m*/*z* 178 (M^+). GC: >99% *Z*. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.23 (s, 1 H, CH═), 5.84 (s, 1 H, Ar-H), 3.50 (s, 2 H, NCH_2C═), 2.69 (s, 4 H, CH_2 CH_2), 2.46 (s, 3 H, NCH_3), 2.28 (s, 3 H, Ar-CH_3). Anal. ($C_{10}H_{14}N_2O$, $C_2H_2O_4$) C, H, N.

(*E*)-16. Mp: 170−173 °C. MS: *m*/*z* 178 (M^+). GC: >99% *E*. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.28 (s, 1 H, CH═), 5.90 (s, 1 H, Ar-H), 3.30 (s, 2 H, NCH_2C═), 2.65 (s, 4 H, CH_2 CH_2), 2.42 (s, 3 H, NCH_3), 2.28 (s, 3 H, Ar-CH_3). Anal. ($C_{10}H_{14}N_2O$, $C_2H_2O_4$) C, H, N.

Method D. 3-(3-Methyl-5-isoxazolyl)methyleneazetidine (18). The free base 1-benzhydryl-3-(3-methyl-5-isoxazolyl)methyleneazetidine (**15**) (0.78 g, 2.5 mmol) was dissolved in toluene (20 mL) and cooled to 0 °C. 1-Chloroethyl chloroformate (0.815 g, 5.7 mmol) was added, and the reaction mixture was stirred for 20 min. The reaction mixture was evaporated in vacuo, then redissolved in methanol (20 mL), and heated at reflux for 1 h, and evaporated in vacuo. The crude material was suspended in diethyl ether and the precipitated crystals collected by filtration, giving the hydrochloride salt of the title compound in 370 mg yield (80%). Mp: 168−169 °C (dec). MS: *m*/*z* 150 (M^+). ^{1}H NMR (300 MHz, DMSO): δ 9.88 (s, 2 H, NH_2^+), 6.56 (s, 1 H, CH═), 6.38 (s, 1 H, Ar-H), 4.87 (s, 2 H, NCH_2), 4.76 (s, 2 H, NCH_2), 2.23 (s, 3 H, Ar-CH_3). Anal. ($C_8H_{10}N_2O$, HCl) C, H, N.

Compounds **19** and **20** were prepared according to method D.

Method E. (*Z*)-3-Acetonylidene-1-azabicyclo[2.2.2]octane (22). Dimethyl 2-oxopropylphosphonate (4.9 g, 30 mmol) was added dropwise to a solution of 1-azabicyclo[2.2.2]octan-3-one (2.0 g, 16 mmol) and KOH (1.93 g, 30 mmol) in water (7.7 mL) at −5 °C. The reaction mixture was stirred at −5 °C for 90 h, then quenched with 1 N HCl (50 mL), rinsed three times with diethyl ether, made alkaline (K_2CO_3), and extracted with dichloromethane (5 × 50 mL). The solvent was removed after drying ($MgSO_4$), and the crude compound was crystallized as the hydrochloride salt from ethanol and then recrystallized from ethanol to give (*Z*)-3-acetonylidene-1-azabicyclo[2.2.2]-octane (**22**) hydrochloride in 21% yield. Mp: 189−190 °C. MS: *m*/*z* 165 (M^+). ^{1}H NMR (300 MHz, DMSO): δ 6.38 (s, 1H, CH═), 4.31 (s, 2H, NCH_2C═), 3.35−3.19 (m, 4H, NCH_2), 2.75−2.66 (m, 1H, CH), 2.20 (s, 3H, CH_3), 2.18−1.97 (m, 2H, CH_2), 1.85−1.75 (m, 2H, CH_2). Anal. ($C_{10}H_{15}NO$, HCl, CH_3CH_2OH) C, H, N.

Compounds **21** and **23** were prepared according to method E.

