HETEROCYCLES, Vol. 71, No. 1, 2007, pp. 105 - 115. © The Japan Institute of Heterocyclic Chemistry
Received, 12th October, 2006, Accepted, 30th November, 2006, Published online, 1st December, 2006. COM-06-10913

STRUCTURAL AND CONFORMATIONAL STUDIES ON 5-[1'-METHYLPYRROLIDIN-2'-YL]-1,3-OXAZOLIDIN-2-ONE FREE BASE AND HYDROCHLORIDE FORM

Fiorella Meneghetti, Roberto Artali, Marco Pallavicini, Ermanno Valoti, and Gabriella Bombieri*

Institute of Pharmaceutical and Tossicological Chemistry "P. Pratesi", Viale Abruzzi, 42-20131 Milano, Italy

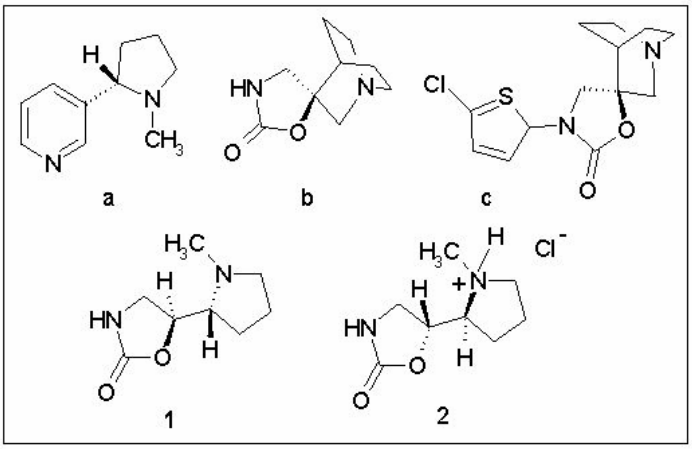
Abstract – The molecular structures of (5*R*,2'*S*)-5-[1'-methylpyrrolidin-2'-yl]-1,3-oxazolidin-2-one free base (**1**) and its enantiomeric hydrochloride salt (**2**) have been determined in order to understand their interaction at neuronal acetylcholine receptor. The molecules are in a bent conformation with the pyrrolidine and the oxazolidinone rings nearly at 60° to each other. The molecular assembly is characterized by the formation of chains joined via hydrogen bonds N-H…N in **1** and N-H…Cl in **2**. The solid state structures have been compared with the theoretical conformations and docked into the crystal structure of Acetylcholine Binding Protein (AChBP), homolog of the ligand binding domain of nAChR. A closer analogy between the receptor bound conformation and the solid state has been found in the hydrochoride form with respect to the free base. This latter (**1**) forms an hydrogen bond with Trp 6702, while **2** beside two additional interactions with Trp 6702 is linked also to Ile 118.

INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels (LGICs) exerting their effects in the central nervous system (CNS). In particular neuronal nAChRs are implicated in modulating neurotransmission, cognition and anxiety, so they have been proposed as potential therapeutic targets for the treatment of pain, epilepsy and a wide range of neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Tourette's syndrome, schizophrenia, anxiety and depression.[1,2] The heterogeneity of the neuronal nAChRs subtypes in the CNS presents major possibilities as well as challenges in terms of developing therapeutics targeted at these receptors. On this respect, significant efforts have been made in designing novel selective agents with proper pharmacologies, leading to drug

candidates with specific profiles without affecting other aspects of the cholinergic neurotransmission.[3] Prerequisite of this rational drug design is the understanding of the specific macromolecular target architecture. Since the subtype receptors binding sites are still unknown the nicotinic structure-activity relationships are the basis for the synthesis of new ligands.

In this paper we report the crystallographic structures of the recently synthesized[4] 5-[1'-methylpyrrolidin-2'-yl]-1,3-oxazolidin-2-one free base (**1**) and hydrochloride salt (**2**). These compounds were designed as possible analogues of the nAchR agonist nicotine (**a**),[5] where an oxazolidinone ring replaces the pyridine, as made for other oxazolidin-2'-one derivatives (**b**[6] and **c**[7]) (see Scheme 1). The importance of the latter pharmacophoric moiety is confirmed by compounds (**b**) and (**c**), which are potent and selective full agonists for α7 nicotinic receptors; changes in the oxazolidinone ring reduce the affinity. Further, the electronic nature and the location of the carbonyl functionality are crucial to mimicking acetylcholine in the α7 receptor. It is important to underline that for these agonists exist a clear stereogenic preference in the receptor selectivity for the (-) *versus* the (+) enantiomer.



