6-Chloropyridazin-3-yl Derivatives Active as Nicotinic Agents: Synthesis, Binding, and Modeling Studies†

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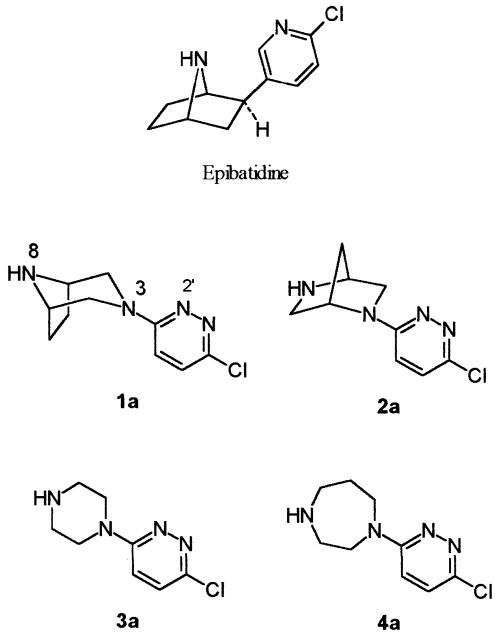
3,8-Diazabicyclo[3.2.1]octane (**1**), 2,5-diazabicyclo[2.2.1]heptane (**2**), piperazine (**3**), and homopiperazine (**4**) derivatives, substituted at one nitrogen atom with the 6-chloro-3-pyridazinyl group while the other nitrogen atom was either unsubstituted or mono- or dimethylated, were synthesized and tested for their affinity toward the neuronal nicotinic acetylcholine receptors (nAChRs). All of the compounds had K_i values in the nanomolar range. A molecular modeling study allowed location of their preferred conformations, the energies of which were recalculated in water with a continuum solvent model. Some of the compounds showed, in their populated conformations, only pharmacophoric distances longer than the values taken into consideration by the Sheridan model for nAChRs receptors. Thus, this SAR study gives support to the hypothesis that these longer distances are still compatible with affinity for α4β2 receptors in the nanomolar range.

Introduction

The discovery of compounds that can safely treat both acute and chronic pain without the side effects of drug dependency is highly desirable in pain management. In this respect, suggestions have been made that selective neuronal nicotinic acetylcholine receptor (nAChR) agonists may be useful. Nicotine itself has long been known to have antinociceptive properties, but a variety of side effects (mainly on the gastrointestinal tract and the cardiovascular system) make it a poor therapeutic choice.[1−4] The discovery of epibatidine[5−7] (Chart 1), a potent analgesic and nAChR modulator, brought about a renewed interest for compounds acting through nAChRs. These receptors are widely distributed through the central and peripheral nervous system. In particular, a major subtype in brain is composed of the α4β2 subunits combination, whereas ganglionic-type nAChRs are thought to contain α3 in combination with β2 or β4 and possibly other α units. Because ganglionic-type nAChRs are believed[2] to at least partially mediate several side effects of nicotinic agonists, the α4β2 subunits have become the target of our research. In a previous paper[8] we reported on the binding and analgesic properties of 3-(6-chloro-3-pyridazinyl)-3,8-diazabicyclo[3.2.1]octane (**1a**, Chart 1), which we prepared as a possible analogue of epibatidine. In the same study,[8] we performed molecular modeling investigations at the semiempirical AM1 level on the protonated form of **1a**. Four minimum energy conformations were located, among which the most stable one was found to have a geometry similar to that of epibatidine. This conformation of **1a** appears to be stabilized by an intramolecular hydrogen bond between the aromatic N2′ atom and one of the two hydrogen atoms present on the positively charged N8. However, the ^{1}H NMR spectrum of **1a** recorded in water was in disagreement with a significant contribution of this conformation to the overall population.[8] To confirm, or to rule out, the importance of such conformation stabilized by the hydrogen bond, we decided to synthesize other models, either bicyclic (**2a**) or monocyclic (**3a**, **4a**) (Chart 1), structurally related to the 3,8-diazabicyclo[3.2.1]octane system; moreover, we prepared the mono- or dimethyl derivatives **1b**−**4b** and **1c**−**4c** (Scheme 1) to set aside the possibility of the hydrogen bond by replacement of

Chart 1



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† Dedicated to Professor Paola Vita Finzi on the occasion of her 70th birthday.

