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STRUCTURAL AND CONFORMATIONAL STUDIES OF 3-(6-CHLOROPYRIDAZIN-3-YL)-*N*,*N*-DIMETHYLPIPERAZINIUM IODIDE AND 3-(6-CHLOROPYRIDAZIN-3-YL)-*N*,*N*-DIMETHYL-HOMOPIPERAZINIUM IODIDE WITH CENTRAL NICOTINIC ACTION

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Abstract - The structural characteristics of 3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylpiperazinium iodide (**3c**) and 3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylhomopiperazinium iodide (**4c**) have been investigated in order to understand their actions at the nAchR receptor. The results of the X-Ray molecular structures and of the conformational analysis have been compared with the structure of epibatidine that represents one of the most potent nicotinic agonist.

INTRODUCTION

Central nicotinic cholinergic receptors (nAchRs) are implicated in neurodegenerative disorders such as Parkinson's and Alzaheimer's desease,[1,2] where the nicotinic receptors are less than those present in the brain of healthy individuals. They belong to the superfamily of ligand-gated ion channels (LGICs).[3] The neuronal type, located on both pre- and postsynaptic nerve terminals, mediate the positive effects of nicotine on cognition, memory and attention. Several subtypes are present in the CNS, because nAChRs are pentameric combinations of α and β subunits, then it is important to develop new selective ligands specially for the $\alpha4\beta2$ and $\alpha7$ subtypes, which have demonstrated prominent importance.[4] For the compounds object of this research, 3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylpiperazinium iodide (**3c**) and

3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylhomopiperazinium iodide (**4c**), the receptor target is the neuronal α4β2 subunit.

These compounds were synthesized as possible analogues of the nAchR modulator epibatidine and the analgesic 3-(6-chloro-3-pyridazinyl)-3,8-diazabicyclo[3.2.1]octane (**2a**) [5] and showed affinity for the nAChR in the nanomolar range. The molecular modelling studies [6] of the protonated form of a 6-chloropyridazin-3-yl derivative (**1a**) indicated that the most stable conformations having a similar geometry to that of epibatidine, were stabilized by an intramolecular hydrogen bond between the aromatic N2' and one of the two hydrogen atoms present on the positively charged N(8) (Figure 1).

The *N*-dimethyl iodide derivatives are unable to cross the blood-brain-barrier, but are able to interact with high affinity with nicotinic receptors,[7] in particular 3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylpiperazinium iodide (compound (**3c**)). The latter is particularly useful in order to characterize nAchR and to draw up a model for ligands interactions. For the nicotinic receptor, the pharmacophoric model proposed by Sheridan,[8] is formed by three essential groups, usually a cationic centre (A), an hydrogen bond acceptor (B) and an atom (C) forming a dipole with B.

In this paper the X-Ray structures of compounds (**3c**) and (**4c**) are reported and the Sheridan's pharmacophoric distances are compared. They differ more than 100 times in activity [7] and they could be a reliable model for studying the subtle structural differences important in the receptor interactions.

