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Conformational and biological studies for a pair of novel synthetic AT_1 antagonists: stereoelectronic requirements for antihypertensive efficacy

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Abstract

One of the major systems which interferes with the disease of hypertension, is the Renin Angiotensin Aldosterone System (RAS). The octapeptide hormone angiotensin II is the active product of RAS which causes vasoconstriction when binds to the AT_1 receptor. In the last years, there has been a development of drugs which block the Angiotensin II from binding the AT_1 receptor and are called AT_1 antagonists. In an effort to comprehend their stereoelectronic features, a study has been initiated to compare the conformational properties of drugs already marketed for the treatment of hypertension and others which are designed and synthesized in our laboratory possessing structural characteristics necessary for antihypertensive activity. In this study, two synthetic non-peptide AT_1 antagonists, are structurally elucidated and their conformational properties and bioactivity are compared to the prototype and first approved drug of this category in the market; losartan (trade name: COZAAR).

Keywords: Antihypertensives; NMR; AT_1 antagonists; Molecular modeling; Losartan

1. Introduction

The Renin-Angiotensin-Aldosterone System (RAS) is one of the most important regulators of the blood pressure. Several types of antihypertensives were designed to block the RAS system in different stages. Renin is the first enzyme in the RAS cascade and has been targeted for drug development. The synthetic molecules were very effective in animal testing but lacked duration of action and oral bioavailability.

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Another generation of synthetic drugs, approved for the market during the last 15 years, are the angiotensin-converting-enzyme (ACE) inhibitors. The ACE inhibitors constitute an important advance in cardiovascular medicine and are still widely used for the treatment of hypertension and lowering the morbidity and mortality in congestive heart failure [1,2]. The major disadvantage of these drugs is that together with the blockage of the angiotensin II synthesis they prevent the metabolism of other important peptides such as bradykinin, which is the contributor to two of the most characteristic side effects of ACE inhibition: persistent cough and angioedema [3–6]. The cough is thought to be due to kinin accumulation, possibly through the generation of thromboxane A_2 [7,8]. The pathogenesis of angioedema is still unclear, but increased levels of bradykinin have been observed during acute attacks [3]. Apart from the ACE which is responsible for the production of angiotensin II in the RAS system, there are alternative enzymatic pathways which can also convert angiotensin I to II. However, how much these other enzymes contribute to the generation of angiotensin II is not clear yet [9].

These reasons led the scientists to develop more specific drugs than ACE inhibitors. AT_1 antagonists constitute the most recent class of drugs which act in the RAS [10]. They block the AT_1 receptor in a competitive or insurmountable way so that angiotensin II cannot bind on the receptor and cause vasoconstriction [11]. These antagonists are designed to mimic the C-terminal segment of angiotensin II. The first available AT_1 blocker approved in 1995 was losartan (COZAAR). When absorbed, losartan is metabolized in the liver to EXP3174, which is the active metabolite responsible for most of the drug's actions. Many other antagonists, derivatives of losartan (such as irbesartan, telmisartan, candesartan, valsartan and tasosartan) appeared for the treatment of hypertension in the last 5 years.

Our work is focused on examining the conformations of these drugs in different solvent environments, their interactions with biological membranes and finally their binding properties to the AT_1 receptor. Comparative studies with drugs possessing different antihypertensive efficacy give clues about the relation of conformation and bioactivity [12,13].

In the present study two angiotensin II antagonists (BZI8 and V12), synthesized in the Laboratory of Organic Chemistry of the University of Patras are structurally elucidated and their conformational properties are compared to those of losartan (Fig. 1). Their biological response is also tested on rabbits and compared to the corresponding response of the prototype drug losartan.

The structural features which determine the pharmacophoric segments of losartan have been examined in a previous study [14]. These are: (a) conformation of the biphenyl tetrazole moiety; (b) orientation of the hydroxymethylimidazole relative to the biphenyl template; and (c) the butyl chain conformation and orientation toward the biphenyl group. Losartan was superimposed with C-terminal region of AT_1 antagonist sarmesin since its design was based on its similarity with side groups that compose the C-terminal part of angiotensin II. Superimposition of losartan with AT_1 antagonist sarmesin showed that: (i) losartan's hydroxymethylimidazole matched with sarmesin's imidazole of His^6; (ii) losartan's butyl chain was in a spatial proximity with butyl chain of sarmesin's Ile^5 carbon chain; (iii) losartan's tetrazole was in close vicinity with sarmesin's isosteric carboxylate of Phe^8 and (iv) losartan's spacer phenyl ring matched with Sarmesin's pyrrolidine group of Pro^7. Interestingly, losartan showed to mimic the γ-turn formed around Pro^7 in sarmesin.

