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STRUCTURAL AND CONFORMATIONAL STUDIES ON TWO DIASTEROMERIC DIHYDROISOXAZOLYL CYCLOPROPANE DERIVATIVES

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Abstract – The molecular structures of 2-(3'-bromo-4',5'-dihydroisoxazol-5'-yl)-1-*tert*-butoxycarbonylaminocyclopropanecarboxylic acid (±)-**10a** and 1-amino-2-(3'-hydroxy-4',5'-diydroisoxazol-5'-yl)cyclopropanecarboxylic acid (±)-**11b** were determined by single crystal X-ray diffraction method. The geometrical features and the intermolecular interactions of the two diastereoisomers have been compared evidencing a different conformation of the dihydroisoxazole ring: in (±)-**10a** is almost planar, while in (±)-**11** adopts an envelope shape. Intermolecular hydrogen bonds of OH…O and NH…O type in (±)-**10a** determine the formation of a three-dimensional network, whereas in (±)-**11b** polimeric chains due to NH…O interactions are obtained. These compounds are key intermediates of conformationally constrained glutamic acid homologues and the opposite stereochemistry of C(3) leads to different pharmacophoric distances, important for the ligand-receptor interaction. The results of the X-ray molecular structures have been complemented by theoretical calculations.

INTRODUCTION

L-Glutamic acid (**Glu**, Figure 1) is the main excitatory neurotransmitter in the central nervous system (CNS) of vertebrates. It plays an important role for neurotransmission and in synaptic plasticity of processes such as learning, memory and cognition.[1] The over-stimulation of the glutamatergic pathway leads to neurotoxicity associated with acute and chronic neurodegenerative disorders, i.e. cerebral ischemia, epilepsy, amyotrophic lateral sclerosis, Parkinson's and Alzheimer's diseases.[2]

Glu influences the neuronal transmission through its activation of both ligand-gated ion channel (iGluRs) and G-protein coupled metabotropic receptors (mGluRs).[3] These two families are composed of different receptor classes and subtypes. The fast excitatory effects of **Glu** are mediated by three subclasses of iGluRs,

which have been pharmacologically classified and named depending on the interaction with selective ligands into *N*-methyl-D-aspartic acid (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainic acid (KA) receptors.[4,5] On the other hand, eight subtypes of mGluRs, whose activation produces metabolic changes in the postsynaptic cells, have been cloned and categorized into three groups (I-III) according to their sequence homology, second messenger coupling and pharmacology.[6] Nowadays, glutamatergic modulators have been recognized therapeutically useful drugs for the treatment of a number of neurodegenerative disorders.[7]

Since **Glu** is a flexible molecule, a number of constrained analogues have been designed and tested with the aim to uncover the conformational requirements needed to activate the different receptor classes. In particular, it was established that folded conformation is necessary to fit the binding sites of iGluRs, while an extended conformation is required for the mGluRs interaction.[8] A selective interaction with the different receptor subtypes is achieved increasing the distance between the proximal and the distal acidic groups of **Glu** and this usually leads to NMDA antagonists.[9] It is interesting to underline that the eutomer of the majority of NMDA ligands has opposite absolute configuration at the amino acidic stereogenic center with respect to the natural endogenous neurotransmitter.[10] Recently, in order to understand the critical pharmacophoric features for the interaction at the NMDA receptor, novel conformationally constrained compounds bearing the cyclopropane ring together with the 3-hydroxyisoxazoline ring as bioisosterical replacement of the distal carboxylate have been published (compounds (±)-**1a** and (±)-**1b**, Figure 1).[11]

Previously, the crystallographic studies of related cyclic[12] and byciclic NMDA ligands have been published.[13] Now, to thorough characterize the structure activity relationships within these substances and the role of conformational freezing of the amino acid chain, the crystal structure determinations of compounds (±)-**1a** and (±)-**1b** were undertaken, securing the assignment of configuration. As the cyclopropane derivatives are excellent mimics of the bound state of their flexible analogues, the analysis of their geometrical features with the relative orientation of the substituents with respect to the cyclopropane ring is of a particular interest. In fact, the introduction of a cyclopropyl moiety induces chirality and reduces the conformational flexibility allowing a most selective interaction with the receptor.

