Conformation and Bioactivity. Design and Discovery of Novel Antihypertensive Drugs

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Abstract: Peptidomimitism is applied in the medicinal chemistry in order to synthesize drugs that devoid of the disadvantages of peptides. AT1 antagonists constitute a new generation of drugs for the treatment of hypertension designed and synthesized to mimic the C-terminal segment of Angiotensin II and to block its binding action on AT1 receptor. An effort was made to understand the molecular basis of hypertension by studying the conformational analysis of Ang II and its derivatives as well as the AT1 antagonists belonging to SARTANs class of molecules. Such studies offer the possibility to reveal the stereoelectronic factors responsible for bioactivity of AT1 antagonists and to design and synthesize new analogs. An example will be given which proves that drugs with better pharmacological and financial profiles may arise based on this rational design.

INTRODUCTION

The Nobel Prize winners J. D. Watson and F. Crick (1962) who published the structure of the biomolecule of deoxyribose nucleic acid (DNA) realized that it had novel structural features. They reported in their published brief article in Science [1] that it had not escaped their notice that the specific pairing observed in the salt of DNA suggested a possible copying mechanism for the genetic material. This is the first example as far as we know which relates activity of a biomolecule with the stereoelectronic properties and the spatial arrangement of its constituted atoms.

The relationship between the spatial arrangement of atoms and their conformational properties of bioactive molecules with their pharmacological profile has been well documented in the Medicinal Chemsitry with several reports in journals related to Medicine and Pharmacy. The few following examples are sufficient to show the effects of stereoelectronic factors in determining the bioactivity of the molecule. However, the aim of this review article is to explain how the knowledge of the relationship between bioactivity and conformation may lead to novel drugs with better pharmacological profile in the field of hypertension.

1. Stereochemistry of the Drug and Analgesia

Ibuprofen is approved as an anti-inflammatory agent and is given to patients as racemic mixture. However, the enantiomers that constitute the racemic mixture are not of equivalent potency. For example, only the (-)S-enantiomer of the analgetic anti-inflammatory drug ibuprofen is very active Fig. (**1**) [2].

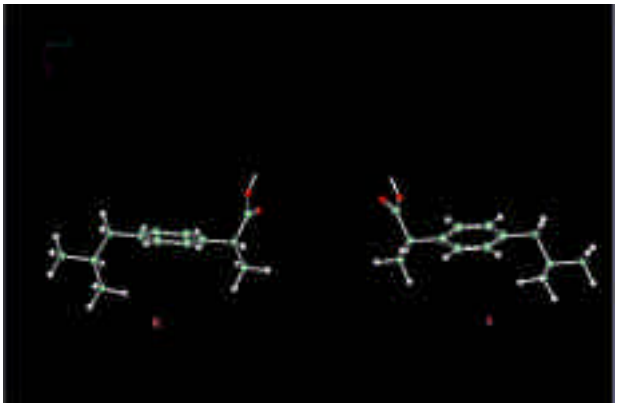

Fig. (1). Chemical structures of ibuprofen's enantiomers.

2. Stereoselectivity of the Taste receptor

If a molecule has more chiral centers then it is probable that only one of the diastereomers has the desired properties. Sensory evaluation of Aspartame (Nutra Sweet) showed that only its S,S isomer tastes sweet (100 times sweeter than sucrose), while S,R and R,S are bitter and R,R has a hot, bitter, slightly sweet taste. Molecular modeling studies showed that only S,S isomer adopted an L shape which is a strict requirement for binding to the receptor. It is apparent

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from these results that the taste receptor is stereoselective [3] Fig. (**2**).

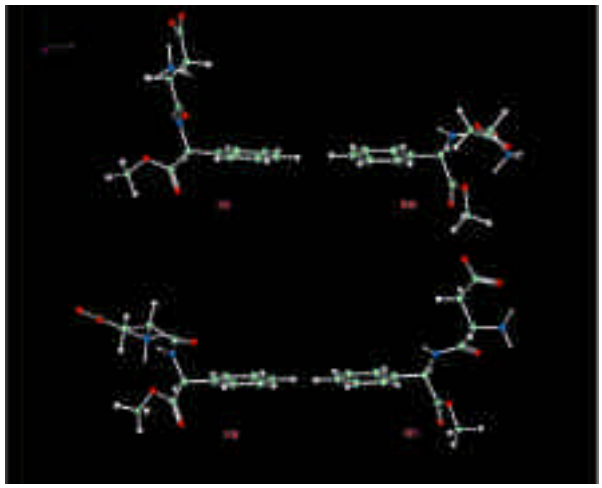


Fig. (2). The four diastereomers of aspartame. The four diastereomers are obtained using energy minimization algorithms of first and second derivative. As it can be observed only SS diastereomer adopts an L shape which may trigger the taste receptor resulting a sweet taste.

3. Amphipathicity of the Drug and Psychotropic Activity

Extensive studies on the structure-function correlation of psychomimetic cannabinoids have shown that the phenolic hydroxyl group has a pivotal role in determining the pharmacological properties of these molecules. Phenolic hydroxyl methylation gives the *O*-methyl ether results in analogs that devoid of biological activity.

We have used a combination of biophysical methods to study the effects in membranes of a pair of drugs (-)-Δ^8-tetrahydrocannabinol (Δ^8-THC) and its ether analog (-)-O-methyl-Δ^8-tetrahydrocannabinol (Me- Δ^8-THC) that differ in their amphipathic properties Fig (**3**).

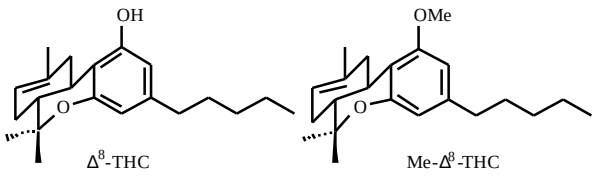


Fig. (3). Chemical structures of cannabinoids (-) -Δ^8-THC and (-)-Me- Δ^8-THC.

The bioactive psychomimetic drug Δ^8-THC anchors itself at the membrane interface, presumably through hydrogen bonding with the phospholipid ester groups or with water molecules present at the interface. In this location, the drug molecule can induce bilayer perturbations most effectively. Conversely, the almost inactive analog Me-Δ^8-THC locates itself deeper in the bilayer away from the interface, a property which accounts for its decreased ability to perturb the membrane. Their different effects in membrane bilayers may reflect their different diffusion and consequently may explain their pharmacological profile [4,5].

4. Geometry of the Drug

As it can be observed from their structures Fig. (**4**), alphaxalone (5α-pregnane-3α-ol-11,20-dione) which has potent anaesthetic properties and was used clinically as the main active component in the commercially available anaesthetic Althesin differs from Δ^{16}-alphaxalone (5α-pregn-16-ene-3α-ol-11,20-dione) which lacks anaesthetic activity only in the C-16 position .

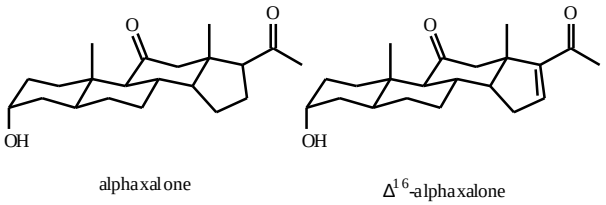


Fig. (4). Chemical structures of Alphaxalone and Δ^{16}-alphaxalone.

Conformational analysis of the anesthetic steroid alphaxalone and its inactive congener Δ^{16}-alphaxalone along with their interactions in membrane bilayers showed that their differences are attributed in their different molecular geometry of D ring between the two drugs [6,7].

5. Role of the Conformation in the Hypertension

Hypertension is growing in our times and it threatens mostly the developed societies. It is estimated that 1/5 of the Greek population suffers from hypertension.

Details of this disease from the medicine point of view [8-10] will be covered by the other authors that contribute in this special issue. In this manuscript we will focus only on Angiotensin II (Ang II), the primary active hormone in the Renin-Angiotensin System which is the major vasoconstrictor implicated in the cause of hypertension.

Research efforts for the treatment of hypertension are focused in blocking Ang II release and more recently in competing Ang II binding on AT_1 receptors. This latest approach generated the synthesis of losartan [32] and promoted it in the pharmaceutical market (COZAAR). Other derivative drugs which fall into SARTAN's class followed, Fig (**5**) [33]. Losartan is converted *in vivo* to its active metabolite EXP-3174. To comprehend in the stereoelectronic requirements that may lead to the better understanding of the molecular basis of hypertension, the stereochemical features of angiotensin II, its peptide antagonists sarmesin and sarilesin, synthetic peptide analogs, AT_1 non-peptide antagonists commercially available as well as synthetic ones were explored. AT_1 antagonists are designed to mimic the C-terminal part of Ang II. In this aspect, it is proposed that the butyl chain of losartan may mimic the isopropyl chain of Ile, the tetrazole ring mimics the C-terminal carboxylate group and the imidazole ring the corresponding imidazole ring of His^6 [34-36]. This mimic however can be revised if future literature shows unequivocally that AT1 antagonists possessing tetrazole may anchor in a different aminoacid of AT1 receptor than C-carboxylate terminal of Ang II. [10].

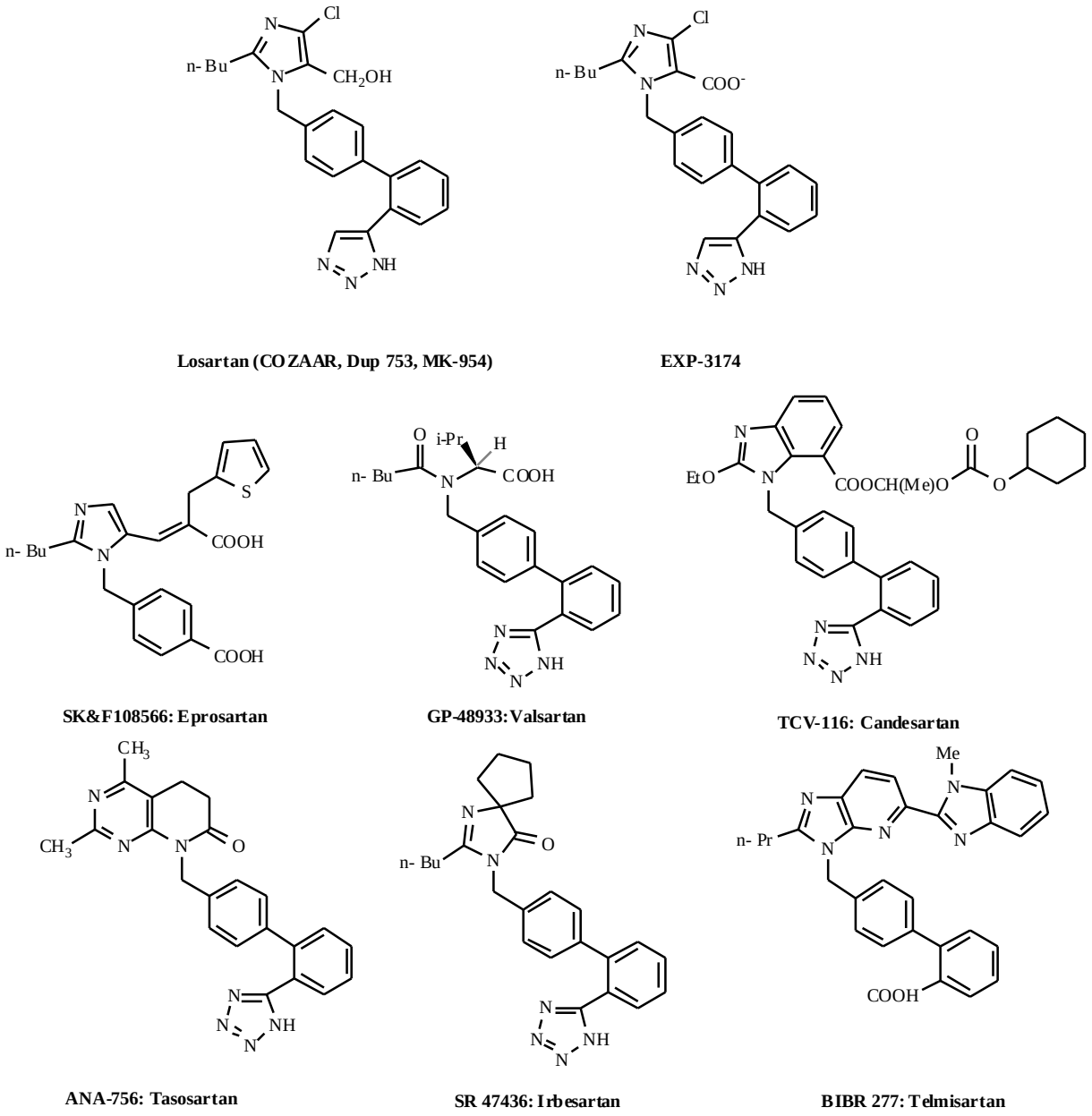


Fig. (5). Structures of AT1 antagonists.