Scheme 1

For the nicotinic receptor common pharmacophoric elements are a cationic centre (N^+) and a suitably distanced hydrogen bond acceptor and/or an π-electron rich moiety (HBA/π). A thorough knowledge of the hydrogen bond acceptor site structure (distances, conformation…), selectivity and characteristics (length, linearity and directionality) is clearly important to understand the structure-activity relationships of the ligands, in particular for new potential agonists with one rotatable bond between N^+ and HBA/π. These structural studies together with docking experiments contribute to a better characterization of the nicotinic drugs binding site. Deepen in this investigation, compounds (**1** and **2**) are analyzed as novel potential nicotinoids useful as ligand template. In a first part we will describe the geometrical characteristics of **1** and **2** and in a second part the results of the X-ray crystallographic analysis will be complemented by theoretical and docking calculations in order to evaluate the possible conformational differences between **1** and **2**, which could be relevant for the receptor binding.

RESULTS AND DISCUSSION

Perspective views of (5*R*,2'*S*)-5-[1'-methylpyrrolidin-2'-yl]-1,3-oxazolidin-2-one free base **1** and its enantiomeric hydrochloride salt (**2**) are shown in Figure 1 with the labelling scheme.

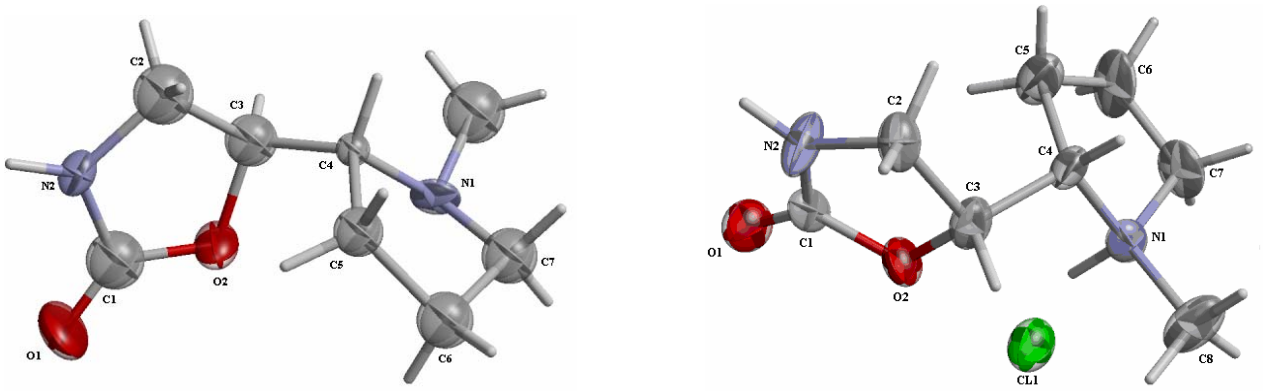


Figure 1. ORTEP [9] of compounds (**1**) (left) an (**2**) (right), showing the atom-numbering scheme (ellipsoids are at the 40% probability).

Compound (**2**) is in the ionized form confirmed by the N(1)-C(7) lengthening, due to the nitrogen protonation.[10] The configuration of the C(3) and C(4) chiral atoms are *S* and *R* in compound (**1**), while in **2** is *R* and *S*. The absolute stereochemistry was defined by the synthetic pathway for their preparations, using *R*-proline in **1** and *S*-proline in **2** as starting reagents and by the X-ray analysis results.

Despite the low data set of **1** due to the poorly diffracting samples, a clear molecular conformation was obtained with geometrical parameters in good agreement with those reported for related compounds.[11] The two molecules are characterized by the equatorial position of the N(1) methyl substituent, i.e. an *anti* orientation with respect to the oxazolidinone ring. The overall conformation of the compounds is defined by the torsion angles O(2)-C(3)-C(4)-N(1) of 61(1)° in **1** and -58(1)° in **2**, indicative of the bent orientation of the oxazolidinone with respect to the rest of the molecule. The reciprocal orientation the two rings in both compounds are less perpendicular with respect to the orientation of the pyridine and pyrrolidine rings in nicotine.[12] The oxazolidinone moiety in **1** is almost puckered with C(3) deviating by 0.23(1)Å from the other ring atoms, while it is about planar in **2**. In both compounds the pyrrolidinyl moieties have about an envelope conformation. The five membered ring asymmetry is quantitatively described by the puckering parameters[13] q_2=0.40(1)Å and φ_2=74(1)° for **1** and q_2=0.38(1)Å and φ_2=-57(1)° for **2**, where q is the puckering amplitude and φ is the phase angle. The deviations from the pyrrolidine plane is of –1.18(1)Å for the atom C(7) in **1**, while in **2** N(1) is out of the mean plane of 0.571(8)Å. The different ring conformation in the latter seems related to the protonation, which influences the intermolecular contacts and consequently the crystal packing.

