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# Structural Comparison Between Type I and Type II Antagonists: Possible Implications in the Drug Design of AT1 Antagonists

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**Abstract**—Analogues of sarilesin (type I AT1 antagonists), and sarmesin (type II AT1 antagonists) with homoserine (hSer) at position 8 were prepared and bioassayed. The presence of a Tyr[4]-Ile[5]-His[6] bend found in sarmesin but not in sarilesin was identified. The obtained results coupled with conformational analysis studies, using a combination of NMR spectroscopy and computational chemistry, propose important conformational and stereoelectronic properties for agonist and antagonist activity at AT1 receptors.


The octapeptide angiotensin II (AII, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) acts at receptors of peripheral and central sites to produce increased vascular resistance and extracellular fluid volume and has been implicated in blood pressure regulation in both normotensive and hypertensive states.[1] As a result of its biological importance, AII has been extensively studied since its discovery several years ago. In particular, the spectroscopic investigations led to models in which the AII carboxyl terminal region was used to design peptidomimetic analogues. The synthetic effort for such analogues was rewarded with losartan and other derivatives (SARTANs) that entered the market, while others are in a clinical trial.[2]

Our laboratory has been engaged for more than a decade in the conformation analysis study of AII, its superagonist [Sar[1]]AII, and structurally similar peptide agonists and antagonists. The accumulated experimental evidence for AII and [Sar[1]]AII in DMSO supports a bioactive conformation characterized by (a) a Tyr[4]-Ile[5]-His[6] bend, (b) a major His[6]-Pro[7] *trans* conformer, (c) a cluster of the side chain aromatic rings of the triad key amino acids Tyr[4], His[6], Phe[8] which drives the formation of a charge relay system between Tyr[4] hydroxyl, His[6] imidazole and Phe[8] carboxylate, analogous to that found in serine proteases. This relay system appears to be responsible for AII and [Sar[1]]AII biological activity.[3] 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 charge relay system possibly interacting through cation-π interactions.[4] The charge relay conformation is supported by the synthesis and conformational analysis 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.[5] This cyclic analogue was found to possess agonistic activity when tested in the rat uterus assay and in anesthesized rabbits. Structure–activity relationships demand the presence of Phe[8], His[6], Tyr[4] and Sar[1] for [Sar[1]]AII to possess biological activity. Therefore, it can be inferred that the aromatic ring cluster and consequently the charge relay system formation of [Sar[1]]AII may be the key stereoelectronic molecular features to exert its biological activity. Substitution of Asp at position 1 with Sar results in an increase in potency of both agonist and antagonist analogues of AII — an effect that may be attributed to an increased binding affinity as well as an

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increased biological half-life of the peptide. Removal of Sar[1] resulted in a dramatic loss in biological activity of AII agonists and antagonists.

Two types of antagonist analogues have been identified from structure–activity studies. Type I antagonists are obtained by replacing the Phe[8] residue by an aliphatic one (e.g. Ala, Ile) and are characterized by slow receptor dissociation rates. The conformational properties of these analogues differ from those of AII because they lack the Tyr[4]-Ile[5]-His[6] bend.[5,6] Type II antagonists are produced by methylating or omitting the Tyr hydroxyl group in [Sar[1]]II and are reversible and competitive antagonists. [Sar[1],Tyr(Me)[4]]AII (sarmesin) is a prototype type II antagonist.[7]

In a previous study it was found that sarmesin adopts a folded conformation characterized by a His[6]-Pro[7] *trans* conformer, a clustering of the side chain aromatic rings of aminoacids Tyr(Me)[4], His[6] and Phe[8] and a proximity of Sar[1] or Arg[2] with the cluster.[8] Removal of Sar[1] resulted in [des[1]]sarmesin which showed the presence of an aromatic ring cluster and a dramatic loss in biological activity in comparison with sarmesin. Substitution of the Arg[2] residue of sarmesin with Sar abolished antagonist activity.[9] However, the ability of sarmesin to form Tyr[4]-Ile[5]-His[6] bend as it was observed with AII was not investigated. Based on these data, new molecules were designed and synthesized with the aim to get more information on the conformational properties of the C-terminal domain. In the present paper the C-terminal aminoacid of sarmesin or sarilesin were replaced with hSer and the pharmaceutical significance of the C-terminal domain was further investigated.

