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Structural studies and NMDA activity of an enantiopure dihydroisoxazole derivative

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Abstract—The enantiopure 5-(2-amino-2-carboxyethyl)-4,5-dihydroisoxazole-3-carboxylic acid (−)-**1b** and its racemic *tert*-butoxycarbonylamino (Boc) precursor (±)-**11** were structurally characterized by the single crystal X-ray diffraction technique. The geometrical features and the intermolecular interaction of the pure (3*S*,5*S*)-aminoacid are compared with the racemic derivative. This analysis has shown a different conformation of the dihydroisoxazole ring: (±)-**11** adopts an envelope shape, while in (−)-**1b** it is almost planar. The intermolecular assembly is characterized by hydrogen bonds of an NH···O type in (±)-**11** with the formation of polymeric chains, whereas in (−)-**1b** the hydrogen bonds determine a three dimensional network. The tight intermolecular interactions of (−)-**1b** could be responsible for the short distances between ionizable groups, which are important as pharmacophoric parameters for NMDA activity.

1. Introduction

Glutamate (**Glu**, Fig. 1) is the main excitatory neurotransmitter in the mammalian central nervous system (CNS) and mediates neurotransmission across most excitatory synapses, being involved in a variety of CNS functions, such as learning and memory.[1–3]

Glutamatergic hyperactivity leads to neurotoxicity typically associated with acute and chronic neurodegenerative diseases, that is, cerebral ischaemia, epilepsy, amyotrophic lateral sclerosis, Parkinson's and Alzheimer's diseases.[1–3]

The effects of Glu are mediated by ligand-gated ionotropic receptors (iGluRs) and G-protein coupled metabotropic receptors (mGluRs):[1–6] both families are composed of different receptor classes and subtypes. The fast excitatory effects of Glu are mediated by three subclasses of iGluRs, which are transmembrane cation channels. These 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] The modulation of the glutamatergic pathways may represent a relevant therapeutic approach for the treatment of a number of neurodegenerative pathologies, neuropsychiatric diseases, as well as learning and memory impairments.[7–9] As a result, novel high affinity ligands endowed with family and subtype selectivity are required to better characterize the physio-pathological role of

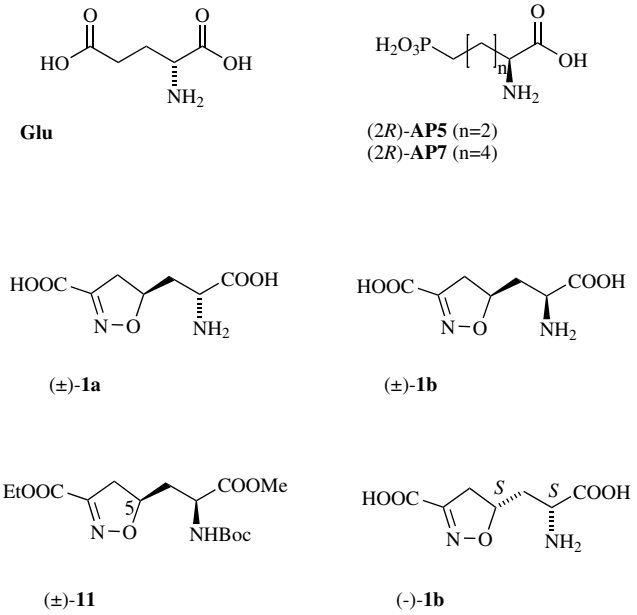

Figure 1.

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iGluRs and mGluRs and, consequently, to uncover specific targets for pharmacological intervention.[10–12]

Selectivity for iGluRs versus mGluRs can be achieved through the choice of an appropriate conformation. Since Glu is a flexible molecule, a number of constrained analogues have been designed and tested with the aim of uncovering the conformational requirements needed to activate the different Glu receptor classes. It has been shown that an extended conformation is required for the interaction with mGluRs, whereas a folded conformation is necessary to fit the binding sites of iGluRs.[13]

Homologation of the glutamate chain is a strategy in order to achieve selectivity in the interaction with the different receptor subtypes.[14] When considering the interaction of ligands with NMDA receptors, an increase in the distance between the proximal and the distal acidic groups of Glu usually leads to NMDA antagonists, with the most potent NMDA antagonists bearing a chain of four or six carbon atoms linking the two acidic groups, that is, (2*R*)-2-amino-5-phosphonopentanoic acid (2*R*)-**AP5** and (2*R*)-2-amino-7-phosphonoeptanoic acid (2*R*)-**AP7** (Fig. 1).