RESULTS

Approach used to study the conformation of angiotensin II, peptide derivatives and non-peptide antagonists

First, the molecules under study were structurally elucidated using 1D and 2D COSY, NOESY and ROESY NMR spectroscopy. NOESY and ROESY experiments were used to determine the distances between the atoms arranged in spatial proximity. These distance constraints are then incorporated into computational analysis using conformational search methods (Monte Carlo, Boltzmann Jump, Dynamics). The procedure used to determine the low energy conformers consistent with NOE data is shown in Fig. (**6**).

A. Conformational Analysis of Ang II

The chemical structure and the proposed bioactive conformation of Ang II are shown in Fig. (**7**).

The above model was based on structure-activity relationships, NMR spectroscopy and nanosecond time-resolved tyrosinate fluorescence life time studies It is characterized by a backbone bend at the sequence Tyr-Ile-His, a clustering of the aromatic rings Tyr^4-His^6-Phe^8, and a charge relay system involving the triad phenolic hydroxyl group of Tyr^4, imidazole of His^6 and carboxylate of Phe^8. The clustering is the result of the backbone bend which brings the aromatic rings into spatial proximity. The

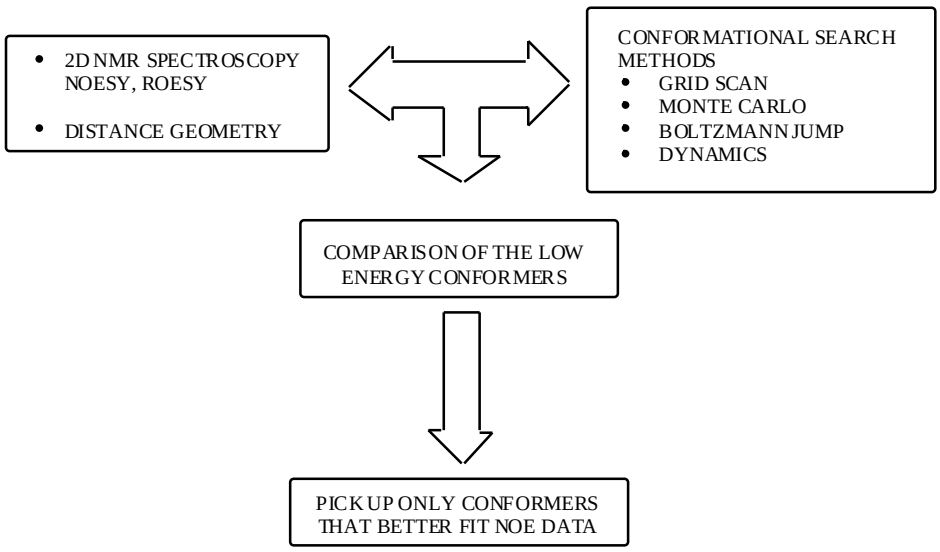


Fig. (6). Flow-chart showing the procedure used to calculate the conformations of peptides and non-peptide mimetics under study.

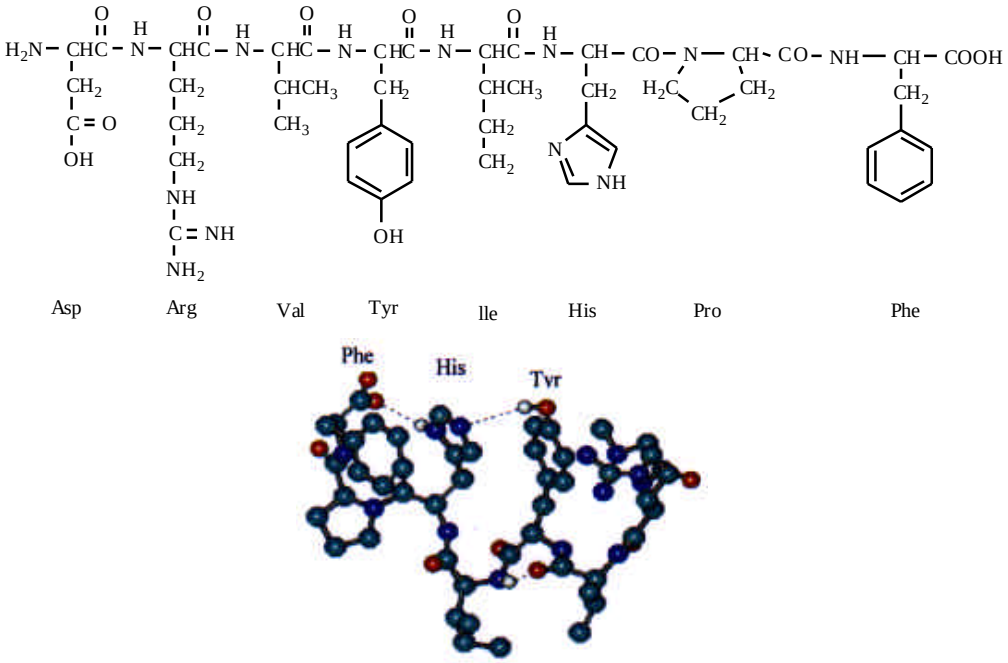


Fig. (7). Chemical structure of Angiotensin II and its proposed bioactive conformation. Notice the major conformational feature of the molecule which is the cluster and relay system between the side groups of Phe[4]-His[6]-Phe[8].

clustering is shown in 1D NOE difference spectra shown in Fig. (**8**). Arginine is also protruding towards this cluster by interacting with tyrosine. The key aminoacids Tyr[4], His[6], Phe[8] form a relay system through electrostatic interactions. This form of a relay system is energetically favoured and it may be a key requirement for Ang II to interact with the active site of the receptor productively [11-13]. This model is recently supported by Carpenter *et al.* [14] who performed conformational analysis experiments in micelles and phospholipid bilayers. Thus, DMSO may simulate the amphoteric environment of membrane bilayers. Other models derived in different environments using physical chemical methods and theoretical calculations are reported in the literature [15-31].

B. Conformational Analysis of Synthetic Peptides

Several peptide analogs have been synthesized in order to comprehend and confirm the model proposed for Ang II. *Conformational analysis of Sarmesin.* Sarmesin is a competitive antagonist (type I) and resembles structurally the Ang II. Thus, sarmesin has methylated the phenolic hydroxyl group of Tyr[4] aminoacid and Sar[1] instead of Asp[1].

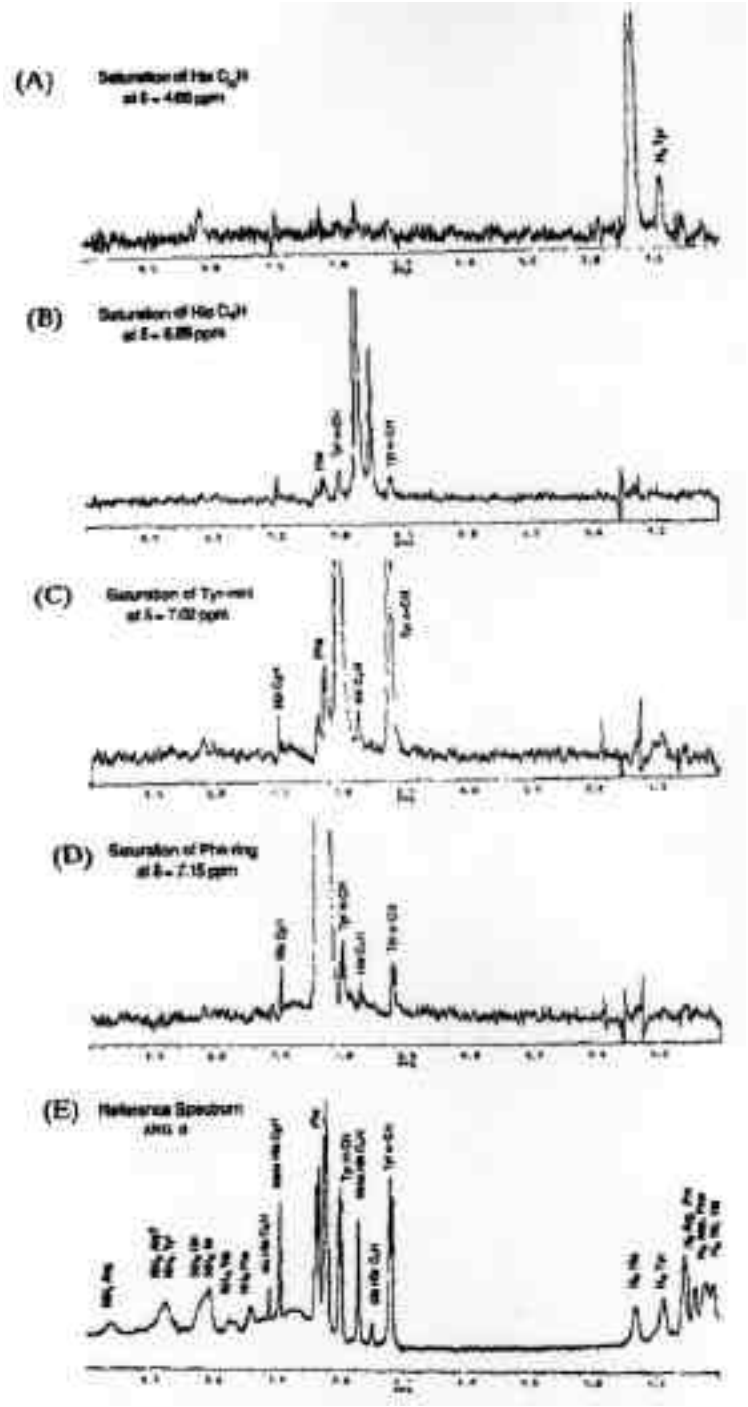


Fig. (8). Reference spectrum (E) and NOE difference spectra for Ang II obtained upon saturation of proton lines for CaH (A), C4H (B), Tyr-mH (C) Phe ring (D).

