An Effort To Understand the Molecular Basis of Hypertension through the Study of Conformational Analysis of Losartan and Sarmesin Using a Combination of Nuclear Magnetic Resonance Spectroscopy and Theoretical Calculations

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Losartan is the first recently approved drug against hypertension disease that competes with the biological action of angiotensin II (AII) at the AT1 receptor. Its design was based on the mimicry of the C-terminal segment of AII. Due to the biological significance of Losartan, its structure elucidation and conformational properties are reported as determined by NMR spectroscopy and computational analysis. In addition, molecular modeling of the peptide Sarmesin [Sar^{1}Tyr(OMe)^{4}AII], a competitive antagonist of AII, was also developed based on NMR and computational analysis data. Sarmesin's C-terminal was used as a template for superimposition with specific molecular features of interest in the structure of Losartan such as the conformation of biphenyltetrazole, the *n*-butyl chain, and the orientation of hydroxymethylimidazole relative to the biphenyl template. The major conclusions derived from this study are the following: (a) Sarmesin, like the AII superagonist [Sar^1]AII, adopts a conformation which keeps in close proximity the key amino acids Sar^1 (or Arg^2)-Tyr(OMe)^4-His^6-Phe^8. (b) Losartan favors a low-energy conformation in which imidazole and tetrazole rings are placed in the opposite site relative to the spacer phenyl ring plane; the hydroxymethyl group is placed away from the spacer phenyl ring, the alkyl chain is oriented above the spacer phenyl ring, and the two phenyl rings deviate approximately 60° from being coplanar. (c) Overlay of the C-terminal region of Sarmesin with Losartan using equivalent groups revealed an excellent match. (d) Interestingly, the matching between enantiomeric structures of Losartan was not equivalent, proposing that the chirality of this molecule is significant in order to exert its biological activity. These findings open a new avenue for synthetic chemists to design and synthesize peptidomimetic drugs based on the C-terminal segment of the proposed model of Sarmesin. The new candidate drug molecules are not restricted to structurally resemble Losartan as the design is hitherto focused.

Introduction

Angiotensin II (AII) is an octapeptide hormone (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) that is well-known to be implicated in the cause of hypertension, a recently widespread and growing disease due to the stressful and diet life style of modern civilized societies.[1−4] As a result of its biological importance, AII has been extensively studied since its discovery several years ago.[5] These studies have included theoretical,[6−10] physicochemical,[11−14] and spectroscopic investigations.[15−21] In particular, the spectroscopic investigations led to models in which the AII carboxyl terminal region was used to design peptidomimetic analogues.[22] The synthetic effort for such analogues was rewarded with Losartan that recently entered the market, while several other molecules are in a clinical trial.[23]

Our laboratory has been engaged for more than a decade in the conformational analysis study of AII, its superagonist [Sar^1]AII, and structurally similar peptide agonists and antagonists. The accumulated experimental evidence for AII in DMSO supports a bioactive conformation characterized by a Tyr^4-Ile^5-His^6 bend, a major His^6-Pro^7 trans conformer, a cluster of the side chain aromatic rings of the triad key amino acids Tyr^4, His^6, Phe^8, and a charge relay system between Tyr^4 hydroxyl, His^6 imidazole, and Phe^8 carboxylate, analogous to that found in serine proteases, which appears to be responsible for its biological activity.[24−26] Arg^2 and Sar^1 which have been shown from SAR studies to contribute essentially to the biological activity of [Sar^1]-AII protrude also in the relay system. The charge relay conformation is supported by the synthesis and conformational study of the novel constrained AII cyclic analogue *c*-[Sar^1,Lys^3,Glu^5]AII, which is designed to have as a major molecular feature the integrity of the aromatic ring cluster.[27] This cyclic analogue was found to possess agonistic activity when tested in the rat

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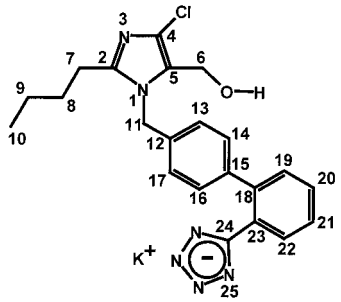
Chart 1



uterus assay and in anesthesized rabbits.[27,28] Structure−activity relationships demand the presence of Phe[8], His[6], Tyr[4], Arg[2], and Sar[1] for [Sar[1]]AII to possess biological activity. Therefore, it can be inferred that the ability of [Sar[1]]AII to form a ring cluster and consequently a charge relay system may be the key stereoelectronic molecular features to exert its biological activity.[26,27] Last year, a published article by Carpenter and coworkers showed that the octapeptide AII adopts a well-defined structure in a phospholipid environment which resembles the one proposed by our group. This points out that DMSO may simulate the amphoteric environment of lipid bilayers.[29]

In this research article computational analysis was performed on Sarmesin (Sar-Arg-Val-Tyr(OMe)-Ile-His-Pro-Phe) in order to develop a molecular model which may represent its bioactive conformation. Sarmesin ([Sar[1]Tyr(OMe)[4]]AII) is the prototype of type II fully competitive and reversible antagonists that differs structurally from the superagonist [Sar[1]]AII only on the Tyr[4] amino acid.[30] In particular, the phenolic hydroxyl group of Tyr[4] amino acid, which serves as the driving force in the formation of the proposed charge relay system, is methylated. Such methylation is anticipated to interrupt the charge relay system but not necessarily to break the proximity of the key amino acids Arg[2] or Sar[1]-Tyr(OMe)[4]-His[6]-Phe[8] in Sarmesin. In a previous publication, this proximity has been suggested on the basis of NMR spectroscopy in the receptor-simulating environment provided by DMSO.[31] These data were used as distance constraints to obtain information on the conformation of Sarmesin using calculations that simulate the solvent's environment ($\epsilon = 45$).