V12 is an almost equipotent molecule with losartan. The positions of butyl alkyl chain and hydroxymethyl substituents in the imidazole ring are reversed in comparison with losartan. The imidazole ring of V12 devoid of chlorine. BZI8 has more significant structural changes when it is compared with losartan. Imidazole ring at positions 4 and 5 is fused with a phenyl ring. This study aims to examine the significance in the conformational analysis of the orientation of: (a) the hydroxymethyl group; and (b) to give some evidence on the role of the substituent chlorine atom. The tetrazole group of the two molecules under study is substituted with a benzyl ring. This

substitution aimed to increase the lipophilicity of the molecules which may in turn lead to better metabolic properties.

2. Materials and methods

2.1. Materials

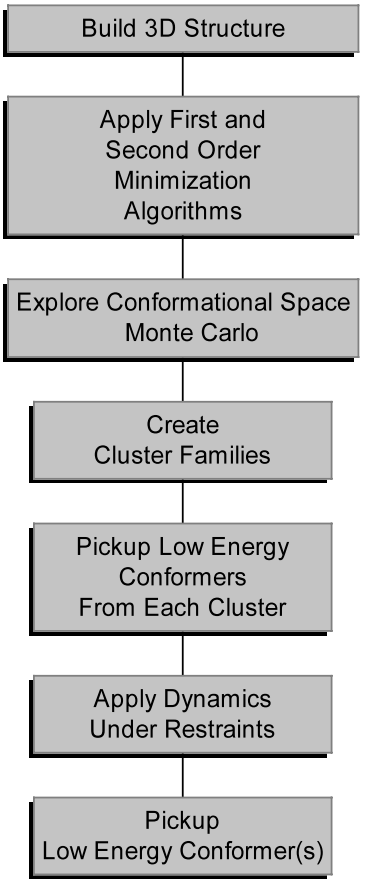
DMSO-d_6 and ultra precision NMR tubes Wilmad 535–5 mm (SPINTEC ROTOTEC) were used for the NMR experiments.

2.2. Nuclear magnetic resonance spectroscopy

BZI8 and V12 molecules were dissolved in DMSO and a series of experiments were performed using Varian INOVA 600 MHz and Bruker AC 300 instruments at 298 K. All data were collected using pulse sequences and phase-cycling routines provided in the Bruker and Varian libraries of pulse programs. The DQF-COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments were performed with gradients [15–17]. The ROESY experiment was recorded using standard pulse sequence in the phase-sensitive mode and was measured at 150 ms mixing time [18]. The ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra were run with ^{13}C spectral width 20 000 and 30 000 Hz, respectively.

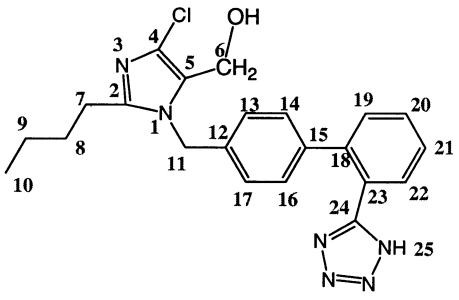
2.3. Molecular dynamics

Computer calculations were performed on a Silicon Graphics using QUANTA software purchased from Molecular Simulation Incorporated (MSI). BZI8 and V12 were first minimized and then subjected to Molecular Dynamics. The dielectric constant (e) set in minimization and Molecular Dynamics was 45. A time step of 1 fs was employed for the MD simulation. The simulation protocol consisted of two minimization cycles (steepest descent and conjugate gradients), first with the solute fixed and then with all the atoms allowed to move freely. The NMR derived distance restraints with a force constant of 10 Kcal mol^{-1} $Å^{-1}$ were applied during the complete simulation. A Monte Carlo conformational search without constraints aided and preceded Molecular Dynamics experiment under constraints in an attempt to expand the conformational space and increase the probability to generate lower energy conformers which agree with the ROE data. Chart 1 includes the methodology used in our study.