Sarmesin's conformation is found closely related to that of Ang II. NMR spectroscopy coupled with computational analysis showed a Tyr^4 (OMe)-His^6-Ile^8 bend and a major His^6-Pro^7 peptide trans conformation similar to that observed with Ang II. More specifically, the low energy conformer of sarmesin is characterized by a cluster of the key tetrads of aminoacids Sar^1-$Tyr(OMe)^4$-His^6-Phe^8 Fig. (**9**) [37-39]. The obtained data showed that only aromatic chain interactions observed in Ang II or its superagonist Sar^1 [Ang II] are missing. Therefore, it can be concluded that these interactions are essential for agonist activity.

Conformational Analysis of Cyclic and linear Analogs of Ang II

The model of Ang II is supported by the design and synthesis of a cyclic Ang II analogue. The amide linked

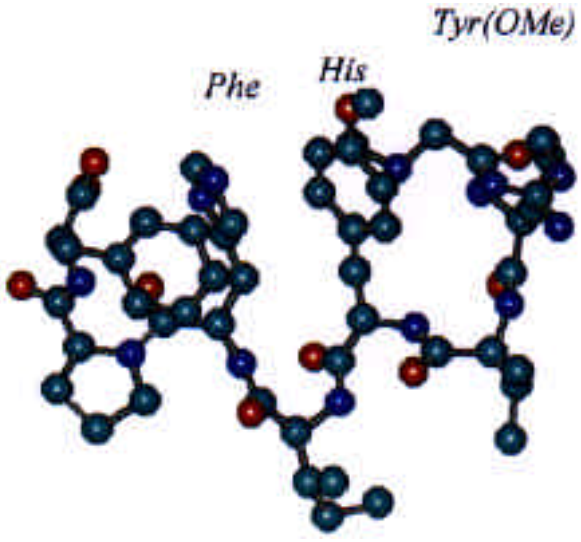


Fig. (9). Low energy-conformer of sarmesin derived from a combination of NMR data and theoretical calculations. The model is characterized by a clustering of the key tetrad amino acids Sar[1]-Tyr(OMe)[4]-His[6]-Phe[8].

cyclic angiotensin II analogue, cyclo (3,5)-[Sar[1], Lys[3], Glu[5]] Ang II was synthesized in an attempt to test the ring clustering and the charge relay conformation. Thus, the three key aminoacids Tyr[4]-His[6]-Phe[8] were enforced to be in a spatial proximity. Indeed, cyclo (3,5)-[Sar[1], Lys[3], Glu[5]] Ang II was found to be a potent agonist in the rat uterus assay and in anaesthesized rabbits strengthening our hypothesis that the aromatic side chains must be able to cluster together with the C-terminal carboxylate, and are the essential pharmacophoric groups for receptor activation, Fig. (**10**) [40-43]. Interchange of the cyclization between the aminoacids 3 and 5 gave also the agonist cyclo (3,5)-[Sar[1], Glu[3],Lys[5],] Ang II showing that the major role of Glu[3] and Lys[5] may be attributed to the proper backbone conformation and the steric influence of these side chains.

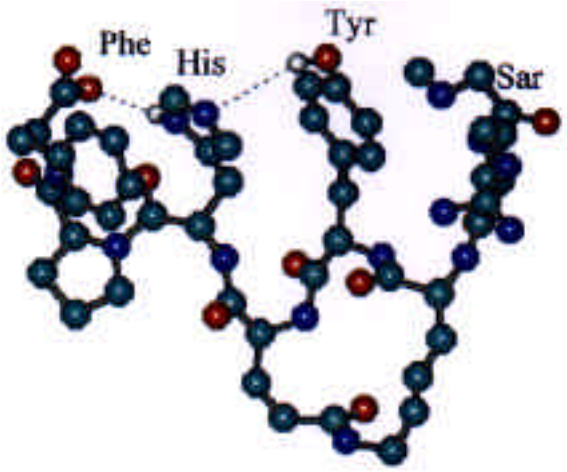


Fig. (10). Proposed model of c-[Sar[1],Lys[3],Glu[5]] Ang II generated by distance geometry and molecular dynamics. Distance constraints were obtained from 1D-NOE data. A cluster of the three aromatic rings of aminoacids (Tyr[4], His[6], Phe[8]) and the Arg[2] guanidino group is favoured.

Conformational analysis of Sarilesin and analogs

Sarilesin is a non competitive antagonist (type II) showing a more extended conformation. The constrained cyclic analogue of sarilesin cyclo(3,5)-[Sar[1]-Lys[3]-Glu[5]-Ile[8]] ANG II with a lactam amide bridge linking the Lys-Glu pair at positions 3 and 5 and possessing Ile at position 8 was synthesized by solution procedure using the maximum protection strategy, Fig.(**11**) and was found to be also an inhibitor of Ang II as expected [44]. Analogues of sarilesin and sarmesin with homoserine (hSer) at position 8 were prepared and bioassayed. The results showed that agonists have a Tyr[4]-Ile[5]-His[6] bend, a major His[6]-Pro[7] trans peptide conformation, an extended side chain cluster between Tyr[4], His[6], Phe[8], Sar[1] and or Arg[2] which is accompanied by a relay system between Tyr[4], His[6], Phe[8]. The antagonists have only part of these molecular features (like Sarmesin) or a less ordered conformation (like Sarilesin and analogs) [45].

C. Mimicry with AII and Sarmesin

Conformational Analysis and Superimposition of AT1 Drug Antagonists with Sarmesin.

a) Losartan

Assignment of losartan proton resonances was achieved from inspection of the chemical shifts of the ^{1}H NMR spectrum and the 2D DQF-COSY and ROESY spectra, Fig. (**12**). Conformational analysis using the ROE constraints and applying different computational methods revealed different low energy conformers. AT1 antagonist losartan was superimposed with peptide antagonist 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]. From the candidate structures of losartan tested for superimposition the enantiomeric conformations D3 and D3' shown in Fig. (**13**) had the best fit. The matching between the conformational enantiomers of losartan was not equivalent (D3 showed a better matching) , proposing the significance of the chirality for the molecule to exert its biological activity. Interestingly, losartan mimics the bend (γ-turn) formed around Pro[7] in sarmesin, Fig. (**14**).

The conformational study of losartan was also sought in other amphiphilic environments (D_2O, micelles). 1D NMR spectra of losartan in these environments are shown in Fig. (**15**). The ROE data clearly show that the conformation of losartan is not affected significantly by the environment. This is a significant observation because it shows that low energy conformers of AT1 antagonists may be insensitive to the environment. This is not the case for Ang II where

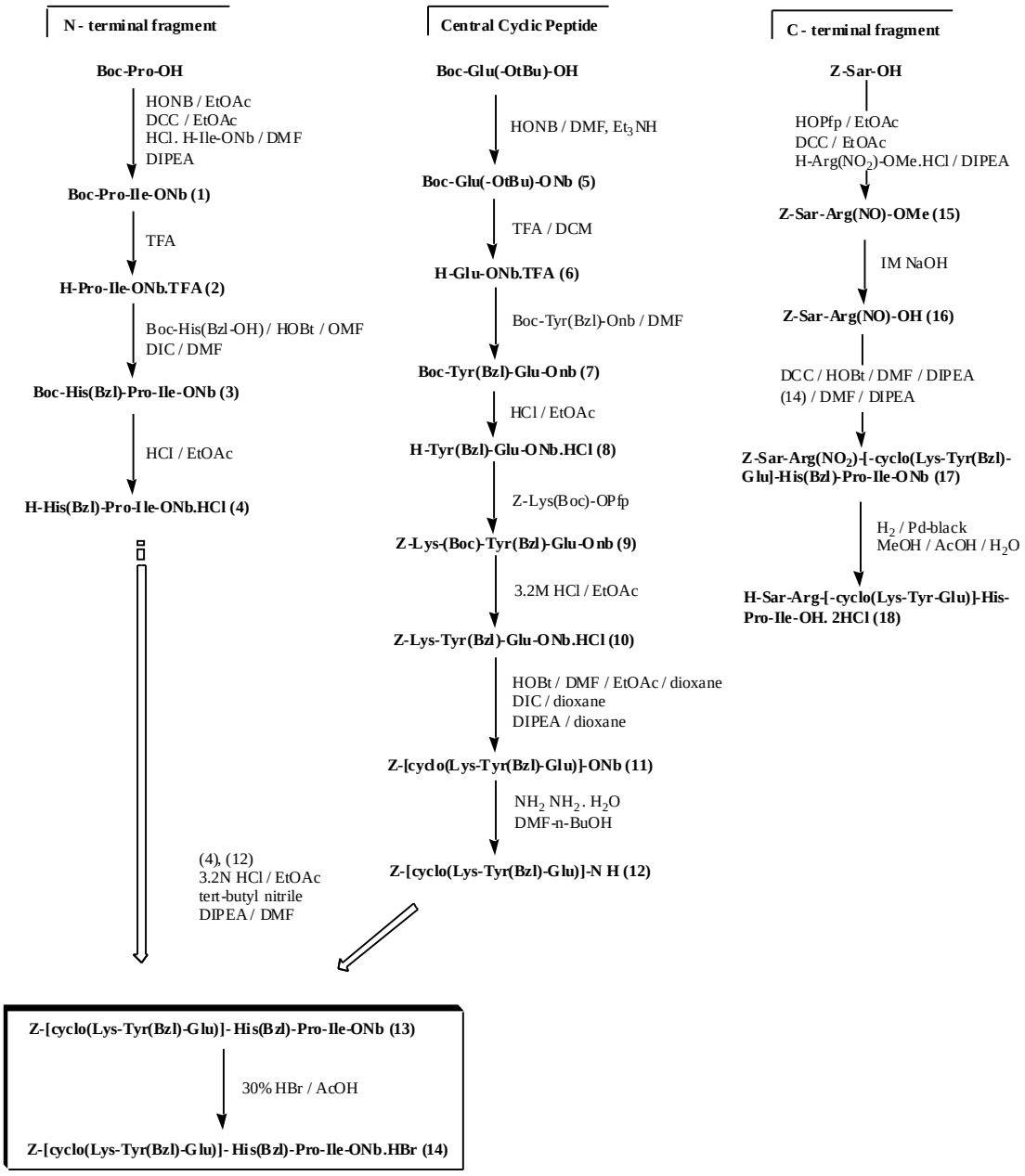


Fig. (11). Synthesis of the cyclic analog H-Sar-Arg-[-cyclo(Lys-Tyr-Glu)]-His-Pro-Ile-OH. 2HCl.

several models have been proposed as already mentioned that varied depending on the temperature, the solvent and pH of the experimental conditions.

(b) Eprosartan

Fig. (**16**) depicts the ^{1}H NMR spectrum of eprosartan in DMSO. The proton chemical shifts were assigned using homonuclear COSY, NOESY, HSQC and HMBC 2D NMR experiments. The assignment of the peaks is shown on the top of the spectrum. Using conformational analysis of eprosartan, eight low energy conformers were derived, Fig. (**17**). These were superimposed with losartan. The superimposition involved the following equivalent pharmacophore segments: (i) eprosartan's nitrogen atoms of the imidazole ring with the corresponding ones of hydroxymethylimidazole ring of losartan; (ii) eprosartans' and losartans' terminal methyl groups; (iii) eprosartans' carboxylate group with the isosteric tetrazole of losartan; (iv) eprosartans' carboxyl group with losartans' hydroxymethyl group. The pair of class I showed the best overlapping.