In addition, a conformational analysis of Losartan, the prototype peptidomimetic antagonist of AII, was sought. Its conformational properties were studied using a combination of 2D NOESY spectroscopy coupled with computational analysis. Computational analysis was also performed in a DMSO-simulating environment. Specific structural features of interest in Losartan were the conformation of biphenyltetrazole, the orientation of hydroxymethylimidazole relative to the biphenyl template, and the *n*-butyl chain conformation. The chemical structure of Losartan and its critical angles that define the above-mentioned stereoelectronic features are shown in Chart 1.

Sarmesin was used as a template for superimposition with Losartan. Such overlays are essential to define and compare the similarities and differences between the two competitive AII antagonists under study. They also test the validity of the proposed model and motivate synthetic chemists to design, synthesize, and test novel molecules in an attempt to develop drugs with better biological profile vis a vis Losartan.

Table 1. ^{1}H NMR (500.13 MHz) Chemical Shift Assignments of Losartan in DMSO Solution at 300.0 K

hydrogen	chemical shift (ppm)	no. of hydrogens	multiplicity
10	0.82	3	t
9	1.26	2	m
8	1.49	2	m
7	2.5	2	t
6	4.32	2	s
11	5.22	2	s
OH	5.29	1	br s
13/17	6.91	2	d
14/16	7.1	2	d
19	7.31	1	m
20/21	7.37	2	m
22	7.55	1	m

Results and Discussion

Hitherto, only succinct theoretical work has been performed to describe the conformational properties of AII prototype peptidomimetic antagonist Losartan using fragment analysis.[22] In these studies information on the conformation of *n*-butyl and hydroxymethyl groups of Losartan, which are key pharmacophoric groups for its biological activity, is lacking. In addition, all the attempted overlays of peptidomimetic antagonists with the AII proposed model by Fermandjian are achieved assuming an extended alkyl chain conformation oriented away from the biphenyl scaffold.[22,23]

Here are provided results using a combination of NMR and computational analysis of AII nonpeptide antagonist Losartan and the peptide AII antagonist Sarmesin. Such studies provide more precise and valuable information for their low-energy conformers because they are determined on the basis of the maximum agreement between experimental and theoretical results. Therefore, proper superimpositions of equivalent pharmacophore groups may be of biological relevance.

a. Structure Assignment of Losartan. Assignment of Losartan proton resonances was achieved from integral inspection of and chemical shifts of the ^{1}H NMR spectrum, from the 2D COSY spectrum, and from the observed through-space proton correlations in the 2D NOESY spectra. Table 1 shows ^{1}H chemical shift assignments of Losartan in DMSO-d_6 solution at 300.0 K.

b. Conformational Analysis of Losartan. (i) NMR Results. The through-space dipolar−dipolar correlations provide important information on drug conformations since their magnitudes are inversely proportional to the sixth power of the interproton distance in space. The most important observed NOEs are shown in Figure 1. The NOEs between protons 7 and 8 with protons 11 and between protons 7 and 9 with aromatic protons 13/17 are important because they establish vicinity between the *n*-butyl chain and the methylene attached to the spacer phenyl ring as well as the phenyl spacer. The strong NOE between protons 7 and 10 is informative for the preferred values that dihedral angle φ_3 can adopt. The observed strong upfield shifts of 7−10 proton resonances relative to 2-*n*-butyl-4-chloro-5-(hydroxymethyl)imidazole[32] provided evidence for the preferred orientation of the *n*-butyl chain. The strong NOEs

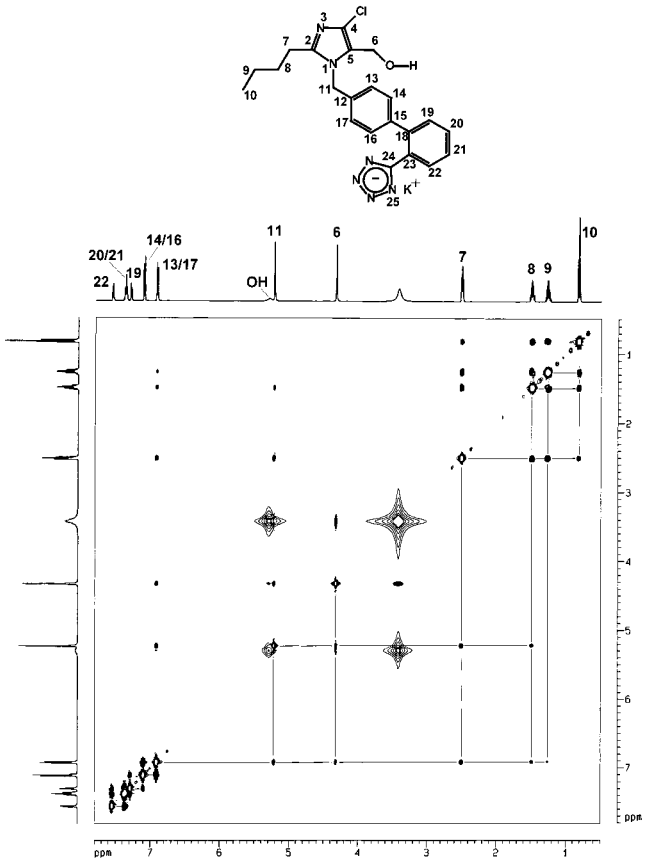


Figure 1. 2D phase-sensitive NOESY/TPPI spectrum of Losartan in DMSO at 300.0 K recorded on a Bruker Avance 500 MHz. The lines indicate the observed nuclear Overhauser enhancements due to through-space interproton couplings.

between protons 6 with protons 13/17 and the absence of any NOE for the hydroxyl proton are informative for the conformation of the hydroxymethyl group and the orientation of the imidazole heterocycle relative to the spacer phenyl ring. The NOE observed between protons 14/16 and 19 establishes the vicinity of the two phenyl rings. Experiments at higher temperatures showed an additional NOE between methyl protons 10 and aromatic proton 19. This is attributed to the higher flexibility of the methyl group in Losartan. This observed NOE is however significant because it imposes imidazole and tetrazole rings to have an anti orientation relative to the spacer phenyl ring.