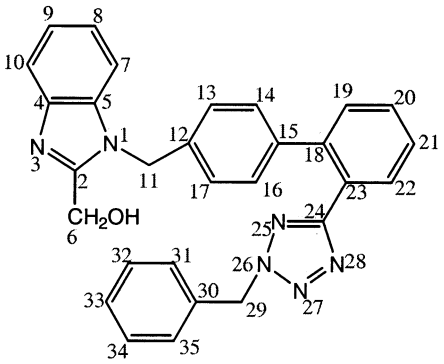


2.4. Biological response

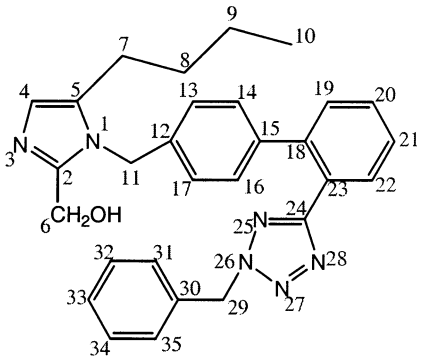
Adult normotensive male New Zealand White rabbits weighing between 2.5 and 3.3 kg were used in the study. The animals were anesthetized by pentobarbitone (30 mg kg^{-1}), intubated and mechanically ventilated with 100% oxygen using a respirator for small animals. The used tidal volume was 15 ml and the rate was adjusted to keep blood gases within the normal range. Two polyethylene catheters were inserted, one in the



LOSARTAN



BZI 8



V 12

Fig. 1. Chemical structures of the AT_1 antagonists losartan, BZI8 and V12.

carotid artery for continuous blood pressure monitoring via a transducer attached to a multichannel recorder and the other in the jugular vein for the administrations of the solutions made by diluting angiotensin II and its antagonists losartan, V12 and BZI8 in 5% dextrose at final concentration of 5 and 50 μg ml^{-1}, respectively. Each dose was given in random sequence after a wash-out period of 30 min.

The hypertensive responses to angiotensin II infusion at 1, 2 or 3 μg min^{-1} were recorded continuously. Angiotensin II-dependent hypertension was then induced and maintained by a constant infusion of angiotensin II via a syringe pump at a rate of 0.2 ml min^{-1} (1 μg min^{-1}). Five minutes after the establishment of hypertension, boluses of different doses of losartan (0.5 mg, 1 mg and 1 mg), BZI8 (1 mg, 1.5 mg) and V12 (1 mg, 2 mg) were given via an ear vein in random sequence and the drop in mean blood pressure was recorded.

3. Results and discussion

3.1. Structure elucidation of BZI8 and V12

Fig. 2 depicts the ^1H NMR spectra of BZI8 and V12 in DMSO. This solvent was used to simulate the receptor environment [14]. Observed peaks are referenced to TMS. The assignment of the peaks is shown on the top of the spectrum. The proton