In these conformers aromatic groups i.e. phenyl, thiphenyl rings and alkyl chain are clustering to favor attracting van der Waals interactions. In the first overlay (top) with the conformer I, the four pharmacophore segments

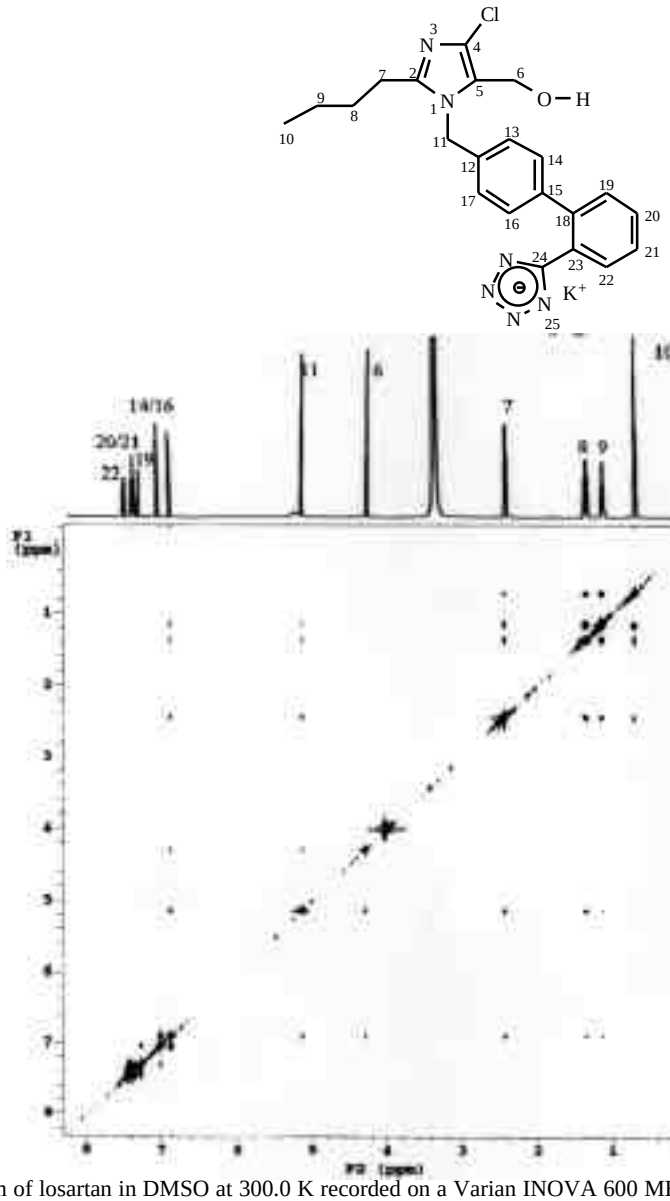


Fig. (12). 2D ROESY spectrum of losartan in DMSO at 300.0 K recorded on a Varian INOVA 600 MHz. The WATERGATE sequence has been used for effective water suppression.

under comparison were matched nicely (RMSD=1.31). In this comparison thiophene appeared with no other equivalent group. In the second overlay (conformer II), the matching of the four pharmacophores was inferior (RMSD=1.97). However, the thiophene ring was in a close spatial proximity with the biphenyl system, Fig. (**18**) [46].

The two low energy conformers best approximated the four pharmacophore segments of losartan were superimposed with similar in feature aminoacid segments of sarmesin. The best overlapping was achieved with conformer II of class I. In this superimposition the following equivalent groups were matched: (i) imidazole rings of His^6 and eprosartan; (ii) butyl chain of eprosartan with Ile^5 alkyl chain; (iii) acrylic acid group of eprosartan with phenolic hydroxyl group of Tyr^4. (iv) eprosartan's phenyl carboxyl group with Phe^8 carboxyl group. The RMSD found during the superimposition of the two molecules was 2.53 Å, Fig. (**19**).

Fig. (13). Proposed bioactive enantiomeric conformers of losartan.

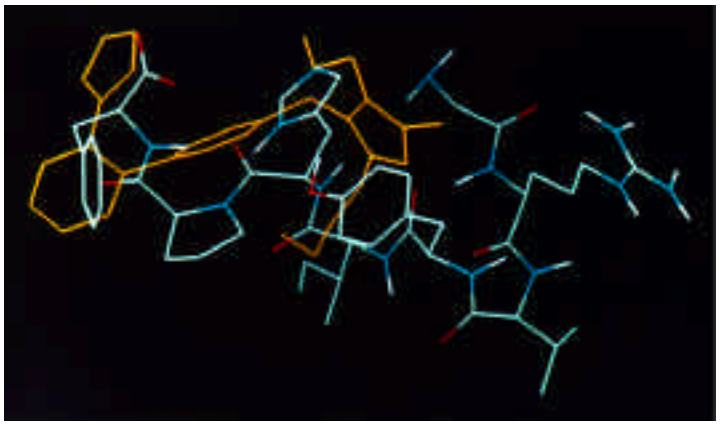


Fig. (14). Superimposition of sarmesin with a proposed bioactive conformer of losartan.

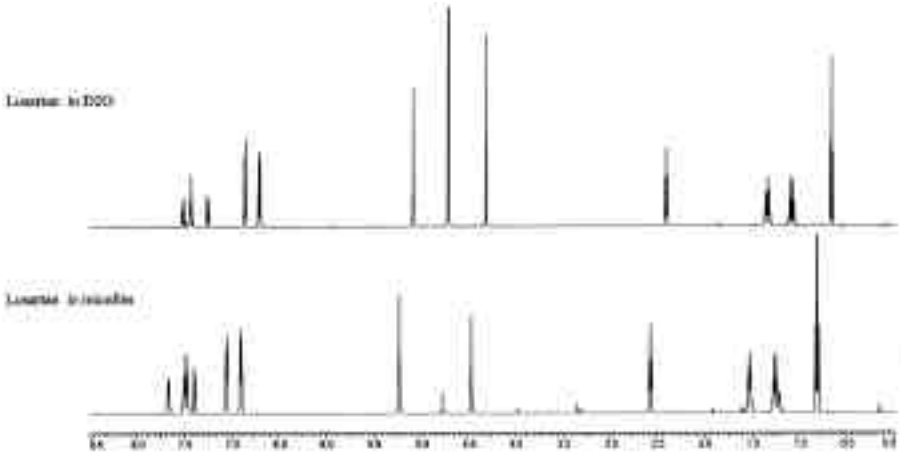


Fig. (15). 1D ^{1}H-NMR spectra of losartan in D_2O and micelles.

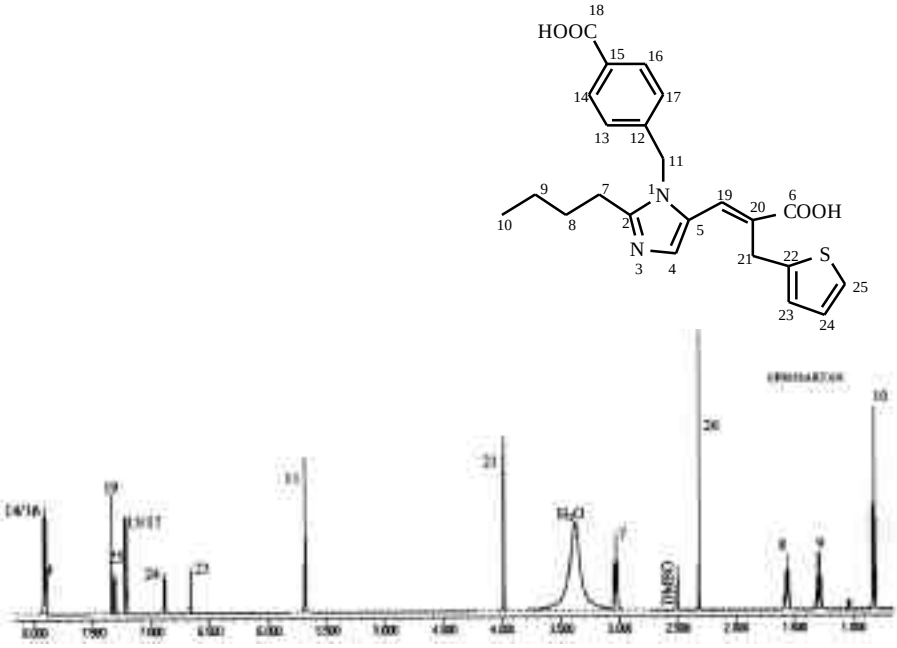


Fig. (16). ^{1}H NMR spectra of eprosartan in DMSO at 300.0 K, recorded on a Varian INOVA 600 MHz spectrometer.

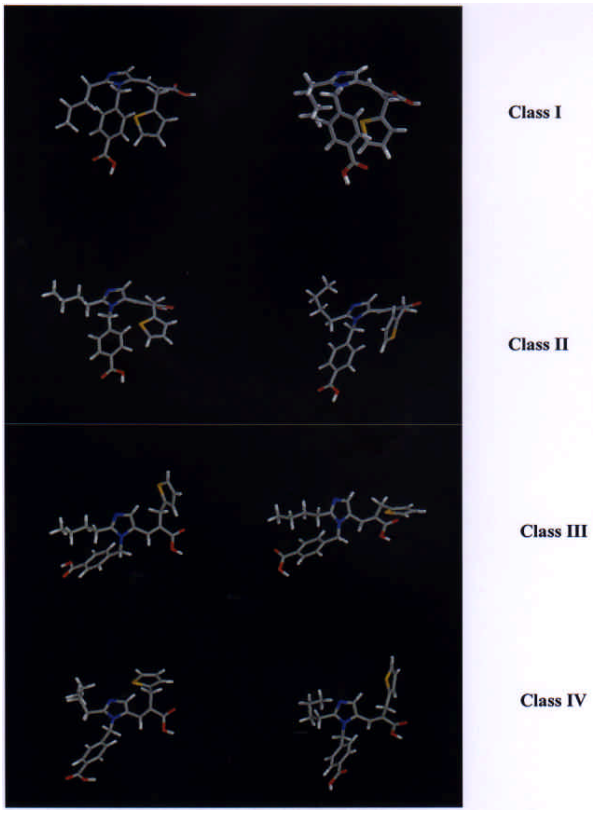


Fig. (17). Lowest energy conformers of eprosartan, derived using a combination of molecular dynamics under constraints and Monte Carlo conformational searches.

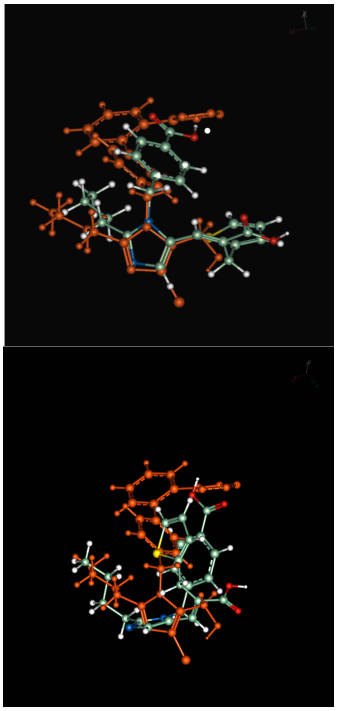


Fig. (18). Superimposition of the low energy conformers of eprosartan with losartan (orange).