In **1** the carbonyls are aligned opposite to each other to overcome the dipole-dipole interactions and/or van der Waals repulsions and the molecules are joined by infinite chains parallel to the *b* axis having the

amide hydrogen atom at a distance of 2.10(1)Å, angle 155(1)° (N(1)-H…N(2)', 'at 1-x, y-1/2, 1-z) with the adjacent pyrrolidinyl nitrogen (Figure 2).

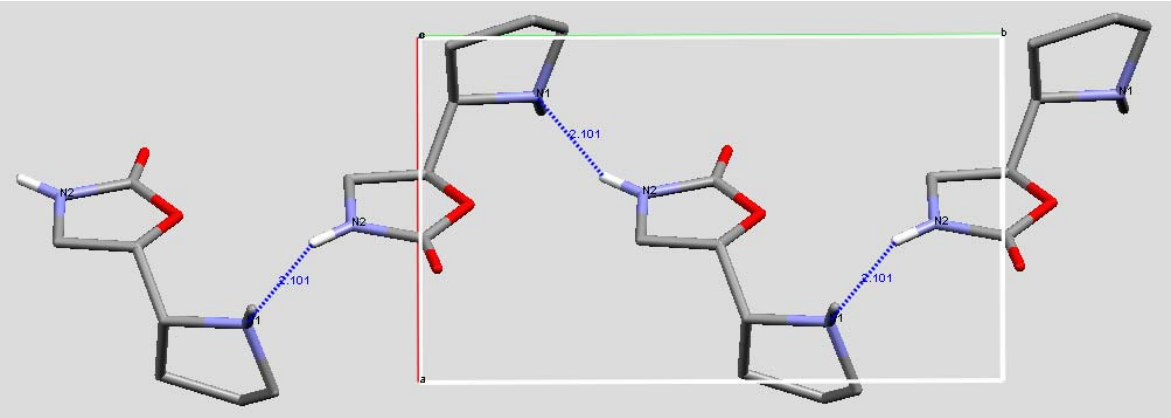


Figure 2. Hydrogen bond of **1** showing the chain formation along the *b* axis (H-bond distances are reported).

In **2** the chlorine anion participates in two significant interactions: a salt bridge with the protonated N(1) and a hydrogen bond with N(2)' ('at x-1,y,z) of 2.34(1)Å, leading to molecular chains formation along the *a* axis (Figure 3). The observed H…Cl distance is in agreement with the literature values.[14]

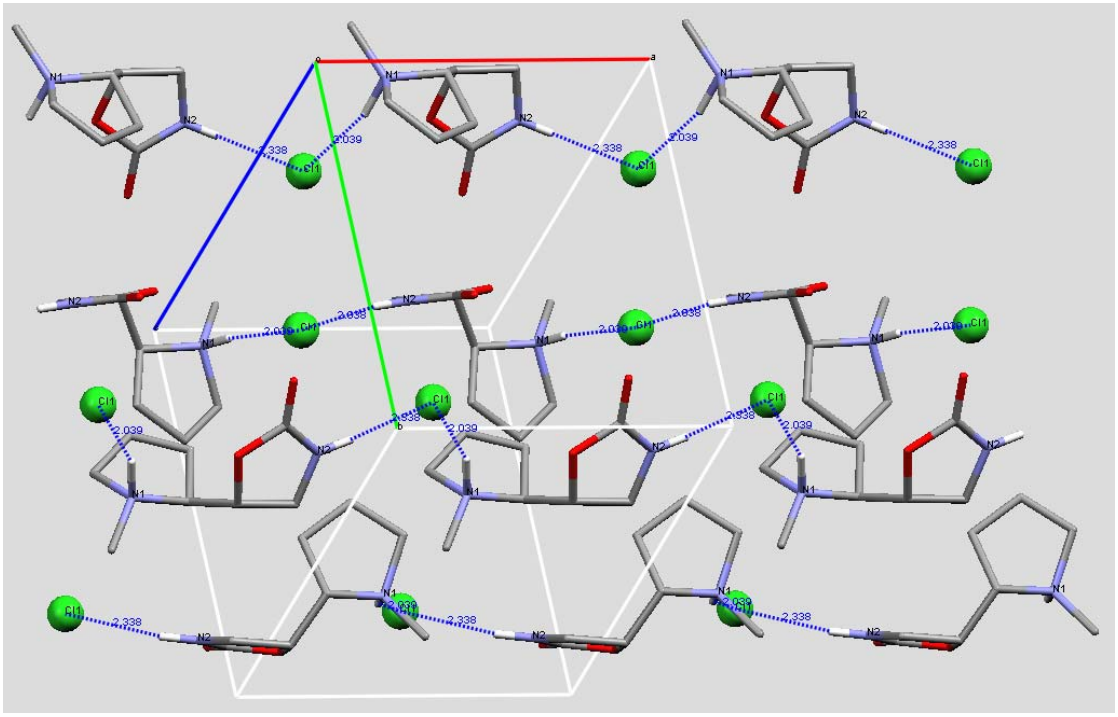


Figure 3. Crystal packing of **2** with evidenced the molecular chains along *a*.

