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X-RAY STRUCTURAL CHARACTERIZATION OF SR 142948, A NOVEL POTENT SYNTHETIC NEUROTENSIN RECEPTOR ANTAGONIST

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Abstract: SR 142948 is an original and extremely potent neurotensin receptor antagonist developped in a promising approach to novel antipsychotic drugs. The X-ray structure was elucidated and compared to SR 48692 and levocabastine, providing new informations about the possible recognition process of NT receptor subtypes.

Neurotensin (NT), a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), has a wide range of pharmacological effects in peripheral tissues and in the central nervous system.[1] It acts as a neuromodulator in the brain and as a gastrointestinal hormone in the periphery. Concerning its neuromodulator role, the possibility of a subtle and complex modulation of the dopaminergic system offers the exciting possibility of a new treatment strategy for certain psychotic disorders.[2-5] In the last few years, the discovery of a number of non-peptide NT antagonists has been reported.[6] All but one series, based on SR 48692,[7] exhibit weak affinities for human NT receptors. For some years major effort has been directed toward the rational design of improved synthetic NT analogues, based on the structural requirements of SR 48692 and its analogues.[8-10]

The recent discovery of pharmacological and biochemical characteristic properties of SR 142948,[11] a compound chemically related to SR48692 with binding affinities in the nanomolar range, motivated a new structural study of analogues bearing a bisubstituted phenyl ring (Scheme 1). Actually, SR 142948 retains the properties of SR 48692 (no intrinsic agonist activity, oral bioavailabality, long duration of action and good brain access) but displays a wider spectrum of activity than SR48692, notably by antagonizing NT-induced hypothermia, analgesia and dopamine release.[11] These effects are believed to be due to the inhibition of still uncharacterized NT receptor (NTR) subtypes.

It is well described[12] that, in adult rat brain, NT binds to two distinct sites distinguishable by (i) their sensitivity to levocabastine, a histamine H1 receptor antagonist, and (ii) their different affinity to neurotensin. The high affinity NTR is levocabastine-insensitive and has been cloned from rat[13] and human[14]. It is worth noting that this receptor subtype NTR1, (which represents less than 30% of the NT binding sites in the rat adult brain[2]) does not seem to mediate the NT-induced hypothermia and analgesia. Actually SR48692 inhibits the

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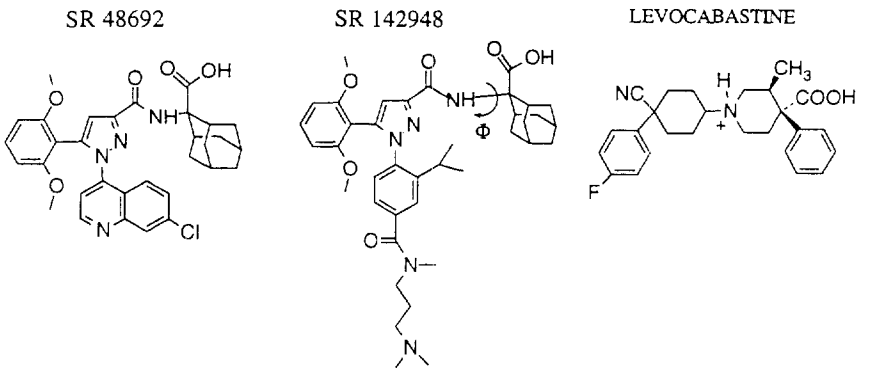
binding of NT to the cloned receptor without affecting those NT-induced effects.[11] Another NTR subtype (NTR2) has been recently cloned from rat[12] and mouse.[15] NTR2 seems to correspond to the levocabastine-sensitive NT site previously described. Indeed binding studies show that levocabastine can compete efficiently with NT for occupancy of this site. Interestingly, SR 142948, unlike SR 48692, is unable to discriminate between the high affinity NT receptor and the low affinity binding site described as levocabastine-sensitive in mice and rats.[11] Finally, the fact that NTR2 subtype has been shown not to be involved in either analgesia or hypothermia induced by NT raises the possibility of identifying other NTR subtypes.