The present study has been performed on the two (±)-**10a** and (±)-**11b** 1-*tert*-butoxycarbonylamino (BOC) intermediates, due to the poor quality of (±)-**1a** and (±)-**1b** crystals not suitable for X-ray diffraction analysis. These results provided an opportunity to investigate the effect of mixed donor-acceptor substitution on the cyclopropane ring geometry, affording relevant information on the stereoelectronic interactions. This could define foreseeable orientations of the ω-acidic group (OH) with respect to the α-aminoacid moiety, allowing the assessment of the conformational requirements of the receptors subtypes interactions. The structure-based investigations have been complemented by

theoretical calculations in order to evaluate the possible conformational differences which could be relevant for the receptor binding and then have been correlated to the pharmacological activities in order to gain further insight into the structural features productive for the interaction with the NMDA receptors.

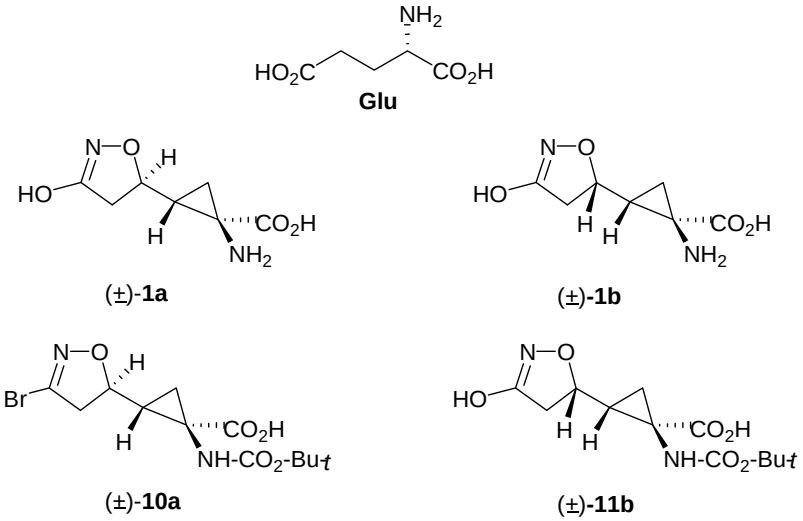

Figure 1. Chemical schemes

RESULTS AND DISCUSSION

The 2-(3'-bromo-4',5'-dihydroisoxazol-5'-yl)-1-*tert*-butoxycarbonylaminocyclopropanecarboxylic acid (±)-**10a** structure is shown in Figure 2, as perspective view. The compound is a racemate and the three chiral centres are either (S)/(S)/(S) and (R)/(R)/(R).

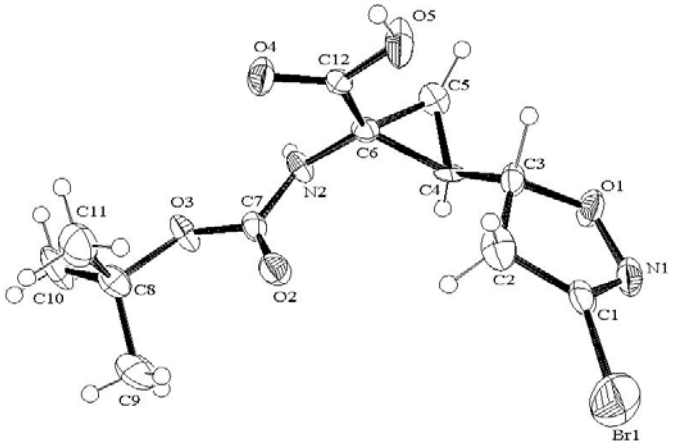

Figure 2. ORTEP[14] view of (±)-**10a**, showing the atom-numbering scheme (ellipsoids are at the 40% probability and H atoms are as spheres of arbitrary radii)

The dihydroisoxazole ring, defined by the puckering parameters[15] Q=0.119(8)Å and φ=76.7(1)°, has a rather distorted planar conformation with the C(2) out of the mean plane of the remaining four of –0.183(7)Å. The mean plane of the five-membered ring forms a dihedral angle with the cyclopropane moiety of 75.0(1)° and the torsion angle O(1)-C(3)-C(4)-C(6) is -165(1)°.