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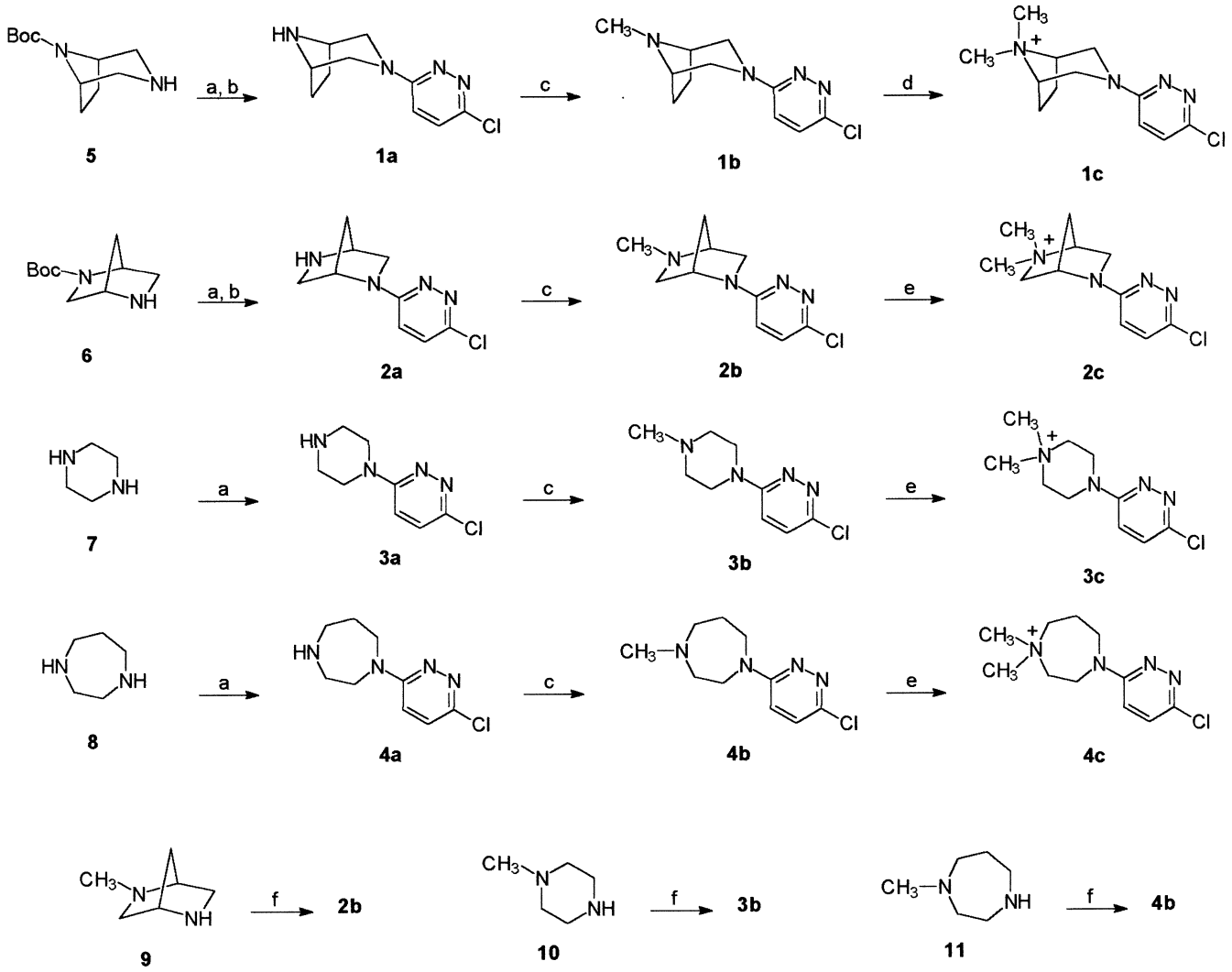
Scheme 1[a]



[a] (a) 3,6-Dichloropyridazine, NEt_3, toluene, reflux. (b) 1, HCl, Et_2O; 2, 6 N NaOH. (c) 1, HCOOH, 40% HCHO, 110 °C; 2, aqueous $NaHCO_3$. (d) CH_3I, PMP, DMF, rt. (e) CH_3I, Et_2O, rt. (f) 3,6-Dichloropyridazine, 160 °C.

one or both the ammonium hydrogen atoms with methyl groups. We report here their synthesis together with the binding affinity for the $\alpha4\beta2$ nAChR subtype. Moreover, modeling studies of all the compounds, including also the influence of a continuum solvent model, are reported.

Chemistry

Compounds **1**−**4** were synthesized according to Scheme 1. The required diazaderivative either protected as a Boc-derivative, as in the case of the diazabicyclo compounds (**5**, **6**),[8−10] or unprotected (**7**, **8**) was condensed with 3,6-dichloropyridazine in refluxing toluene and in the presence of triethylamine to give **1a**−**4a**. In the case of **1a** and **2a** the necessary deprotection step was easily performed by treatment with a solution of hydrochloric acid in diethyl ether. N-Methylation was then achieved by treatment of **1a**−**4a** with HCOOH/HCHO at 110 °C for 2.5 h to afford **1b**−**4b**. It should be noted that the *N*-methyl derivatives **9**−**11** are commercial compounds. Therefore, compounds **2b**−**4b** could also be obtained by treating them with 3,6-dichloropyridazine as described for the corresponding *nor*-analogues. Finally, stirring of **1b**−**4b** with iodomethane in diethyl ether at room temperature for 18 h led to the desired quaternary salts **1c**−**4c**. However, in the case of **1c** better yields were obtained by repeated treatment of **1b** with iodomethane and 1,2,2,6,6-pentamethylpiperidine (PMP) in dimethylformamide at room temperature.[11]

Table 1. Radioligand Binding Properties of Compounds **1**−**4**

compd	K_i (nM)[a]	compd	K_i (nM)[a]
1a	4.1	**3a**	32
1b	39	**3b**	14
1c	2.3	**3c**	0.44
2a	8.8	**4a**	1.5
2b	65	**4b**	220
2c	4.3	**4c**	120

[a] [^{3}H]Cytisine competition assay. Each experiment was performed in triplicate. K_i values were from three experiments, which agreed within 10%.

In Vitro Assays. Compounds were tested in binding studies in [^{3}H]cytisine competition assays. Rat cerebral cortical membranes were used.[12] K_i values are reported in Table 1.