(ii) Computational Analysis. The first step in the conformational analysis of Losartan was to construct a preliminary structure that fits the observed NOEs through manipulation of its critical dihedral angles. In particular, the alkyl chain of this conformer was oriented above the spacer phenyl ring in anti-gauche(−) conformation ($\varphi_1 = 104°$, $\varphi_2 = 179°$, $\varphi_3 = 72°$), while imidazole was imposed to an anti orientation relative to the tetrazole ring. This conformer was energy-minimized and was further subjected to an exhaustive grid scan and random sampling search as well as molecular dynamics combined with minimization procedures in order to explore its conformational properties. The most important features we sought to explore in the structure of Losartan were (i) the conformation of the biphenyl scaffold and the orientation of the tetrazole relative to the imidazole heterocycle; (ii) the orientation of the tetrazole relative to the terminal phenyl; (iii) the relative orientation of the imidazole and spacer phenyl rings; (iv) the orientation of the hydroxymethyl group; (v) the conformation and mobility of the alkyl chain.

The final aim was to detect other possible bioactive low-energy conformers[33] which would fit the NOE data and achieve the best superimposition with the proposed model of Sarmesin.

(iii) Conformation of the Biphenyl Ring. It has been proposed that the biphenyl template is the scaffold upon which the pharmacophoric groups are mounted. The critical torsion angle τ_2 describes the conformational behavior of the biphenyl template by rotation around the C18−C15 bond and defines the relative orientation of the tetrazole and imidazole rings.

The potential energy profile as a function of torsion angle τ_2 is shown in Figure 2. This profile shows that the planes of the two phenyl rings are twisted. Two syn orientations A and B ($\tau_2 = -113°, -67°$) and two anti orientations C and D ($\tau_2 = 62°, 118°$) of tetrazole relative to the imidazole ring are observed. The conformers that adopt a syn relative orientation between the two heterocyclic rings are almost isoenergic in comparison with the ones which are more consistent with the observed

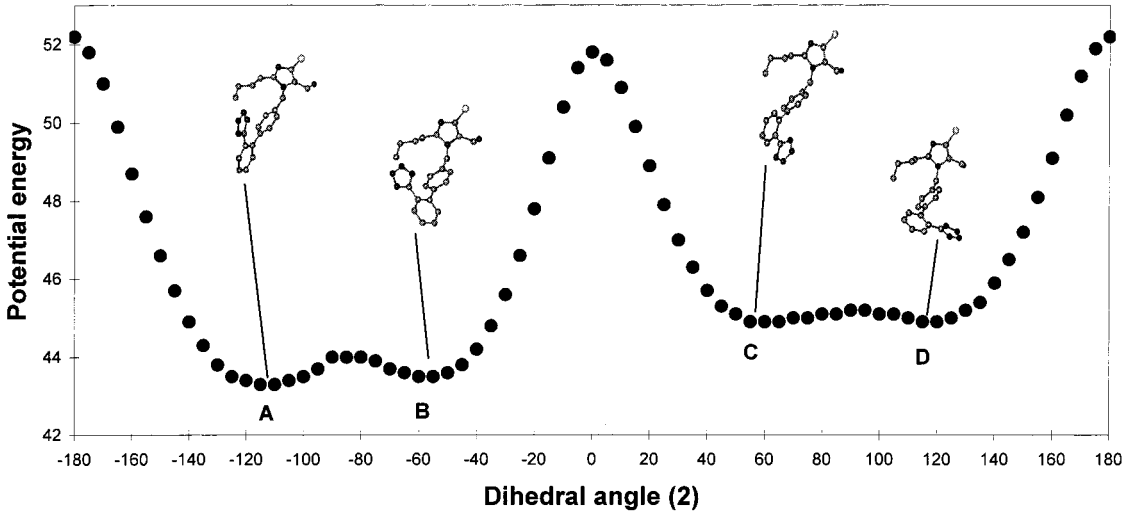


Figure 2. Conformational energy profile (kcal mol^{-1}) as a function of the C18−C15 dihedral angle (τ_2) in the biphenyl ring of Losartan obtained from a grid scan with increments of 5°.

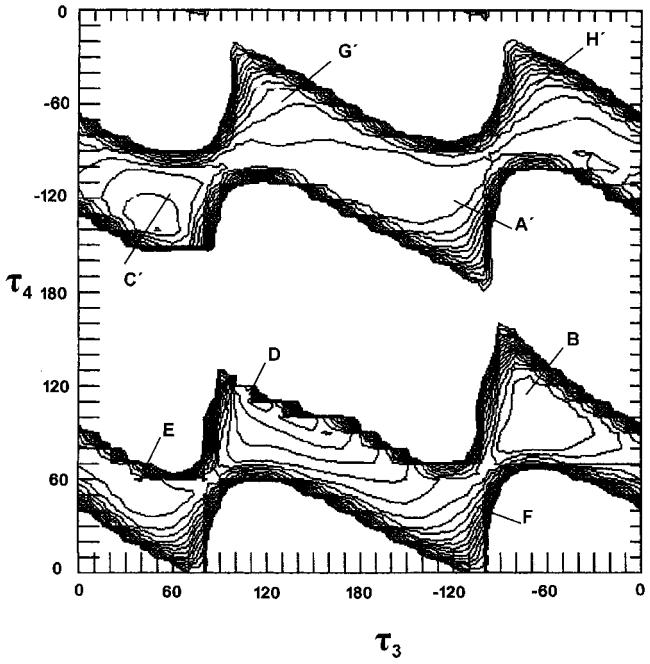


Figure 3. Contour plot as a function of τ_3 and τ_4 dihedral angles of Losartan obtained from a conformational grid search analysis with increments of 10°.

