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REFINEMENT OF A MOLECULAR MODEL OF ANGIOTENSIN II (AII) EMPLOYED IN THE DISCOVERY OF POTENT NONPEPTIDE ANTAGONISTS

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Abstract: A novel conformational model of AII, Model II, had been employed previously in the design of potent benzylimidazole AII antagonists (*J. Med. Chem.* **1991**, *34*, 1514-1517). This paper considers this model in relation to the recently described potent AII analogs [hCys^{3,5}]-AII, and [Sar^1,hCys^{3,5},Ile^8]-AII. Conformational analysis of Ac-S,S-cyclo-(hCys-Ala-hCys)-NH_2 suggests a family of modified conformations for AII, Model III, which retains the topological arrangement of functional groups in Model II that had been employed in nonpeptide analog design.

Introduction. We recently described[1,2] the use of a molecular model of angiotensin II, Model II, Figure 1, as a template for modifying the weak benzylimidazole AII antagonist 1[3] by the addition of certain binding groups from AII. Through the use of this overlay hypothesis, the acrylic acid SK&F 108566 6, Table I, was discovered[1] which has 40,000x greater affinity and 13,000x greater *in vitro* antagonist potency than 1. In fact, the *in vitro* potency and affinity of 6 surpasses saralasin, [Sar^1,Ala^8]-AII.[1] Subsequent to our development of acrylic acid analogs of 1, the AII analogs [hCys^{3,5}]-AII, and [Sar^1,hCys^{3,5},Ile^8]-AII, where hCys = homocysteine, appeared in the literature.[4,5] These analogs contain a cyclic structure between residues three and five and retain potent agonist and antagonist activity respectively. As originally proposed,[2] Model II is not consistent with the high potencies of the hCys analogs, since the sidechains in position three and five of Model II point in opposite directions, Figure 1, preventing formation of a disulfide bridge between homocysteines in these positions. Since the spatial relationship of positions four and five to position eight is a critical component of Model II, an important test of our overlay hypothesis would be to attempt revision of Model II into a conformation that would be consistent with the hCys analogs and yet retain a good overlay with the benzylimidazoles. This paper considers refinement of Model II to account for the hCys analogs and examines the effect of such modifications upon our overlay hypothesis. First, the manner in which Model II was developed and employed in nonpeptide AII antagonist design will be reviewed.

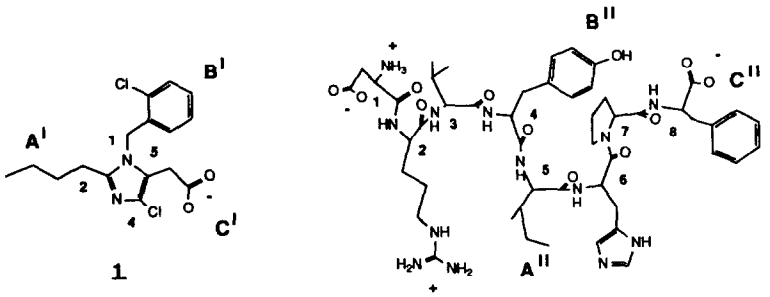
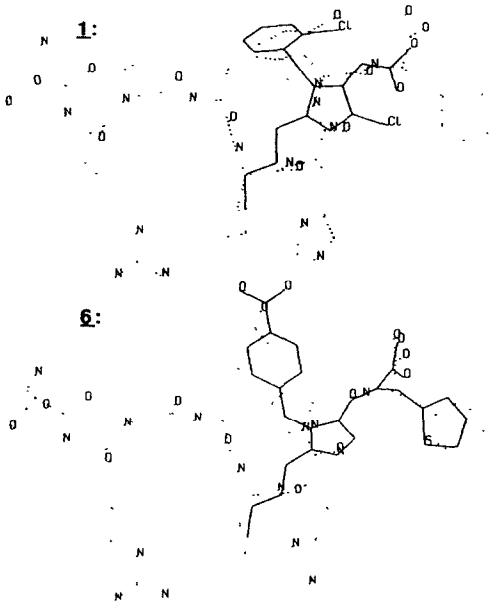


Figure 1. Correlation of sidechains in benzylimidazole 1: A^I,B^I,C^I with sidechains in AII Model II: A^{II},B^{II},C^{II}