Table 1 shows the biological activities of the various sarmesin and sarilesin analogues synthesized in the present study. As it is evident from the obtained data for the nine peptides listed in Table 1, the N-terminal residue sarcosine is required for antagonist activity since its removal results in inactive peptides.

In the type I antagonist Sarilesin replacement of Ile[8] by hSer results in a strong antagonist ($pA_2 = 7.8$) indicating the ability of the receptor to accommodate a polar hydroxyl group at the C-terminus side chain.

Type I/II hybrid AII antagonists are produced by the simultaneous substitution of Phe[8] by an aliphatic amino acid and methylation of phenolic hydroxyl group of Tyr[4]. Whereas [Sar[1],Tyr(Me)[4],Ile[8]]AII is a weak antagonist ($pA_2 = 6.6$), [Sar[1],Tyr(Me)[4],HSer[8]]AII has furthermore reduced antagonist potency ($pA_2 = 5.7$) indicating that the receptor cannot tolerate two changes at positions 4 and 8.

**Table 1.** Rat uterus biological activities of sarilesin, sarmesin and their analogues containing homoserine at position 8

| Analogue | Antagonist activity ($pA_2$) |
|---|---|
| [Sar[1], Ile[8]]AII (sarilesin) | 8.1 |
| [Sar[1], hSer[8]]AII | 7.8 |
| [hSer[8]]AII | 6.7 |
| [Des[1], hSer[8]]AII | < 4.8 |
| [Sar[1],Tyr(Me)[4]]AII (sarmesin) | 7.5 |
| [Sar[1],Tyr(Me)[4], Ile[8]]AII | 6.6 |
| [Sar[1],Tyr(Me)[4], hSer[8]]AII | 5.7 |
| [Tyr(Me)[4], hSer[8]]AII | 5.4 |
| [Des[1],Tyr(Me)[4], hSer[8]]AII | < 4.8 |

The investigation of the presence of a Tyr[4]-Ile[5]-His[6] bend was preliminarily investigated in the two prototype antagonists sarmesin and sarilesin by 1D NOE spectroscopy. Thus, selective irradiation of sarmesin His CaH resulted in intensity enhancement of the Tyr(Me) CaH. Interestingly, the dipolar correlation between His CaH and Tyr(Me) CaH was also found in [des[1]]sarmesin a peptide in which the first aminoacid Sar is missing. An analogous NOE was missing for sarilesin.

The activities of the designed molecules were found as anticipated based on proposed models of AII, sarmesin and sarilesin. According to the proposed model of sarmesin there is a *trans* His[6]-Pro[7] conformation, a Tyr(Me)[4]-Ile[5]-His[6] bend and a side chain cluster between Tyr(Me)[4], His[6] , Phe[8], Sar[1]. The Tyr(Me)[4]-Ile[5]-His[6] bend and the aromatic ring cluster between Tyr(Me)[4], His[6] and Phe[8], is not affected by the presence of Sar[1] as NOE results point out the same cluster in [des[1]]sarmesin. This illustrates the significance of the extended side chain cluster between Tyr(Me)[4], His[6], Phe[8], Sar[1]. Substitution of Phe[8] with other aminoacids resulted in the loss of Tyr[4]-Ile[5]-His[6] bend and aromatic ring cluster formation and therefore in activity as it is observed in the novel synthetic compounds (Fig. 1).[10]

Replacement of AII Phe[8] with other aliphatic aminoacids leads to the type I antagonists with different biological profile than type II antagonists. The biological importance of aminoacid 8 is not restricted in type II antagonists but also in type I antagonists. In the prototype anatagonist sarilesin the Tyr[4]-Ile[5]-His[6] bend was not observed and the aromatic ring cluster is not present. Peptide antagonists of type I seem to adopt a more loose conformation and may act through a different subsite of a receptor (Fig. 2).