NOE data and adopt an anti relationship. The highest energy in the biphenyl system is observed when the two phenyl rings are coplanar.

(iv) Orientation of Tetrazole Relative to Terminal Phenyl. The negatively charged acidic group of tetrazole is a crucial pharmacophore of Losartan, and it has been suggested to interact with a positively charged site on receptor binding.[23] Thus, in the mapping of Losartan with Sarmesin, the relative orientation of the imidazole ring has received special attention because it matches the C-terminal part of the peptide. A second grid scan search was performed through rigid rotation around the C23−C24 bond to describe the potential energy changes as a function of the dihedral angle τ_1. The conformers **A**−**D** were used as starting structures. The obtained potential energy profiles were found to be similar for all searches. Two conformational minima were observed in all conformers located around 100° and −80°. In these conformers tetrazole is inclined relative to the terminal phenyl plane possibly to avoid peri interaction with the terminal phenyl ring and to favor possible $\pi^*-\pi^*$ interactions with the spacer phenyl ring. The energy barrier of rotation was found to be ∼1.5 kcal mol^{-1} suggesting a completely free rotation of the tetrazole ring about the C23−C24 bond.

(v) Relative Orientation of Imidazole and Spacer Phenyl Rings. Conformers **A**−**D** were then used as starting structures for two-bond rotary search through grid scan conformational analysis on τ_3 and τ_4 torsion angles. Dihedral angle τ_3 defines the relative orientation of imidazole to the spacer phenyl ring, while τ_4 dihedral angle describes the transposition of *n*-butyl and hydroxymethyl groups of the imidazole ring. The four conformers **A**−**D** gave similar energy contour plots, and therefore only the representative conformer **D** is plotted (Figure 3). The contour levels shown in Figure 3 represent the lowest energy levels and those which are higher in energy by as much as 6 kcal/mol. We assumed that these levels may best simulate the bioactive conformations of Losartan. While the details of the analysis of each level are not of special interest, they define the relative energy barriers of the molecule to adopt possible bioactive conformations. Each of the two distinct areas of contour energy levels spread all over possible τ_3 dihedral angles observed in Figure 3 include four low-energy regions. From these regions the corresponding minima **B, D, E, F** and **A′, C′, G′, H′** were obtained as described in Table 2. Structures **C′** and **A′** are enantiomeric to **C** and **A**, respectively. A comparison of the dihedral angles of the two enantiomeric conformers shows that they have $\varphi_1-\varphi_3$ and $\tau_4-\tau_6$ angles equal and opposite and τ_1-τ_3 dihedral angles complementary. Such symmetry in their dihedral angles is a result of their mirror image relationship. From the obtained conformers only **A′, B, C′**, and **D** can fit the NMR results. The performance of all four grid scans resulted in the conformers **A**−**H** and their enantiomeric structures **A′**−**H′**. From these conformers only **A**−**D** and **A′**−**D′** fit the NMR results.

Table 2. Conformational Descriptors for τ_3 and τ_4 Torsion Angles and Relative Energies of Conformational Minima Obtained from Grid Scan Conformational Analysis

conformers	τ_3 (deg)	τ_4 (deg)	relative energy (kcal mol^{-1})
E	40	62	45.03
D	130	111	44.34
F	−137	60	45.27
B	−64	104	42.42
A′	−132	−112	42.66
C′	50	−110	44.29
G′	123	−68	45.29
H′	−42	−76	45.02

According to a previous fragment analysis for *N*-(phenylmethyl)imidazole,[34,35] two possible low-energy orientations were proposed and designated as H-1 and H-2. In the H-1 conformer, the spacer phenyl plane is nearly parallel to the N1−C5 bond, while in the H-2 conformer, it is parallel to the N1−C2 bond. In these studies the H-1 conformer was found to be 0.1 kcal mol^{-1} lower in energy than H-2.

Our results from grid scan conformational analysis on torsion angles τ_3 and τ_4 also identified the presence of these conformers. Specifically, conformers **E**−**H** and **E′**−**H′** with an H-2 orientation and a syn or anti relative orienation of imidazole to the tetrazole ring are higher by ≈1−3 kcal mol^{-1} than conformers **A**−**D** and **A′**−**D′** that adopt the H-1 orientation. This is attributed to their hydrophobic interactions between the carbon chain and biphenyl scaffold present in the H-1 but not H-2 orientation. Furthermore, the H-2 orientation is not consistent with the observed NOE between methyl 10 and proton 19 and the upfield shifts observed in the *n*-butyl chain protons.