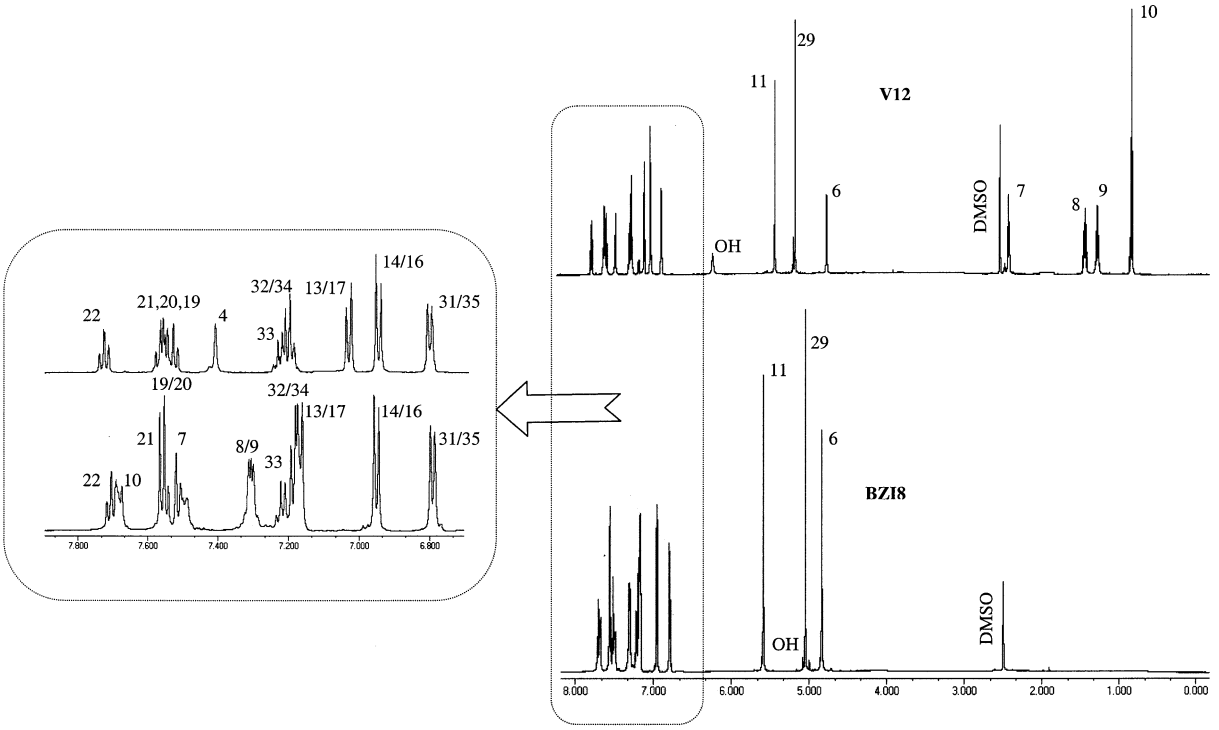


Fig. 2. ^{1}H-NMR spectra of V12 (top) and BZI8 (bottom), recorded on a Varian INOVA 600 MHz spectrometer at a temperature of 27 °C. Inset shows an expansion of the aromatic ring.

chemical shifts are assigned following standard procedures and using homonuclear DQF-COSY and ROESY in combination with ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments (Table 1).

3.2. Conformational properties of BZI8 and V12

The ROEs which govern the conformational properties of the two molecules are shown in Table 2. BZI8 has shown a critical ROE between H7 and H29, which suggested a clustering between the benzyl and the phenyl rings. This proximity is due to π*–π* interactions. The butyl chain of V12 appears to be in a close special vicinity with the ring of the biphenyl system as it is observed with losartan. Likewise, the two rings of the biphenyl system for the two molecules are not linear to each other which was also observed with the antihypertensive drugs, losartan, eprosartan and irbesartan [12,14].

3.3. Conformational search

Random conformers were generated by Monte Carlo method for BZI8 and V12 molecules and were divided into different classes after minimization. Some of the lowest energy conformers are shown in Fig. 3 and their critical dihedral angles in Table 3. The low energy conformers were subjected to molecular dynamics experiments, using distance constraints from ROE data. The conformers for both molecules consistent with experimental ROE data, are opposed in Fig. 4 and their critical dihedral angles are shown in Table 4.

3.4. Superimposition of BZI8 with V12

The best overlapping of the two studied molecules is depicted in Fig. 5. The superimposition involved matching of corresponding nitrogen (1, 3) and carbon (2, 4, 5) atoms of the imidazole ring. This overlay resulted in a nice match of their