Modeling Studies. Compounds **1a**,**b**−**4a**,**b**, in their protonated form, and **1c**−**4c**, as cations, were submitted to a modeling study through theoretical calculations performed in two steps; first, the conformational space was completely explored at the semiempirical AM1

Table 2. Relative Energy and Selected Geometrical Features of the B3LYP/6-31G(d) Calculated Minimum Energy Conformations of Compounds **1**−**4**[a]

conformation	E_{rel} (kcal/mol)	E_{rel}(aq) (kcal/mol)	A−B (Å)	A−C (Å)	conformation	E_{rel} (kcal/mol)	E_{rel}(aq) (kcal/mol)	A−B (Å)	A−C (Å)
1a-A	0.00	12.08	3.89	3.94	**4a-A**	0.00	6.40	3.85	3.98
1a-B	3.51	**0.00**	**6.22**	**5.57**	**4a-B**	0.33	7.08	3.85	4.00
1a-C	3.65	**2.11**	**5.93**	**5.38**	**4a-C**	1.37	6.88	3.83	3.89
1a-D	6.01	7.05	5.86	5.44	**4a-D**	7.79	**0.00**	**5.46**	**5.17**
1b-A	0.00	14.07	3.93	3.99	**4a-E**	8.77	**2.12**	**5.16**	**4.87**
1b-B	2.29	**0.00**	**6.26**	**5.58**	**4a-F**	10.42	**2.09**	**5.81**	**5.17**
1b-C	2.42	**1.89**	**6.03**	**5.44**	**4a-G**	10.54	**2.61**	**5.39**	**4.88**
1b-D	4.24	**1.54**	**6.29**	**5.61**	**4a-H**	10.89	5.83	5.05	4.74
1b-E	4.69	4.38	6.00	5.43	**4a-I**	11.13	4.04	5.55	5.27
1b-F	4.88	7.37	5.87	5.44	**4a-J**	11.48	4.57	5.93	5.04
1b-G	6.83	10.23	5.85	5.44	**4a-K**	12.61	**0.26**	**6.53**	**5.63**
1c-A	0.00	**0.00**	**6.29**	**5.61**	**4b-A**	0.00	7.83	3.89	4.02
1c-B	0.65	**2.72**	**6.12**	**5.51**	**4b-B**	0.16	8.51	3.87	4.04
1c-C	2.69	8.90	5.82	5.43	**4b-C**	0.98	8.15	3.86	3.93
2a-A	0.00	10.66	4.05	4.07	**4b-D**	6.50	**0.00**	**5.48**	**5.20**
2a-B	2.36	**0.00**	**6.13**	**5.44**	**4b-E**	7.76	**2.06**	**5.20**	**4.91**
2a-C	3.33	**0.21**	**6.18**	**5.48**	**4b-F**	8.83	4.97	4.89	4.69
2b-A	0.00	11.26	4.09	4.11	**4b-G**	8.92	**2.30**	**5.51**	**5.22**
2b-B	2.42	**0.00**	**6.14**	**5.45**	**4b-H**	9.23	**2.44**	**5.80**	**5.16**
2b-C	2.66	7.48	4.84	4.66	**4b-I**	9.48	3.60	5.19	4.90
2b-D	3.33	**0.28**	**6.18**	**5.48**	**4b-J**	9.61	**2.95**	**5.43**	**4.92**
2b-E	3.46	**1.14**	**6.17**	**5.49**	**4b-K**	9.62	3.18	5.50	5.27
2b-F	4.33	**1.06**	**6.23**	**5.52**	**4b-L**	9.88	5.90	5.08	4.77
2c-A	0.00	5.58	4.96	4.71	**4b-M**	9.97	4.87	5.95	5.06
2c-B	0.35	**0.00**	**6.16**	**5.48**	**4b-N**	10.05	4.19	5.58	5.30
2c-C	1.19	**0.39**	**6.22**	**5.51**	**4b-O**	10.22	7.86	4.77	4.65
3a-A	0.00	11.13	3.86	3.91	**4b-P**	10.65	7.14	5.15	4.95
3a-B	4.80	8.52	4.87	4.78	**4b-Q**	11.24	4.23	5.43	4.92
3a-C	5.20	**0.00**	**6.26**	**5.58**	**4b-R**	11.27	**0.18**	**6.55**	**5.65**
3a-D	9.05	5.07	5.81	5.16	**4b-S**	11.67	**2.89**	**6.06**	**5.45**
3b-A	0.00	12.37	3.89	3.95	**4b-T**	12.55	5.98	5.37	4.85
3b-B	4.14	8.33	4.89	4.81	**4b-U**	13.72	**2.54**	**6.58**	**5.67**
3b-C	4.24	**0.00**	**6.29**	**5.61**	**4c-A**	0.00	**0.00**	**5.53**	**5.25**
3b-D	6.76	**2.18**	**6.31**	**5.62**	**4c-B**	0.76	3.33	4.99	4.79
3b-E	6.81	10.91	4.88	4.80	**4c-C**	0.76	**1.92**	**5.20**	**4.94**
3b-F	8.81	6.03	5.99	5.25	**4c-D**	1.03	**1.45**	**5.56**	**5.33**
3b-G	10.38	11.59	4.93	4.66	**4c-E**	1.85	6.08	4.87	4.75
3b-H	11.05	6.77	6.10	5.39	**4c-F**	2.47	5.13	5.25	5.05
3c-A	0.00	**0.00**	**6.34**	**5.65**	**4c-G**	2.71	**2.28**	**5.45**	**4.94**
3c-B	0.18	8.49	4.89	4.82	**4c-H**	3.02	**0.89**	**6.21**	**5.60**
3c-C	4.57	9.71	5.05	4.75	**4c-I**	3.66	6.50	5.07	4.77
3c-D	4.71	5.09	6.14	5.44	**4c-J**	4.09	3.96	5.45	4.92
					4c-K	4.82	**0.32**	**6.61**	**5.70**