Remarkably, the eutomer of the majority of NMDA ligands has an absolute configuration at the amino acidic stereogenic centre opposite to that of natural Glu, the endogenous neurotransmitter. On the basis of these findings, we have previously designed and synthesized two homologues of Glu in which the amino acid chain is embedded in a cyclic system [(±)-**1a** and (±)-**1b**, Fig. 1]. These two diastereoisomeric aminoacids turned out to be very potent and selective NMDA antagonists endowed with a remarkably neuroprotective effect.[15] Compounds (±)-**1a** and (±)-**1b** are characterized by partial rigidification: the chain appended at the 5-position of the isoxazoline moiety is still flexible and is able to assume several different conformations.

Previously we have reported[16] the structure of bicyclic derivatives in order to understand the critical pharmacophoric features. Herein we report the geometrical characterization in the solid state of the correlated compounds (±)-**11** and (−)-**1b** (Fig. 1). This could help in defining foreseeable orientations of the ω-acidic group (COOH) with respect to the α-aminoacid moiety, allowing the assessment of the conformational requirements of the interactions at the Glu receptors subtypes. The structure-based investigations are correlated to pharmacological activities to demonstrate the molecular features for the interaction with the NMDA receptor.

2. Results and discussion

The molecular structures of (±)-**11** and (−)-**1b** were determined by X-ray crystal analysis. The skeleton of these compounds consists of a five-membered dihydroisoxazole ring attached via a methylene group to the Cα atom. The absolute configuration of (−)-**1** was previously assigned by chemical correlation with the enantiopure dipolarophile (*S*)-(+)-*N*-Boc allylglycine methyl ester as (3*S*,5*S*)[15] but was not unambiguously confirmed by X-ray data, due to the absence of significative anomalous scatterers.

Figure 2 represents the molecular structure of 1-*tert*-butoxycarbonylamino-2-methoxy-carbonylethyl 4′,5′-dihydro-isoxazole ethyl ester (±)-**11** with atom numbering. This is a racemic molecule with the same stereochemistry at both stereogenic centres C(3) and C(5). The crystal structure of (±)-**11** was determined with data collected at room temperature in order to have a direct comparison of the geometrical features of the two compounds not discussed before.[15]

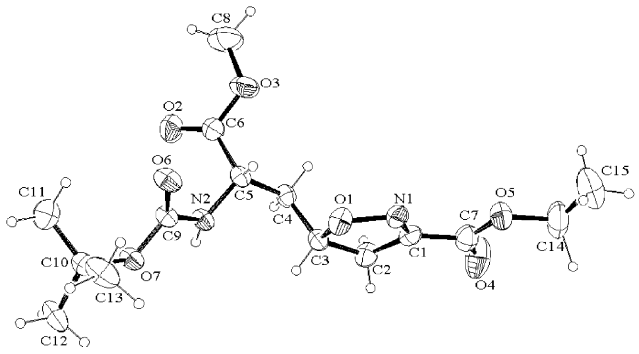

Figure 2. ORTEP[17] view of compound (±)-**11**, showing the atom-numbering scheme (ellipsoids are at the 50% probability and H atoms are as spheres of arbitrary radii).

The dihydroisoxazole ring has a distorted envelope conformation with C(2) out of the plane formed by C(1), O(1), N(1), C(3) of 0.122(1) Å. This plane is almost perpendicular to that formed by C(5), C(6), O(2), O(3) (dihedral angle 83.0(3)°). The structure presents an extended conformation as suggested by theoretical calculation of its derivative (±)-**1b**[15] and confirms the absence of an intramolecular hydrogen bond between the aminic nitrogen and the isoxazolic oxygen.

The molecular packing is characterized by infinite molecular chains along the *a* axis (Fig. 3), due to the presence of an intermolecular hydrogen bond between the aminic nitrogen and the carboxylic oxygen of Boc moiety (N(2)–H···O(6)^{I}, distance of 1.97(1) Å, angle 171(1)°, ^{I}at $1/2 - x$, $y + 1/2$, z).

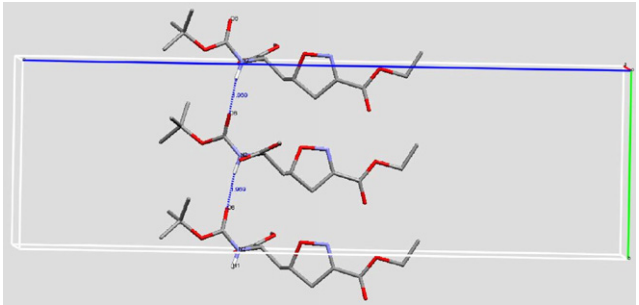

Figure 3. Chain formation in compound (±)-**11** via intermolecular hydrogen bonds along the *a* axis.