The crystal packing of (±)-**10a** is characterized by a tight three-dimensional array, stabilized mainly by a network of intermolecular OH…O and NH…O hydrogen bonds, as it is shown in Figure 3. Two molecules related by a crystallographic symmetry centre are linked by strong hydrogen bonds via the O(5)…H-O(4)^{I} interaction (distance of 1.79(1)Å angle 174(1)°, ^{I}at 1-x,-1-y,-z) with dimer formation, resembling the common H-bonding motif found for the carboxylic acids.[16] In addition, the dihydroisoxazole ring is stacked parallel onto the symmetric ones at 1-x,-y,-z in a "head to tail" manner. The adjacent molecular layers are approximately 3.9(1)Å apart and these non-conventional π-π interactions between the rings contribute to hold the intermolecular hydrogen bonding network in the crystal cell. The O(1) of the ring is then hydrogen bonded to N(2) at 3/2-x,y-1/2,z with a distance of 2.57(1)Å and an angle of 119(1)°. The last is a loose hydrogen type interaction, but in this case could be relevant in maintaining this molecular conformation in the crystal packing, as previously reported.[17]

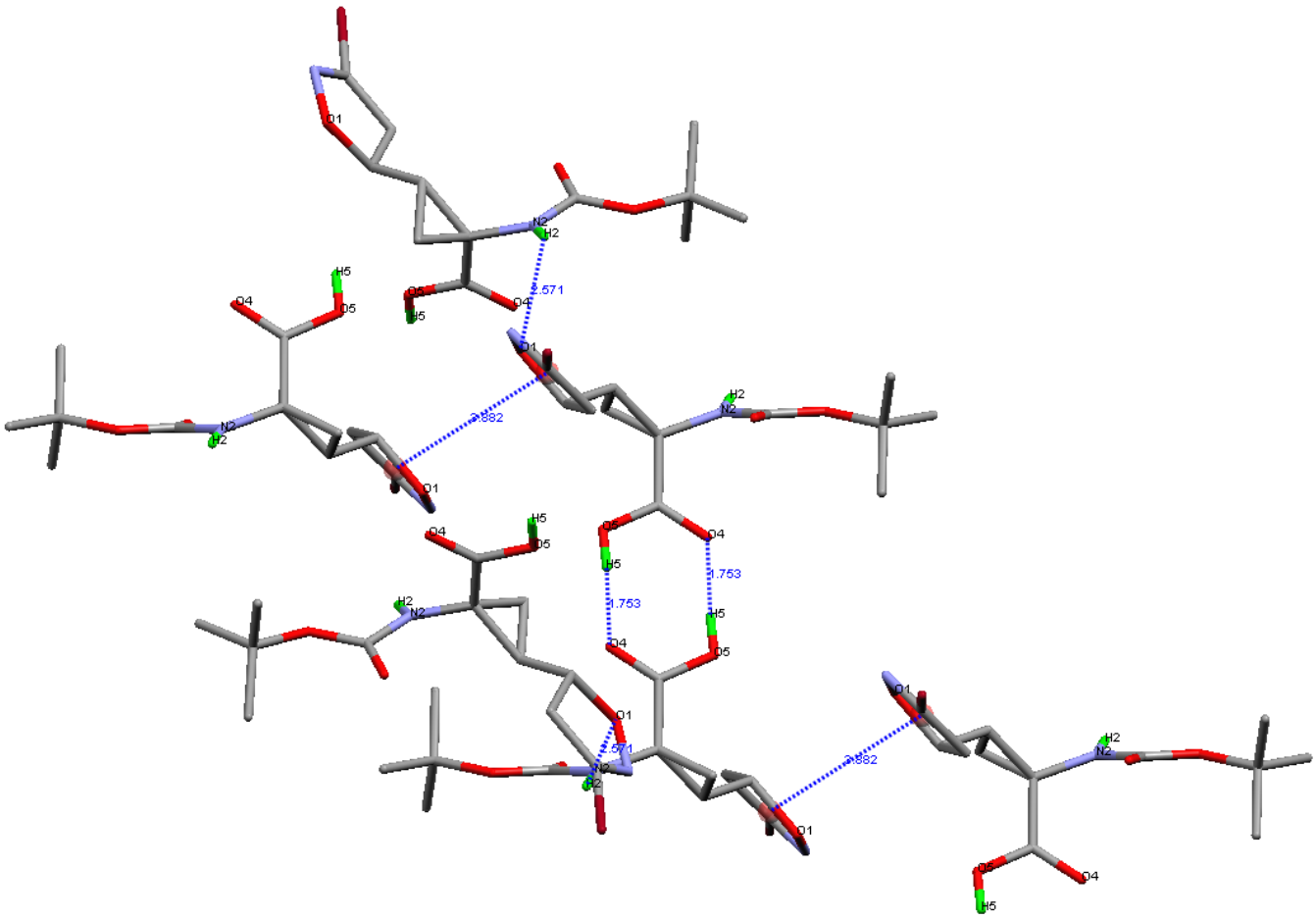