In an attempt to design potent antagonists and agonists the results showed the strict molecular requirements for peptide agonist activity. In particular, peptide AII agonists must have a Tyr[4]-Ile[5]-His[6] bend, 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]. In the type II antagonists a Tyr[4]-Ile[5]-His[6] bend and an extended side chain cluster between Tyr(Me)[4], His[6] , Phe[8], Sar[1] and or Arg[2] is observed. Absence of or partial formation of the above mentioned molecular clustering results in abolishment or decrease of their antagonist activity. In the type I antagonists among the above molecular feature only a partial clustering can be observed i.e., between Tyr[4] and His[6].[6] This points out different structural requirements between the two types of antagonists.

The significance of these observations is evident. Based on the stereoelectronic properties for agonist and antagonist

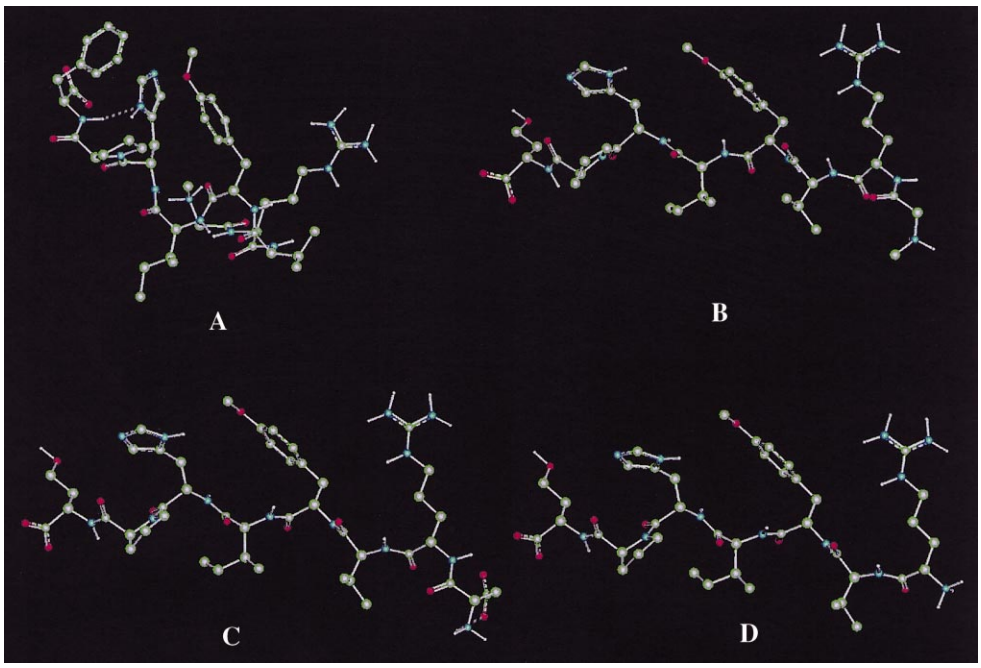


**Figure 1.** Molecular models of (A) sarmesin, (B) [Sar$^1$,Tyr(Me)$^4$,hSer$^8$]AII, (C) [Tyr(Me)$^4$,hSer$^8$]AII, (D) [Des$^1$,Tyr(Me)$^4$,hSer$^8$]AII based on NMR spectroscopy and computational analysis results.