Utilization of a Conformational Model of AII in the Development of Potent Nonpeptide AII Antagonists. Elements of 1 appeared reminiscent of AII: 2-Butyl (A^I) ~ Ile^5 (A^{II}); 1-(o-Cl)benzyl (B^I) ~ Tyr^4 (B^{II}) and 5-CH_2CO_2H (C^I) ~ AII C-terminus (C^{II}), Figure 1. A unique AII conformation was created[2] by folding AII around 1 in such a way that A^{II}-B^{II}-C^{II} was aligned with A^I-B^I-C^I , Figure 2, while retaining consistency with constrained AII analog SAR[6]. Model II was then employed in the design of potent AII antagonists. Examination of the overlay of 1 onto Model II and subsequent SAR development resulted in analog 3, bearing the conformationally restricted 5-CH=$CHCO_2H$ extension of the carboxylate group. Compound 3 was found to exhibit enhanced affinity and potency, relative to 1, Table 1.



Backbone Torsion Angles for 6

Angle	Asp	Arg	Val	Tyr	Ile	His	Pro	Thi
ϕ	---	-73	-134	75	-83	68	-60	-73
ψ	152	89	145	89	115	71	110	---
χ_1		-172		-88	-177	-41		-140

Figure 2: A II, Model II Overlayed with Benzylimidazoles 1, 3, and 6

No.	Subst. 1	Subst. 4	Substituent 5	K_B (uM) Rab. Aorta[a]	IC_{50} (uM) Binding (Mes)[b]	ID_{50} (mg/kg) ANG II-Rat[c]
1:	-(o-Cl)Bn	-Cl	-CH_2CO_2H	2.70	43.0	30.0
2:	"	-H	-CH_2CO_2H	1.90	12.0	22.5
3:	"	"	-CH=$CHCO_2H$*	0.81	8.9	15.0
4:	"	"	-CH=$C(Bn)CO_2H$*	0.064	2.60	14.0
5:	"	"	-CH=$C(CH_2Thi)CO_2H$*	0.051	0.44	3.60
6:	-(p-CO_2H)Bn	"	-CH=$C(CH_2Thi)CO_2H$*	0.00021	0.001	0.08

Table I: 1-Benzyl-2-butylimidazole Analogs. *Trans. a) Inhibition AII induced rabbit aorta constriction b) Inhibition ^{125}I- AII binding rat mesenteric membranes, c) IV Inhibition AII pressor response conscious normotensive rats. N=3-5.a-c

Model II also suggested appending a benzyl group alpha to the carboxylate group, eg. 4, Figure 2, which displays greater affinity and potency than acrylate 3. The thienyl analog 5 was even more potent in all assays, which is consistent with the enhanced potency of [Sar^1,(β-Thienyl)Ala^8]-AII over [Sar^1]-AII.[1] The design of aryl acrylate benzylimidazoles is an instance where molecular modeling of a nonpeptide with a peptide has played a key role in the discovery of nonpeptides with enhanced activity. Further modifications of 5 led to the aforementioned potent AII antagonist 6. Model II was subsequently modified to overlay the p-OH of Tyr^4 with the p-CO_2H of 6, Figure 2, (Tyr^4 χ^1 -140° to -90°).[2] SAR correlation between these groups is under examination.

Model III: Refinement of Model II to Account for the Potent hCys^{3,5} Analogs of AII. To evaluate the influence of the macrocycle created by the disulfide bridge in [hCys^{3,5}]-AII, a full conformational search was conducted[7] on the model tripeptide Ac-S,S-cyclo-hCys-Ala-hCys-NH_2. Four types of conformation were found for this peptide, Table II, (The high degree of flexibility for this peptide has been reported recently).[8] The alanine residue, which would correspond to Tyr in AII, adopts an α_L conformation in conformer X, congruent with earlier findings that [(αMe)Tyr^4]-AII retains high potency[9] (αMe-amino acids are constrained to alpha helical conformations[10]). In all four conformer types, the sidechains of both hCys^3 and hCys^5 point roughly in the same direction, due to the disulfide bridge.