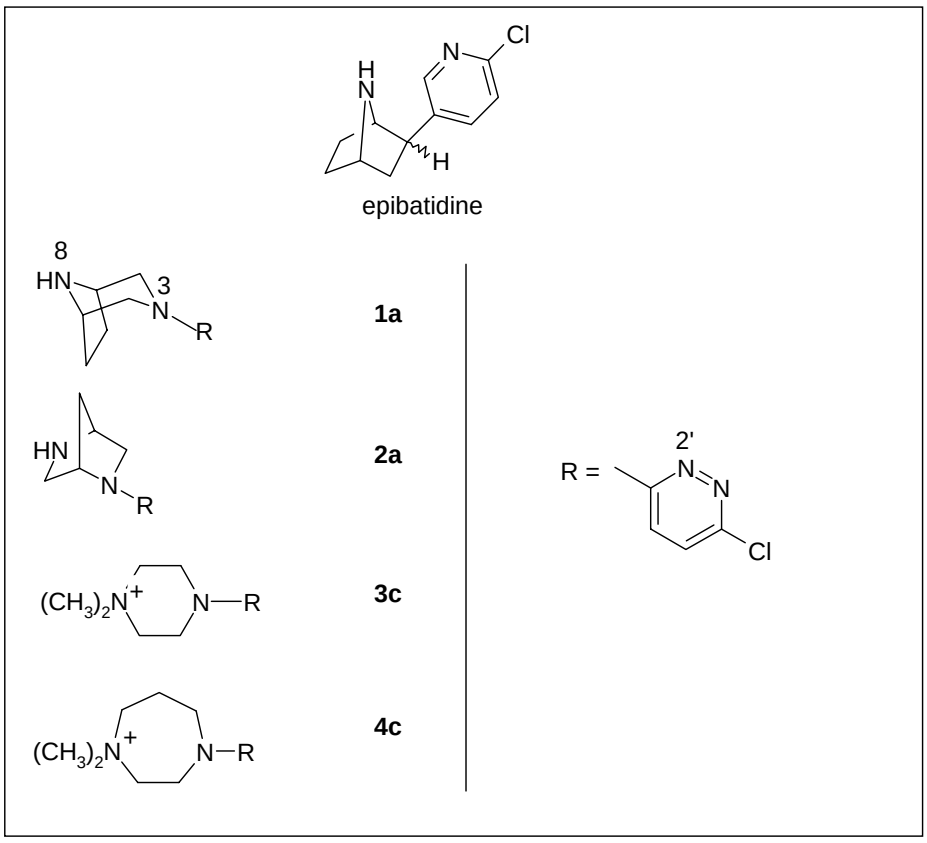

Figure 1

RESULTS AND DISCUSSION

ORTEP [9] views of the molecular structures of [3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylpiperazinium] (**3c**) and [3-(6-chloropyridazin-3-yl)-*N*,*N*-dimethylhomopiperazinium] (**4c**) are shown in Figures 2 and 4 respectively.

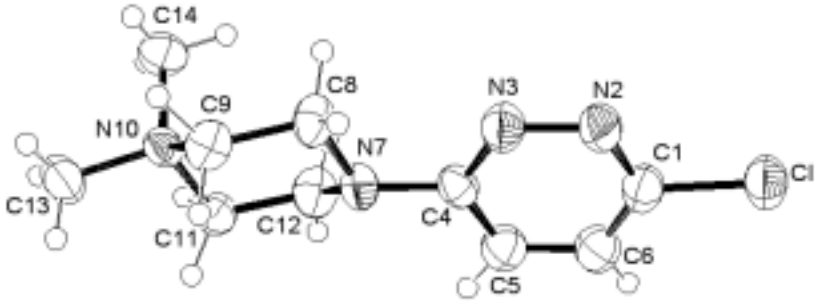

Figure 2: ORTEP drawing of the **3c** cation (Ellipsoids are at 50% probability).

The molecular structure of **3c**, which represents the more active compound, is characterized by a chair conformation of the piperazine ring, with an A-B distance of 6.28(1)Å (N(10) cationic centre and N(2) hydrogen bond acceptor) and the A-C distance of 5.59(1)Å (referred to the gravity centre of the pyridazine ring), and by the dihedral angle between the aromatic system and the chair plane of 28.6(4)°. The C(4)-N(7) bond distance of 1.41(1)Å maintains the single bond character and the values of the angles around N(7) indicate a sp^3 hybridization (the sum of the angles around N(7) is 346.7(6)°). The crystal packing (Figure 3) is characterized by molecular stacking, being the two centrosymmetrically related pyridazine moieties faced each other at a distance of about 3.4Å, forming a kind of "dimeric" arrangement controlled by π interactions. The quaternary group and iodine atoms are located in the crystal cell with contact distances I…N(10) ranging from 4.40 to 5.06Å. Each iodine is surrounded by four quaternary groups of the "dimeric" molecules in a infinite tridimensional network. The increased affinity towards the receptor, the absence of hydrogen bond interactions, the values of the A-B and A-C distances, suggest the possibility that the steric interactions could play a special role in the quaternary ammonium compound.

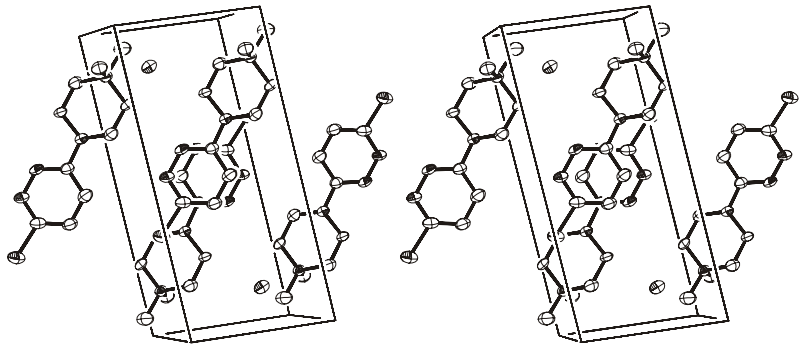

Figure 3: Stereoview of the crystal packing of compound (**3c**) (Hydrogen atoms are omitted for sake of clarity).