Figure 3. Intermolecular interactions in the crystal packing of (±)-**10a** with evidenced the distances in Å

The crystal structure of 1-amino-2-(3'-hydroxy-4',5'-diydroisoxazol-5'-yl)cyclopropane carboxylic acid (±)-**11b** is depicted in Figure 4. It crystallizes in a centrosymmetric group, than both the (R)/(S)/(S) and the (S)/(R)/(R) stereoisomers are present.

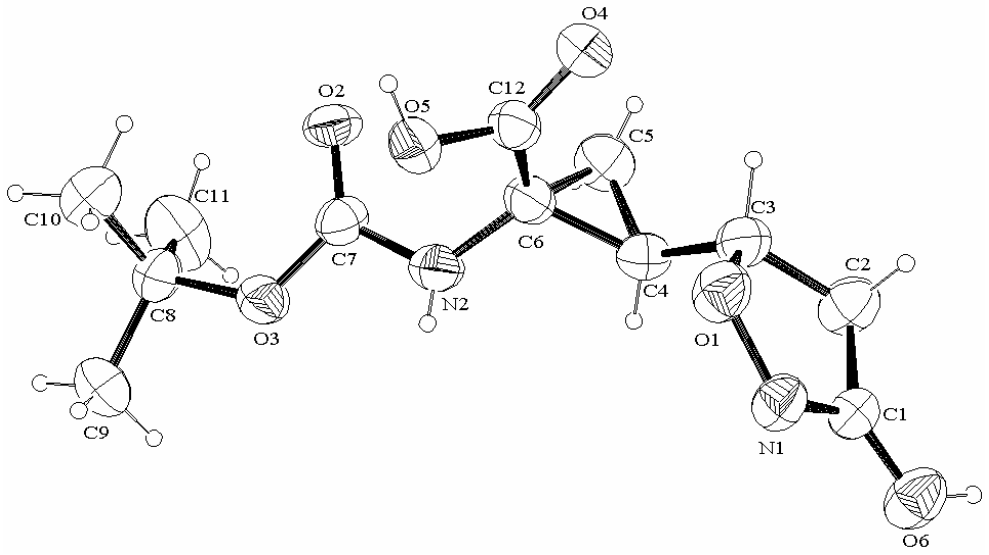

Figure 4. ORTEP[14] view of compound (±)-**11b**, showing the atom-numbering scheme (ellipsoids are at the 40% probability and H atoms are as spheres of arbitrary radii)

The envelope conformation of the (±)-**11b** dihydroisoxazole ring is defined by the puckering parameters: Q = 1.910(8)Å and φ = 69.4(3)°,[15] with the C(3) atom at the flap, out of -0.324(9)Å from the plane of the remaining atoms. This ring is inclined with respect to the adjacent ciclopropane moiety of 21.0(4)° with a torsion angle O(1)-C(3)-C(4)-C(6) of 63(1)°. The molecular packing (Figure 5) is determined by the presence of hydrogen bonds N(2)-H(2A)…O(3)^{I} with distance of 2.16(1)Å angle 149(1)° (^{I} at 1/2-x, y-1/2, 1/2-z) leading to chain formation along the *b* axis.

