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A CONFORMATIONAL STUDY OF LIGANDS FOR OMEGA MODULATORY SITES OF $GABA_A$ RECEPTORS BY NOESY NMR SPECTROSCOPY AND DISTANCE GEOMETRY.

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Abstract : Conformational analysis by NOESY NMR spectroscopy and Distance Geometry of an ω_1 selective (zolpidem) and a non selective (saripidem) ligand of ω modulatory sites suggests that the single set of conformations observed with zolpidem could be account for its ω_1 selective properties. The methodology applied in this study appears to be a powerful technique to explore conformational space of non-peptidic ligands of $GABA_A$ receptor subtypes. © 1997 Elsevier Science Ltd.

Introduction

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system. It exerts its main actions through $GABA_A$ receptors which belong to the superfamily of ligand-gated ionic channels. Molecular cloning experiments have revealed the existence of five different families (α_{1-6}, β_{1-3}, γ_{1-3}, δ_1 and ρ_{1-2}) of subunits which constitute the $GABA_A$ receptor. This provides the basis for structural diversity of $GABA_A$ receptors[1]. It has been shown that the $GABA_A$ receptor possesses a pentameric structure with five subunits arranged to enclose a chloride channel[2]. Each subunit possesses four transmembrane domains. In addition to a binding site for the neurotransmitter, the $GABA_A$ receptor also has a recognition site for benzodiazepines, the so-called "benzodiazepine receptor" or "ω modulatory site". $GABA_A$ receptors sensitive to benzodiazepines are formed by a combination of three subunits namely α_m, β_n and γ_p. On the basis of binding assays performed on native receptors in different brain regions, two subtypes of modulatory sites have been identified : benzodiazepine type I (BZ_1 or ω_1) and type II (BZ_2 or ω_2). The α subunit appears to be determinant for the pharmacology of the receptor subtypes. The BZ_1 or ω_1 subtype corresponds to α_1-subunit containing receptors. The BZ_2 or ω_2 subtype was found to be heterogeneous and corresponds to a mixture of α_2-, α_3- or α_5-subunit containing receptors. A large number of compounds from several chemical series possess affinity for ω sites, but most of them are non selective. Only a few chemical series (eg. the triazolopyridazines, the imidazopyridines and the β-carbolines[3]) provide selective compounds.

The aim of this work is to study the conformations in solution of a ω_1 selective and a non selective ligand belonging to the same chemical series with the purpose of identifying the structural and conformational requirements for selectivity and affinity for the ω_1 receptor subtype.

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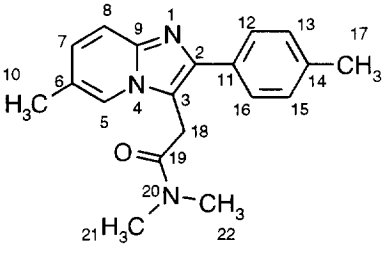
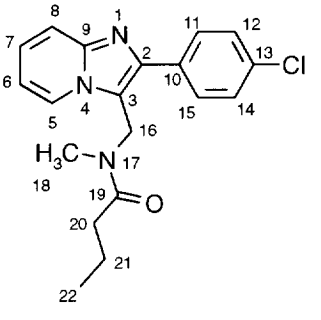
Zolpidem, an ω_1 (α_1-containing receptor subtype) selective ligand[4], and saripidem[5], a non selective ligand (ω_1 *versus* ω_5 (α_5-containing receptor subtype)) (table 1), were used as prototype molecules because they differ mainly in terms of the amide side chain (figure 1).

Figure 1 : Structures and numbering



ZOLPIDEM



SARIPIDEM

Table 1 : Binding profile of zolpidem and saripidem

DISPLACEMENT OF [^{3}H]FLUMAZENIL				
Compounds	IC_{50} (nM)			
	Cerebellum	Spinal cord	Recombinant receptors	
			$\alpha_1\beta_2\gamma_2$	$\alpha_5\beta_2\gamma_2$
Zolpidem[8,6]	14	130	46.3	≥10000
Saripidem[7,9]	2.7	4.6	1.1	33

Results obtained from Faure-Halley et al.[6], Benavides et al.[7,8], D. Graham[9].

To reach our objectives, we applied a methodology usually used for generating conformations of peptides: 2D-NMR spectroscopy coupled to distance geometry calculations. In the last decade, the techniques developed for NMR studies of proteins were applied to peptides. As compared to proteins, the conformational analysis of peptides is rendered somewhat difficult for several reasons : there is a lower density of NOEs, it is rare that peptides have a single rigid conformation and the observed NOEs lead to a time-averaged conformation[10]. Nevertheless, these conformational studies are feasable and a large number of publications concerning peptide conformations appear in the literature. On the other hand, we found very few studies concerning conformations of non peptidic molecules by NMR spectroscopy coupled to distance geometry[11,12]. The potential of this methodology remains to be evaluated as illustrated in the present study.