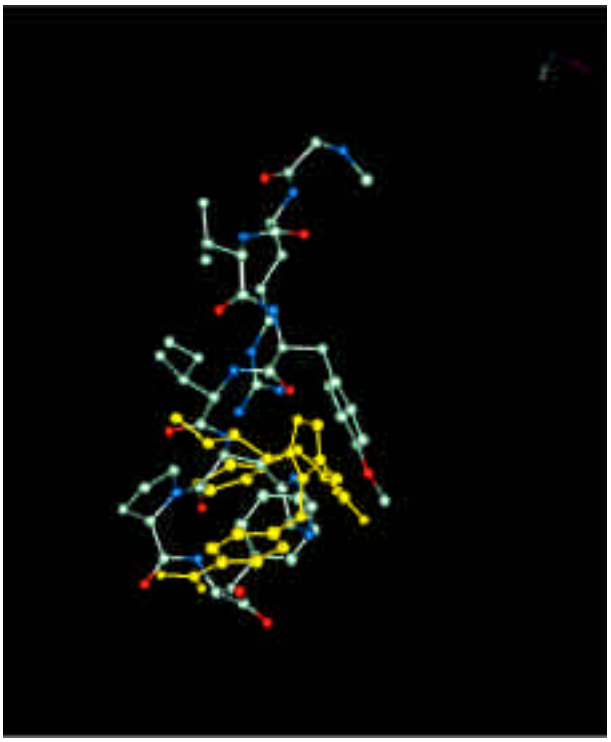


Fig. (19). Superimposition of the best-fit conformer of eprosartan (yellow) with sarmesin.

(c) Irbesarta

The proton chemical shifts of irbesartan are assigned following standard procedures and using homonuclear DQF-COSY and NOESY in combination with heteronuclear ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC 2D experiments. The assignment was in agreement with data obtained by Bauer *et al*. [47]. Interestingly, ^{1}H NMR spectrum of aromatic region of irbesartan at 7.1 ppm shows only one single peak assigned to 13,17 and 14,16 protons in the temperature range of 20-100 ^{o}C. However, phenyl rings of losartan and eprosartan attached to imidazole ring showed two doublets resonated at 6.9 ppm (13/17), 7.1 (14/16) ppm and at 7.20 ppm (13/17), 7.9 ppm (14/16) correspondingly, Fig. (**20**). This probably reflects the different chemical environment surrounded the phenyl rings of the three AT_1 antagonists. To give some evidence on this, experiments of irbesartan in micelle environment were run. Two doublets were observed as with losartan and eprosartan. The conformational properties of irbesartan were explored using computational analysis based on observed NOEs. The derived low energy conformer of irbesartan is shown in Fig. (**21**). Superimposition of the low energy conformer of losartan with the corresponding low energy conformer of irbesartan reveal the structural similarities and differences between the two molecules, Fig. (**22**). One major difference between the two AT_1 antagonists is that the butyl chain of irbesartan is not oriented towards the biphenyl ring as in losartan. Similar orientation of the biphenyl rings of the two AT_1 antagonists is observed. The carbonyl oxygen in irbesartan mimics the hydroxymethyl group of losartan. The two nitrogens of the imidazole rings are found to have almost identical spatial position. Chlorine

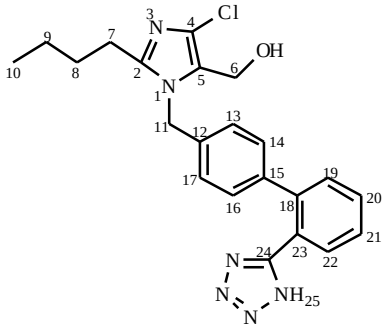
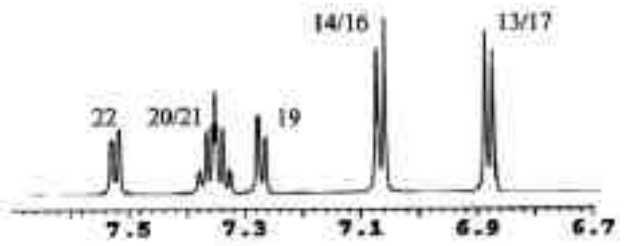
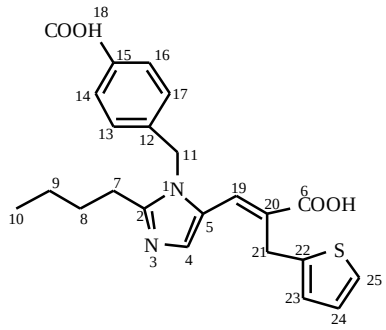
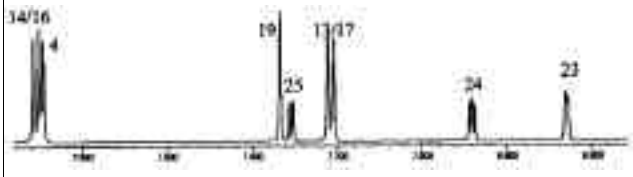
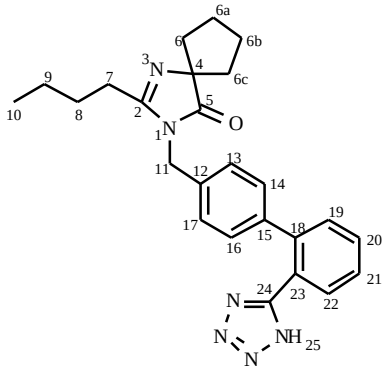
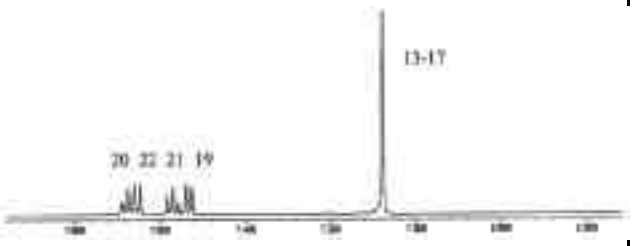
DRUG	STRUCTURE	AROMATIC REGION OF ^{1}H NMR SPECTRUM
LOSARTAN		
EPROSARTAN		
IRBESARTAN		

Fig. (20). ^{1}H NMR spectrum of the aromatic region of the AT_1 antagonists losartan, eprosartan and irbesartan, obtained at 27° C.

of losartan orients towards the hydrophobic cyclopentane ring. Thus, pharmacophoric segments of irbesartan show a good superimposition with that of losartan and correspondingly with C-terminal segment of sarmesin. AT_1 antagonists show also a good superimposition with C-terminal segment of Ang II since as it is pointed out the two peptides have similar conformer properties in their C-terminal segments.

Literature search showed that superimpositions were sought between the pharmacophore groups of C-terminal segment of Ang II and AT_1 antagonists [34] . These superimpositions while were proved successful for the drug design were mainly based on theoretical conformations for AT_1 antagonists. The contribution of this work is based on the use of conformations derived for sarmesin and AT_1 antagonists using a combination of NMR spectroscopy and computation analysis. This rationale opens new avenues for designing drugs with better biological profile.

D. Conformational Analysis of Synthetic Non-Peptide Mimetics Analogs. Superimposition with Losartan.

Two avenues were explored in an attempt to design and synthesize novel AT1 antagonists. The first avenue is based on losartan structural modifications while the second one does not use biphenyl segment as a template.

a. Derivatives of Losartan

Various structurally similar to losartan synthetic analogues are prepared. The synthesis of a pair of analogues with different bioactivity is shown in Fig. (**23**).

V12 is an almost equipotent molecule with losartan. The positions of butyl alkyl chain and hydroxymethyl

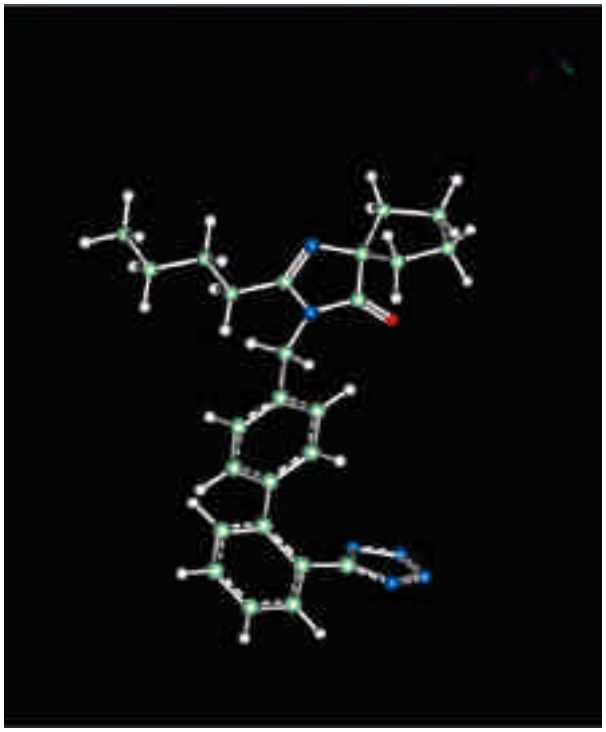


Fig. 21. Low energy conformer of irbesartan drived using a combination of computational analysis and Nuclear Magnetic Resonance Spectroscopy.

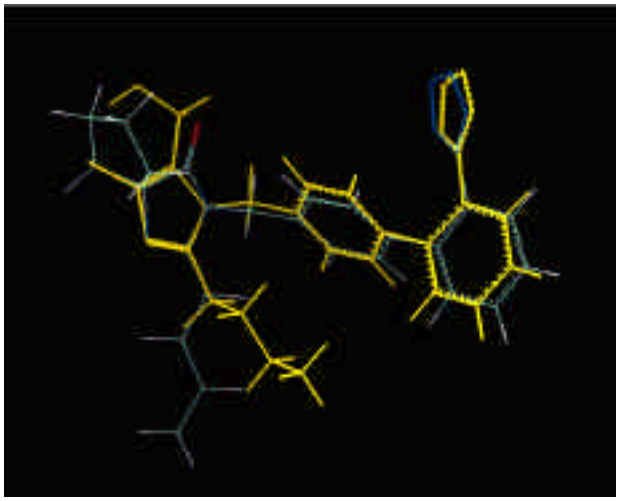


Fig. (22). Superimposition between losartan (yellow) with irbesartan using equivalent pharmacophore segments.

substituents in the imidazole ring were reversed in comparison with losartan. Based on the superimposition models such a substitution would optimize the spatial vicinity of butyl chain with isopropyl group of Ile[5]. The imidazole ring of V12 devoids of chlorine. BZI8 has more significant structural changes. Imidazole ring at positions 4 and 5 is fused with the phenyl ring to give a benzoimidazole ring. Therefore, this study aims through the use of conformational analysis to examine the significance of the spatial location of hydroxymethyl group. It may also provide a molecular basis of the role of the constituent chlorine atom. The two molecules under study contain a substitution of benzyl ring in tetrazole ring. This substitution aims to increase the lipophilicity of the molecules which may in turn lead to better metabolic properties.

^{1}H NMR spectra of BZI8 and V12 in DMSO solvent are depicted in Fig. (**24**). Their assignment shown on the top of the spectrum is based on a standard procedure using homonuclear DQF-COSY and ROESY in combination with ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments.

A critical ROE which governs the conformational properties of BZI8 is between H7 and H29. This suggests a clustering between the benzyl and the phenyl rings. The butyl chain of V12 appears to be in a close vicinity in space with the ring of the biphenyl system attached to imidazole as it is observed with losartan. Likewise, the two rings of the biphenyl system for the two molecules are not coplanar which was also observed with the antihypertensive drugs, losartan, eprosartan and irbesartan.

The conformers for both molecules consistent with experimental ROE data, are apposed in Fig. (**25**). The best overlapping of the two studied molecules is depicted in Fig. (**26**). This overlay resulted in a nice match of their biphenyl imidazole rings (RMSD value is 0.05). However, the tetrazole rings of the two molecules favor a spatial arrangement extending at the two opposite sides of the biphenyl template. Interestingly, the overlay of the more potent analog V12 with losartan showed a nice matching with an RMSD value equal to 0.07 as shown in Fig. (**27**).