Since [Cys^{3,5}]-AII displays considerably lower activity than [hCys^{3,5}]-AII, Ac-S,S-cyclo-Cys-Ala-Cys-NH_2 was also modeled. The same four conformer families W-Z were also found for this molecule. The main difference between these two model cyclic tripeptides resides in the Cys-Cα to Cys-Cα distance (mean ± S.D. for conformers within 5 kcal of minimum): Cys-Cys 5.12 ± 0.20 Å, hCys-hCys 5.76 ± 0.32 Å . Since the same types of conformers appear in both model tripeptides, the difference between the activities of Cys vs. hCys AII analogs may lie in the Cys-Cα to Cys-Cα distance which could affect the relative orientations of the important Arg^2 sidechain. Since Arg^2 is not constrained in either of these peptides we cannot determine what those relative orientations would be with the data in hand.

Family No.	Conformer Type	Alanine Torsion Angle Phi	Psi	Lowest Energy kcal/mole
W	extended	-130.8	99.8	4.86
X	α_L helix	58.5	78.2	5.69
Y	α_R helix	-90.1	-30.1	5.77
Z	$C7_{eq}$	-90.8	81.6	6.72

Table II. Conformers of Ac-S,S-cyclo-hCys-Ala-hCys-NH_2, (The lowest energy conformers from each family are shown).

Four analogous AII conformations were constructed from Model II through incorporation of the backbone angles of the lowest energy conformers from each conformer family of Ac-S,S-cyclo-hCys-Ala-hCys-NH_2 into residues three through five of AII Model II, Table III. These are refered to collectively as Model III. Residues 6 through 8 in these conformers remained unchanged from Model II. A high degree of overlap occured among the Ile^5 sidechain in these Model III conformers with the Ile^5 sidechain in Model II. The net result of this exercise, which is summarized in Figure 3, is that the N-terminal tripeptide is flipped to orient the Val^3 sidechain on the same side of the backbone and roughly parallel to Ile^5. The Tyr phenolic groups in the conformers of Model III adopt similar orientations as that of Model II, Figure 4.

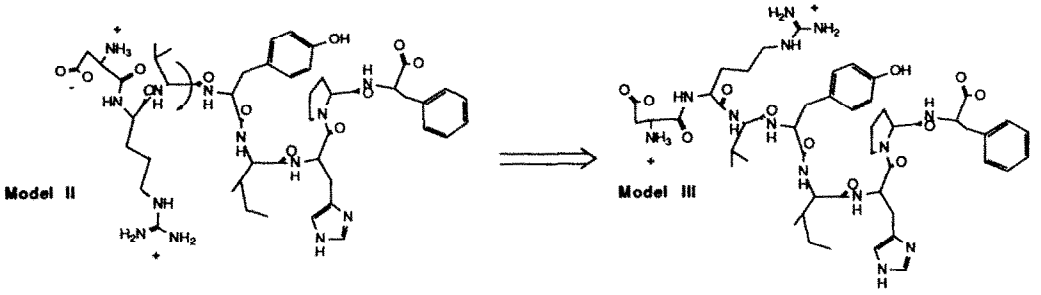


Figure 3. Refinement of Model II to Account for [hCys^{3,5}]-AII: Model III.

Position in AII	Torsion Angle	Model II	Model III, Conformers W	X	Y	Z
Asp	ϕ	---				
1	ψ	150.0				
	ω	-178.6				
Arg	ϕ	-74.0				
2	ψ	89.0				
	ω	174.3				
Val	ϕ	-133.5		-60		
3	ψ	145.6	163.6	-57.6	99.2	133 9
	ω	-175.4	174.9	179.8	175.9	180
Tyr	ϕ	74.8	-130.8	58.5	-90.1	-90.8
4	ψ	87.8	99.8	78.2	-30 1	81.6
	ω	175.8	195.1	175.9	184.9	181 3
Ile	ϕ	-81 8	-73.9	-80.2	69.2	-116.9
5	ψ	117.6			60	
	ω	-177 4				
His	ϕ	67.1				
6	ψ	72.4				
	ω	174.7				
Pro	ϕ	-58.8				
7	ψ	118.6				
	ω	-171.9				
Phe	ϕ	-86.2				
8	ψ	---				
Distance (Å)						
Tyr_{OH}-Arg_{guan}		14.4	10.9	2.44	8.79	8.6