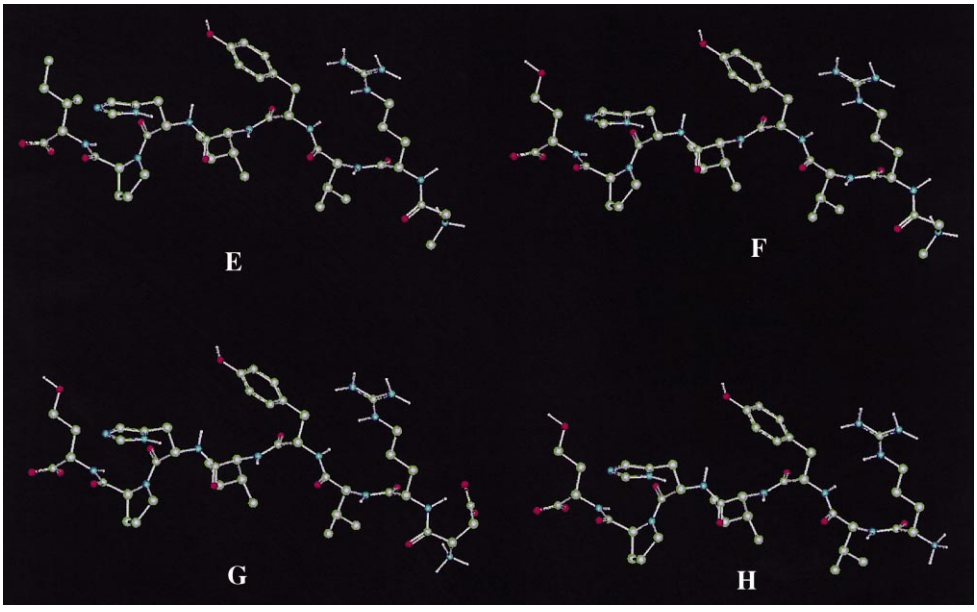


**Figure 2.** Molecular models of (E) sarilesin, (F) [Sar$^1$,hSer$^8$]AII, (G) [hSer$^8$]AII, (H) [Des$^1$, hSer$^8$]AII based on NMR spectroscopy and computational analysis results.

activities, novel synthetic peptidomimetic compounds can be synthesized that their molecular architecture can deviate from those of losartan. It remains to the synthetic chemists to open a new avenue and take advantage of the new findings.

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## References and Notes

1. Sealey, J. E.; Laragh, J. H. In *Hypertension: Pathophysiology, Diagnosis and Management*; Laragh, J. H.; Brener, B. M., Eds.; Raven: New York, 1990; pp 1287–1317.

2. Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D.; Timmermans, P. B. M. W. M. *J. Med. Chem.* **1996**, *39*, 625 and references there in.
3. (a) Matsoukas, J. M.; Hondrelis, J.; Keramida, M.; Mavromoustakos, T.; Makriyannis, A.; Yamdagni, R.; Wu, Q.; Moore, G. J. *J. Biol. Chem.* **1994**, *269*, 5303. (b) Carpenter, A. K.; Wilkes, C. B.; Schiller, P. W. *Eur. J. Biochem.* **1998**, *251*, 448.
4. Dougherty, D. A. *Science* **1996**, *271*, 163.
5. Matsoukas, J. M.; Polevaya, L.; Ancans, J.; Mavromoustakos, T.; Kolocouris, A.; Roumelioti, P.; Vlahakos, D. V.; Yamdagni, R.; Wu, Q.; Moore, G. J. *Bioorg. Med. Chem.* **2000**, *8*, 1.
6. Matsoukas, J. M.; Agelis, G.; Wahhab, A.; Hondrelis, J.; Panagiotopoulos, D.; Yamdagni, R.; Wu, Q.; Mavromoustakos, T.; Maia, H. L. S.; Ganter, R.; Moore, G. J. *J. Med. Chem.* **1995**, *38*, 4660.
7. Matsoukas, J. M.; Agelis, G.; Hondrelis, J.; Yamdagni, R.; Wu, Q.; Ganter, R.; Smith, J. R.; Moore, D.; Moore, G. J. *J. Med. Chem.* **1993**, *36*, 904.
8. Mavromoustakos, T.; Kolocouris, A.; Zervou, M.; Roumelioti, P.; Matsoukas, J. M.; Weisemann, R. *J. Med. Chem.* **1999**, *42*, 1714.
9. Matsoukas, J. M.; Cordopatis, P.; Belte, U.; Goghari, M. H.; Ganter, R. C.; Franklin, K. J.; Moore, G. J. *J. Med. Chem.* **1988**, *31*, 1418.
10. Models were generated using the QUANTA package and CHARMm force field. Low energy conformations A-H were resulted using a combination of molecular dynamics and NOE distance constraints. For details see ref 8.