(vi) Orientation of Hydroxymethyl Group. It is generally accepted that the Losartan hydroxymethyl group plays the vital role of hydrogen bonding at the AT1 receptor site. To analyze the conformational properties of the hydroxymethyl group, the grid search on the torsion angles τ_5 and τ_6 was performed for all derived low-energy structures. The obtained contour plots for all starting conformers were found identical. Therefore, only a representative contour plot for conformer **D** is shown in Figure 4. In general, the conformational

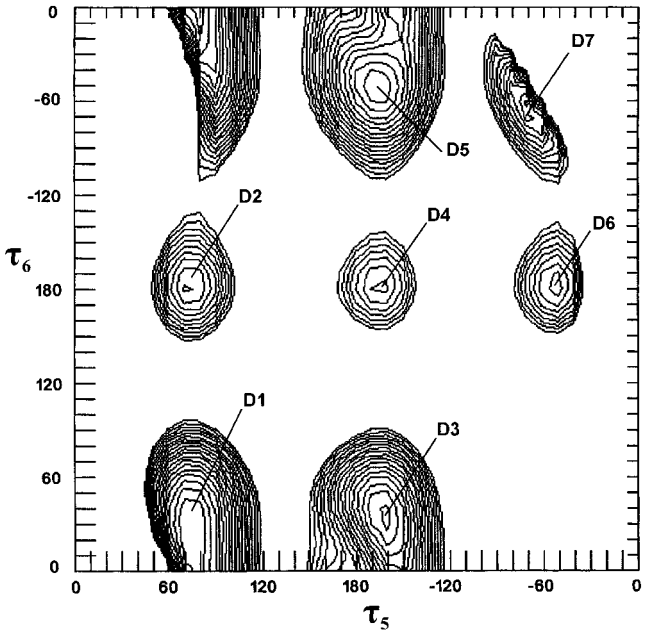


Figure 4. Contour plot as a function of τ_5 and τ_6 dihedral angles of Losartan obtained from a conformational grid search analysis with increments of 10°.

Table 3. Conformational Descriptors for τ_5 and τ_6 Torsion Angles and Relative Energies of Conformational Minima Obtained by Grid Scan Conformational Analysis around τ_5 and τ_6 Torsion Angles Using **D** Conformer as the Starting Structure[a]

conformers	τ_5 (deg)	τ_6 (deg)	H6−H13 (Å)	OH−H13 (Å)	relative energy (kcal mol^{-1})
D1	71	34	2.52	3.01	42.60
D2	90	−179	2.51	3.20	44.41
D3	−158	37	2.45	4.38	43.02
D4	−160	−178	2.27	4.43	46.12
D5	−165	−51	2.46	4.52	44.34
D6	−70	179	3.99	3.26	44.41
D7	−78	−138	4.0	3.40	42.80

[a] The distances between H6−H13 and OH−H13 protons were calculated in order to identify the NOE consistent conformers.

mobility of the hydroxymethyl group is restricted by its steric interactions with protons 11 and spacer phenyl proton 13. In Table 3 are shown the low-energy conformers obtained from this grid scan search.

The lowest energy conformers in the energy profile of hydroxymethyl group correspond to the ones with eclipsed C6−H and C4−C5 bonds (**D(1−2)**, **D(6−7)** conformers) and with eclipsed C−O and C4−C5 bonds (**D(3−5)** conformers). In **D(1−2)** and **D(6−7)** conformers the hydroxyl group is oriented away and close to the spacer phenyl ring. From the 2D NOESY spectra, the dipolar correlation between 6 and 13 protons is informative for the hydroxymethyl conformation suggesting that **D(1−5)** conformations are preferable. In addition, the absence of any NOE between O-H and H-13/17 identified **D(3−5)** conformers as the most consistent with NMR results. Correspondingly, conformers **A(3−5)−C(3−5)** and **A′(3−5)−C′(3−5)**, obtained from **A−C** conformers, are consistent with the through-space correlations observed for the hydroxymethyl group.

Hydrogen bonding between hydroxyl and tetrazole can be observed only when a syn orientation of tetrazole with the imidazole ring is assumed. Besides, such an orientation should result in a space correlation between hydroxyl and spacer phenyl protons which was not observed experimentally. For the above two reasons such a hydrogen bonding is unlikely to be favored.

(vii) Monte Carlo Random Sampling. A Monte Carlo conformational analysis was performed through φ_1, φ_2, and φ_3 dihedral angles that define the conformation of the *n*-butyl chain. In the lowest energy structures the carbon chain is oriented above the phenyl ring plane ($\varphi_1 = 90-135°$). In these structures all nine low-energy conformers with φ_2 and φ_3 adopting either anti or gauche conformations were obtained. However, taking into account: (i) the observed NOE between protons 7 and 9 and 13/17, (ii) the observed NOE between protons 7 and 10 which suggests a gauche conformation around ϕ_3 dihedral angle, and (iii) the dipolar correlation between 10 and 14/16 or 19 protons, the dihedral angles $\varphi_2-\varphi_3$ are imposed to most probably adopt an anti-gauche(−) or gauche(+)-gauche(−) conformation.

NOE data combined with the observed paramagnetic shielding of protons 7−9 and 10 by the spacer phenyl ring suggest that the carbon chain is oriented above the spacer phenyl ring. Indeed, the lowest energy structures resulting from systematic search, random sampling, and molecular dynamics conformational analysis confirmed that the *n*-butyl chain is oriented above the spacer phenyl ring.

The conformational descriptors for the most important conformers derived from the above analysis are shown in Table 4. Among them, representative conformers **A3−D3** or **A3′−D3′** best account for the NOE data and theoretical calculations. Figure 5 shows **A3−C3** conformers, and Figure 6 shows the enantiomeric pair of the conformers **D3−D3′**.

c. Conformational Analysis of Sarmesin. The SAR work for Sarmesin has identified residues 1, 2, 4, 6, and 8 to be critical for biological activity and 3, 5,