The compound (**4c**) is characterized by the homopiperazine ring, in chair conformation, with three atoms, respectively N(7) –1.185(7)Å, N(10) 0.622(6)Å and C(13) –1.004(8)Å, out of the chair plane (C(8), C(9), C(11), C(12)). The total puckering value of the molecule is 0.858(8)Å.[10] The dihedral angle between the chair plane and the pyridazine ring is 71.7(3)°. The A-B and A-C distances are 6.37(2)Å and 5.42(2)Å respectively (A is N(10), B is N(2) and C is the gravity centre of the pyridazine ring). Differently from (**3c**) the N(7)-C(4) bond distance is of 1.37(1)Å, showing partial conjugation with the adjacent aromatic system, with a sp^2 hybridization of the involved nitrogen, confirmed by the value of 360.0(6)° of the angles around N(7). The crystal packing is characterized by molecular stacking of the aromatic rings that are facing two by two and by short interactions involving the C(5)-H proton and the adjacent N(3)" nitrogen ("at x+½, y, 1-z, distance of 2.347(6)Å, angle 169.2(5)°), as shown in Figure 5. In the crystal cell each iodine is connected to four molecules through the nitrogen of the quaternary group N(10) with a range of distance 4.52(2) to 5.17(7)Å.

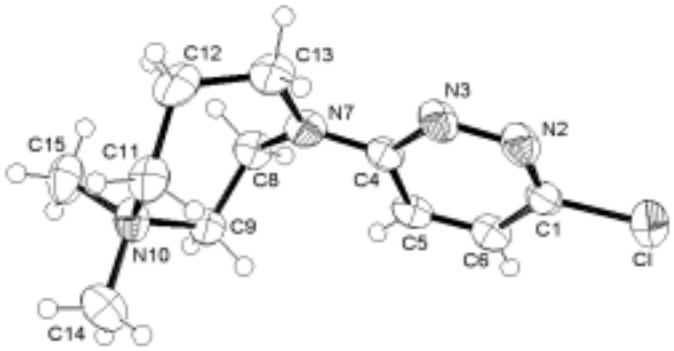

Figure 4: ORTEP drawing of **4c** cation (Ellipsoids are at 50% probability).