The conformational differences of the structures (**1** and **2**) are shown in Figure 4 by the superimposition

of the respective oxazolidinyl moieties evidencing the different chiralities at the two stereocentres. In attempts to map the pharmacophoric requirements for nicotinic activity parameters as the distance of the nitrogen atoms and the hydrogen bond acceptors are used.[8] In the free base this distance is more extended than in the hydrochloride salt (4.63Å *versus* 4.51Å).

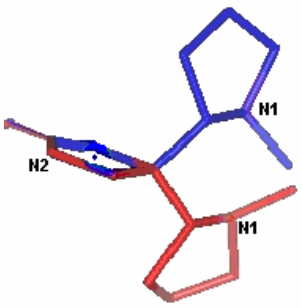


Figure 4. Superimposition of **1** (red) and the cation of **2** (blue), evidencing the considered pharmacophoric elements.

The resulting crystallographic structures represent only one of the possible molecular conformations, not enough for the definition of the bioactive form that elicits the biological response. Interesting is the correlation of the solid state conformations with those present at the receptor binding site. On this respect, the structural data of **1** and **2** have been used to explore their *in vacuo* conformational space and the docking with the Acetylcholine Binding Protein (AchBP), as nAChRs model.

A systematic variation of the torsion angle O(2)-C(3)-C(4)-N(1) in the range -180÷180° (step of 5°) shows a quite specular behavior for the two compounds, with high rotational barriers approximatively at -150° and 150° for **1** and **2** respectively (Figure 5).

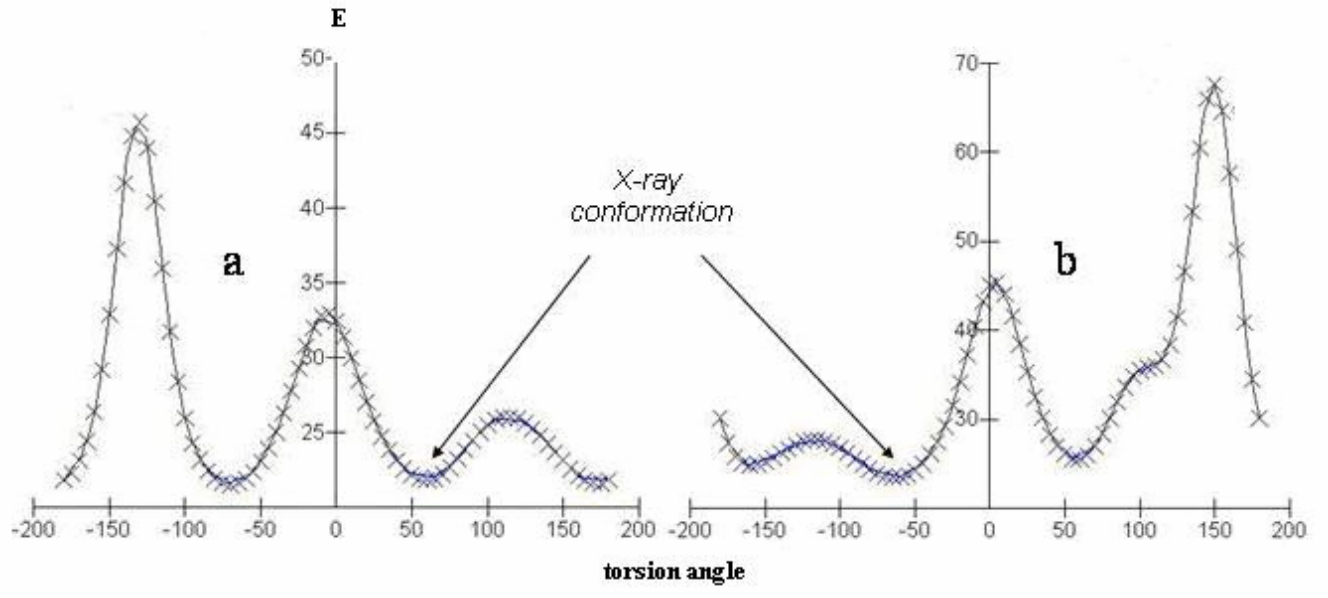


Figure 5. Conformational profile of O(2)-C(3)-C(4)-N(1) torsion angle for **1** (**a**) and **2** (**b**). Bond lengths and angles were fixed at their experimental values.[15]

According to these qualitative results, the conformational analysis gives three different minima for both compounds: **1A**, **1B** and **1C** and **2A**, **2B** and **2C** as shown in Table 3, where the root mean square deviations (RMSD) from the crystallographic data, energy, torsion angle τ_1 O(2)-C(3)-C(4)-N(1) are also reported. The **D** columns show the most stable conformations obtained with the flexible automated docking procedure with the ligands inserted in the Epibatidine binding pocket, as reference compound (see Experimental).