[a] Boldface data represent conformations with E_{rel}(aq) in a range of 3 kcal/mol above the global minimum. For compounds **4** only the most significant conformations from the AM1 analysis have been reoptimized at the B3LYP/6-31G(d) level.

level;[13] successively, all of the minimum energy conformations thus located for **1**−**3** were reoptimized at the higher B3LYP/6-31G(d) level.[14,15] For **4**, reoptimization was limited to the most significant conformations found with AM1. Moreover, the solvation energy was calculated with continuum solvent models, AM1-SM5.4[16] and C-PCM,[17] to take into account the strong influence of water on the behavior of these compounds that bear a positive charge under physiological conditions.

Table 2 reports the B3LYP calculated gas-phase and water-solvated energies together with selected geometrical features of the various conformations of the compounds studied. Figure 1 reports the three-dimensional plots of the global energy minimum conformations.

Discussion

The binding data reported in Table 1 indicate for all of the compounds an affinity in the nanomolar range as already shown for the model compound **1a**. The influence of methylation was similar in the series of compounds **1** and **2**: addition of the first methyl group leads to an ∼10-fold decrease in affinity, whereas addition of the second one restores it; indeed, quaternarization slightly improves K_i with respect to the parent nonmethylated compounds. Substantially different is the behavior of the series of compounds **3** and **4**; in the piperazine series **3** double methylation to the quaternary ammonium salt **3c** improves K_i by 2 degrees of magnitude, approaching the subnanomolar range; conversely, in the homopiperazine series methiodide **4c** shows a 100-fold decrease in affinity with respect to **4a**. The monomethyl derivatives **3b** and **4b** in both series stand close to the less active compound.

In binding assays, typical nicotinic ligands exhibit diverse structure−activity patterns with respect to N-substitution; whereas the affinity of *S*-nicotine is ∼20-fold higher than that of its *nor*-analogue,[18] N-methylation decreases by 2-fold the affinity of (−)-epibatidine[19] and by several hundreds that of (+)-anatoxin.[20] The effects of the N-substitution have been discussed in a recent review.[21] The hypothesis has been made that it may evoke indirect conformational effects in addition to the exclusive steric effects usually taken into consid-

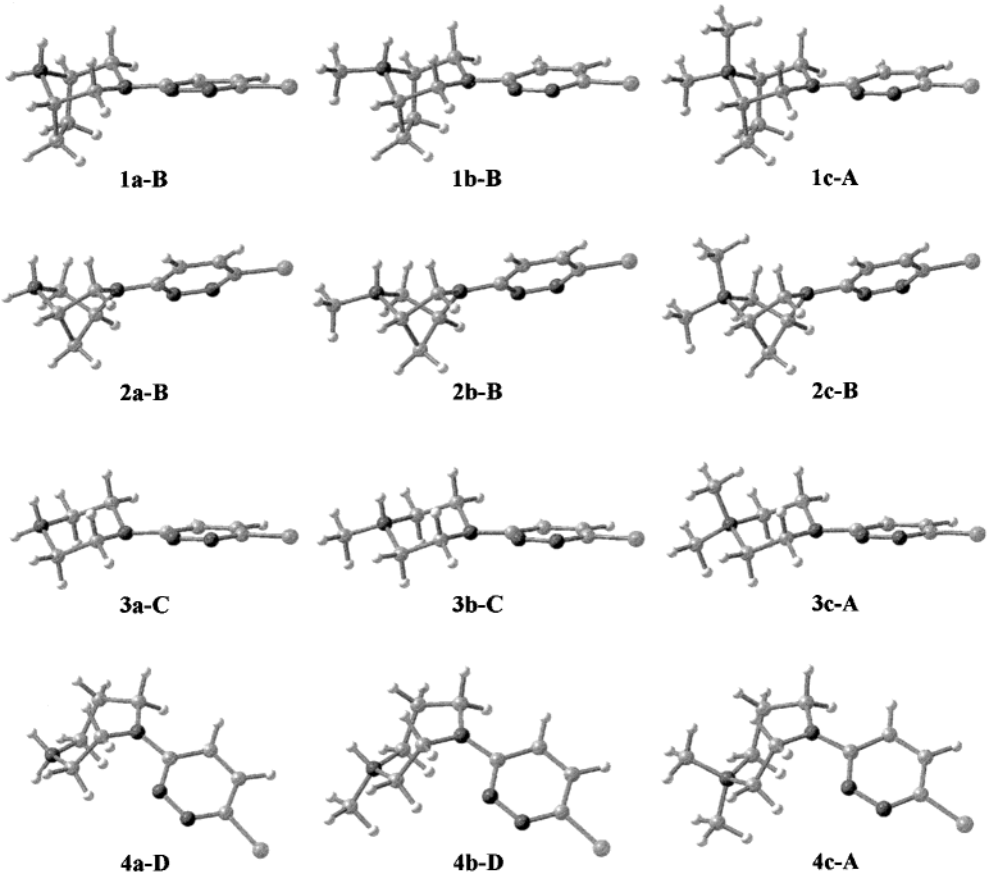


Figure 1. Three-dimensional plots of the global energy minimum conformations of compounds **1**−**4** as optimized at the B3LYP/6-31G(d) level and recalculated in water using the C-PCM model.