The perspective view of the enantiopure 5-(2-amino-2-carboxylethyl)-4,5-dihydro-isoxazole-3-carboxylic acid (−)-**1b**, crystallized as inner salt zwitterions, is shown in Figure 4.

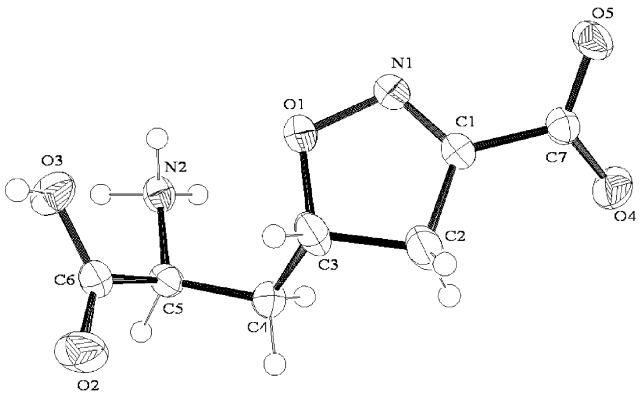

Figure 4. ORTEP[17] view of compound (−)-**1b**, showing the atom-numbering scheme (ellipsoids are at the 50% probability and H atoms are as spheres of arbitrary radii).

The atom N(2) adopts a tetrahedral geometry due to the presence of the additional hydrogen atom consequent to the ionization. The C–O and C–OH bond lengths in the α and ω-carboxyl groups compare well with those usually found in carboxylic acid structures.[18] The carboxylate C(7)–O(5)–O(6) and the carboxylic groups C(6)–O(3)–O(4) are out of the plane of the dihydroisoxazole ring. They are inclined to one another by −60(1)°, and with respect to the dihydroisoxazole ring by 42(1)° and 55(1)°, respectively.

The dihydroisoxazole ring is almost planar and the maximum deviations from the least-square plane through the ring are 0.018(4) Å for C(2) and −0.012(3). This is in contrast with the conformation observed in previously reported structures[19–21] and in compound (±)-**11**. The loss of the envelope conformation is probably due to the electronic delocalization of the carboxylate group. This is confirmed by the shortening of the C(1)–C(7) and C(1)–C(2) distances, as reported in Table 3 (see Experimental).

The comparison of the 'peptide' torsion angles has evidenced that the two correlated compounds present comparable torsion angles φ N(2)–C(5)–C(4)–C(3) and ψ O(3)–C(6)–C(5)–N(2) with values of −60(1)° in (±)-**11** and −72(1)° in (−)-**1b** for φ, and 163(1)° in (±)-**11** and 9(1)° in (−)-**1b** for ψ, respectively. On the contrary, the torsion angle ω C(5)–C(4)–C(3)–O(1) is opposite in the two compounds, (−)-**1b** has a value of 55(1)° compared with −57(1)° present in (±)-**11**.

The main conformational differences are shown by the superimposition of the two compounds presented in Figure 5.

The crystal packing in (−)-**1b** is mainly stabilized by a network of intermolecular OH···O and NH···O hydrogen bonds as shown in Figure 6.

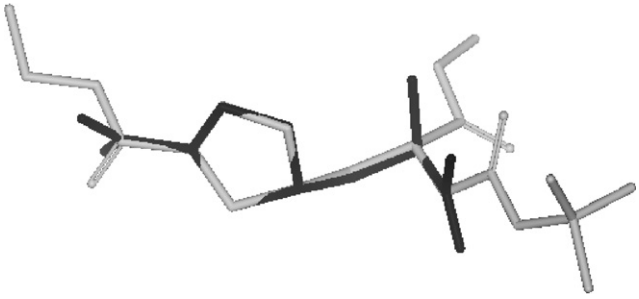

Figure 5. Superimposition of the precursor (±)-**11** (grey) and the aminoacid (−)-**1b** (black).