Table 3. Relevant torsion angles (°), Energies (kcal/mol)[a] and RMSD (Å, heavy atoms) of **1** and **2**.

	X-ray	**1A**	**1B**	**1C**	**1D**	X-ray	**2A**	**2B**	**2C**	**2D**
RMSD	-	0.368	1.776	1.324	1.103	-	0.211	1.354	1.497	0.343
Energy	19.22[b]	19.13	19.66	19.67	25.35[b]	18.92[b]	18.89	19.44	19.53	26.27[b]
τ_1=O(2)-C(3)-C(4)-N(1)	60.6	53.3	-78.2	157.9	169.3	-57.7	-53.5	-158.1	76.7	-85.8

a) Calculated at the semiempirical level of theory.
b) Values obtained from a single point calculation.

Compound (**1**) presents the first energetically favorable conformation **1A** comparable with the crystallographic structure, with an RMSD of 0.368Å and a difference of 7.3° for τ_1. Differently, the second and third minima conformations **1B** and **1C** are far from the crystallographic one, with RMSD values of 1.776Å (**1B**) and 1.324Å (**1C**) and τ_1 of 138° and 97° for **1B** and **1C** respectively. For compound **2**, conformation **2A** is in good agreement with the crystal structure, (RMSD of 0.211Å and torsion angle difference of 4.2°), while the conformations of the second **2B** and third minima **2C** have RMSD values of 1.354Å and 1.497Å respectively and τ_1 of 138° and 97°.

In solution **1** and **2** could exist as a mixture of the different forms in an unknown ratio, the crystallographic and theoretical conformations have been then related to those obtained from docking calculations at the AChBP binding site.[16] These latter show the most stable conformations of **1** and **2** located inside the aromatic cluster of the AChBP binding pocket, but with different orientation. In particular, **1D** docked structure evidences a noticeable departure of the torsion angle τ_1 of about 100° with respect to the value **1A**, while **2D** presents a torsion angle τ_1 comparable to the value of **2A**. These findings could be related to the presence of the charged N(1) in **2** that leads to a tight binding at the receptor active site.

The respective superimpositions of the crystallographic structures of **1** and **2** onto the theoretical (**1A** and **2A**) and the docked conformations (**1D** and **2D**) are drawn in Figure 6.

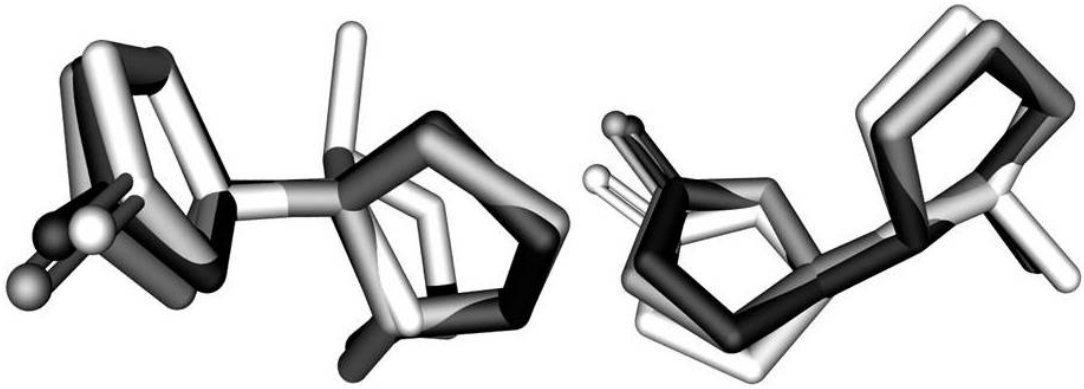


Figure 6. Superimposition of **1** (left) and **2** (right) X-ray structures (black) with the respective theoretical (**1A** and **2A**, dark grey) and docked (**1D** and **2D**, light grey) conformations.

The analysis of the ligand-receptor interactions shows that the docked conformations of **1** and **2** are characterized by hydrogen bonds with Trp6702 of the B subunit, but **1** presents only one hydrogen bond interaction, while compound (**2**) has three additional hydrogen bonds: two with Trp6702 and one with Ile118 (Figure 7). The docked conformation **2D** is in agreement with the crystallographic one with a value of RMSD of 0.343Å, that it is significantly higher (1.103Å) in **1D**.