Scheme 1. Molecular structure of SR 48692, SR 142948 and levocabastine

In this way, structural comparison between SR 142948 and SR 48692, is required for the rational design of new antagonists and understanding the ligand-specificity of these G-coupled receptors. Herein we report the crystallisation and the determination of the crystal structure of SR 142948.[16]

The conformation and packing of SR 142948 are shown in Figure 1. The N(1)-C(6) bond, 1.457(5) Å, between isopropylphenyl group and the pyrazole ring is significantly longer than expected for a standard N(sp^2)-C(ar.) bond (1.371(16)Å)[17]. Such a bond length indicates the lack of a significant orbital overlap between N lone pair and aromatic ring as the result of steric hindrance wich forces the isopropylphenyl moiety to be nearly perpendicular to the pyrazole ring [N(2)-N(1)-C(6)-C(7)=-110.5(4)°]. The 2,6-dimethoxyphenyl group shows a similar behavior: a bond length C(5)-C(41) of 1,472(6) Å between the two aromatic rings and a torsion angle value of -89.9(5)° for N(1)-C(5)-C(41)-C(42). The two methoxy groups have a nearly coplanar orientation with respect to the phenyl ring.

On the other hand, the amidic function in position 3 on the pyrazole is nearly coplanar with the heterocycle as shown by the torsion angle N(2)-C(3)-C(25)-N(27)=5.8(6)°.This arrangement results in good overlaping between the pyrazole ring and the amidic moiety.

The existence of an intramolecular hydrogen bond between N(2) and N(27), [N(2)···N(27)=2.684(5), H(27)···N(2)=2.127 Å, N(27)-H(27)···N(2)=116.2°], in a five-membered ring N(2)-C(3)-C(25)-N(27)-H(27) increases the rigidity of the molecule.

The carboxyl moiety is situated *gauche*$^-$ with respect to the amidic carbonyl as shown by the torsion angle C(25)-N(27)-C(28)-C(29)=-54.9(5)°. We reported previously that the interaction from an adamantyl group on the α C(28) carbon atom leads to this conformation.[10]

Beside these features common with SR 48692, SR142948 displays the following structural characteristics. SR 142948 exists as a zwitterion form as demonstrated by the similar bond lenghts C(29)-O(30), 1.246(8) and C(29)-O(31), 1.255(9) Å and the protonation of N(22) of the tertiary amine. This basic function and the carboxylic moeity are H-bonded (charge assisted) through intermolecular contacts.

The plane of the tertiary amide substituent forms an angle of 73.5(5)° (C(8)-C(9)-C(15)-O(16)) with the plane of the phenyl ring. The dimethylaminopropyl chain adopts a *trans, gauche*$^+$, *gauche*$^+$, *trans* conformation as indicated by the values of the torsion angles: C(9)-C(15)-N(17)-C(19)=179.4(6)°, C(15)-N(17)-C(19)-C(20)=94.0(6)°, N(17)-C(19)-C(20)-C(21)=52.4(7)° and C(19)-C(20)-C(21)-N(22)=160.6(5)°.

The crystal packing reveals the presence of a strong hydrogen bond reinforced by an ionic bridge between the carboxylic oxygen O(31) and the protonated nitrogen N(22) [N(22)···O(31)=2.582(6), H(22)···O(31)=1.528.Å, N(22)-H(22)···O(31)=163.5°].

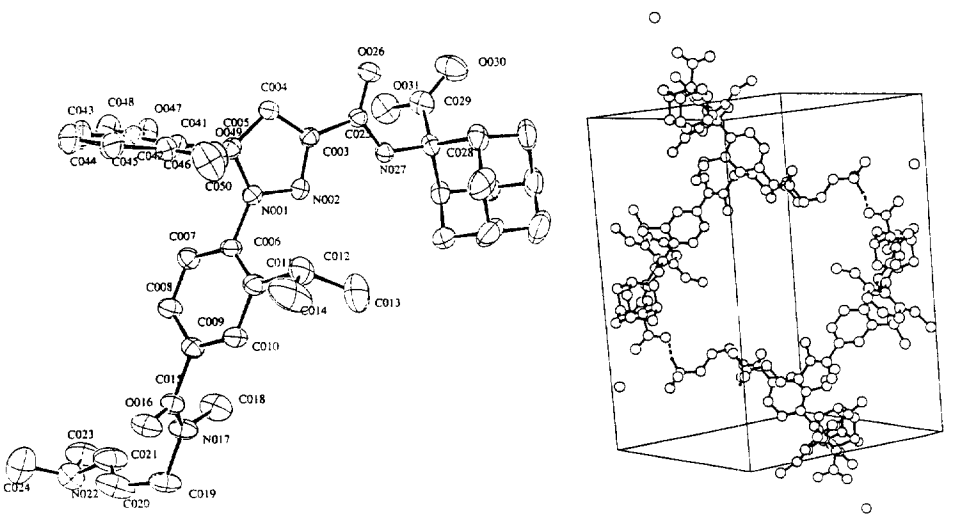


Figure 1. (a) Crystal conformation and (b) stereoscopic view of the crystal packing of SR 142948. Dotted lines represent intermolecular hydrogen bonds

Moreover, the three dimensionnal arrangement is stabilized by π–π interactions leading to an aromatic stacking between the dimethoxylphenyl rings with a distance (centroïd to centroïd) of 4.06(5) Å. Finally, an intermolecular hydrogen bond is observed between the carboxylic oxygen atom O(30) and a water molecule of cocrystallization O(51) (H(O51) non localized) [O(30)···O(51)=2.707(16)Å].