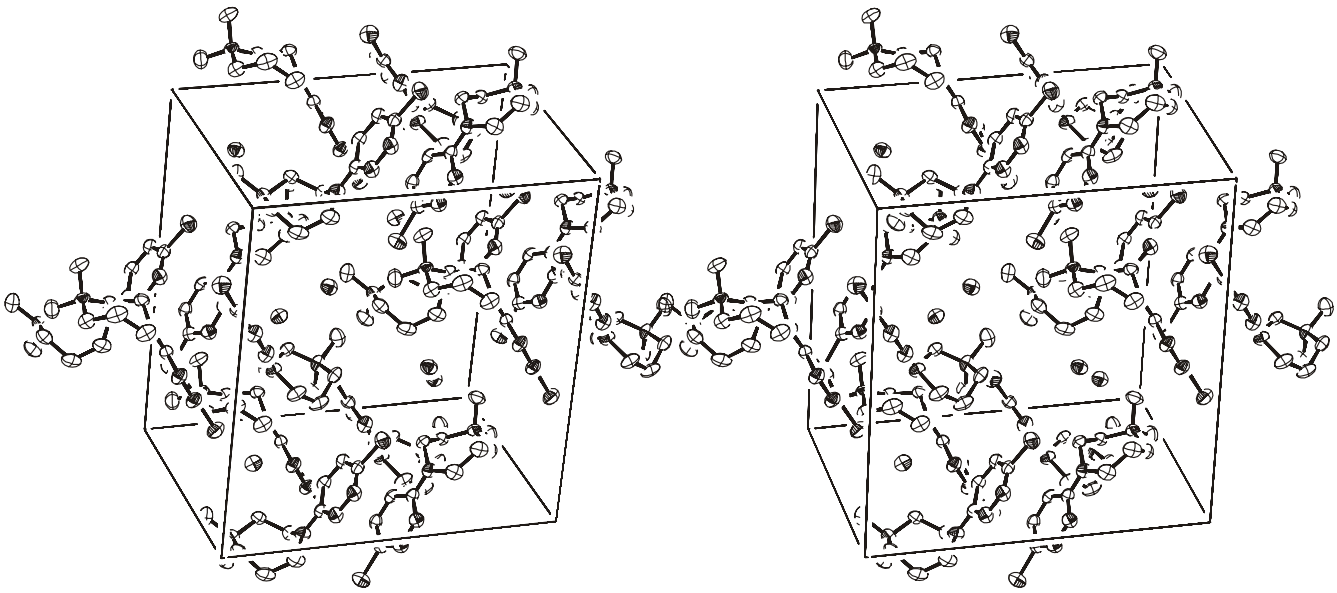

Figure 5: Stereoview of the crystal packing of compound (**4c**) (Hydrogen atoms are omitted for sake of clarity).

A comparison between the two crystal structures is reported in Figure 6, where the two Cl-pyridazine moieties are superimposed. The main difference resides in the torsion angles C(8)-N(7)-C(4)-C(5) 176.9(7)° **3c** versus the corresponding C(13)-N(7)-C(4)-C(5) 170.3(7)° **4c** and C(12)-N(7)-C(4)-N(3) 139.0(8)° **3c** compared with C(8)-N(7)-C(4)-N(3) 170.6(7)° **4c**.

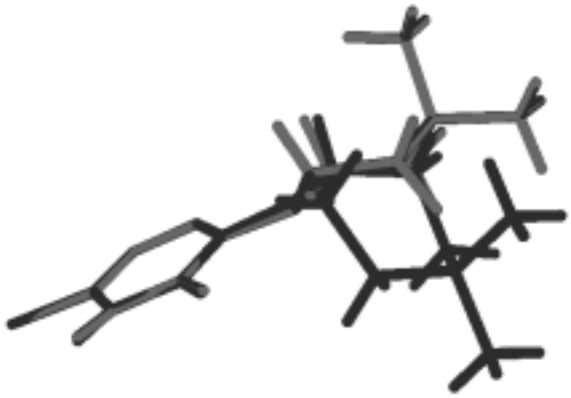

Figure 6: Superimposition of the crystal structure of **3c** (light grey) and **4c** (dark grey).

The decrease in the binding activity of **4c** has been related to the larger molecular volume 275Å^3 with respect to 257Å^3 in **3c** (epibatidine 234Å^3).[7] The corresponding volumes as deduced from X-Ray follow the same trend being 364Å^3 for **4c** and 336Å^3 for **3c**. The important difference with epibatidine is the presence of a second nitrogen in the aromatic ring (pyridazine ring) capable of interaction with the charged cationic centre (N(10)) that can influence the orientation of the aromatic ring.

The A-B and A-C distances of 6.28Å (6.34Å) and 5.59Å (5.65Å) in **3c** compare well with those energetically favoured found from modelling studies,[7] indicated in parentheses, while in **4c** they are different 6.37Å (5.53Å) and 5.42Å (5.25Å) respectively, closer to the values of another energetic minimum (differing of 0.89 Kcal/mol from the previous one) of 6.21Å and 5.60Å respectively.

Compounds (**3c**) and (**4c**) were analysed with the MOPAC program[11] to study their electronic characteristics with respect to epibatidine, as reference compound. The structures have been minimized at the PM3 level (convergence criteria 0.0001 Kcal mol^{-1}), starting from the crystallographic coordinates of **3c** and **4c**, while for epibatidine the starting structure was the most stable conformer obtained with the module annealing of the program TINKER.[12]

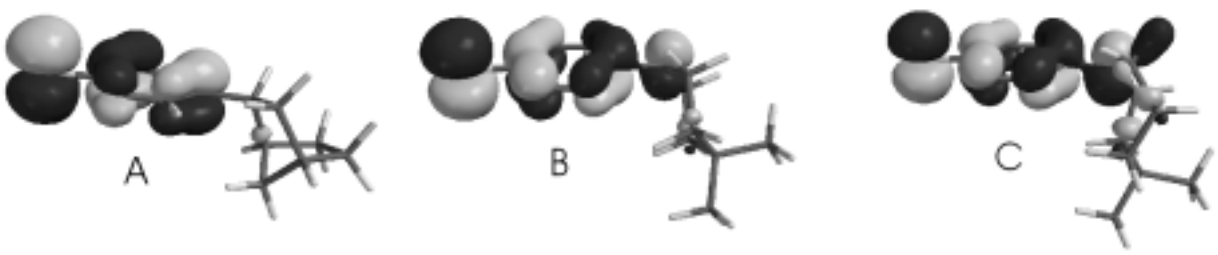

Figure 7: HOMO-orbitals of epibatidine (A), **3c** (B) and **4c** (C).