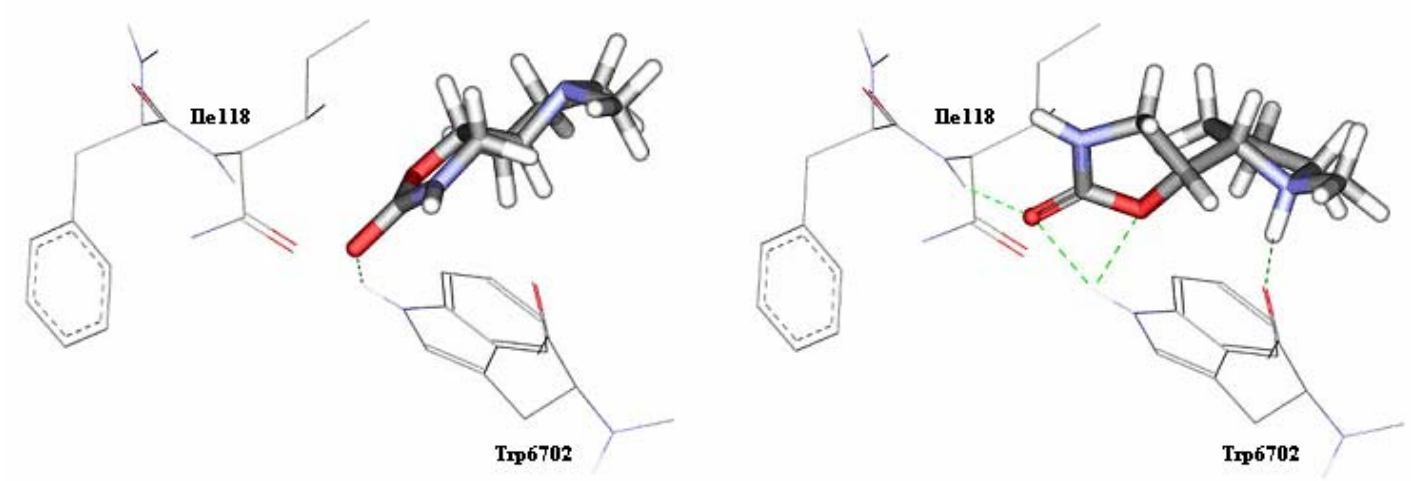


Figure 7. Docked conformations of **1** (left) and **2** (right) inside the AChBP binding pocket. The hydrogen bond interactions are evidenced in dotted lines.

The molecular structures allow the identification of the essential pharmacophoric chemical groups recognized by a single nicotinic receptor.

In particular, the comparison of the neutral form (**1**) and hydrochloride salt (**2**) structures shows a higher hydrogen acceptor strength of the oxazolidinone nitrogen over the pyrrolidine one and gives reliable input geometric parameters useful for further differentiation of the steric and electronic requirements of a receptor target.

The theoretical and docking results are in agreement with the AChBP binding mode of the nicotinic agonists. There is a closer correspondence between crystallographic and docked conformations of the hydrochloride form with respect to the free base, showing the capability of the crystal packing of the salt to mimic the binding site environment.

The analysis of the structural differences between the neutral and the hydrochloride form underlines the importance of the charged nitrogen to obtain a nicotinic action, with the pyrrolidine nitrogen located inside the receptor aromatic cluster.

In conclusion these nicotinoid analogs are a good probe for further refinement of pharmacophore models allowing the development of new and better designed pharmaceutical compounds and improve the knowledge of the structure-function relationship of the nAChRs, that constitutes an invaluable tool for the studies of the LGIC transmembrane domain.

EXPERIMENTAL

Crystallography

Crystals were obtained as yellow prisms from toluene for **1** and ethanolic for **2** solutions at room temperature. They were mounted on an Enraf Nonius CAD-4 diffractometer using MoKα ($\lambda_{MoK\alpha}$=0.71073Å) radiation at room temperature (293K).

The lattice parameters were determined by least-squares refinements of 25 high angle reflections. The structures were solved by direct methods[17] and the refinements were carried out by full-matrix least-squares. Oxygen and nitrogen atoms of **1** and all non-H-atoms of **2** and were refined anisotropically. The H-atoms positions were detected in a difference Fourier synthesis and refined with isotropic thermal factors, or introduced in calculated positions in their described geometries and allowed to ride on the attached carbon atom with fixed isotropic thermal parameters ($1.2U_{eq}$ of the parent carbon atom). Refinements were performed with SHELX-97.[18]

A summary of the crystal data, data collection, and structure refinement is presented in Table 1; selected bond lengths and angles are reported in Table 2. Geometrical calculations were carried out with the program PARST.[19]

The absolute configuration of **1** was assigned on the basis of the known chirality of a precursor molecule[4] and for **2** confirmed by the X-ray refinement.

CCDC numbers 616375 (**1**) and 623083 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

Table 1. Summary of crystal data and structure refinement for **1** and **2**.