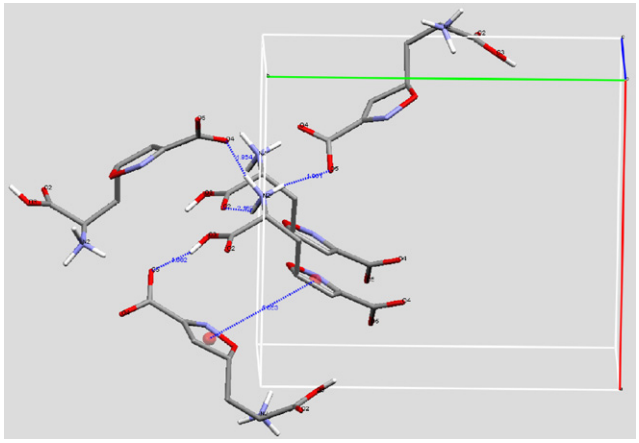

Figure 6. Intermolecular hydrogen bonds and stacking interaction of compound (−)-**1b**.

Symmetry-related molecules are linked in a three-dimensional hydrogen-bond network: this involves the N(2) atom, which is a trifurcated donor of H-bonds, the O(4) and O(5) atoms of the carboxylate and the O(2) of the carboxylic acid. Details of the interactions are given in Table 1.

The dihydroisoxazoline rings of adjacent molecules, faced together with a centroid distance of 5.05 Å, contribute to hold the intermolecular hydrogen bonding network in the crystal cell. It is interesting to note the intramolecular interaction occurring between the O(1) of the ring and the H(23) of the aminic moiety of 2.40(4) Å. This hydrogen bond is characterized by a suboptimal value of the N(2)–H(23)···O(1) angle of 107(1)°, but in this case could be relevant to keep the folded conformation of the aminoacid together with the other considered intermolecular interactions, as previously reported.[22]

It is worthwhile noting that this compound, at least in the solid state, is heavily involved in interactions with adjacent molecules, lowering the rotational freedom of the dihydroisoxazole substituents. This could limit the flexible accommodation of this compound at the receptor binding site. In fact, (−)-**1b** does not have a significant NMDA activity,[23] which contributes to lower the activity of the racemate.

Table 1. Intermolecular hydrogen bonds in compound (−)-**1b**

Atoms	Distance (Å)	Angle (°)	Symmetry operator
N(2)–H(21)···O(4)^{I}	1.86(1)	148(1)	^{I}At $1-x, y+1/2, 1/2-z$
N(2)–H(22)···O(5)^{II}	1.91(1)	168(1)	^{II}At $x-1/2, 3/2-y, 1-z$
N(2)–H(23)···O(2)^{III}	2.17(1)	143(1)	^{III}At $x, y, z+1$
O(3)–H(3)···O(5)^{IV}	1.66(1)	163(1)	^{IV}At $3/2-x, 2-y, z-1/2$

In order to explain the enantiopharmacology, the structure–activity relationship given by the Hutchinson's model[24] has been considered and the pharmacophoric distances have been compared. The significant pattern of this model is represented by three ionizable centres N^+, C_α and C_ω, with distances d_1 N^+–$C_\omega = 5.55$ Å and d_2 C_α–$C_\omega = 5.54$ Å, which correspond in the molecules examined with the atoms N(2), C(6) and C(1), respectively. These values are $d_1 = 5.22$ Å and $d_2 = 4.79$ Å in (±)-**11**, while in the aminoacid are $d_1 = 4.68$ Å and $d_2 = 4.59$ Å; therefore, the racemic compound fits better with this model than (−)-**1b**, due to a more extended conformation. In fact, compound (−)-**1b** is characterized by shorter distances among the ionic groups and the not optimal values of d_1 and d_2 could justify the lower potency of (−)-**1b** with respect to the antagonists[13] that better fit the model considered. Nevertheless, this folded conformation justifies the previously studied enantiopharmacology at NMDA receptor of these compounds, and then the hydrogen bond formation ability, besides stabilizing the molecular structure, must have a significant effect on the molecular interaction at the receptor site. The constraints at the receptor binding site should force an extended conformation of the compounds, as previously asserted by docking experiments,[23] in which the distances between the acidic position (distal function) bounded to the dihydroisoxazole ring and the aminoacidic carboxylic group (proximal function) are important for the antagonist properties. They should be larger than those accepted at the glutamate binding site. In the synthetic precursor (±)-**11**, they are in a range compatible with this pharmacophoric model and its extended conformation could be related to the hydrophobic repulsion of the Boc moiety in the crystal packing.

3. Conclusion

The main result of this study was the structural characterization of the novel (3*S*,5*S*)-dihydroisoxazole aminoacid (−)-**1b** and of its racemic synthetic precursor (±)-**11**, acting at the glutamate receptor.

In (±)-**11** the Boc moiety forms chains connected by hydrogen bonds along the *a* axis. In the zwitterions (−)-**1b**, the carboxylate group leads to electronic delocalization towards the dihyroisoxazole ring. The absence of the protective group allows tight intermolecular interactions in the crystal packing.