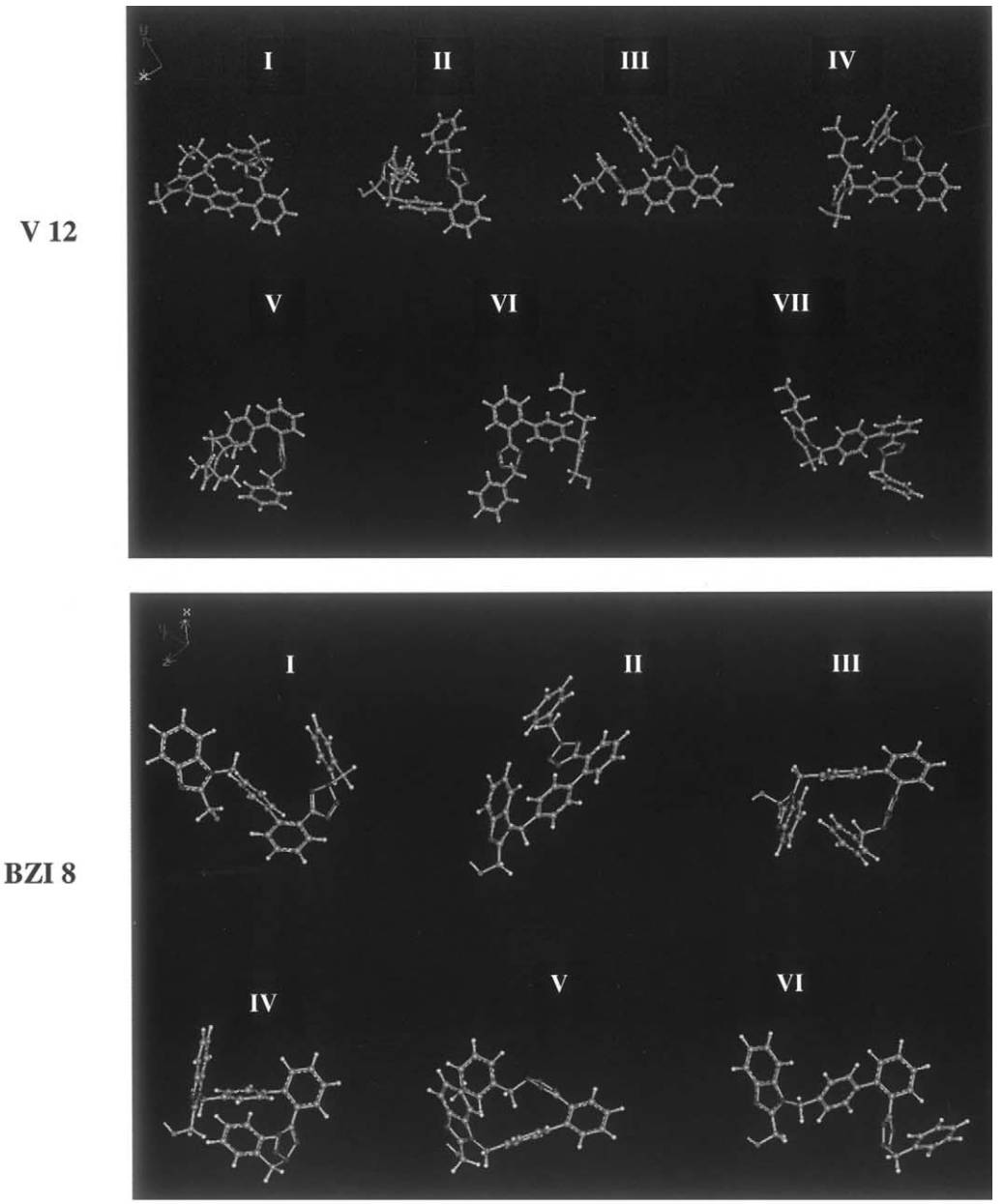


Fig. 3. Different conformers generated with Monte Carlo conformational search algorithm for V12 and BZI8 molecules.

Table 1
Peak assignment of the proton NMR spectra at 600 MHz for V12 and BZI8

V12				BZI 8			
Peak no.	ppm	Peak no.	ppm	Peak no.	ppm	Peak no.	ppm
10	0.80	31/35	6.84	DMSO	2.49	33	7.21
9	1.24	14/16	6.98	6	4.83	8/9	7.30
8	1.40	13/17	7.06	29	5.05	7	7.49
7	2.38	32/34	7.22	–OH	5.09	19/20	7.52
DMSO	2.49	33	7.24	11	5.59	21	7.56
6	4.72	4	7.43	31/35	6.79	10	7.68
29	5.13	21,20,19	7.57	14/16	6.95	22	7.71
11	5.39	22	7.73	13/17	7.16		
–OH	6.18			32/34	7.19		

biphenyl imidazole rings (RMSD value is 0.05). However, the tetrazole rings of the two molecules favor a spatial arrangement extending at the two sides of the biphenyl template.

3.5. Superimposition of V12 with losartan

The overlay of the more potent analog V12 with losartan included the matching of three atoms from the imidazole rings (N1 of both molecules, C5 of V12 with C2 of losartan and C2 of V12 with C5 of losartan). In this superimposition, all pharmacophore segments of the two molecules matched nicely with an RMSD value equal to 0.07 as shown in Fig. 6.

3.6. Superimposition of BZI8 with losartan

The superimposition of the less potent analog BZI8 with losartan is shown in Fig. 7. N1, N3 and C15 atoms of BZI8 were matched with corresponding N1, N3 and C15 atoms of losartan. RMSD value was 0.22 and as it can be seen the two tetrazole rings are oriented at the opposite side of the biphenyl template. The benzyl group attached to the tetrazole ring of BZI8 is located towards the butyl chain of losartan.

3.7. Biological data

The biological results which are summarized in Table 5 and depicted in Fig. 8, represent the mean of three experiments. As expected, losartan produced a significant dose-dependent hypotensive response, when it was given as a bolus in our model of angiotensin II-dependent hypertension in anesthetized rabbits. V12 appears to be a potent compound but less active than the prototype losartan. Finally, a low hypotensive response was observed when boluses of the BZI8 were given in the same experimental model.

Table 2
ROEs observed for the two novel antagonists

Protons	ROE effect
V 12	
7–11	s
6–11	s
6–13/17	s
11–32/34	m
29–14/16	s
29–19/20	m
BZI 8	
6–11	vs
6–13/17	s
29–14/16	s
29–7	m
11–7	s

vs: very strong (2.0–2.5Å). s: strong (2.5–3.0 Å). m: medium (3.0–3.5 Å).