Identification	**1**	**2**
Empirical formula	$C_8 H_{14} N_2 O_2$	$C_8 H_{15} N_2 O_2 Cl_1$
Formula weight	170.21	206.67
Crystal system	Monoclinic	Orthorhombic
Space group	P 2_1	P 2_1 2_1 2_1
Unit cell dim.(Å,°)	a = 6.464(5)	a = 8.497(2)
	b = 10.571(4)	b = 10.113(3)
	c = 6.572(9)	c = 12.361(3)
	β = 105.2(1)	
Volume ($Å^3$)	445.1(9)	1099.3(6)
Z	2	2
Density calc. Mg/m^3	1.269	1.295
F(000)	184	440
Crystal size (mm^3)	0.5 x 0.3 x 0.2	0.7 x 0.5 x 0.2
θ range (°)	3.13 to 21.97	2.60 to 23.97
Index ranges	-6≤h≤6;-1≤k≤11;-1≤l≤7	-1≤h≤10;-1≤h≤11;-1≤l≤14
Reflections collected	843	1376
Independent reflections	649	1237
Completeness to θ	100.0 %	99.9 %
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/param.	649 / 1 / 44	1237 / 0 / 131
Final R indices [I>2σ(I)]	R1 = 0.066 (wR2 = 0.131)	R1 = 0.065 (wR2 = 0.159)

Table 2. Selected bond lengths [Å] and angles [°] for **1** and **2**.

	1	**2**
O(1)-C(1)	1.21(2)	1.202(9)
O(2)-C(1)	1.39(2)	1.35(1)
O(2)-C(3)	1.51(1)	1.44(1)
N(1)-C(4)	1.54(1)	1.52(1)
N(1)-C(7)	1.48(2)	1.57(1)
N(1)-C(8)	1.47(1)	1.48(1)
N(2)-C(1)	1.36(2)	1.29(1)
N(2)-C(2)	1.39(2)	1.42(1)
O(1)-C(1)-O(2)	121(2)	120.7(9)
O(1)-C(1)-N(2)	127(2)	129.8(9)
O(2)-C(1)-N(2)	106(1)	109.5(8)
O(2)-C(3)-C(2)	104(1)	105.2(8)
O(2)-C(3)-C(4)	109.1(9)	111.5(9)
N(1)-C(4)-C(3)	111(1)	111.7(8)
N(1)-C(4)-C(5)	100.9(9)	102.7(9)
N(1)-C(7)-C(6)	106.1(9)	104.0(9)
N(2)-C(2)-C(3)	101.9(6)	100.5(9)
C(1)-O(2)-C(3)	110.2(9)	109.0(7)
C(1)-N(2)-C(2)	115.0(9)	115.5(8)
C(4)-N(1)-C(7)	107.4(9)	103.9(8)
C(4)-N(1)-C(8)	106.1(9)	115.2(9)
C(7)-N(1)-C(8)	116.5(9)	114.4(8)

Computational methods

The conformational analysis of **1** and **2** was done starting from their X-ray conformations using TINKER.[20] The molecular conformation was optimized with respect to all the torsion angles and convergence was assumed when the energy changes in two subsequent cycles of the minimization procedure was less than 0.5kcal/mol.

MM2 force-field[21] was used for the calculation of the conformational energies, while atomic charges and the energies of the **1A-C** and **2A-C** conformers were determined with the semiempirical molecular orbital software MOPAC 7.0.[22]

Representative models of the binding site of the AChBP were constructed from the crystal structure coordinates (PDB entry 2BYQ,[16] www.rcsb.org) by including only the subunits A and B. All possible hydrogen atoms were added to the edited crystal structure with their standard geometry and partial atomic charges were assigned to the protein. The resulting structure was then equilibrated for 100ps to remove bad contacts between hydrogen atoms, using a positional restrained molecular dynamics protocol (GROMACS).[23]

eHits[24] was used for docking purpose, with the ligands positioned in the binding pocket of the Epibatidine molecule as reference compound [16] in order to minimize any bad steric interactions and to ensure starting conditions congruent with the interactions of the pharmacophoric model. Each docking run produces a molecular database with 25 docked configurations for each structure. At the end of each simulation, the conformation with the lowest interaction energy (**1D** and **2D**) was chosen as the "best structure" and this conformation was energy minimized in order to refine the orientation of the substrate in the AchBP active site, taking into account the aminoacids mobility within a 10.0Å radius around the ligands. All calculations were done on a dual-Pentium III workstation, running SGI-SMP version of Linux RedHat 7.3 Professional.