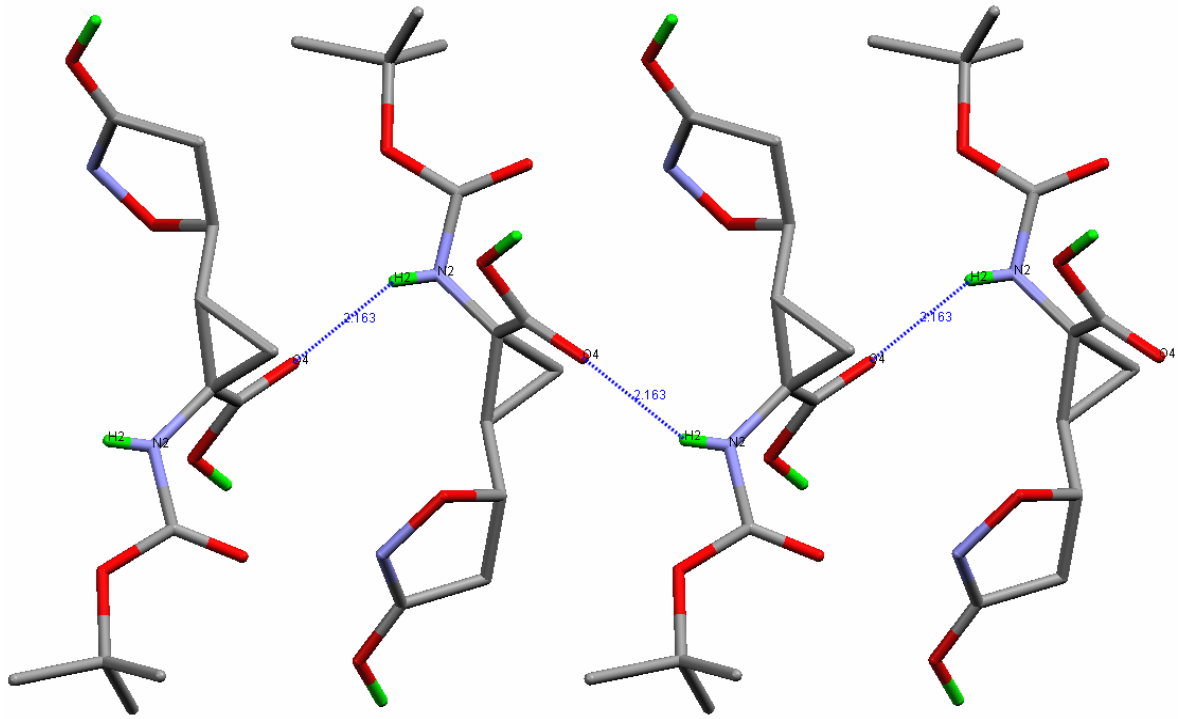

Figure 5. Chains formation in (±)-**11b** due to intermolecular hydrogen bonds with evidenced the distances in Å

The superimposition of (±)-1**0a** and (±)-**11b** shows the different stereochemistry at C(3) and the main conformational differences (Figure 6).