Table 4. Conformational Descriptors for the Most Important Derived Conformers Obtained from the Combination of Experimental and Computational Analysis

conformers	relative orientation of tetrazole to imidazole ring	τ_1 (deg)	τ_2 (deg)	τ_3 (deg)	τ_4 (deg)	τ_5 (deg)	τ_6 (deg)	φ_1 (deg)	φ_2 (deg)	φ_3 (deg)	relative energy (kcal mol^{-1})
starting structure	anti	100	118	130	111	−165	−51	104	179	72	44.34
A3	syn	93	−113	−48	112	−158	37	106	180	73	41.32
A3′	syn	87	−67	−132	−112	158	−37	−106	−180	−73	41.32
B3	syn	111	−67	−64	104	−158	37	106	178	72	41.08
B3′	syn	69	−113	−114	−104	158	−37	−106	−178	−72	41.08
C3	anti	79	60	130	110	−158	37	103	179	71	42.95
C3′	anti	121	120	50	−110	158	−37	−103	−179	−71	42.95
D3	anti	100	118	130	111	−158	37	104	179	72	43.0
D3′	anti	80	62	50	−111	158	−37	−104	−179	−72	43.0

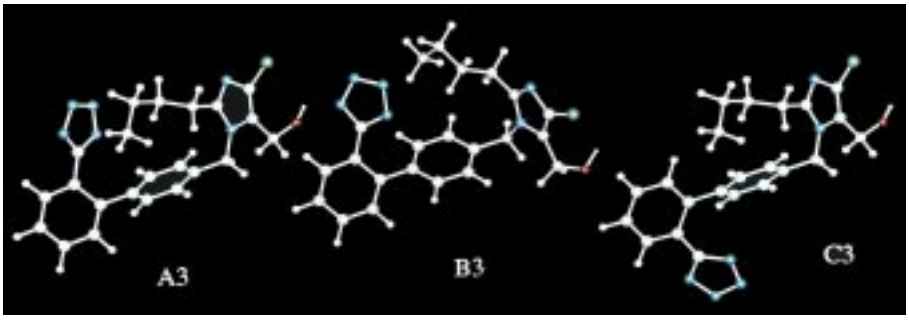


Figure 5. Proposed bioactive conformers **A3**−**C3** of Losartan used for superimposition with Sarmesin as they are depicted from exhaustive grid scan search, Monte Carlo, and molecular dynamics experiments.

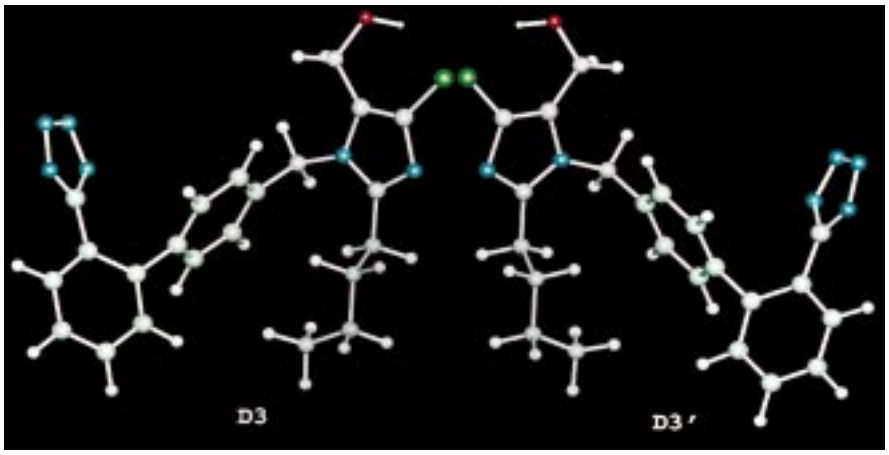


Figure 6. Proposed bioactive enantiomeric conformers **D3** and **D3′**.

and 7 for proper backbone orientation. Our previous NMR data[31] coupled with presently performed computational analysis suggest that Sarmesin can adopt a low-energy conformation characterized by a trans His^6-Pro^7 bond, a Tyr(OMe)^4-Ile^5-His^6 bend, and a cluster of side chain aromatic rings of key amino acids Tyr(OMe)^4, His^6, and Phe^8 (Figure 7). A spatial proximity of His^6 with Sar^1 indicates a turn of Sarmesin N-terminal region. Such a conformation is similar to that proposed for AII or [Sar^1]AII. Unlike [Sar^1]AII, Sarmesin lacks the proposed AII charge relay system because of the methylation of the phenolic hydroxyl group in the Tyr^4 amino acid. The Tyr^4 phenolic hydroxyl group is the driving force for the charge relay system formation in [Sar^1]AII because it can transfer its proton through His^6 imidazole to Phe^8 carboxylic group. In the Sarmesin model, hydrogen bonding between Phe^8 carboxylate and His^6 imidazole N-H is possible which may enhance the stability of the aromatic ring cluster formation. The stereoelectronic requirements for type II antagonist activities are difficult to assess by studying the conformation of only one peptide. It is, however, of importance that the antagonist Sarmesin keeps in close proximity the tetrad of the amino acid Arg^2 or Sar^1-Tyr^4-His^6-Phe^8 as in our proposed model of [Sar^1]AII.[26,28]

d. Superimposition of Losartan with Sarmesin. Losartan was superimposed with Sarmesin in order to reveal the similarities of its C-terminal segment with specific structural features of Losartan such as the conformation of biphenyltetrazole, the *n*-butyl chain, and the orientation of hydroxymethylimidazole relative to the biphenyl template. From the different possible overlays, the best superimposition between the two molecules was achieved when the following groups were matched: (i) Losartan's hydroxymethylimidazole with Sarmesin's imidazole of His^6, (ii) Losartan's *n*-butyl chain with Sarmesin's Ile^5 carbon chain, (iii) Losartan's tetrazole with Sarmesin's isosteric carboxylate of Phe^8, and (iv) Losartan's spacer phenyl ring with Sarmesin's pyrrolidine group of Pro^7. Interestingly, Losartan mimics the γ-turn formed around Pro^7 in Sarmesin.