In conclusion, the conformation of SR 142948 presents the common structural features characterizing a potent neurotensin antagonist i.e. orientation of aromatic moieties and amidic function respectively perpendicular and planar to a pyrazole ring, and a torsion angle value around -60° for Φ of the terminal amino acid. The novel properties, associated with the structure, originate from the specific interactions of the *para*-substituent of the isopropylphenyl group with the target receptors. This characteristic adds to the NT antagonist relevant pharmacophoric elements, especially a basic tertiary amine. To fulfill intermolecular interaction requirements of the packing, the long lateral chain does not adopt an expected all-*trans* conformation suggesting a high flexibility of this fragment. It is therefore questionable to propose this packing conformation as a bioactive conformation of the ligand. Nevertheless superimposition studies with the rigid molecule levocabastine[18], reveals common elements between these two NT ligands. The similarities (in the white square of Figure 2) include a tertiary nitrogen (H donor (N+)), an aromatic ring (Arom.), a hydrogen bond acceptor (H accept. (C=O)) and two aliphatic groups (Aliph.1 and Aliph.2). This observation, supported by the fact that SR 142948 -in contrast to SR 48692- binds effectively to the levocabastine-sensitive NTR, may be of particular importance for the design of new NT antagonists. Does this common region represent a key pharmacophoric element for targeting the stereospecific low-affinity binding site?

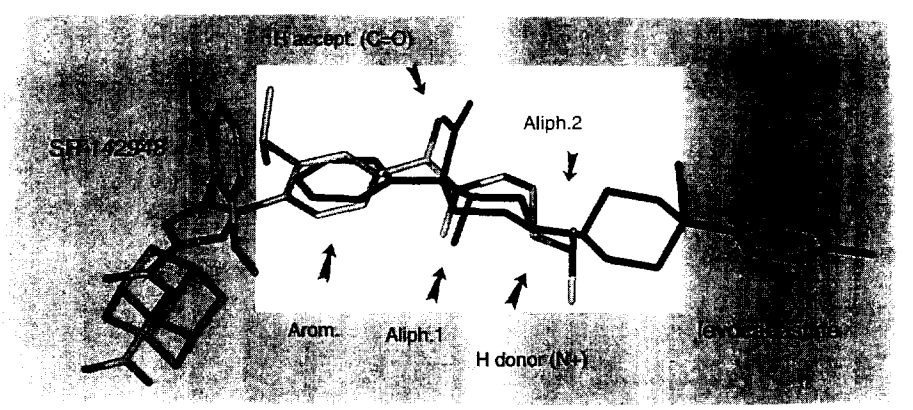


Figure 2. Superimposition of the crystallographic structures for SR 142948 and levocabastine; the structurally similar parts of these two NTR ligands are enclosed in the white square[19]

We are currently developping 3D models of NTR1-NTR2 subtypes in order to investigate the molecular recognition process of NT ligands. The present structure determination of SR 142948 is an important step in this direction. Based on this accurate molecular picture and on recent mutagenesis data, modelling and docking experiments are now under investigation.

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16. Crystals of SR 142948, 2-{[5-(2,6-dimethoxyphenyl)-1(4-(N-(3-dimethylaminopropyl)-N-methylcarbamoyl)-2-isopropylphenyl)-1H-pyrazole-3-carbonyl]amino}adamantane-2-carboxylic acid, were obtained by slow evaporation of a methanolic solution at room temperature. A colourless prism of dimensions 0.20 x 0.20 x 0.10 mm was used for all X-ray measurements and was found to be monoclinic, space group $P2_1/c$ with a = 14.628(5), b = 22.806(5), c = 12.434 (5) Å and $\beta = 108.21(5)°$. X-ray diffractometer data were collected using Cu Kα radiation to a θ angle of 72°. The structure was solved by direct method (SIR92)[20] and refined by full-matrix least-squares fit on F^2 (7749 data) with the SHELXL93 program[21] to an R_1 factor ($I > 2.0\ \sigma(I)$) of 0.089. Tables of atomic coordinates, bond lengths and angles, and thermal parameters for the non-hydrogen atoms of SR 142948 are available upon request.
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