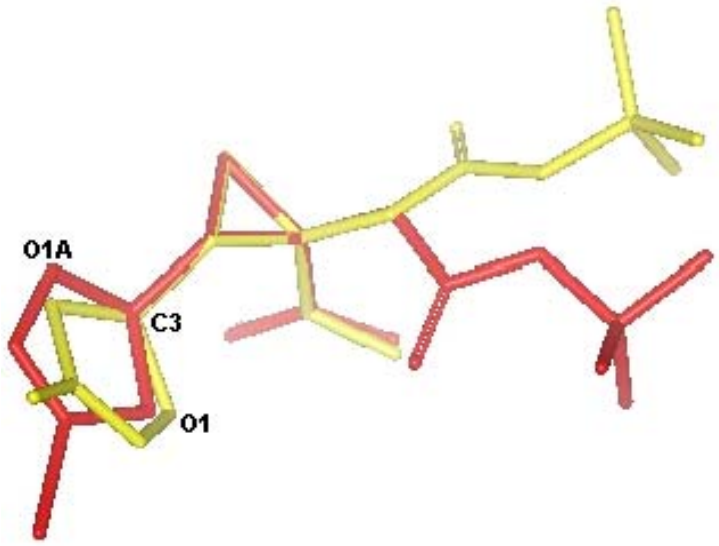

Figure 6. Superimposition of (±)-**10a** (red) and (±)-**11b** (yellow)

In the examined compounds the BOC and the carboxylic moieties are about *trans* and *cis* to the dihydroisoxazole ring respectively, as indicated by the torsional angles C(4)-C(6)-N(2)-C(7) of 86(1)° for (±)-**10a** and –150(1)° for (±)-**11b** and C(3)-C(4)-C(6)-C(12) of 1(1)° likewise for (±)-**10a** and (±)-**11b**. It is interesting to note that in both compounds the cyclopropane ring shows significant bond-length asymmetry induced by the substituents, as in other cyclopropyl derivatives,[18-20] where the carbonyl group induces distal bond shortening and adjacent bond lengthening (Table 1). The exocyclic bond angle N(2)-C(6)-C(12) are 112(1)° in (±)-**10a** and 117(1)° in (±)-**11b** deviating significantly from the tetrahedral value, for the steric congestion around the cyclopropane, which forces the BOC and the carboxylate moiety to be remoted from each other. The torsion angles defined by the cyclopropane atoms N(2)-BOC and C(12) are –107(1);113(1)° and –109(1);117(1)° respectively, indicating an *anticlinal* conformation of the ring substituents. The oxygen atom O(4) eclipses the cyclopropane ring: the torsion angle M-C(6)-C(12)-O(4) is 178(1)° for (±)-**10a** and 13(1)° for (±)-**11b** (M is the midpoint of the distal bond C(4)-C(5)), in agreement with a *synperiplanar* conformation.[20] The BOC chain and the carboxylic moiety adopt *antiperiplanar* conformation defined by the torsions C(6)-N(2)-C(7)-O(3) of 160(1)° for (±)-**10a** and -167(1)° for (±)-**11b**, and C(6)-C(12)-O(5)-H(5) of –180(1)° for (±)-**10a** and of –140(1)° for (±)-**11b**. The values of torsion angle N(2)-C(6)-C(12)-O(5) in both derivatives (179(1)° in (±)-**10a** and 8(1)° in (±)-**11b**) reflect the widening of N(2)-C(6)-C(12), in order to release close contacts between atoms of neighbouring residues.[21-24] The cyclopropane is almost perpendicular to the plane of N(2)-C(6)-C(12) (dihedral angles 88(1)° for (±)-**10a** and 89(1)° for (±)-**11b**).

Table 1. Selected bond lengths (Å) and angles (°) for (±)-**10a** and (±)-**11b**

	(±)-**10a**	(±)-**11b**
Br(1)-C(1)	1.865(8)	
O(1)-N(1)	1.419(8)	1.394(7)
O(1)-C(3)	1.482(8)	1.443(8)
O(6)-C(1)		1.218(8)
N(1)-C(1)	1.254(9)	1.314(8)
C(1)-C(2)	1.494(9)	1.496(9)
C(2)-C(3)	1.538(9)	1.528(9)
C(3)-C(4)	1.464(9)	1.490(9)
C(4)-C(5)	1.513(9)	1.499(9)
C(4)-C(6)	1.514(9)	1.525(9)
C(5)-C(6)	1.496(9)	1.502(9)
Br(1)-C(1)-N(1)	118.7(6)	
O(1)-N(1)-C(1)	109.3(6)	113.8(6)
O(1)-C(3)-C(2)	103.9(5)	103.9(6)
O(1)-C(3)-C(4)	107.5(5)	111.2(7)
O(6)-C(1)-N(1)		124.9(8)
N(1)-O(1)-C(3)	109.2(5)	106.5(5)
N(1)-C(1)-C(2)	115.8(5)	108.7(7)