The candidate structures of Losartan tested for superimposition were **A(3**−**5)**−**D(3**−**5)** and their enantiomeric conformations **A′(3**−**5)**−**D′(3**−**5)**. The best fit was achieved by conformer **D(3′**−**5)** (Figure 8). Interestingly, the match between conformational enantiomers of Losartan was not equivalent, proposing the significance of the chirality for the molecule to exert its biological activity.

Due to the conformational similarities of the superagonist [Sar^1]AII with Sarmesin,[26] it is anticipated that Losartan also fits equally well in the agonist molecule. This excellent superimposition of Losartan with both [Sar^1]AII and Sarmesin simply expresses the possible structural similarities between an agonist and antagonist peptides which are also reflected in the peptidomimetic drugs. For example, the structurally resembled molecules of L-162,313 and L-162,389 have an agonist versus an antagonist relationship.[23] The obtained results are clearly better than our previously reported preliminary results related to the superimposition of a low-energy conformation of Losartan derived solely from pure theoretical calculations with AII. In addition, the present results involve a more appropriate comparison of the conformational properties of the C-terminal of Sarmesin with Losartan. Other superimposition studies referred to in the literature are related to the superimposition of a low-energy conformation of Losartan

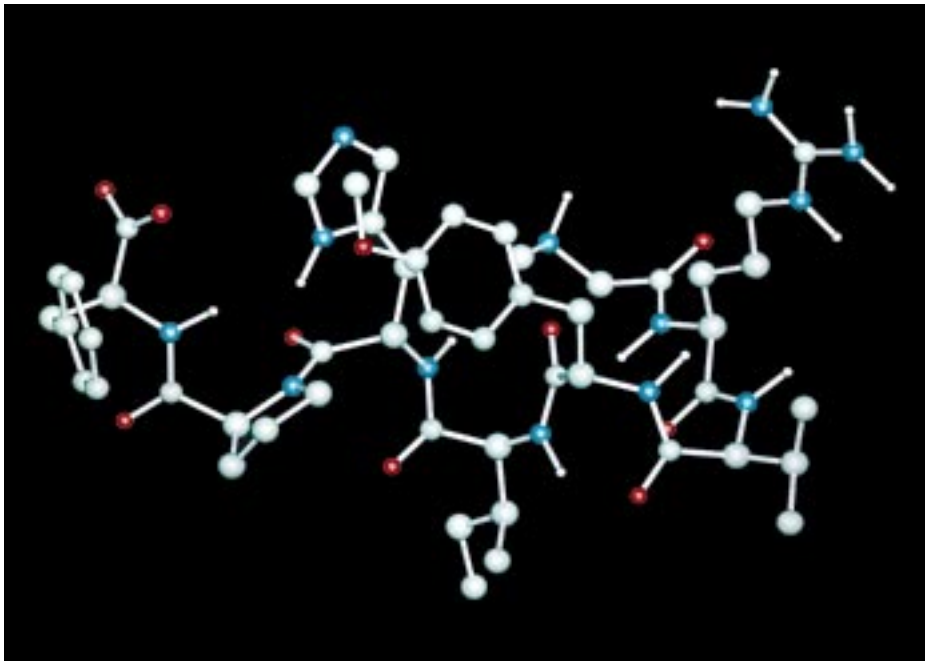


Figure 7. Low-energy conformer of Sarmesin derived from a combination of NMR data and theoretical calculations. The model is characterized by a clustering of the key tetrad amino acids Sar[1]-Tyr(OMe)[4]-His[6]-Phe[8].

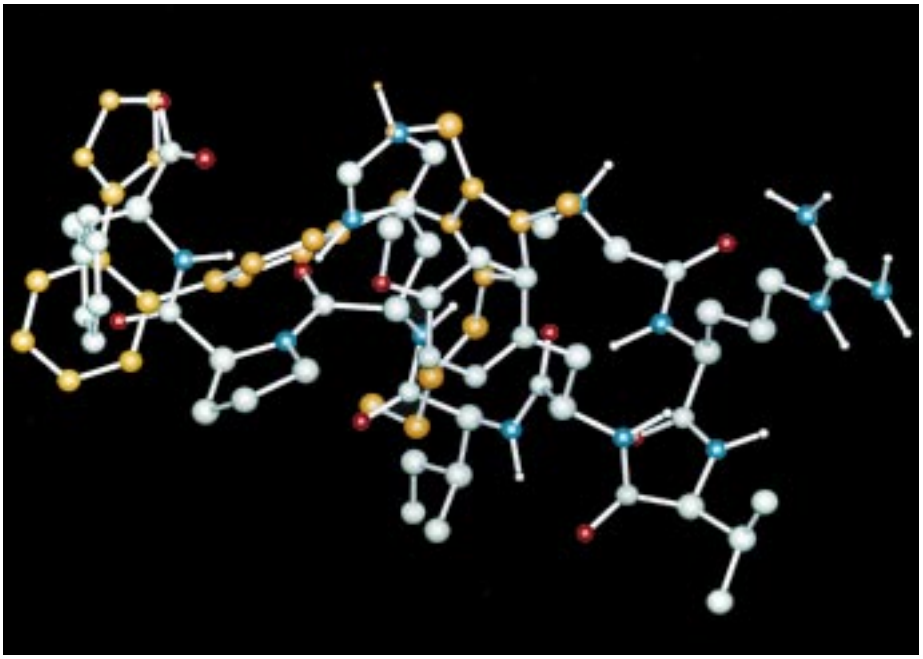


Figure 8. Superimposition of Sarmesin with a proposed bioactive conformer **D3′** of Losartan.

derived solely from purely theoretical calculations with AII or that of Valsartan with [Sar[1],Ile[8]]AII.[36−38] The use of [Sar[1],Ile[8]]AII as a template is not considered a good choice since previous conformational analysis has shown that this peptide antagonist of AII has profound stereoelectronic differences from those observed in AII or Sar[1][AII].[39]