Table 3
Dihedral angles of the lowest energy structures, derived using conformational search of random sampling for V12 and BZI8

Class	Pot. energy (kcal mol^{-1})	t_1	t_2	t_3	t_4	t_5	t_6	t_7
I	26.572	98.30	−124.58	−128.90	−48.86	132.00	44.55	78.35
II	30.875	−33.37	−33.05	−57.41	64.82	9.96	−39.08	−77.23
III	25.267	95.07	79.36	88.18	−126.79	−100.16	32.92	56.40
IV	26.669	45.21	66.01	28.04	75.09	−37.20	−59.51	80.45
V	23.536	29.24	−76.04	118.16	−121.06	−125.52	169.86	−23.42
VI	27.410	−37.95	−77.76	110.00	129.62	127.11	−40.94	106.30

Class	Pot. energy (kcal mol^{-1})	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9	t_{10}	t_{11}
I	47,0001	111,82	−144,605	64,3344	47,8955	−93,3714	68,1111	50,7601	−113,669	−148,913	53,8196	96,8938
II	46,1178	−118,495	134,005	−64,4161	168,886	−171,033	179,836	122,786	−127,945	49,4515	−168,883	−129,019
III	46,1474	96,8085	62,2713	−63,9708	−55,3662	177,798	−60,0352	59,0508	−55,1682	−76,1032	162,975	71,3147
IV	47,2286	−108,07	101,195	−74,6579	−169,519	175,825	−60,2716	157,91	−60,3743	−41,5228	88,2696	−101,659
V	44,9128	−123,664	−85,9419	−74,5292	−58,6655	173,514	−179,325	153,269	−135,963	−117,834	174,295	47,0876
VI	50,422	93,8452	−94,6715	−64,8603	−169,636	−175,996	60,4355	27,6997	53,2633	43,1196	152,969	70,8634
VII	49,8687	86,7821	85,1597	−178,973	−177,109	179,396	−179,37	−91,9882	−106,839	3,07982	54,5565	−87,4903

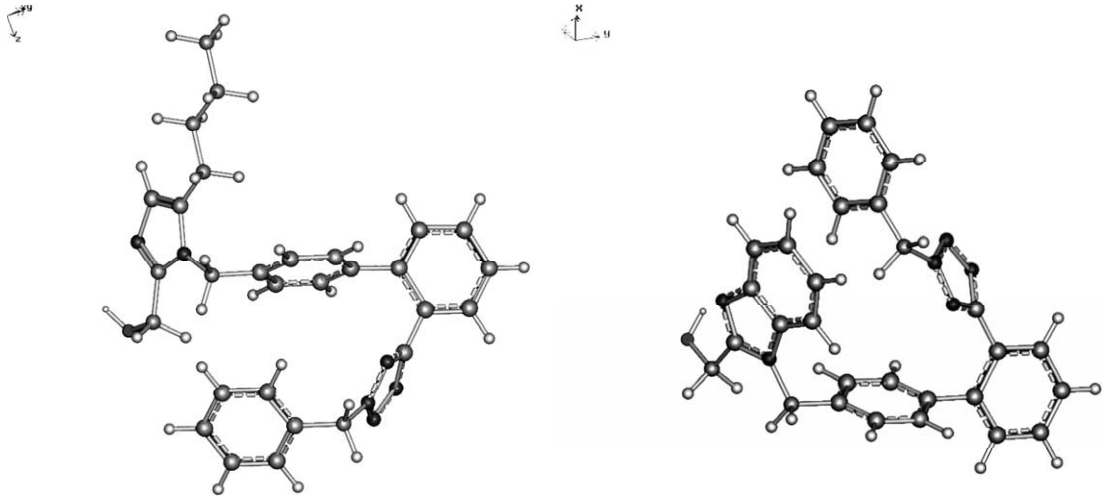


Fig. 4. Low energy conformers of V12 (left) and BZI8 (right) consistant with ROE data.

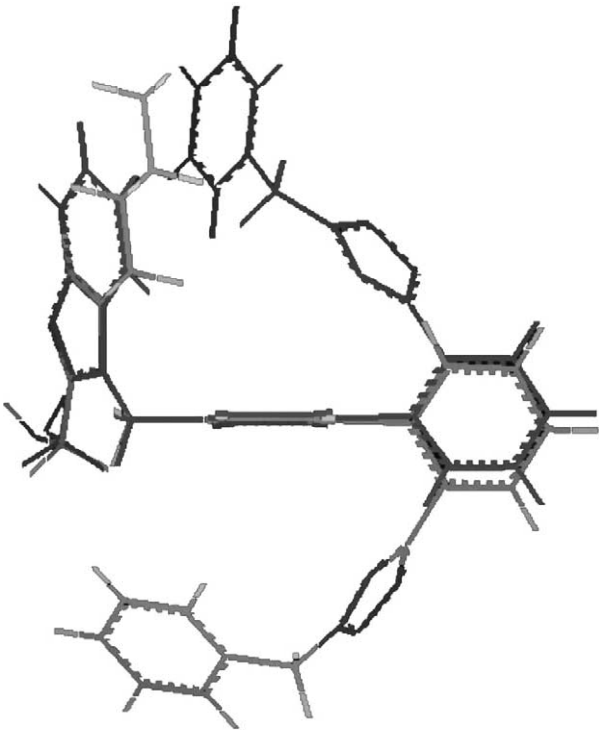


Fig. 5. Superimposition of V12 with BZI8 (yellow).

