Effects of D-Amino Acid Substitution on Antagonist Activities of Angiotensin II Analogues†

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The synthesis and biological activities of angiotensin II (AII) analogues are described and compared to the literature. D-Amino acid substitution was employed to search for novel AII antagonists that would also display reduced partial agonist activity. Substitution of D-amino acids into the interior positions 2–7 of [Sar^1,Ile^8]-AII gave rise to inactive compounds or weak antagonists. Substitution of D-amino acids into position 8 gave rise to potent antagonists in vivo including [Sar^1,D-Phe^8]-AII 8, [Sar^1,D-(αMe)Phe^8]-AII (**35**), [Sar^1,D-Trp^8]-AII (**32**), [Sar^1,D-Phg^8]-AII (**29**), [Sar^1,D-Peg^8]-AII (**30**), and [Sar^1,D-Phe^8]-AII-NH_2 (**31**). The structural requirements for D-AA^8 analogues (antagonists) showed similarities with those of L-AA^8 analogues (agonists). The latter three analogues, **29–31**, were considerably more potent in vivo than their in vitro affinities would indicate, suggesting that these analogues may resist carboxypeptidase-like degradation. While partial agonist activity was not removed by D-AA^8 substitution, [Sar^1,D-Phe^8]-AII-NH_2 (**31**) displays lower partial agonist activity than [Sar^1,Ile^8]-AII. A receptor model is presented that highlights the difference between [L-AA^8]-AII analogue agonist activity and [D-AA^8]-AII analogue antagonist activity.

Of the more than 300 analogue of angiotensin II (AII) described in the literature,[1,2] only a small percentage of these analogues were designed to probe the structural requirements for AII antagonists. Potent antagonists to AII have been obtained by substituting aliphatic amino acids in place of phenylalanine in position 8. Two of these analogue, [Sar^1,Ala^8]-AII (saralasin) (**3**) and [Sar^1,Ile^8]-AII (**4**), have been shown to lower blood pressure in human hypertensives with high renin levels. These peptides display sufficient residual agonist action to severely limit their use as antihypertensive agents.[3,4]

In order to eliminate intrinsic agonist activity in AII antagonists, alternate strategies are needed in the search for improved antagonists. In the present study we have pursued the approach of D-amino acid substitution into various positions of angiotensin II.

D-Amino acid substitution can be a useful tool for increasing potency in a peptide or for changing its activity from agonist to antagonist.[5] The potency and duration of action of naturally occurring peptides has often been dramatically increased by D-amino acid substitution. In these cases enhanced properties were ascribed to either increased resistance to proteolysis or stabilization of bioactive conformation.

A number of naturally occurring peptides have been transformed into antagonists by substitution of D-amino acids for L-amino acids in certain positions of the native sequences.[5] In these cases D substitution presumably stabilizes conformations that bind to the receptor but do not provoke receptor stimulation. On the basis of such examples, the substitution of D-amino acids into angiotensin analogues appears to be a reasonable approach in the search for ways to increase antagonist potency and duration and for unique modifications that generate antagonist activity.

Analogues of angiotensin II bearing D-amino acids in positions 1–8 have been reported previously (analogues 1–8) (Table I).[1,6-12] D substitution in position 1 of AII, analogue 1, was reported to enhance agonist activity by stabilizing against aminopeptidase action.[10] Leduc et al.[9] reported that D substitution in position 8 of [Sar^1]-AII gave an analogue, **8**, that lacked significant agonist activity and displayed potent antagonism. Also previously reported were analogues **2**, **3**, **5**, **6**, and **7** bearing D-amino acids in the interior positions of the AII sequence.[1,6-8,11] These were weak agonists with no reported antagonist effects.