A conformational study carried out on the isolated molecules shows that the lowest-energy conformer of (±)-**10a** is characterized by values of the torsion angles C(6)-C(4)-C(3)-O(1) and C(4)-C(6)-N(2)-C(7) that slightly differ (8° and 25°, respectively) with respect to that of the X-ray conformation, while the most significant deviation found is the 115° rotation of the carboxylic group. This latter could be a consequence of the crystallographic packing effects: in the solid state the carboxylate points in the direction of a symmetry related molecule, leading to the formation of intermolecular contacts forcing in some way the molecular bending. Compound (±)-**11b** instead presents the major experimental and theoretical conformational differences in the above considered torsion angles (97° and 156°, respectively), and the carboxylic group is rotated of 133° (Figure 7).

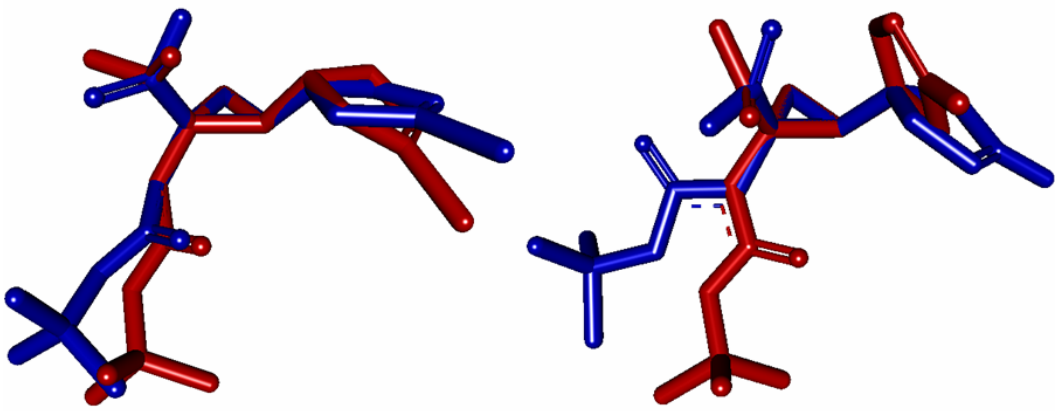

Figure 7. Superimposition of the experimental (blue) and theoretical (red) conformations of (±)-**10a** (left) and (±)-**11b** (right)

The influence of the crystal packing effects has been also analyzed on the light of the electronic properties. For (±)-**10a** a significant HOMO-LUMO difference, related to the absolute electronegativity (χ) of the molecule, with respect to the X-ray conformation which presents a lower χ value (characteristic of soften acids) has been observed, while for (±)-**11b** no variation between the crystallographic and the theoretical conformations has been found. This behaviour confirms the importance of the crystal packing effects for (±)-**10a**, which leads to an acidity enhancement consequent to the carboxylate intermolecular contacts.
The relationships between activity at NMDA and distance among the pharmacophoric moieties have been analyzed and compared with the Hutchinson's model,[25] whose pharmacophoric pattern is formed by three ionizable centres N^+, C_α and C_ω (distances d_1 N^+-C_ω=5.55Å and d_2 C_α-C_ω=5.54Å). Suboptimal values of d_1 and d_2 have been found: 5.58 and 5.08Å in (±)-**10a** and 5.32 and 5.09Å in (±)-**11b**, as shown by the correspondence of N^+, C_α and C_ω with N(2), C(12) and C(1) respectively. Their less extended conformation, leading in particular to short d_2 C_α-C_ω distances, could justify the significant drop in activity of (±)-**10a** and (±)-**11b** with respect to the potency of other ligands which fit better the Hutchinison's model[11] (and references therein).