Method F. (*Z*)-3-(5-Isoxazolyl)methylene-1-azabicyclo-[2.2.2]octane (25). (*Z*)-3-Acetonylidene-1-azabicyclo[2.2.2]-octane (**22**) (0.36 g, 2.2 mmol) was dissolved in dimethyl formamide-dimethyl acetal (DMF-DMA) (3 mL), and the solution was stirred overnight at 90 °C under N_2. The mixture was evaporated to dryness, and the residue was dissolved in glacial acetic acid (10 mL). Upon addition of hydroxylammonium chloride (0.50 g, 7.2 mmol), the solution was stirred at 90 °C for 60 min. The brownish mixture was evaporated to dryness, whereupon the residue was dissolved in water, made alkaline with K_2CO_3, and extracted with diethyl ether (2 × 50 mL). The combined organic layers were dried and concentrated in vacuo to a brown oil. Column chromatography (dichloromethane/methanol/ammonium hydroxide, 25% in water: 80:20:0.5) gave 150 mg (37%) of the desired product as a yellow oil. Crystallization with oxalic acid in acetone/diethyl ether gave 180 mg of (*Z*)-3-(5-isoxazolyl)methylene-azabicyclo-[2.2.2]octane ((*Z*)-**25**) oxalate as white crystals. Mp: 122−124 °C. MS: *m*/*z* 190 (M^+). GC: 87% *Z*. ^{1}H NMR (300 MHz, DMSO): δ 8.60 (s, 1 H, Ar-3-H), 6.60 (s, 1 H, CH═), 6.45 (s, 1 H, Ar-4-H), 4.37 (s, 2 H, NCH_2C═), 3.42−3.22 (m, 4 H, NCH_2), 2.86 (s, 1 H, CH), 2.11−1.80 (m, 4 H, CH_2). Anal. ($C_{11}H_{14}N_2O$, 1.5 $C_2H_2O_4$, $^1/_4C_3H_6O$) C, H, N.

Compounds **24** and **26** were prepared according to method F.

(*Z*/*E*)-3-(1-Azabicyclo[2.2.2]oct-3-ylidene)-3-fluoro-propionic Acid Ethyl Ester (27). Triethyl-2-fluoro-2-phosphonoacetate (4.9 g, 20 mmol) was dissolved in dry DMF (20 mL) at 0 °C under N_2, whereupon potassium *tert*-butoxide (2.5 g, 22 mmol) was added over a period of 5 min. The reaction mixture was stirred for 1.5 h at 0 °C, upon which 1-azabicyclo-[2.2.2]octan-3-one (2.5 g, 20 mmol) dissolved in dry DMF (10 mL) was added dropwise. Stirring was continued for 30 min at 0 °C, followed by 30 min at 20 °C. TLC (dichloromethane/methanol/ammonium hydroxide, 25% in water: 80/20/0.5) showed that no starting material was left. The alkaline reaction mixture was neutralized with glacial acetic acid (1 mL) and concentrated in vacuo. The resulting oil was dissolved in dichloromethane (100 mL) and washed with 10% $NaHCO_3$ (3 × 100 mL) and water (100 mL). Drying ($MgSO_4$) and evaporation gave the crude product (4.6 g). Crystallization with oxalic acid in acetone gave (*Z*/*E*)-3-(1-azabicyclo[2.2.2]oct-3-ylidene)-3-fluoro-propionic acid ethyl ester (**27**) as the oxalate salt (4.8 g, 79%). The product was used without further purification. ^{1}H NMR (300 MHz, DMSO): δ 4.27 (quartet, 2H, J = 7 Hz, OCH_2), 4.11 (d, 2H, J = 3 Hz, NCH_2C═), 3.85−3.75 (m, 1H, CH), 3.38−3.17 (m, 4H, NCH_2), 2.09−1.96 (m, 2H, CH_2), 1.90−1.78 (m, 2H, CH_2), 1.28 (t, 3H, J = 7 Hz, CH_3).

(*E*)-3-(Fluoro-(3-methyl-5-isoxazolyl)methylene)-1-azabicyclo[2.2.2]octane (28). To a solution of acetone oxime (590 mg, 8.0 mmol) in dry THF (10 mL) was added *n*-butyllithium (1.6 M in hexanes, 10 mL, 16 mmol) under N_2 at 0 °C. The viscous solution was stirred for 45 min at 0 °C, whereupon (*Z*/*E*)-3-(1-azabicyclo[2.2.2]oct-3-ylidene)-3-fluoro-propionic acid ethyl ester (**27**) (1.1 g, 5.0 mmol) in THF (15 mL) was added dropwise. The mixture was stirred at 0 °C for 5 min, and then the temperature was kept at 5 °C overnight. The following day stirring was continued at 50 °C for 3 h, upon which the temperature was lowered to 0 °C and concentrated sulfuric acid (6 mL) was added dropwise. After being stirred at 20 °C