Table III. Conformers of AII, Model III (Torsion angles in conformers W-Z are displayed only if different from Model II)

The backbone torsion angles of the N-terminal Asp-Arg dipeptide remains unchanged in Model III. Due to different conformations in the Val-Tyr-Ile region, however, the orientations of these groups vary relative to the His-Pro-Phe region in each Model III conformer when compared with Model II. Since the Arg^2 alkylguanidine sidechain is an important binding group in both AII agonists and antagonists,[11] the relative position of this group constitutes an important difference between Model II and the set of conformers in Model III. As seen in Figure 4, each Model III conformer places the Arg^2 guanidine group much closer to the Tyr^4 phenol than in Model II. Since the overlays with benzylimidazole antagonists do not involve the Arg^2 guanidine group, neither Model II nor Model III should be expected to define the orientation of that group. Constrained AII analogs which define the relationship between positions one and two with the rest of the peptide might help to "increase the resolution" of our models. It is interesting to note that in Model III three of the four major binding groups (Arg^2, Tyr^4, and the C-terminal carboxylate but not His^6) appear on one face of the molecule.

Of the four conformers, Conformer X, in which Tyr occurs in a helical conformation, is the most consistent with experimental data, eg. [αMe)Tyr^4]-AII.[9] Most important is the fact that our overlay hypothesis is upheld with Model III, since a good overlay may still be obtained with the benzylimidazole antagonist, Figure 5. (An overlay with Conformer X is displayed in Figure 5 although comparable overlays may be obtained with all four conformers). Model III may be one step closer to defining a bioactive conformation, since it can accomodate a macrocyclic ring via hCys in positions three and five. Nonetheless, these conformational models must be considered working hypotheses and templates that have served the chemist well in the design of potent nonpeptide.AII antagonists.[1]

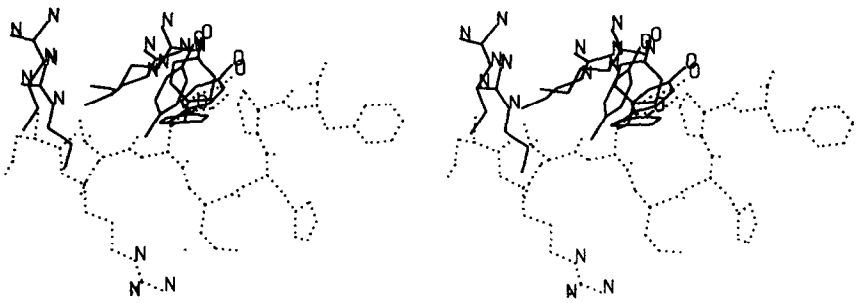


Figure 4: Relationship between Arg and Tyr sidechains in each Model III Conformer. The Arg-alkylguanidine and Tyr-phenol sidechains in Model III Conformers W-Z (solid lines) are superimposed upon Model II (dots) to highlight the most important differences between Model II and Model III. Were the entire structures to be shown, complete overlap of the Model III conformers with Model II from the carbonyl of Tyr^4 to the C-terminus (Ile-His-Pro-Phe) would be evident, as well as the lack of overlay of the Asp^1 and Val^3 sidechains.

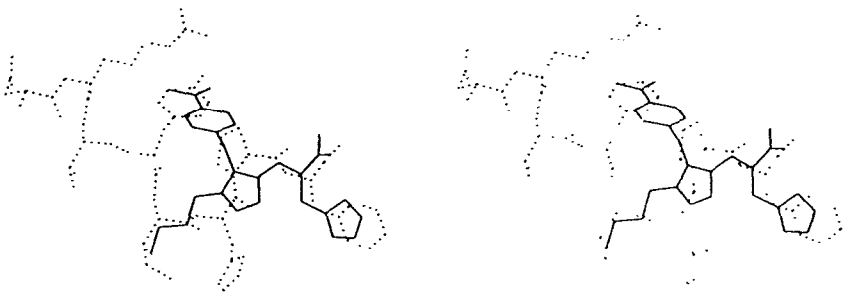


Figure 5: Overlay of Benzylimidazole 6 with Model III Conformer X, stereo plot.

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