eration. *N*-Methyl derivatives **1b**−**4b** were always weaker ligands than their nonmethylated and/or dimethylated analogues. This suggests that, in our case, protonated tertiary amines are not able to profit from the efficient binding modes of the protonated secondary amines and quaternary ammonium compounds.[22]

The best compounds in the **1**−**4** series show affinity for α4β2 nAChR in the same range as nicotine, and the question arises whether they interact with the receptor similarly to nicotine or epibatidine. Over the years, on the basis of the structures of nicotine and other nicotinic agonists, several pharmacophoric elements have been proposed; the Beers and Reich model[23] suggested a distance of 5.9 Å between the center of positive charge (N^+) and a hydrogen acceptor (the lone pair of a pyridine nitrogen atom or of a carbonyl oxygen). Subsequently, Sheridan et al.[24] proposed a model based on three pharmacophoric elements, the cationic center N^+ (A), an electronegative atom capable of accepting a hydrogen bond (B), and a dummy atom that defines the line along which the hydrogen bond may form (C): the Sheridan model indicates for a good affinity an A−B distance of 4.8 ± 0.3 Å, an A−C distance of 4.0 ± 0.3 Å, and a B−C distance of 1.2 Å. Subsequently, a longer A−B distance was proposed[25] on the basis of the fact that epibatidine presents minimum energy conformations with an A−B distance of 5.5 Å. This finding was also supported by the discovery of azetidinylmethoxypyridine A-85380,[4] almost equipotent to epibatidine, which shows an even longer A−B distance of 6.1 Å in its minimum energy conformation. Actually, epibatidine possesses other low-energy conformations with an A−B distance of 4.5 Å, and also A-85380, being a rather flexible molecule, presents conformers with A−B distances of <6.1 Å. Moreover, when epibatidine is considered as a reference compound, it should be noted that the barriers to rotation of the pyridine ring are very low, 2.0−2.5 kcal/mol as calculated by Campillo et al.[26] at various levels of calculations; none of its four energy minima can be taken as a rigid model for α4β2 receptor agonists, and it is only possible to say that the active conformation has an A−B distance somewhere in the range of 4.5−5.5 Å. The discovery[3] of a series of derivatives of A-85380 variously substituted by a halogen atom on the pyridine ring gave again support to the original Sheridan model as only compounds able to present low energy conformations with A−B of ∼4.4 Å possess affinity comparable to that of A-85380. However, a comprehensive review[21] on nicotinic agonists and antagonists has shown several cases, in addition to those reported above, of good affinity still compatible with pharmacophoric distances higher than that of the Sheridan model.

The strong influence of the water solvation on the energy of the various conformers in the case of charged species should be noted. Table 3 shows as an example how the energy values vary for the four conformations of protonated epibatidine calculated with the B3LYP/6-31G(d) method in gas phase and in water with a continuum solvent model (see also Figure 2). Some reranking of the conformations can be observed, which, however, remain close in energy in a range of a few kilocalories per mole.

As far as compounds **1**−**4** are concerned, the considerations reported above for epibatidine should also apply

Table 3. Relative Energy and Selected Geometrical Features of the B3LYP/6-31G(d) Calculated Minimum Energy Conformations of Protonated Epibatidine

conformation	dihedral angle C1−C2−C3′−C2′ (deg)	E_{rel} (kcal/mol)	E_{rel}(aq) (kcal/mol)	A−B (Å)	A−C (Å)
epi-A	13	0.95	0.00	4.57	4.26
epi-B	85	0.00	1.38	4.51	4.13
epi-C	−90	1.03	2.49	5.30	4.21
epi-D	−170	2.27	1.52	5.49	4.32

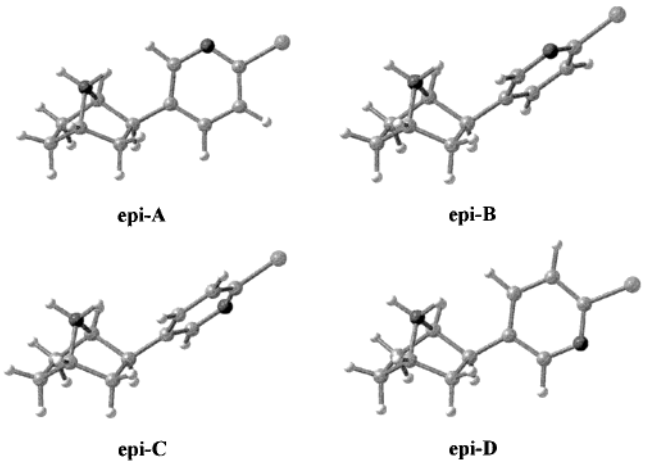


Figure 2. Three-dimensional plots of the energy minimum conformations of epibatidine as optimized at the B3LYP/6-31G-(d) level.