The Mulliken population analysis, together with the HOMO and LUMO distributions, indicate a well defined separation between the nucleophilic and the electrophilic reactive portions of the three molecules, represented respectively by the aromatic ring and the nitrogen (N(10)) of the heterocyclic aliphatic moieties. However, the main differences in the electronic structure reside in the HOMO orbitals extension: in epibatidine the HOMO is localized only on the aromatic moiety (Figure 7), while for **3c** and **4c** it extends over the N(7) of the piperazine and homopiperazine rings respectively. This can be explained assuming for **3c** and **4c** a partial sp^2 hybridisation of the aliphatic N(7), with consequent restriction of the rotation around the C(4)-N(7) bond.

EXPERIMENTAL

Crystals of **3c** and **4c** were obtained by slow evaporation of an ethanolic solution at room temperature. After four days yellow crystals in platelets shape precipitated.

The crystal were mounted on an Enraf Nonius CAD-4 diffractometer using MoKα radiation. The lattice parameters were determined by least-squares refinements of 25 reflections and the space groups were determined from the observed systematic absences. The structures were solved by direct methods (SIR-92 [13]) and the refinements were carried out by full-matrix anisotropic least-squares for non-hydrogen atoms. The hydrogen atoms were included at their calculated positions, riding on their parent atoms, and refined isotropically. Refinements were carried out by using SHELX-97 package [14] and by the WINGX [15] suite for the molecular graphics.

Crystallographic and refinement data for the two structures are presented in Table 1; selected bond lengths (Å) and angles (°) are reported in Table 2.

Table 1. Crystal data and structure refinement for **3c** and **4c**.

Identification code	**3c**	**4c**
Empirical formula	$C_{10}H_{16}N_4ClI$	$C_{11}H_{18}N_4ClI$
Formula weight	354.62	368.64
Temperature(K)	293(2)	293(2)
Wavelength(Å)	0.71073	0.71073
Crystal system	triclinic	orthorhombic
Space group	$P\bar{1}$ (2)	$I2cb$ (45)
Unit cell dimensions(Å),(°)	a=6.403(2) b=7.649(1) c=13.830(2) α=93.17(2)	a=11.047(2) b= 15.488(2) c=17.040(2)

	β=96.78(4) γ=90.99(2)	
Volume(A^3)	671.4(2)	2915.5(7)
Z	2	8
Calc. Density(g/cm^3)	1.754	1.680
Absorption coeff.(mm^{-1})	2.565	2.366
F(000)	348	1456
Crystal size(mm)	0.5 x 0.7 x 0.8	0.7 x 0.3 x 0.8
θ (°) range data coll.	2.67 to 24.9	2.39 to 26.99.
Limiting ind.	-7≤h≤7,-9≤k≤9,0≤l≤16	-14≤h≤13,-16≤k≤19,17≤l≤21
Refl.Coll./unique	7389/2356[R(int)=0.010]	27828/3081[R(int)=0.047]
Completeness to θ	100.0 %	99.9 %
Refinement method	Full-matrix least-squares on F^2	
Data/restr./param.	2356/0/188	3081/1/101
Goodness-of-fit on F^2	1.18	1.15
Final R ind[I>2σ(I)]	R1=0.059, wR2=0.170	R1=0.051, wR2=0.129

Table 2 . Bond lengths [Å] and selected angles [°] for **3c** and **4c**.[16]