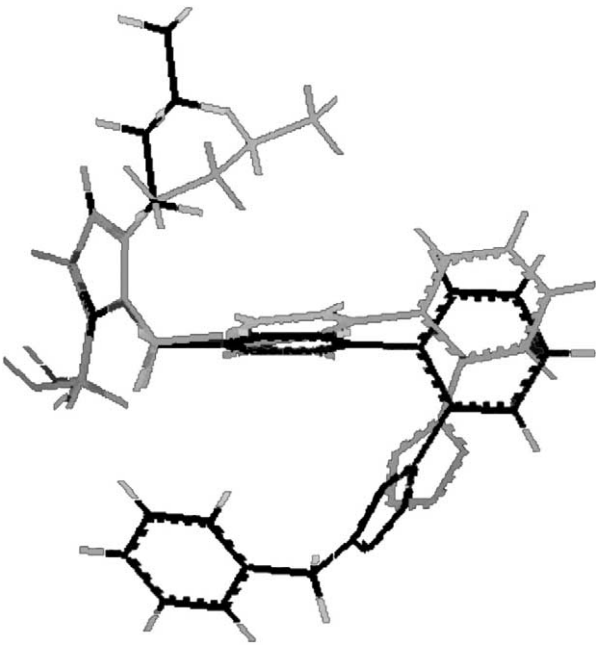
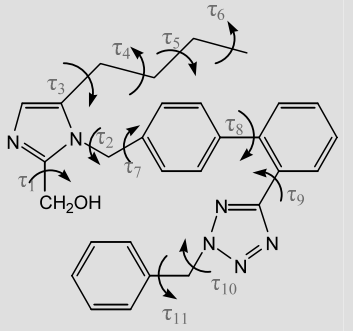


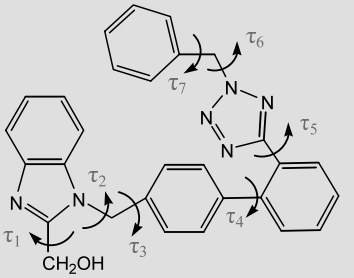
Fig. 6. Superimposition of V12 with losartan using as equivalent atoms the N1, C2 and C5 of their corresponding imidazole rings.

Table 4
Dihedral angles for the low energy conformers shown in Fig. 4.

V12

Dihedral	Value	Dihedral	Value	E=59.27 kcal/mol
τ_1	7.3	τ_7	-50.6	
τ_2	105.7	τ_8	-79.0	
τ_3	-178.6	τ_9	-64.1	
τ_4	179.3	τ_{10}	108.1	
τ_5	174.9	τ_{11}	86.5	
τ_6	59.8			

BZI8

Dihedral	Value	Dihedral	Value	E= 36.32 kcal/mol
τ_1	17.1	τ_5	-133.7	
τ_2	-72.6	τ_6	-163.3	
τ_3	126.7	τ_7	75.2	
τ_4	-111.9			

4. Conclusions

In an effort to comprehend the stereoelectronic properties for antihypertensive activity, we have explored the conformational properties of AT_1 antagonists with different pharmacological profile. Using NMR spectroscopy and computational chemistry we aim to: (i) explore the conformations of the AT_1 antagonists; (ii) study their interactions with the membrane bilayers using biophysical techniques such as X-ray diffraction, DSC and solid state NMR; and finally (iii) simulate the docking of these molecules on the AT_1 receptor in order to determine the structural requirements for better activity.

The obtained results show conformational similarities of all pharmacophore segments between the prototype losartan and V12 which possesses considerable hypotensive activity. Despite V12 and losartan had hydroxymethyl and butyl groups