with an important difference: the presence of a second nitrogen atom (N2′) in the heterocyclic ring limits the rotational freedom; in fact, an interaction should exist between this atom and the positively charged center. This interaction could stabilize a particular orientation of the aromatic ring as a consequence not only of the possible existence of a hydrogen bond between N2′ and one N−H hydrogen atom but also of the influence that the solvent has on the strength of this bond. Moreover, the orientation of the aromatic ring can be influenced by the resonance with the lone pair of the nitrogen atom to which it is connected, so that conformations that make possible this conjugation should be preferred. The results previously reported by us[8] on compound **1a** were in some respect contradictory: whereas gas-phase AM1 calculations indicated as favored the conformation that presents the intramolecular hydrogen bond N8−H···N2′ and a boat conformation of the piperazine ring, vicinal coupling constants data from the ^{1}H NMR spectrum of compound **1a** registered in D_2O support a chair conformation of the piperazine ring. Reinvestigation of the behavior of **1a** at the B3LYP level confirmed conformation **1a-A**, presenting the intramolecular hydrogen bond, as the gas-phase global energy minimum. Three other conformations, **1a-B**−**D**, more compatible than **1a-A** with the ^{1}H NMR data, were located; they all present a chair conformation of the pyperazine ring, and two of them, **1a-B** and **1a-C**, show the aryl ring correctly oriented for a good conjugation with the N3 nitrogen lone pair. When the energy of the four conformations of **1a** was recalculated in water with a continuum solvent model,[16,17] conformation **1a-A** became a very high energy conformer, less stable than the global energy minimum **1a-B** by >10 kcal/mol, in agreement

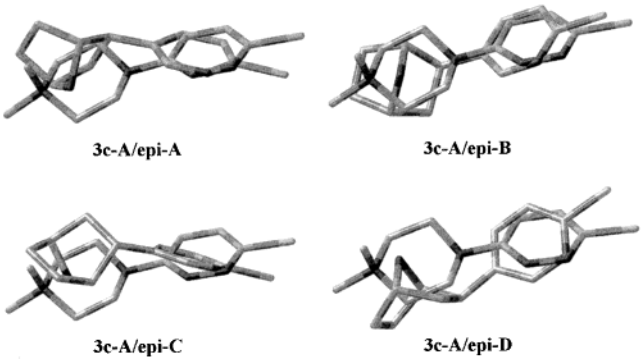


Figure 3. Superimposition of conformation **3c-A** of compound **3c** with the four energy minimum conformations of epibatidine.

with experimental NMR data. Conformers **1a-B**−**D** differ only in the orientation of the aryl ring and present similar A−B (5.9−6.2 Å) and A−C (5.4−5.6 Å) distances. The former distance appears in the upper limit for good $\alpha4\beta2$ agonists and in disagreement with the Sheridan model; also, the latter distance does not conform to the model as it is ∼1.5 Å longer.

Compound **1b** presents seven energy minima; the first one, **1b-A**, corresponds to **1a-A**, and the other six correspond to the three conformers **1a-B**−**D** each doubled by the two possible orientations of the *N*-methyl group. In compound **1c** the possibility of a hydrogen bond is precluded by the presence of two *N*-methyl groups; hence, only the three conformers **1c-A**−**C**, resembling the **1a-B**−**D** conformations of **1a**, could be located. This fact suggests that the conformations stabilized by a hydrogen bond are not a necessary requisite for good binding properties as the affinity of compound **1c** is even higher than that of **1a**. For **1c** the global minimum conformation is the same in water and in the gas phase and resembles the best "solvated" conformations of **1a** and **1b**. This suggests that also for **1a** and **1b** the "solvated" conformations should be more relevant for a good affinity. In fact, although it is generally accepted that ligands bind receptors after desolvation, probably the bound conformation of compounds **1** resembles the "solvated" conformations. On these bases, we were induced to focus our discussion on the energy data in water.

These considerations may be extended to compounds **2a**−**c** and **3a**−**c**, which also show, in their preferred "solvated" conformations, A−B and A−C distances of 6.1−6.3 and 5.4−5.6 Å, respectively. It is worth pointing out that **3c**, the compound with the highest affinity in our four series, presents a largely preferred conformation, **3c-A**, with A−B and A−C distances significantly high. However, a superimposition of this conformation with the four conformations of epibatidine (Figure 3) shows that the similarity of the molecules is greater than was indicated by the analysis of the pharmacophoric distances. This suggests that the three-point pharmacophore is useful only for a preliminary comparison. In fact, additional binding interactions may be present and may derive from the two methyl groups that appear able to superimpose to the ethylidenic bridge of epibatidine in some of the pictures reported in Figure 3.