In summary, the structural characteristics of two diastereomeric diydroisoxazolyl-cyclopropane derivatives (±)-**10a** and (±)-**11b** have been analyzed, with the aim to explore the orientation of the functional groups important for the interaction at NMDA receptor. In (±)-**11b** the BOC moiety forms chains connected by hydrogen bonds along the *b* axis, while in (±)-**10a** the different conformation of the cyclopropane substituents gives rise to tight intermolecular interactions in the crystal packing. The comparison of the structural features of the considered compounds with their relative affinities give reason at the importance of the structure-activity relationships proposed by the Hutchinson's model for the recognition by the **Glu** receptor, in particular the value of d_2 C_α-C_ω. In addition, these findings suggest that for the cyclopropyl constrained analogues of glutamic acid the bioactive conformations could depend upon the nature and the torsion angles of the substituents of the cyclopropane ring.

EXPERIMENTAL

Crystallography

Single crystals suitable for X-ray structure analysis of (±)-**10a** and (±)-**11b** were obtained by slow evaporation at room temperature of a methanolic solution as colourless prisms. An Enraf Nonius CAD-4 diffractometer was used for data collection at room temperature (MoK_α radiation).

The lattice parameters were determined by least-squares refinements of 25 high angle reflections. The structures were solved by direct methods using Sir92[26] and the refinements were carried out by full-matrix

least-squares with SHELX-97.[27] All non-H-atoms were refined anisotropically. The H-atoms positions were introduced in calculated positions in their described geometries and allowed to ride on the attached carbon atom with fixed isotropic thermal parameters ($1.2U_{eq}$ or $1.5U_{eq}$ of the parent carbon atom) for (±)-**10a** and the methyl group of (±)-**11b**. The other hydrogen of (±)-**11b** were detected in the Fourier map. A summary of the crystal data, data collection, and structure refinement is presented in Table 2.

CCDC numbers 661590 (±)-**10a** and 297225 (±)-**11b** 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 2. Summary of crystal data and structure refinement for (±)-**10a** and (±)-**11b**.

Compound	(±)-**10a**	(±)-**11b**
Empirical formula	C_{12} H_{16} Br_1 N_2 O_5	C_{12} H_{18} N_2 O_6
Formula weight	348.18	286.28
Temperature(K)	293(2)	293(2)
Wavelength(Å)	0.71073	0.71073
Crystal system	Orthorhombic	Monoclinic
space group	P bca	P 2_1/n
Unit cell dimens.(Å,°)	a=10.989(3)	a=11.90(1)
	b=10.982(2)	b=9.257(8)
	c=25.241(4)	c=13.55(1)
		β=96.79(2)
Volume(Å^3)	3046(2)	1482(2)
Z	8	4
Calc. density (Mg/m^3)	1.518	1.283
Crystal size (mm)	0.4 x 0.3 x 0.7	0.3 x 0.2 x 0.2
θ range(°)	3.23 to 30.01	2.16 to 21.03
Limiting indices	0≤h≤15	-12≤h≤12
	-13≤k≤15	-9≤k≤9
	0≤l≤-35	-1≤l≤13
Reflections collected	3400	3579
Independent reflections	3382	1603
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/param.	3382 / 0 / 184	1603 / 0 / 220
Goodness-of-fit	1.391	0.958
Final R ind. [I>2σ(I)]	R1 = 0.097	R1 = 0.062
R indices (all data)	R1 = 0.104	R1 = 0.082

Theoretical calculations

Compounds (±)-**10a** and (±)-**11b** were submitted to a detailed modeling study through theoretical calculations performed in two steps. The conformational space was completely explored using TINKER,[28] then the molecular conformations were optimized considering all the torsion angles and convergence was assumed when the energy change in two subsequent cycles of the minimization procedure was less than

0.5kcal/mol. The MM2 force-field[29] was used to calculate the conformational energies, while atomic charges were determined with the semiempirical molecular orbital software MOPAC 7.0.[30] All the minimum energy conformations thus located were successively re-optimized using the default scheme in Gaussian03[31] at the B2LYP/6-31G(d)[32] level of theory. The solvatation energy was calculated with continuum solvent models (PCM) to take into account the strong influence of water on the behavior of these compounds.

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