We have analysed the conformations of small non peptidic molecules namely zolpidem and saripidem by NMR coupled to distance geometry. An initial set of conformations was generated by distance geometry, constrained by unambiguous NOEs. In the following steps, additional ambiguous NOEs (defined as NOEs with same chemical shift or NOEs observed in presence of rotation like for example NOEs with H16 or H11) were integrated consequently. The conformers were accepted if they had a low final error and then low NOE violations. At each step, it is very important to verify that conformers generated with ambiguous NOEs are in agreement with the whole experimental data. If this is not the case, these NOEs must not be included in the Distance Geometry algorythm. Only final conformations compatible with all of the NOEs were taken into account.

Discussion

After performing 2D-NMR investigations of zolpidem and saripidem (table 2), coupled to structures generation by DGII methodology, it was observed that the ω_1 selective ligand zolpidem presents only one family of conformations (and the corresponding symetrical conformations), namely **Z1** (figure 2).

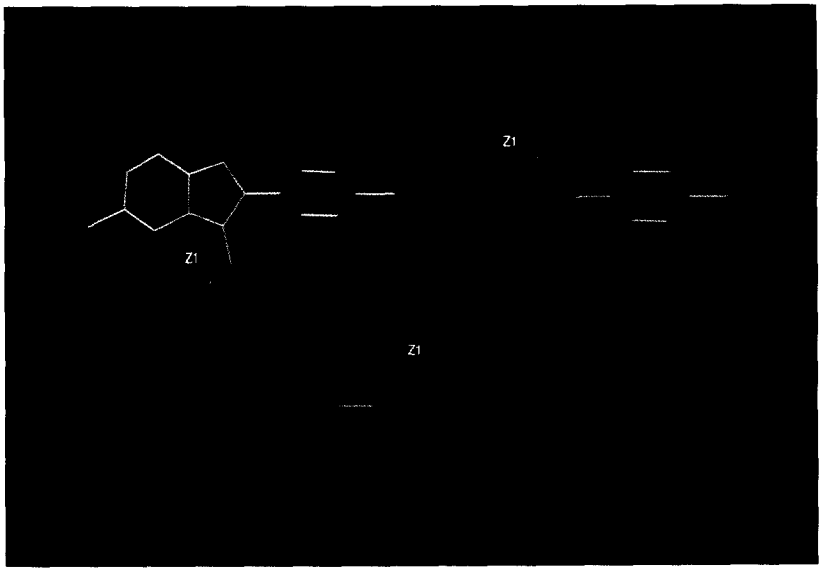


Figure 2 : Orthogonal view of conformational family obtained for zolpidem

Observed NOEs			Observed NOEs (continuation)		
Proton 1	Proton 2	Intensity	Proton 1	Proton 2	Intensity
5	10	m	12-16	18	m
5	18	F	12-16	22	m
5	22	f	12-16	21	f
5	21	f			

Table 2 : Observed NOEs (τ_m equal 400 ms) classified in strong (F), medium (m), and low (f)

On the other hand, the mixed ω_1/ω_5 ligand saripidem presents two families (and corresponding symetrical conformations), namely **S1** and **S2** (figure3).

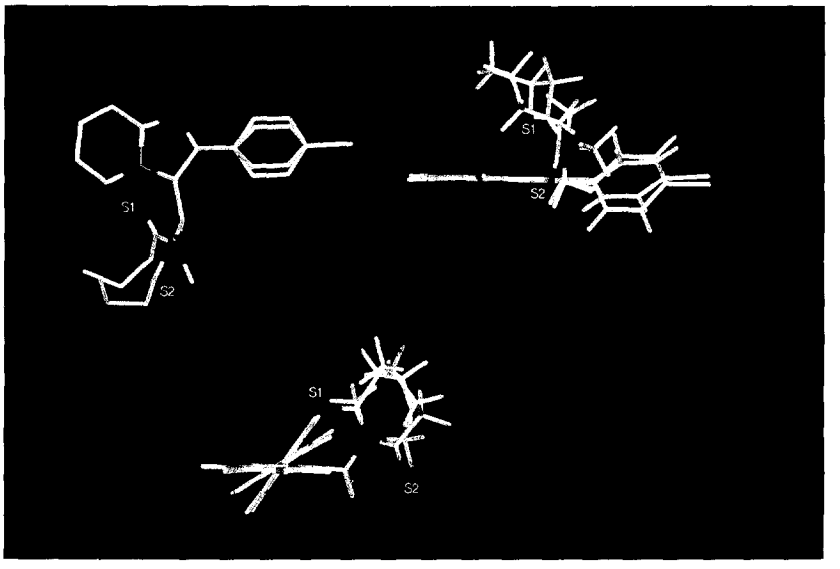


Figure 3 : Orthogonal view of conformational families obtained for saripidem

The superimposition of these three conformational families allows us to show that **Z1** is similar to **S1** (figure 4).