The superimposition of the less potent analog BZI8 with losartan is shown in Fig. (**28**). 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,

The obtained results show conformational similarities of all pharmacophore segments between the prototype losartan and V12 which possesses considerable hypotensive activity. However, the additional hydrophobic segment of benzyl ring attached to the tetrazole, may not be related to the binding on the AT_1 receptor. It is in our future plan to transfer all this obtained information in docking experiments to AT1 receptor. Already, AT1 receptor was modeled and docking of losartan with other AT1 antagonists was attempted [48-65]. However, the docking of the molecules was not based on obtained conformational analysis data of the ligands.

E. Novel Approaches. Drug Design Based on the Mimicry of Aromatic Side Chain Cluster of Sarmesin

What is the significance to study mimicry of AT1 antagonists with sarmesin? Can be of aid in the medicinal chemistry this effort to find novel molecules not resembling to losartan but mimicking the aromatic side chain cluster of sarmesin and Ang II? The answer is yes. Based on this mimicry new molecules can be designed and synthesized having new appropriate scaffolds and deviating from the prototype structure of losartan.

An example of a simple molecule that has characteristics of C-terminal of Sarmesin and mimicks its conformation is shown in Fig. (**29**). In this molecule the aromatic side chains

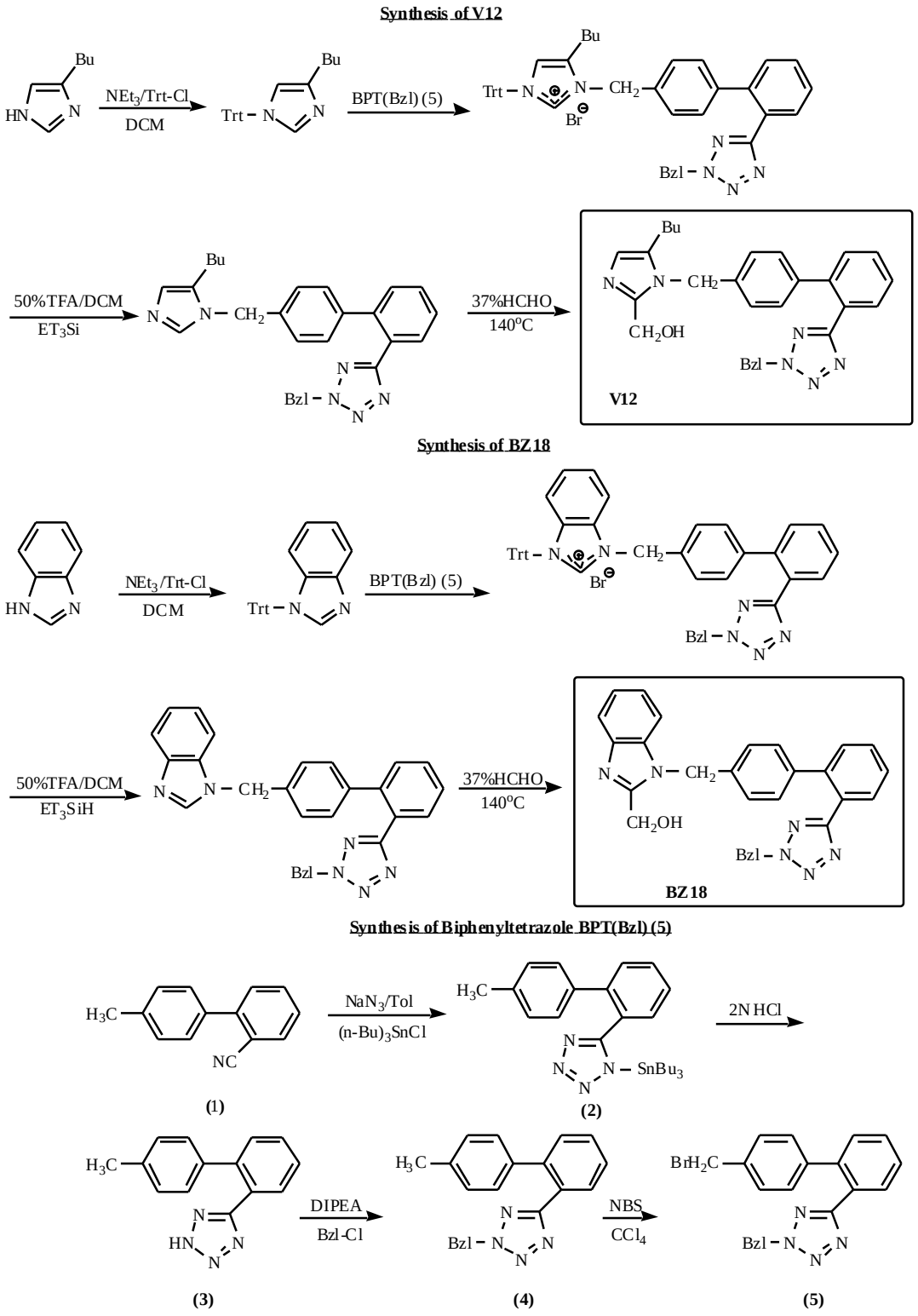


Fig. (23). Synthesis of V12 and BZI8.

of Tyr(OMe)[4] and His[6] are mounted into pyrrolidine scaffold. The pyrrolidine scaffold has already been used as a scaffold for the development of CCK peptide mimetics [66].

MM1 is the first lead compound and many others have been designed and are in the process of being synthesized. It has significant antihypertensive activity (71% compared to

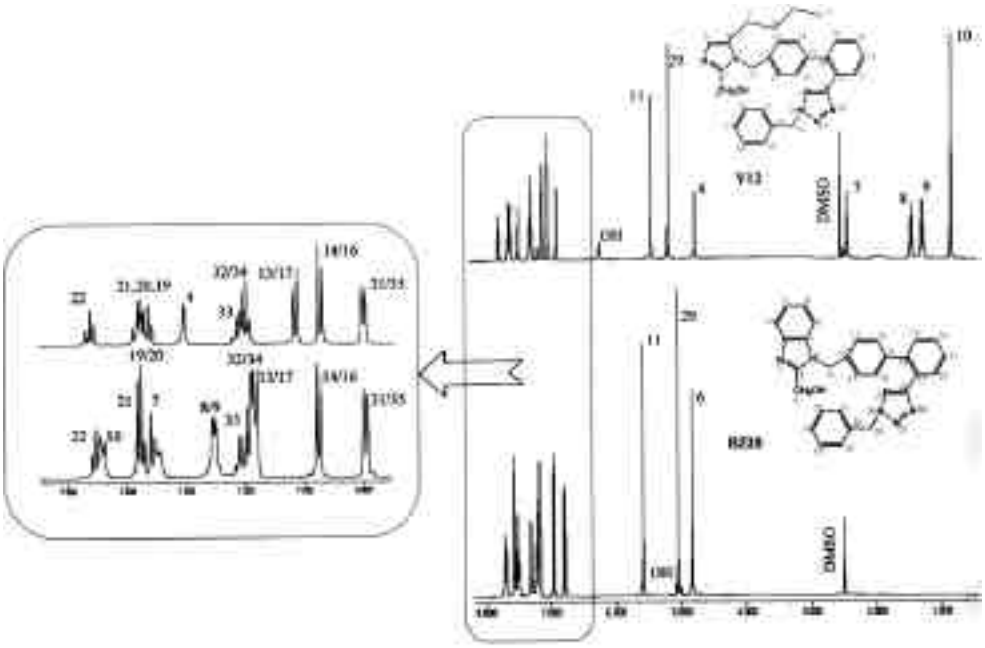


Fig. (24). ^{1}H NMR of V12 and BZI8, obtained using Varian 600 MHz at 27^{o} C.

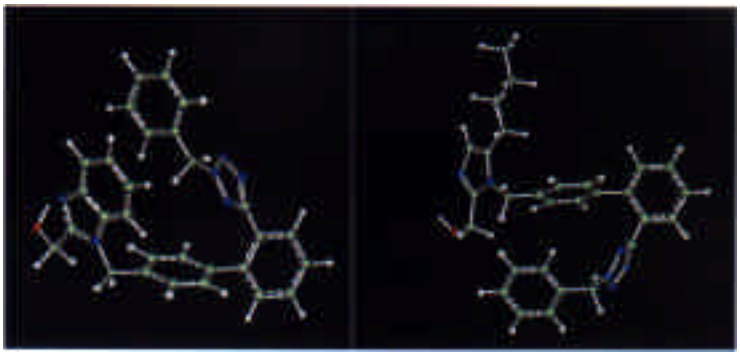


Fig. (25). Low energy conformers for BZI8 (left) and V12 (right) consistent with ROE data.

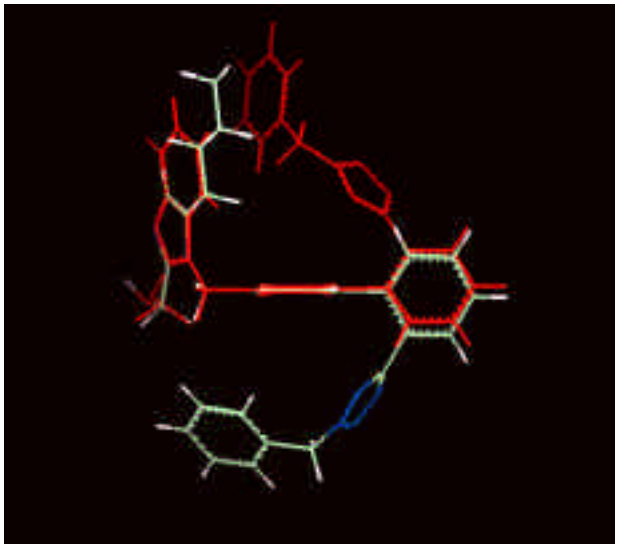


Fig. (26). Superimposition of V12 with BZI8 (red).

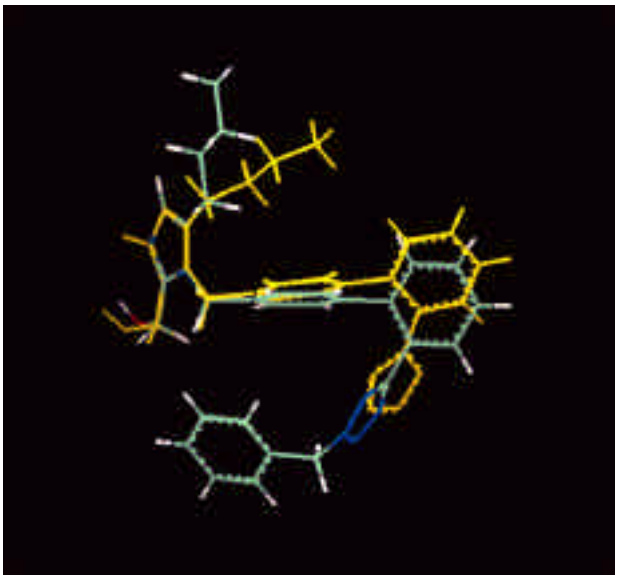


Fig. (27). Superimposition of V12 with losartan (yellow), using as equivalent atoms the N1, C2 and C5 of their corresponding imidazole rings.

Fig. (28). Superimposition of BZI8 with losartan (yellow), using as equivalent atoms the N1, N3 and C15 of their corresponding imidazole and phenyl rings.

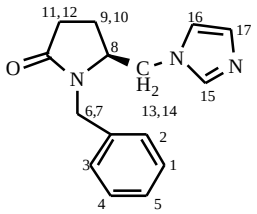


Fig. (29). Chemical structure of MM1.