Finally, the great flexibility of the seven-membered ring in **4a**−**c** allows the existence of several conforma-

Table 4. Physicochemical Properties of Compounds **1-4**

compd[a]	yield (%)	formula[b]	^{1}H NMR (δ)	solvent
1a	46	$C_{10}H_{13}ClN_4$	1.80 (m, 4H + 1H, exch with D_2O), 3.10 (m, 2H), 3.65 (m, 2H), 3.90 (m, 2H), 6.75 (d, 1H), 7.15 (d, 1H)	$CDCl_3$
1b	6	$C_{11}H_{15}ClN_4$	1.70 (m, 2H), 2.00 (m, 2H), 2.35 (s, 3H), 3.30 (m, 4H), 3.80 (d, 2H), 6.80 (d, 1H), 7.15 (d, 1H)	$CDCl_3$
1c	13	$C_{12}H_{18}ClIN_4$	2.20 (m, 2H), 2.45 (m, 2H), 3.20 (s, 3H), 3.35 (s, 3H), 3.80 (m, 2H), 4.10 (m, 4H), 7.35 (d, 1H), 7.55 (d, 1H)	D_2O
2a (1*S*,4*S*)	40	$C_9H_{11}ClN_4$	2.10 (d, 1H), 2.20 (d, 1H), 3.2−3.3 (m, 2H+1H, exch with D_2O), 3.70 (d, 1H), 3.80 (d, 1H), 4.60 (br s, 1H), 5.00 (br s, 1H), 7.30 (d, 1H), 7.70 (d, 1H)	$CDCl_3$
2b (1*S*,4*S*)	64	$C_{10}H_{13}ClN_4$	1.90 (d, 1H), 2.05 (d, 1H), 2.45 (s, 3H), 2.90 (m, 2H), 3.40 (dd, 1H), 3.65 (d, 1H), 3.75 (bs, 1H), 4.65 (bs, 1H), 6.70 (d, 1H), 7.15 (d, 1H)	$CDCl_3$
2c (1*S*,4*S*)	78	$C_{11}H_{16}ClIN_4$	2.52 (br d, 1H), 2.73 (d, 1H), 3.23 (s, 3H), 3.35 (s, 3H), 3.81 (m, 3H), 4.14 (d, 1H), 4.6−5.1 (m, 2H, partly obscured by HOD peak), 7.18 (d, 1H), 7.58 (d, 1H)	D_2O
3a	21	$C_8H_{11}ClN_4$	1.70 (s, 1H, exch with D_2O), 3.00 (t, 4H), 3.65 (t, 4H), 6.90 (d, 1H), 7.25 (d, 1H)	$CDCl_3$
3b	44	$C_9H_{13}ClN_4$	2.35 (s, 3H), 2.55 (t, 4H), 3.65 (t, 4H), 6.90 (d, 1H), 7.20 (d, 1H)	$CDCl_3$
3c	40	$C_{10}H_{16}ClIN_4$	3.20 (s, 6H), 3.60 (t, 4H), 4.00 (t, 4H), 7.55 (d, 1H), 7.75 (d, 1H)	DMSO
4a	42	$C_9H_{13}ClN_4$	1.90 (t, 2H), 2.20 (s, 1H, exch with D_2O), 2.80 (t, 2H), 3.05 (t, 2H), 3.75 (m, 4H), 6.90 (d, 1H), 7.15 (d, 1H)	$CDCl_3$
4b	28	$C_{10}H_{15}ClN_4$	2.00 (m, 2H), 2.40 (s, 3H), 2.55 (t, 2H), 2.75 (t, 2H), 3.65 (t, 2H), 3.90 (t, 2H), 6.75 (d, 1H), 7.15 (d, 1H)	$CDCl_3$
4c	90	$C_{11}H_{18}ClIN_4$	2.35 (m, 2H), 3.24 (s, 6H), 3.62 (m, 2H), 3.67 (m, 2H), 3.72 (t, 2H), 4.19 (m, 2H), 7.26 (d, 1H), 7.52 (d, 1H)	CD_3OD

[a] With the exception of **3b** (mp = 115−116 °C) and the quaternary salts, which, however, decomposed before their melting points, all of the compounds were oils. [b] Elemental analyses for C, H, and N were within ±0.4% of the calculated data.

tions, and also the most stable "solvated" conformations present a large distribution of the A−B (5.2−6.6 Å) and A−C distances (4.9−5.7 Å).

Conclusions

Compounds **1**−**3** give support to the hypothesis[21] that A−B distances longer (∼6.0 Å) than in the Sheridan model are still compatible with affinity for α4β2 receptors in the nanomolar range. Our results put also attention on the A−C distance that, contrarily to epibatidine, in several of our compounds appears by ∼1.5 Å longer than in the Sheridan model. Compounds **4** appear to be more flexible and present larger ranges of the A−B and A−C distances; however, it should be noted that, if the conformations presenting the intramolecular hydrogen bond are excluded, the A−C distance remains longer than in the Sheridan model. The reason only **3c**, and not **1c**, **2c**, or **4c**, shows a significant improvement of the binding affinity with respect to the nonmethylated and monomethylated analogue is not evident from the analysis of the A−B and A−C distances; other factors, such as steric interactions, could play a special role in the case of the quaternary ammonium compounds. The three-dimensional plots of **1c-A**, **2c-B**, and **3c-A** depicted in Figure 1 show a great similarity among them. However, it should be noted that **1c** and **2c**, as well as **4c**, have a molecular volume larger than that of **3c**; because all of the values found for their volumes (V = 283, 267, 257, and 275 Å^3 for **1c**, **2c**, **3c**, and **4c**, respectively) are greater than those of nicotine (208 Å^3) and epibatidine (234 Å^3), it could be hypothesized that the value found for **3c** might represent the maximum tolerance for the receptor.

Experimental Section

Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Analyses indicated by the symbols were within ±0.4 of the theoretical values. ^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported as δ (parts per million) relative to tetramethylsilane. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck; 230−400 mesh) was used for flash chromatography. All commercial compounds were purchased from Aldrich Chemical Co.