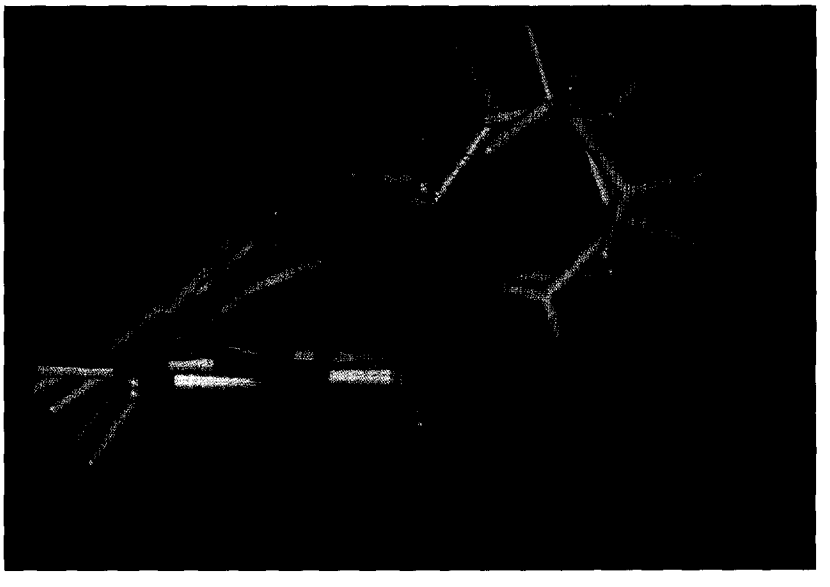


Figure 4 : Superimposition of conformational families obtained for zolpidem and saripidem

In these two conformations (Z1 and S1) the carbonyl bond of the amide function is located at 2 Å above the plane of the heterocycle (and below for symetrical conformations) and oriented towards the pyridine ring. The plane formed by the amide function makes a dihedral angle with that of the heterocycle of 70° for Z1 (N4-C3-C18-C19) and 65° for S1 (N4-C3-C16-N17). The torsion angles C3-C18-C19-N20 and C3-C16-N17-C19 equal 28° and 11° respectively. In the S2 conformation the oxygen atom of the amide function is in the plane of the heterocycle near the pyridine ring. The dihedral angle between the plane of the heterocycle and the plane of amide function is about 65°, and the torsion angle C3-C16-N17-C19 equals 108°.

These observations lead to the following hypothesis :

- The unique family of conformations (Z1) of zolpidem, which is similar to S1 observed for saripidem, could correspond to the appropriate conformation for ω_1 affinity,
- The second set of conformations observed with saripidem (S2) could correspond to the appropriate orientation of the C=O bond for ω_5 affinity,
- The amide function of zolpidem and saripidem can be considered as a proton acceptor site involved in the binding interaction with ω receptor subtypes.

A recent study in Molecular Mecanics shows that Z1, S1 and S2 are conformations which correspond to energy minimum states. On the other hand, there is no energetic barrier between S1 and S2 for saripidem, however zolpidem is unable to attain the S2 conformation[13].

Conclusion

These results allow us to propose, in a series of imidazopyridine derivatives, a conformational model for ω ligands that can explain their affinity either for ω_1 or for ω_1 and ω_5 receptor subtypes. Future studies will be directed to confirm this hypothesis with selective and non selective ω ligands belonging to other chemical series. We have also demontrated that the methodology used (NOESY NMR spectroscopy coupled to distance geometry) is a powerful technique to explore conformational space of non peptidic ligands of ω modulatory sites.

Materials and methods

Zolpidem and saripidem were obtained from the CNS Research Departement, Synthélabo Recherche.

All proton NMR spectra were performed on a Bruker ARX-400 spectrometer in methanol-d4 at 310 K (c=10 mM). 1D and COSYgradient NMR spectra were used to NMR spin system assignement. All 2D NMR spectra were recorded in the phase sensitive mode with mixing times of 200, 400, 600 and 800 ms for NOESY and 600 ms for ROESY (2048 data points in F2 and 512 in F1). Nuclear Overhauser Effect measurements (NOEs) were divided into strong, medium and weak categories based on cross-peak volume measurements. An identical pattern of dipolar effects is also observed in Rotating-frame Overhauser Effect Spectroscopy (ROESY). All DGII calculations were performed using the NMRchitect program package (version 2.3.5) of Biosym. NOEs at 600ms were translated into

interproton distances from reference data fixed by covalent geometry. An additional 0.5 Å was added to the upper distance limits for the NOEs involving methyl protons. DGII calculates a consistent matrix of distances bounds between all atoms, starting from the limited set of distances input to it. It then embeds a subset of these distances into cartesian space and refines the resultant structures, to improve the fit of the structure to the distance matrix. For each compound, 100 conformations were generated. Families **Z1** and **S2** were generated with a final error function value of 10^{-5} while a value of 10^{-3} was found for **S1**.

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