The aminoacidic derivative with a Boc-blocked amino group (±)-**11** is of an extended conformation and it matches better the pharmacophoric Hutchinson's model

Table 2. Summary of crystal data and structure refinement for (±)-**11** and (−)-**1b**

Identification code	(±)-**11**	(−)-**1b**
Empirical formula	$C_{15}H_{24}N_2O_7$	$C_7H_{10}N_2O_5$
Formula weight	344.36	202.17
Temperature (K)	293	293
λ Mo Kα (Å)	0.71073	0.71073
Crystal system	Orthorhombic	Orthorhombic
Space group	*Pcab*	$P2_12_12_1$
Unit cell dimension		
a (Å)	9.847(1)	12.011(3)
b (Å)	12.007(3)	13.368(5)
c (Å)	31.752(2)	5.507(1)
Volume (Å^3)	3754(1)	884(1)
Z	8	4
Density (calcd) (Mg/m^3)	1.219	1.519
F(000)	1472	424
Crystal size (mm)	$0.1\times0.05\times0.09$	$0.6\times0.5\times0.3$
θ Range for data collected (°)	2–22	3–30
Index ranges	$-1\leqslant h\leqslant 10\ 0\leqslant k\leqslant 12\ 0\leqslant l\leqslant 33$	$-1\leqslant h\leqslant 16\ 0\leqslant k\leqslant 18\ 0\leqslant l\leqslant 7$
Reflections collected	2607	1445
Independent reflections [$I>2\sigma(I)$]	2294	1438
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameter	2294/0/291	1438/0/167
Goodness-of-fit on F^2	1.119	1.205
Final *R* indices [$I>2\sigma(I)$]/all data	$R1 = 0.054(0.118)$	$R1 = 0.048(0.049)$

Table 3. Selected bond lengths (Å) and angles (°) for (±)-**11** and (−)-**1b**

Bond lengths	(±)-**11**	(−)-**1b**	Angles	(±)-**11**	(−)-**1b**
O(1)–N(1)	1.404(5)	1.411(3)	O(1)–N(1)–C(1)	108.7(4)	109.2(2)
O(1)–C(3)	1.467(6)	1.469(4)	O(1)–C(3)–C(2)	104.0(4)	105.1(2)
N(1)–C(1)	1.270(6)	1.268(4)	O(1)–C(3)–C(4)	107.9(4)	108.4(2)
C(1)–C(2)	1.469(8)	1.490(4)	N(1)–O(1)–C(3)	109.5(3)	109.3(2)
C(1)–C(7)	1.486(6)	1.500(3)	N(1)–C(1)–C(2)	115.2(2)	115.7(2)
O(4)–C(7)	1.182(6)	1.234(3)	N(1)–C(1)–C(7)	121.3(2)	120.6(1)
O(5)–C(7)	1.327(7)	1.268(3)	O(4)–C(7)–O(5)	125.4(2)	124.4(2)
O(2)–C(6)	1.218(5)	1.208(4)	O(1)–C(3)–C(4)	108.0(2)	108.4(2)
O(3)–C(6)	1.339(5)	1.304(4)	N(2)–C(5)–C(4)	111.0(2)	112.0(1)
N(2)–C(5)	1.439(5)	1.493(4)			

with respect to the aminoacid derivative (−)-**1b**, in which the stereochemical characteristics determine shorter value of the pharmacophoric Hutchinson's distances. That could justify the low activity of this enantiopure NMDA antagonist.

In conclusion, the comparison of the structural features of the investigated compounds reported with their relative affinities here should enhance efforts to design subtype-selective antagonist having improved therapeutic properties and less side effects.

4. Experimental

Single crystals suitable for X-ray structure analysis of (±)-**11** were obtained by slow evaporation at room temperature of ethyl acetate as colourless prisms. Compound (−)-**1b** formed in colourless water crystals of a cubic shape.

An Enraf Nonius CAD-4 diffractometer was used for data collection at room temperature (Mo Kα radiation).

The lattice parameters were determined by least-squares refinements of 25 high angle reflections. The structures were solved by direct methods (Sir 92)[25] and the refinements were carried out by full-matrix least-squares calculations based on F^2 by application of SHELX-97[26] program. All non-H-atoms were refined anisotropically. The hydrogen atoms of both compounds were detected in a difference Fourier map.

A summary of the crystal data, data collection and structure refinement is presented in Table 2; selected bond lengths and angles are reported in Table 3. CCDC numbers 299003 (±)-**11** and 625949 (−)-**1b** 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).

Acknowledgement

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