Table 5
Biological data

Losartan						
	Baseline	AII	1st dose	2nd dose	3rd dose	Stop AII
I	133	183	$(1.085 \times 10^{-3}$ mmol) 153	$(2.169 \times 10^{-3}$ mmol) 133	$(2.169 \times 10^{-3}$ mmol) 130	118
II	95	173	$(1.085 \times 10^{-3}$ mmol) 150	$(2.169 \times 10^{-3}$ mmol) 138	$(2.169 \times 10^{-3}$ mmol) 128	100
III	123	170	$(1.085 \times 10^{-3}$ mmol) 141	$(2.169 \times 10^{-3}$ mmol) 138	$(2.169 \times 10^{-3}$ mmol) 133	115
V12						
	Baseline	AII	1st dose	2nd dose		Stop AII
I	133	167	$(1.687 \times 10^{-3}$ mmol) 147	$(3.375 \times 10^{-3}$ mmol) 140		100
II	130	190	$(1.687 \times 10^{-3}$ mmol) 180	$(3.375 \times 10^{-3}$ mmol) 148		116
III	133	187	$(1.687 \times 10^{-3}$ mmol) 150	$(3.375 \times 10^{-3}$ mmol) 140		107
BZI8						
	Baseline	AII	1st dose	2nd dose		Stop AII
I	153	185	$(2.116 \times 10^{-3}$ mmol) 177	$(3.174 \times 10^{-3}$ mmol) 170		132
II	130	168	$(2.116 \times 10^{-3}$ mmol) 163	$(3.174 \times 10^{-3}$ mmol) 148		136
III	110	150	$(2.116 \times 10^{-3}$ mmol) 143	$(3.174 \times 10^{-3}$ mmol) 133		108

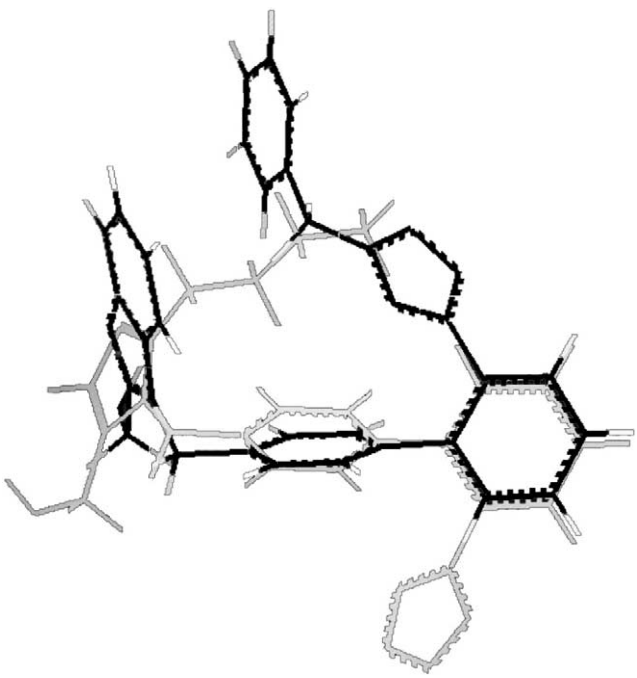


Fig. 7. Superimposition of BZI8 with losartan (yellow) using as equivalent atoms the N1, N3 and C15 of their corresponding imidazole and phenyl rings.

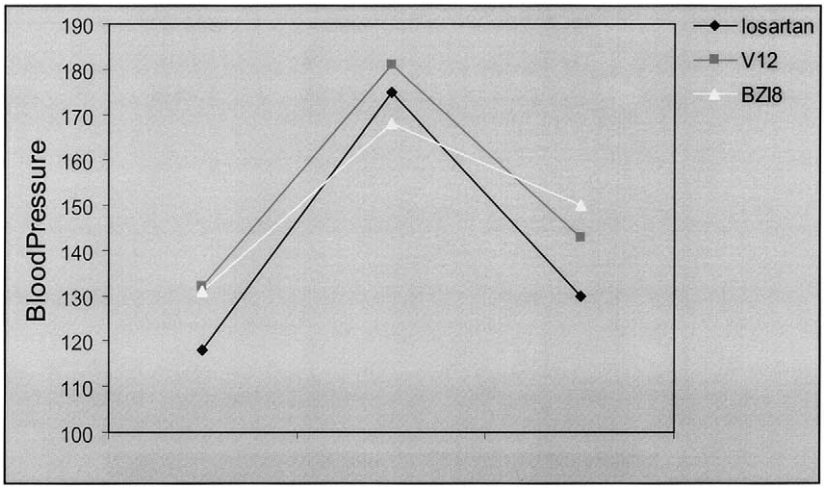


Fig. 8. Biological response of the three AT_1 antagonists.

interchanged on the imidazole ring, they could superimpose nicely. This points out the possibility that the higher activity of losartan may be attributed to the fact that it possesses a chlorine atom.

The superimposition results may also explain the low hypotensive activity observed for the BZI8 molecule. The tetrazole ring of this analog is located at the opposite site, relatively to the biphenyl template when it is compared with the corresponding rings of the potent AT_1 antagonists V12 and losartan.

Acknowledgements

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