REFERENCES

1 R. A. Glennon and M. Dukat, *Pharm. Acta Helv*., 2000, **74**, 103.

2 K. A. Lloyd and M.Williams, *J. Pharmacol. Exp. Ther*., 2000, **292**, 461.

3 M. J. Dart, J. T. Wasicak, K. B. Ryther, M. R. Schrimpf, K. H. Kim, D. J. Anderson, J. P. Sullivan, and D. M. Mayer, *Pharm. Acta Helv*., 2000, **74**, 115.

4 M. Pallavicini, B. Moroni, C. Bolchi, A. Cilia, F. Clementi, L. Fumagalli, C. Gotti, F. Meneghetti, L. Riganti, G. Vistoli, and E. Valoti, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5610.

5 M. N. Romanelli and F. Gualtieri, *Med. Res. Reviews*, 2003, **23**, 393.

6 G. Mullen, J. Napier, M. Balestra, T. DeCory, G. Hale, J. Macor, R. Mack, J. Loch, E. A. Wu, Kover, P. Verhoest, A. Sampognaro, E. Phillips, Y. Zhu, R. Murray, R. Griffith, J. Blosser, D.

Gurley, A. Machulskis, J. Zongrone, A. Rosen, and J. Gordon, *J. Med. Chem*., 2000, **43**, 4045.

7 R. Tatsumi, M. Fujio, H. Satoh, J. Katajama, S. Takanashi, K. Hashimoto, and H. Tanaka, *J. Med. Chem*., 2005, **48**, 2678.

8 R. P. Sheridan, R. Nilakantan, J. S. Dixon, and R. Venkataraghavan *J. Med. Chem*., 1986, **29**, 899.

9 C. K. Johnson, ORTEP 11, Report ORNL-5138, Oak Ridge National Laboratory, TN, 1976.

10 C. T. Lin, P. Y. Siew, and S. R. Byrn, *J. Chem. Soc., Perkin Trans*., 1976, **2**, 957.

11 T. Adachi, J. I. Mizoguche, Y. Hayashi, Y. Yamashoji, N. Kanehisa, Y. Kai, and Y. Jnoue *Acta Cryst*., 1998, **C54**, 1527.

12 R. B. Barlow, J. A. K. Howard, and O. Johnson, *Acta Cryst*., 1986, **C42**, 853.

13 D. Cremer and J. A. Pople, *J. Am. Chem. Soc*., 1975, **97**, 1354.

14 G. A. Jeffrey and W. Saenger, 'Hydrogen bonding in biological structures', Springer, Berlin, 1991, pp. 29-32.

15 H. A. Scheraga, ‘Advances in Physical Organic Chemistry,’ Academic Press (NY), 1968, pp. 103-184.

16 Y. Bourne, T. T. Talley, S. B. Hansen, P. Taylor, and P. Marchot, *Embo J.*, 2005, **24**, 1512.

17 A. C. Altomare, M. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Gagliardi, and G. Polidori, *J. Appl. Crystallogr*., 1994, **27**, 435.

18 G. M. Sheldrick, SHELX-97, University of Göttingen, Germany

19 M. Nardelli, *J. Appl. Cryst*., 1995, **28**, 659.

20 TINKER 4.2: http://dasher.wustl.edu/tinker/ P. Ren and J. W. Ponder, *J. Phys. Chem. B*, 2003, **107**, 5933. R. V. Pappu, R. K. Hart, and J. W. Ponder, *J. Phys. Chem. B*, 1998, **102**, 9725; Y. Kong and J. W. Ponder, *J. Chem. Phys*., 1997, **107**, 481; M. J. Dudek and J. W. Ponder, *J. Comput. Chem.*, 1995, **16**, 791; C. E. Kundrot, J. W. Ponder, and F. M. Richards, *J. Comput. Chem.*, 1991, **12**, 402; J. W. Ponder and F. M. Richards, *J. Comput. Chem.*, 1987, **8**, 1016.

21 MM2 force field: N. L. Allinger, *J. Am. Chem. Soc*., 1977, **99**, 8127; J. C. Tai and N. L. Allinger, *J. Am. Chem. Soc*., 1988, **110**, 2050; J. C. Tai, J. H. Lii, and N. L. Allinger, *J. Comput. Chem*., 1989, **10**, 635.

22 MOPAC 7.0: http://openmopac.net/ MOPAC Version *7.1*, J. J. P. Stewart, Fujitsu Limited, Tokyo, Japan. J. J. P. Stewart, *J Comp. Chem.* 1989, **10**, 209.

23 GROMACS 3.0: http://www.gromacs.org E. Lindahl, B. Hess, and D. van der Spoel, *J. Mol. Model.*, 2001, **7**, 306.

24 eHiTS 5.1: http://www.simbiosys.ca/ehits/ Z. Zsoldos, D. Reid, A. Simon, B. S. L. Sadjad, and J. A. Peter, *Current Protein and Peptide Science*, 2006, **7**, 421.