Compound (**3c**)		Compound (**4c**)	
Cl-C(1)	1.736(8)	Cl-C(1)	1.744(7)
C(8)-N(7)	1.46(1)	C(8)-N(7)	1.46(1)
C(8)-C(9)	1.51(1)	C(8)-C(9)	1.51(1)
N(10)-C(14)	1.49(1)	N(10)-C(14)	1.53(1)
N(10)-C(9)	1.50(1)	N(10)-C(9)	1.508(8)
N(10)-C(11)	1.52(1)	N(10)-C(11	1.52(1)
N(7)-C(4)	1.41(1)	N(7)-C(4)	1.370(9)
N(2)-C(1)	1.32(1)	N(2)-C(1)	1.29(1)
N(2)-N(3)	1.36(1)	N(2)-N(3)	1.349(9)
C(1)-C(6)	1.38(1)	C(1)-C(6)	1.40(1)
C(12)-C(11)	1.50(1)	C(12)-C(11)	1.54(1)
C(4)-N(3)	1.31(1)	C(4)-N(3)	1.339(8)
C(4)-C(5)	1.41(1)	C(4)-C(5)	1.422(9)
C(5)-C(6)	1.37(1)	C(5)-C(6)	1.364(9)
N(10)-C(13)	1.51(1)	N(10)-C(15)	1.49(1)
N(7)-C(12)	1.45(1)	N(7)-C(13)	1.46(1)
		C(12)-C(13)	1.51(1)
N(7)-C(8)-C(9)	110.3(7)	N(7)-C(8)-C(9)	112.7(6)
C(14)-N(10)-C(11)	111.5(7)	C(14)-N(10)-C(11)	106.5(6)
C(9)-N(10)-C(11)	108.3(6)	C(9)-N(10)-C(11)	113.8(6)
C(4)-N(7)-C(8)	115.9(6)	C(4)-N(7)-C(8)	120.5(6)
C(12)-C(11)-N(10)	111.9(7)	C(12)-C(11)-N(10)	114.9(7)
N(10)-C(9)-C(8)	111.6(7)	N(10)-C(9)-C(8)	113.9(6)
C(5)-C(6)-C(1)	117.0(8)	C(5)-C(6)-C(1)	116.7(6)
C(14)-N(10)-C(9)	111.2(7)	C(15)-N(10)-C(9)	110.8(6)

C(14)-N(10)-C(13)	107.3(7)	C(15)-N(10)-C(14)	107.8(6)
C(9)-N(10)-C(13)	109.9(7)	C(9)-N(10)-C(14)	105.4(6)
C(13)-N(10)-C(11)	108.5(7)	C(15)-N(10)-C(11)	112.0(7)
C(4)-N(7)-C(12)	116.7(7)	C(4)-N(7)-C(13)	120.6(6)
C(12)-N(7)-C(8)	114.1(6)	C(8)-N(7)-C(13)	118.9(6)
N(7)-C(12)-C(11)	111.7(8)	N(7)-C(13)-C(12)	112.3(7)
		C(13)-C(12)-C(11)	114.4(7)

The supplementary crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC deposition numbers: 212680 (**3c**), 212679 (**4c**)). Copies can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB 2 1EZ UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac:uk).

CONCLUSION

The 3-D arrangement of the molecules is essential for the determination of the pharmacophoric chemical groups recognized by a single receptor. A model for the nicotinic pharmacophore suggests the optimal distances:[8] A-B 4.7(3)Å, A-C 4.0(3)Å, B-C 1.2(3)Å but recent modelling studies [7] give for the solvated conformations A-B and A-C distances 6.1-6.3Å and 5.4-5.6Å, beyond the range previously described but comparable to those found in the crystal structures of **3c** and **4c** (A-B 6.28Å, 6.37Å and A-C 5.59Å, 5.42Å, respectively). In the compound (**3c**), that binds to nAchR almost equipotently to epibatidine, the charged nitrogen atom is even far apart (5.59Å) from N(2), confirming the possibility to extend the value of the classical pharmacophoric parameters.

The more active **3c** maintains an elongated conformation similar to that of the active epibatidine, while **4c** having a curled conformation, at least in the solid state, seems less favorite. Different factors play a role in the affinity towards the receptor beside the pharmacophoric distances, as the molecular conformation, the presence of lipophilic groups (methyls) with their steric component capable to influence the receptor interaction.

The difference found with the crystallographic results: sp^3 hybridization of N(7) (347° sum of the angles around N(7)) while is 360° in **4c** (sp^2 hybridization) versus a partial sp^2 in both derivatives in gas phase (at PM3 level) but the good convergence with the pharmacophoric distances of the solvate forms [7]are in any case indicative of the importance of an accurate X-Ray structural analysis as reference for modelling studies.

The results obtained provide new insights to the modifications that could lead to a significant alteration of the "bioactive conformation" of the ligands.

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