Conclusions

This study is focused on the conformational analysis of Losartan as determined by a combination of NMR spectroscopy and computational analysis. Conformational analysis was also achieved for the peptide antagonist Sarmesin which structurally differs from the superagonist [Sar[1]]AII only in the methylation of the phenolic hydroxyl group. The major conformational characteristics of the Losartan model can be summarized as follows: (i) the biphenyltetrazole ring system has two phenyl rings deviating by approximately 60° from the coplanar position, while the tetrazole is inclined relative to the adjacent phenyl ring; (ii) imidazole and tetrazole rings are placed in the opposite site relative to the spacer phenyl ring; (iii) the *n*-butyl chain is oriented above the spacer phenyl ring in order to achieve maximum hydrophobic interactions; (iv) the hydroxymethyl moiety is restrained to orient its hydroxyl group away from spacer phenyl ring. The constructed molecular modeling of Sarmesin revealed close

proximity between the four amino acids Sar[1]-Tyr[4]-His[6]-Phe[8] as in the [Sar[1]]AII model.[26,27] However, [Sar[1]]AII possesses a phenolic hydroxyl group which additionally triggers a charge relay system. Sarmesin is used as a template for superimposition with Losartan. Such superimposition gave the stereoelectronic similarities of Losartan important structural features with the C-terminal segment of Sarmesin. The superimposition showed an excellent fit between the equivalent groups of Sarmesin's amino acids Ile[5]-His[6]-Pro[7]-Phe[8]-COO^- with the corresponding alkyl chain, hydroxymethyl imidazole, spacer phenyl ring, and phenyltetrazole groups of Losartan. Additionally, the chirality of the Losartan structure can be important in the way of its interaction with AT1 reseptor binding site. The consequences of such an excellent match do not escape our notice. New molecules can be synthesized based on the proposed model of Sarmesin which do not necessarily resemble the structure of Losartan. Our laboratory has designed and is in the process to synthesize a new class of molecules based solely on the new results. It will be shown soon if the drug design will lead successfully to new drugs with a better biological profile than that of Losartan.

Experimental Section

Materials. Losartan was kindly donated by Merck Research Laboratories. The sample for high-resolution NMR experiments was dissolved in deuterated DMSO (5 mg in 0.4 mL) with TMS used as the chemical shift reference.

NMR Spectroscopy. The high-resolution NMR experiments were performed mainly on a Bruker Avance 500-MHz spectrometer equipped with a 5-mm Inverse Triple Resonance probe with *z*-gradient. All data were collected using pulse sequence and routines provided in the Bruker library of pulse programs. Data processing was performed using Bruker software packages.

Two-dimensional $^1H-^1H$ chemical shift correlation spectrum (COSYGS) was carried out in order to assign proton resonances of Losartan. Two-dimensional phase-sensitive time proportional phase incrementation (TPPI) $^1H-^1H$ nuclear Overhauser enhancement (NOESYTP) was performed to aid the assignment of Losartan and mainly to study its conformational properties. The optimum mixing time in the NOESYTP experiment was found to be 1.0 s by performing a T1 experiment.

Molecular Modeling. Computer calculations[33] were performed on a Silicon Graphics 4D/35 using QUANTA software package. Molecular mechanics calculations were carried out using the CHARMm force field. The various torsion angles of a built three-dimensional structure of Losartan were suitably manipulated and optimized first with steepest descents and then with Newton−Raphson minimization algorithms, using an energy gradient tolerance of 0.01 kcal mol^{-1} $Å^{-1}$, to reach a local minimum which agrees with the observed NOEs. This applied methodology aimed to allow significant freedom to the molecule under study and to perform a detailed theoretical study. To find the preferred torsion angles that correspond to the lowest energy conformers and energy barriers of Losartan, bond rotatory searches (systematic grid scan searches) were used. Intervals of 5° were applied for single bond rotation and 10° for two-bond rotation. During bond rotation searches, the predetermined torsion angle remained constant while minimization using 200 steps of conjugate gradient algorithm was applied to relax the whole molecule. The lowest energy conformers found were further minimized to reach local minima and used as starting structures for the next grid scan search.

The critical *n*-butyl chain dihedrals $\phi_1-\phi_3$ of the derived low-energy conformers were then subjected to Monte Carlo analysis using an angle window of 60° and a number of 216 samples. Minimization using 200 steps of conjugate gradient algorithm was applied during analysis. The several low-energy conformers found were further minimized to reach local minima.

Molecular dynamics simulations on Losartan[33,40] were carried out at 1000.0 K with time steps of 1 fs for 300 ps using an output frequency of 1 ps to sample 300 frames of conformers. After cluster analysis using a torsion angle threshold of 85°, 12 family structures were selected. The obtained low-energy conformers of each cluster were then further minimized. These conformers were compared and agreed with those obtained using a combination of exhaustive grid scan search, Monte Carlo analysis, and molecular mechanics calculations.

The model of Sarmesin was built by modification of the [Sar[1]]AII proposed model.[26] To find a low-energy conformation of Sarmesin, a short molecular dynamics under imposed distance constraints derived from NOE data was applied using the same conditions as in Losartan.[33] The obtained lowest energy structure was then further minimized without imposing constraints to reach a local minimum using steepest descents and modified Newton−Raphson algorithms as well as convergence criterion of 0.01 kcal mol^{-1} $Å^{-1}$. The resulted Sarmesin structure was used for superimposition with Losartan to achieve the optimum overlay. Since the overlay between the two molecules was excellent, only rigid body fit to the target superimposition was applied, which allows translation and rotation of the molecules.

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