General Method for the Synthesis of Compounds 1a—4a. *Method a.* A solution of piperazine (**7**) or homopiperazine (**8**) (5 mmol), 3,6-dichloropyridazine (0.743 g, 5 mmol), and triethylamine (TEA; 0.695 g, 5 mmol) in toluene (10 mL) was refluxed under stirring overnight. The solvent was evaporated under vacuum and the residue purified by flash chromatography (CH_2Cl_2/CH_3OH 75:25) to give the desired compounds **3a** and **4a**, respectively. See Table 4 for data.

Method b. In the case of the diazabicyclo compounds, 8-*tert*-butoxycarbonyl 3,8-diazabicyclo[3.2.1]octane (**5**)[8] and the lower homologue 2-*tert*-butoxycarbonyl-2,5-diazabicyclo[2.2.1]-heptane (**6**)[9,10] were reacted as reported for method a. Final deprotection by treatment with a solution of hydrochloric acid in diethyl ether gave the desired **1a** and **2a** as their hydrochlorides. The corresponding free base could be obtained by treatment with 6 N NaOH and subsequent extraction with Et_2O. See Table 4 for data.

General Method for the Synthesis of Compounds 1b—4b. A mixture of the appropriate **1a**−**4a** (0.47 mmol), 99% HCOOH (0.705 mL, 18.68 mmol), and 40% HCHO (0.034 mL, 0.47 mmol) was stirred at 110 °C for 2.5 h. After cooling, the solvent was evaporated, and the residue was treated with a solution of $NaHCO_3$, extracted with CH_2Cl_2 (3 × 10 mL), and dried over sodium sulfate. After evaporation of the solvent, the product was purified by flash chromatography (CH_2Cl_2/CH_3OH 85:15). See Table 4 for data.

Alternatively, compounds **2b**−**4b** could be obtained by heating the commercially available **9**−**11** with 3,6-dichloropyridazine (molar ratio 1:1.5) at 160 °C for 70 min.

General Method for the Synthesis of Compounds 1c—4c. *Method a.* Methyl iodide (100 μL, 1.4 mmol) was added to a stirred solution of the required **1a**−**4a** (0.10 mmol) in diethyl ether (8 mL). A precipitate begins to form within 1 h, and stirring was continued for 18 h at room temperature. The solid was isolated by filtration, washed well with ether, and dried under vacuum at 50 °C to provide the title compound as an off-white powder. See Table 4 for data.

Method b. In the case of **1c** an alternative procedure[11] was followed, which gave better results. Accordingly, to a solution stirred at room temperature of **1a** (80 mg, 0.356 mmol) in DMF (3.5 mL) were added 1,2,2,6,6-pentamethylpiperidine (PMP; 0.128 mL, 0.712 mmol) and CH_3I (0.044 mL, 0.712 mmol) every 24 h for four times; the last time the mixture was allowed to stir for 72 h. The so-formed precipitate was filtered off by suction and ethyl ether (10 mL) at 0 °C added to the filtrate to give a red solid, which was constituted by a mixture of the

desired product and PMP. The obtained solid was filtered by suction and heated with acetone (15 mL) for a few minutes. After filtration, the solid was further washed with methanol, to give the desired compound in acceptable purity. See Table 4 for data.

Binding Assays. 1. Membrane Preparations. Rat cerebral cortical membranes were purchased from ABS Inc. (Wilmington, DE). Prior to use, the frozen membrane pellets were slowly thawed, washed, and resuspended in 30 volumes of assay buffer (composition, mM: Tris HCl, 50; NaCl, 120; KCl, 5; $MgCl_2$, 1; and $CaCl_2$, 2.5; pH 7.4 at 4 °C). The homogenate was centrifuged at 45000*g* for 20 min at 4 °C and the pellet resuspended in ice-cold buffer.

2. [^{3}H]-(−)-Cytisine Binding. Binding conditions were as previously described.[12] Samples containing 150−200 μg of protein, 0.7 nM [^{3}H]-(−)-cytisine (30 Ci/mmol), and the various concentrations of the nAChR modulators were incubated in a final volume of 500 μL for 75 min at 4 °C in triplicate. Nonspecific binding was determined in the presence of 10 μM (−)-nicotine.

Modeling Studies. Calculations with the semiempirical AM1 method[13] were performed using the PC Spartan Pro molecular modeling program.[27] Molecules were built from the model kit containing the atomic fragments and subjected to a preliminary refinement through the "minimize" option. Compounds in the protonated form or as quaternary ammonium cations were subjected to the conformational search through the conformational distribution option. Moreover, energy profiles for rotation around the single bond connecting the aryl moiety to the molecules were determined to ensure that the conformational search had located all minima. Solvation energy was determined with the AM1-SM5.4/A method.[16] The minimum energy geometries found with AM1 were used as starting geometries for B3LYP/6-31G optimizations performed with the Gaussian 98 package.[28] The energies of the water-solvated conformations were recalculated with the C-PCM approach[17] implemented in the Gaussian 98 package. The A−B distance was directly measured as the distance between the two nitrogen atoms, whereas the A−C distance was measured as the distance between the ammonium nitrogen and the middle point of the segment connecting the C3 and C6 atoms of the heteroaromatic ring. Molecular volumes of a space-filling model were calculated by Spartan Pro.

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