for 18 h, the reaction mixture was poured onto ice, and the solution was made alkaline with NaOH. Dichloromethane (100 mL) was added, and the mixture was filtered through Decalite. Separation of the layers, extraction of the aqueous layer with dichloromethane (2 × 100 mL), washing of the combined organic layers with water (100 mL) and brine (100 mL), drying ($MgSO_4$), and evaporation gave 900 mg (90%) of a yellow oil. Column chromatography (dichloromethane/methanol 9:1) gave 300 mg (30%) of (*E*)-3-(fluoro-(3-methyl-5-isoxazolyl)methylene)-1-azabicyclo[2.2.2]octane (**28**). Crystallization with oxalic acid in acetone gave 410 mg as white crystals. Mp: 162−164 °C. MS: *m*/*z* 222 (M^+). GC: 78% *E*. ^{1}H NMR (300 MHz, DMSO): δ 6.63 (s, 1 H, Ar-H), 4.23 (s, 2 H, $NCH_2C{=}$), 3.38 (s, 1 H, CH), 3.34−3.18 (m, 4 H, NCH_2), 2.29 (s, 3 H, Ar-CH_3), 2.07−1.96 (m, 2 H, CH_2), 1.92−1.81 (m, 2 H, CH_2). Anal. ($C_{12}H_{15}N_2OF$, $C_2H_2O_4$) C, H, N.

Biological Materials and Methods. (a) Nicotinic Receptor Binding. Fresh or frozen cerebral cortex (male Mol: WIST rats (Møllegaard, Ll. Skensved, Denmark)) was homogenized in assay buffer (50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) and centrifuged for 10 min at 40000*g*. Pellets were reconstituted in assay buffer, and an appropriate amount of tissue sample was mixed in tubes with [^{3}H]methylcarbamylcholine ([3H]MCC; NEN Life Sciences, NET-951; final concentration 2 nM) and test drug. The tubes were incubated at 0 °C for 60 min. Unbound ligand was separated from bound ligand by vacuum filtration through GF/B filters presoaked in 0.5% polyethylenimine. Filters were washed three times with 5 mL of wash buffer (50 mM Tris-HCl, pH 7.4) and transferred to vials. Scintillation fluid (4 mL) was added, and the radioactivity was measured by scintillation counting. Nonspecific binding was measured with 10 μM nicotine. IC_{50} values were determined using nonlinear regression (InPlot[41]).

(b) Muscarinic Receptor Binding. The muscarinic receptor binding was determined following a published procedure.[42]

Molecular Modeling. The calculations were performed using Sybyl 6.5.[22] The structures were built by combining and altering standard fragments from Sybyl. Using Gasteiger−Hückel charges, the structures were minimized until a local minimum was reached. The maximum number of iterations was set to 1000 and the termination gradient to 0.001.

(a) Grid Search. The conformational search employing rotation around the single bond connecting the bicycle and the isoxazole was done using the grid search command, which contains an energy minimization method that keeps the torsion angle under examination constant. The torsional scans were performed from 0 to 360° in 5° intervals, and the inherent minimization used the TRIPOS force field and Gasteiger−Hückel charges. The maximum number of iterations was set to 1000 and the termination gradient to 0.001. As the compounds were protonated, the dielectric constant was set to 4 while keeping the cutoff value at 8 Å.

The resulting low-energy structures were then minimized, but this time without any constraints.

(b) Molecular Dynamics. The remaining compounds were investigated using the molecular dynamics option in Sybyl.

In the simulation, the compounds were heated to 1000 K for 100 ps. The step size was 10 fs, and every 500 fs a structure was selected giving a total of 201 structures. The maximum number of iterations was set to 10000. Due to the amount of heat applied, some of the compounds racemized and/or *Z*/*E* interchanged. The 200 structures resulting from a dynamics run were then minimized.[43] The structures were superpositioned using the fit atoms command.

Supporting Information Available: Full results from the conformational analyses, experimental procedure for the syntheses of compounds **2**, **3**, **6**−**11**, **14**, **15**, **17**, **19**−**21**, **23**, **24**, and **26**, and characterization of the nicotinic receptor binding assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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