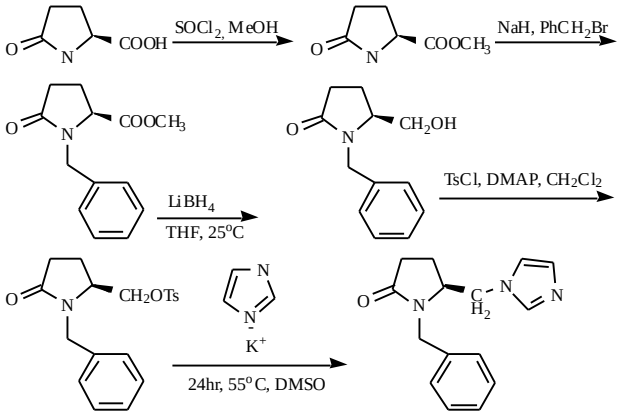


Fig. (30). Chemical synthesis of MM1.

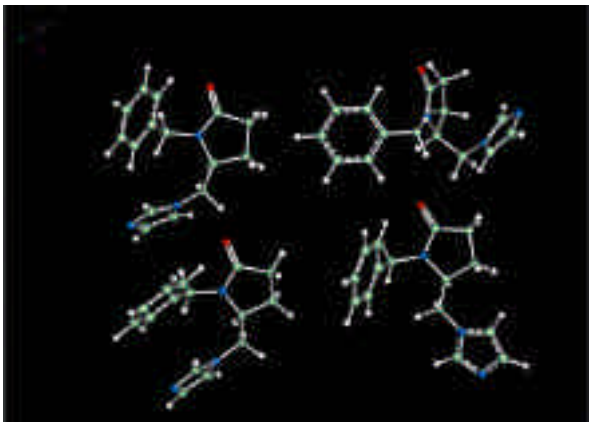


Fig. (31). Low energy conformers of MM1 derived using conformational search procedures.

Fig. (32). Superimposition of MM1 with C-terminal segment of sarmesin [67].

losartan) and has different structural features from losartan. Therefore, a new avenue of candidate antihypertensive drug is opened which uses a pyrrolidinone saffold instead of a

biphenyl ring. These molecules are advantageous over the known SARTANs from two aspects: (a) are easily synthesized and (b) their structures are based on rational drug design. In addition, such a class of molecules further confirm our aromatic side chain ring cluster model of Ang II and Sarmesin.

The simplicity of the synthesis of MM1, a representative molecule in this class is shown in Fig. (**30**). The structure of MM1 was proved unequivocally using 1D and 2D NMR spectroscoopy. The most important NOEs for the MM1 show that there is a spatial proximity between the two aromatic rings. Proton 2 of the phenyl ring is in a close spatial distance with proton 16. NOEs predict a close conformation of the molecule.The use of conformational search procedures provided the four low conformers shown in Fig. (**31**). The four conformers have a diastereomeric relationship (two enantiomeric pairs). The low energy conformer best superimposed in the sarmesin supported also all observed NOEs. The superimposition matched the following equivalent groups. (a) imidazole group of MM1 with imidazole group of His^6; (b) phenyl group of MM1 with Phe group of Phe^8; (c) lactame amide group of pyrrolidinone with amide bond of Phe^8-Pro^7. The superimposition was excellent (RMS 0.71 Å), Fig. (**32**).

CONCLUSIONS

It is apparent from this discussion that mimicry can lead to the rational design and drugs with better pharmacological profile. Understanding the stereoelectronic features responsible for activity in Ang II, Sarmesin and AT_1 antagonists will lead to new classes of molecules with certain advantages over the drugs already existing in the market. Their activity will shed light to the validity of the proposed models. From the reported models of Ang II there is no criterion for favoring a certain conformation. To our opinion a model has the merit if it aids to comprehend in the pharmacophore segments responsible for activity and helps in the design of new bioactive drugs. The model of Ang II proposed by J. Matsoukas and his collaborators appears to be a useful one for the design of new molecules. As a template offers the possibility to organic chemists to use their chemical intuition for synthesis of new molecules that may possess a better pharmacological profile.

REFERENCES

[1] Watson, J.D.; Crick, F.H.C. Molecular Structure of Nucleic Acids. Nature **1953**, *171*, 737-738.

[2] Bando, T.; Namba, Y.; Shishido, K. Lipase-mediated asymmetric construction of 2-arylpropionic acids:enantiocontrolled of S-naproxen and S-ibuprofen. Tetrahedron Asymmetry **1997**, *8*, 13, 2159-2165.

[3] Ebeling, S.C. The Synthesis of Artificial Sweeteners (phenylglycine-analogues of aspartame) in Order to Evaluate Changes in the _-Glycophore Component. **1998**, *1*, 107-112.

[4] Mavromoustakos, T.; A. Yang, D.P.; Charalambous, A.; Herbette, L.G.; Makriyannis, A.: Study of the topography of cannabinoids using x-ray diffraction. *Biochim. Biophys. Acta* **1990**, *1024*,336-344.

[5] Yang D.P.; Mavromoustakos, T.; Beshah, K.; Makriyannis, A.: Amphipathic interactions of cannabinoids with membranes. A comparison of cannabinoids with membranes. A comparison between Δ^8-THC and its O-methyl analog using differential scanning calorimetry, X-ray diffraction and solid state ^{2}H-NMR. *Biochim. Biophys. Acta* **1992**, *1103*, 25-36.

[6] Mavromoustakos,T.; Yang, D.P.; Makriyannis, A.. Topography of Alphaxalone and Δ^{16}-alphaxalone in Membrane Bilayers containing cholesterol *Biochim. Biophys. Acta* **1994**, *1194*, 69-74.

[7] Yang, D.P.; Makriyannis, A.: Effects of the steroids alphaxalone and its inactive analog Δ^{16}-analog on the thermotropic properties of membrane bilayers. A model for membrane perturbation. *Biochim. Biophys. Acta* 1995, *1239(2)*, 257-264.

[8] Burnier, M.; Brunner, M.R. Comparative antihypertensive effects of angiotensin receptor antagonists. *J. Am. Soc. Nephrol.* **1999,** *12S* 278-282.

[9] Birkenhager, W.; De Leeuw, P. Non-Peptide angiotensin type 1 receptor antagonists in the treatment of hypertension. *J. Hypertens.* **1999,** 7873-881.

[10] De Gasparo, M.; Catt, K.J.; Inagami, T.; Wright, J.W. and Unger, T. International Union of Pharmacology. XXIII. The Angiotensin II Receptors. Pharmacol. Rev. **2000**, *52*, 415-472.

[11] Moore, G. J. Kinetics of acetylation-deacetylation of Angiotensin II. Intramolecular interactions of the tyrosine and histidine side chains. *Int. J. Pept. Protein Res.* **1985,** *26*, 469-478.

[12] Turner, R. J.; Matsoukas, J. M. and Moore, G. J. Fluorescence properties of Angiotensin II analogues in receptor-simulating environments: relationship between tyrosinate fluorescence lifetime and biological activity. *Biochim. Biophys. Acta* **1991,** *1065*, 21-28.

[13] Matsoukas, J. M.; Hondrelis, J.; Keramida, M.; Mavromoustakos, T.; Makriyannis, A.; Yamdagni, R.; Wu, Q. and Moore, G. J. Role of the *N*-terminal domain of ANG II and [Sar^1] ANG II on conformation and activity. *J. Biol. Chem.* **1994,** *269*, 5303-5312.

[14] Carpenter, A.K.; Wilkes, C.B.; Schiller, P.W. The octapeptide angiotensin II adopts a well-defined structure in a phospholipid environment. *Eur. J. Biochem.* **1998**, *251*, 448-453.

[15] Nikiforovich, G.V.; Marshall, G.R. Three-dimensional recognition requirements for Angiotensin agonists: A novel solution for an old problem. *Biochem. Biophys. Res. Commun* **1993,** *195*, 222-228.

[16] De Coen, J. L. and Ralston, E. Theoretical conformational analysis of Asn^1, Val^5 Angiotensin II. *Biopolymers* **1977,** *16,* 1929-1943**.**

[17] Rauk, A.; Hamilton, G. and Moore, G. J. Mechanistic consequences of charge transfer systems in serine proteases and Angiotensin: semiempirical computations. *Biochem. Biophys. Res. Commun.* **1987,** *145*, 1349-1355.

[18] Fowler, P. W. and Moore, G. J. Calculation of the magnitude and orientation of electrostatic interactions between small aromatic rings in peptides and proteins: implications for Angiotensin II. *Biochem. Biophys. Res. Commun.* **1988,** *153*, 1296-1300.

[19] Marshall, G. R. and Bosshard, H. E. Angiotensin II, studies on the biologically active conformation. *Circ. Res.* **1972,** *30 and 31 (Suppl. II)*, 143-149.

[20] Smeby, R. R.; Arakawa, K.; Bumpus, F. M. and Marsh, M. M. A. A proposed conformation of isoleucyl^5-Angiotensin II. *Biochim. Biophys. Acta* **1962,** *58*, 550-557.

[21] Printz, M. P.; Nemethy, G. and Bleich. H. Proposed models for Angiotensin II in aqueous solution and conclusions about receptor topography. *Nature (London)* **1972,** *237*, 135-140.

[22] Juliano, L. and Paiva, A. C. M. Conformation of Angiotensin II in aqueous solution. Titration of several peptide analogs and homologs. *Biochemistry* **1974,** *13*, 2445-2450.

[23] Fermandjian, S.; Sakarellos, C.; Piriou, F.; Juy, M.; Toma, F.; Thanh, H. L.; Lintner, K.; Khoslia, M. C.; Smeby, R. R. and Bumpus, F. M. The key role of residue 5 in Angiotensin II. *Biopolymers* **1983,** *22*, 227-231.

[24] Weinkam, R. J. and Jorgensen, E. C. Angiotensin II analogs VIII. The use of free radical containing peptides to indicate the conformation of the carboxyl terminal region of Angiotensin II. *J. Am. Chem. Soc.* **1971,** *93*, 7033-7038; 7038-7044.

[25] Lenkinski, R. E.; Stephens, R. L. and Krishna, N. R. Conformation of Angiotensin II. Evidence for a specific hydrogen bonded conformation. *Biochemistry* **1981,** *20*, 3122-3126.

[26] Liakopoulou-Kyriakides, M. and Galardy, R. E. *s-Cis* and *s-trans* isomerism of the His-Pro peptide bond in Angiotensin and thyroliberin analogues. *Biochemistry* **1979,** *18*, 1952-1957.

[27] Bleich, H. E.; Galardy, R. E. and Printz, M. P. Conformation of Angiotensin II in aqueous solution. Evidence for the _-turn model. *J. Am. Chem. Soc.* **1973,** *95*, 2041-2042.

[28] Deslauriers, R.; Paiva, A. C. M.; Schaumburg, K. and Smith, I. C. P. Conformational flexibility of Angiotensin II. A carbon-13 spin-lattice relaxation study. *Biochemistry* **1975,** *14*, 878-886.

[29] Piriou, F.; Lintner, K.; Fermandjian, S.; Fromageot, P.; Khosla, M. C.; Smeby. R. R. and Bumpus, F. M. Amino acid side chain conformation in Angiotensin II and analogs: correlated results of circular dichroism and ^{1}H nuclear magnetic resonance. *Proc. Natl. Acad. Sci. U.S.A.* **1980,** *77*, 82-86.

[30] Sakarellos, C.; Lintner, K.; Piriou, F. and Fermandjian, S. Conformation of the central sequence of Angiotensin II and anlogs. *Biopolymers* **1983,** *22*, 663-687.

[31] Prendergast, K.; Adams, K.; Greenlee, W. J.; Nachbar, R. B.; Patchett, A. A. and Underwood, D. J. J. Derivation of a 3D pharmacophore model for the angiotensin-II site one receptor. *J. Comput.-Aided Mol. Design* **1994,** *8*, 491-512; references 3, 32, 36 cited there in.

[32] Duncia, J.V.; Carini, D.J.; Chiu, A.T.; Johnson, A.L.; Price, W.A.; Wong, P.C.; Wexler, R.R.; Timmermans, P.B.M.W.M. The discovery of DuP 753, a potent, orally active nonpeptide angiotensin II antagonist. *Med. Res. Rev.* **1992**, *12*, 149-191.

[33] An excellent review on the role of AII in the Renin-Angiotensin system and the classes of pharmaceutical molecules that act on this system is: Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D. and Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II receptor antagonists: the next generation in antihypertensive therapy. *J. Med. Chem.* **1996,** *39*, 625-655.

[34] Samanen, J. M.; Peishoff, C. E.; Keenan R. M.; and Weinstock, J. Refinement of a molecular model of Angiotensin II (AII) employed in the discovery of potent nonpeptide antagonists. *Biorg. Med. Chem. Lett.* **1993**, *3*, 909-914.

[35] Mavromoustakos, T.; Apostolopoulos, V.; Matsoukas, J. Antihypertensive Drugs that Act on Renin-Angiotensin System with Emphasis in AT1 Antagonists. *Mini Reviews in Medicinal Chemistry*, **2001**, *1*, 207-217.

[36] Mavromoustakos, T.; Kolocouris, A.; Zervou, M.; Roumelioti, P.; Matsoukas, J.; Weisemann, R. 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 *J. Med. Chem.* **1999**, *42*(10) 1714-1722.

[37] Matsoukas, J. M.; Agelis, G.; Hondrelis, J.; Yamdagni, R.; Wu, Q.; Ganter, R.; Smith, J.; Moore, D. and Moore, G. J. Synthesis and biological activities of Angiotensin II, Sarilesin and Sarmesin analogues containing Aze or Pip at position 7: conformational properties of [Sar^1,Aze^7] ANG II determined by nuclear overhauser effect (NOE) enhancement spectroscopy. *J. Med. Chem.* **1993,** *36*, 904-911.

[38] Matsoukas, J.M.; Yamdagni, R.; and Moore, G. J. ^{1}H-NMR Studies of Sarmesin and [Des^1]Sarmesin conformation in dimethylsulfoxide by Nuclear Overhauser Effect (NOE) enhancement spectroscopy: folding of the *N*- and *C*- terminal domains. *Peptides.* **1990**, *11***,** 367-374.

[39] Matsoukas, J.M.; Agelis, G.; Wahhab, A.; Hondrelis, J.; Panagiotopoulos, D.; Yamdagni, R.; Wu, Q.; Mavromoustakos, T.; Maia, H.L.S.; Ganter, R.; and Moore, G.J. Differences in Backbone Structure between Angiotensin II Agonists and Type I Antagonists. *J. Med. Chem.* **1995**, *38*, 4660-4669.

[40] Ancans, J.; Biseniece, D.; Myshliakova, N. and Porunkevich, E. A. Cyclic analogues of Angiotensin II, with depressor and histamine-liberating activity. *Bioorg. Khim.* **1986,** *12*, 118-120.

[41] Ancans, J.; Biseniece, D.; Myshliakova, N. and Chipens, G. Synthesis and study of cyclic analogues of Angiotensin. *Bioorg. Khim.* **1990,** *16*, 358-369.

[42] Matsoukas, J.M.; Polevaya, L.; Ancas, J.; Mavromoustakos, T.; Kolocouris, A.; Roumelioti, P.; Vlahakos, D.V.; Yamdagni, R.; Wu, Q.; Moore, G.J.. The Design and Synthesis of a Potent Angiotensin II Cyclic Analogue Confirms the Ring Cluster Receptor Conformation of the Hormone Angiotensin II *Bioorganic and Medicinal Chemistry* **2000**, *8*, 1-10.

[43] Polevaya, L.; Mavromoustakos, T.; Zoumboulakis, P.; Grdadolnik, S. G.; Roumelioti, P.;. Giatas, N.; Mutule, Ilze; Keivish, T.; Vlahakos, D.; Iliodromitis, E.; Kremastinos, D.; Matsoukas J. Synthesis and Study of Cyclic Angiotensin II Antagonist Analogue reveals the role of π^*-π^* interactions in the C-Terminal Aromatic Residue for agonist activity and its structure resemblance with AT_1 non-peptide antagonists. *Bioorg. Med. Chem.* **2001,** 9,1639-16479.

[44] Roumelioti, P.; Polevaya, L.; Zoumpoulakis, P.; Giatas, N.; Keivish, T.; Haritonova, A.; Zoga, A.; Vlahakos, D.; Iliodromitis, E.; Kremastinos, D.; Grdadolnik, S.G.; Mavromoustakos, T.; Matsoukas, J. Design, Synthesis and Biological Evaluation of Cyclic Angiotensin II Analogues with 3,5 Side Chain Bridges: Role of C-Terminal Aromatic Residue and Ring Cluster for Activity and Implications in the Drug Design of AT1 non Peptide Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2627- 2633.

[45] Roumelioti, P.; Tselios, T.; Alexopoulos, K.; Mavromoustakos, T.; Kolocouris, A.; Moore, G.J.; Matsoukas, J.M.. Structural comparison between type I and type II antagonists: possible implications in the drug design of AT-1 antagonists. *Biorganic and Medicinal Chemistry Letters*, **2000**, *10*, 755-758.

[46] Zoumpoulakis, P.; Grdadolnik, S.G.; Matsoukas, J.; Mavromoustakos, T. Structure elucidation and conformational properties of eprosartan a non peptide Angiotensin II AT_1 antagonist. *J. Pharmaceut. Biomed.* **2002**, *28*, 125-135.

[47] Bauer, M.; Harris, R.K.; Rao, C. R.; Apperley, D.C.; Rodger, C.A. NMR Study of Desmotropy in Irbesartan, a Tetrazole-Containing Pharmaceutical Compound. *J. Chem. Soc., Perkin Trans.* **1998**, 2, 475-481.

[48] U. Gether and B.K. Kobilka, G Protein-coupled Receptors, *J. Biol. Chem.* **1998,** *273*, 17979-17982.

[49] Nirula,V.; Zheng, W.; Krishnamurthi, K.; Sandberg, K. Identification of nonconserved amino acids in the AT_1 receptor which comprise a general binding site for biphenylimidazole antagonists, *FEBS Letters* **1996,** *394*, 361-364.

[50] Dascal, D.; Nirula, V.; Lawus, K.; Yoo, S.E.; Walsh, T.F.; Sandberg, K. Shared determinants of receptor binding for subtype selective, and dual endothelin-angiotensin antagonists on the AT_1 angiotensin II receptor, *FEBS Letters* **1998** *423*, 15-18.

[51] Balmforth, A.J.; Lee, A.J.; Warburton, P.; Donnelly, D. and Ball, S.G. The Conformational Change Responsible for AT_1 Receptor Activation Is Dependent upon Two Juxtaposed Asparagine Residues on Transmembrane Helices III and VII, *J. Biol. Chem.* **1997**, *272* 4245-4251.

[52] Schambye, H.T.; Hjorth, S.A.; Weinstock, J.; Schwartz, W. Interaction between the Nonpeptide Angiotensin Antagonist SKF-108,566 and Histidine 256 (HisVI:16) of the Angiotensin Type 1 Receptor, Molec. Pharmac. **1994,** *47*, 425-431.

[53] Nirula, V.; Zheng, W.; Sothinathan, R.; Sandberg, K. Interaction of biphenylimidazole and imidazoleacrylic acid nonpeptide antagonists with valine 108 in TMIII of the AT_1 angiotensin receptor, British J. of Pharmac. **1996**, 119, 1505-1507.

[54] Ji, H.; Zheng, W.; Zhang, Y.; Catt, K.J.; Sandberg, K. Genetic transfer of a nonpeptide antagonist binding site to a previously unresponsive angiotensin receptor, Proc. Natl. Acad. Sci. USA, Pharmacology **1995,** *92*, 9240-9244.

[55] H. Ji, M. Leung, Y. Zhang, K.J. Catt and K. Sandberg, Differential Structural Requirements for Specific Binding of Nonpeptide and Peptide Antagonists to the AT_1 Angiotensin Receptor. *J. Biol. Chem.* **1994**, *269* (24), 16533-16536.

[56] Feng, Y.H.; Noda, K. Y.; Saad, X.P.; Liu, A.; Husain ; Karnik, S.S. The Docking of Arg^2 of Angiotensin II with Asp^{281} of AT_1 Receptor Is Essential for Full Agonism, *J. Biol. Chem.* **1995,** *270*, 12846-12850.

[57] Feng, Y.H. and Karnik, S.S. Role of Transmembrane Helix IV in G-protein Specificity of the Angiotensin II Type 1 Receptor, *J. Biol. Chem.* **1999,** *274*, 35546-35552.

[58] Noda, K.; Saad Y.; Karnik, S.S. Interaction of Phe^8 of Angiotensin II with Lys^{199} and His^{256} of AT_1 Receptor in Agonist Activation, *J. Biol. Chem.* **1995,** *270*, 28511-28514.

[59] Wang, C. Jayadev, S. Escobedo, J.A. Identification of a Domain in the Angiotensin II Type 1 Receptor Determining G_2 Coupling by the Use of Receptor Chimeras, *J. Biol. Chem.* **1995,** *270*, 16677-16682.

[60] Hunyady, L.; Ji, H.; Jagadeesh, G.; Zhang, M.; Gaborik, Z.; Mihalik, B.; Catt, K.J. Dependence of AT_1 Angiotensin Receptor Function on Adjacent Asparagine Residues in the Seventh Transmembrane Helix, *Mol. Pharm.* **1998**, *54*, 427-434.

[61] Hjorth, S.A.; Schambye, H.T.; Greenlee, W.J.; Schwartz, T.W. Identification of Peptide Binding Residues in the Extracellular

Domains of the AT_1 Receptor, J. Biol. Chem. **1994**, *269*, 30953-30959.

[62] Monnot, C.; Bihoreau, C.; Conchon, S.; Curnow, K.M.; Corvol P.; Clauser, E. Polar Residues in the Transmembrane Domains of the Type 1 Angiotensin II Receptor Are Required for Binding and Coupling, *J. Biol. Chem.* **1996**, *271*, 1507-1513.

[63] Miura, S.I.; Feng, Y.H.; Husain, A.; Karnik, S.S. Role of Aromaticity of Agonist Switches of Angiotensin II in the Activation of the AT_1 Receptor, *J. Biol. Chem.* **1999**, *274*, 7103-7110.

[64] Noda, K.; Saad, Y.; Kinoshita, A.; Boyle, T.P.; Graham, R.M.; Husain, A.; Karnik, S.S. Tetrazole and Carboxylate Groups of Angiotensin Receptor Antagonists Bind to the Same Subtype by Different Mechanisms, J. Biol. Chem. **1995,** *270*, 2284-2289.

[65] Yamano, Y. K.; Ohyama, M. Kikyo, T. Sano, Y. Nakagomi, Y. Inoue, N. Nakamura, I. Morishima, D.F. Guo, T. Hamakubo and T. Inagami, Mutagenesis and the Molecular Modeling of the Rat Angiotensin II Receptor (AT_1), J. Biol. Chem. **1995**, *270* 14024-14030.

[66] Giannis, A.; Kolter, T. Peptidomimetics for receptor ligands-discovery, development, and medical perspectives. Angew. Chem. Int. Ed. Engl. **1993**, *32*, 1244-1267.

[67] –NH of the amide bond in sarmesin was converted to $–NH_2$ in order software QUANTA to recognize the structure.