D-Amino acid substitution into [Sar^1,Ile^8]-AII could conceivably stabilize unique antagonist conformations to give antagonists with increased potency or reduced agonist activity. This paper describes the synthesis and biological activities of such analogues and analyzes the structure–activity requirements for AII antagonists, bearing a D residue in position 8. An efficient comparison of new analogue activities with literature analogue activities necessitates construction of data tables bearing both sets of data. Data from the literature is footnoted in each table. The conformational analysis of angiotensin II has been thoroughly reviewed.[12]

† The abbreviations for natural amino acids (AAs) and nomenclature for peptide structures follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* 1971, *247*, 977. Abbreviations for nonnative amino acids include Apb = 3-amino-4-phenylbutyric acid, Apib = 3-amino-3-phenylisobutyric acid, Bph = *p*-(dihydroxyboryl)-phenylalanine, (2)Nal = 3-(2-naphthyl)alanine, Peg = phenethylglycine, Phg = phenylglycine, and Sar = sarcosine. Other abbreviations in this paper include AA = amino acid, Boc-ON = 2-[(*tert*-butoxycarbonyloxy)imino]-2-phenylacetonitrile.

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Table I. Effects of D-Amino Acid Substitution on Activities of Angiotensin Analogues

	primary structure								biological activities			
									in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
	1	2	3	4	5	6	7	8				
no.	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe	AII-like[c]	pA_2	AII-like[d]	ID_{50}[e]
1[i]	β-D-Asp[1]							Phe[8]			200	
2[j]	Asp[1]	D-Arg[2]						Phe[8]			5	
3[k]	Asn[1]			D-Tyr[4]	Val[5]			Phe[8]			0.03	
4[l]	Asn[1]				Pro[5]			Phe[8]			10	
5[l]	Asn[1]				D-Pro[5]						0.05	
6[m]	Asp[1]					D-His[6]		Phe[8]			4.0	
7[n]	Asp[1]						D-Pro[7]	Phe[8]	0.1 (RU)[f]	<5.0 (RU)[f]		
8	Sar[1]							D-Phe[8]	0	9.0 8.19[h]	*g*	12.5 ± 2.3
9	Sar[1]							Ile[8]	0	9.1	*g*	10.0 ± 2.0
10[i]	β-D-Asp[1]							Leu[8]		8.2		15.0
11	Sar[1]	D-Arg[2]						Ile[8]	0	7.8		150.0 ± 25.5
12	Sar[1]		D-Val[3]					Ile[8]	0	6.0	*g*	
13	Sar[1]			D-Tyr[4]				Ile[8]	0	<6.0		
14	Sar[1]				D-Val[5]			Ile[8]	0	<6.0		
15	Sar[1]					D-His[6]		Ile[8]	0	<6.0	0	>1000
16[n]							D-Pro[7]	Ile[8]	0.1 (RU)	<5.0 (RU)		
17	Sar[1]							D-Val[8]	0	7.25	*g*	50 ± 4.2
18[m]								D-αIle[8]		7.33	0.72	
19	Sar[1]	D-Arg[2]		D-Tyr[4]				Ile[8]	0	<6.0		
20	Sar[1]		D-Val[3]		D-Val[5]			Ile[8]	0	<6.0	0	>1000
21	Sar[1]			D-Tyr[4]		D-His[6]		Ile[8]	0	<6.0		
22					D-Val[5]		D-Ala[7]	Ile[8]	0	<6.0		0
23	Sar[1]					D-His[6]		D-Phe[8]	0	5.7	0	0
24	Sar[1]					D-His[6]		D-Val[8]	0	6.0		
25[m]						D-His[6]		D-αIle[8]		7.08	0.014	

[a] "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[36] AII antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the AII dose–response curve in vitro at high doses, but not at low doses.[37] The "pA_2" values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[37] [b] Residual agonist "AII-like" activity and antagonist activity, ID_{50}, were measured in vivo in the rat blood pressure assay described by Regoli et al.[38] [c] AII-like activity is expressed as percent activity relative to AII. [d] AII-like activity is expressed as percent activity relative to AII. [e] ID_{50} in ng/rat per min (using 250-g rats), the dose of antagonist that requires a twofold increase in the dose of AII to produce the same elevation in blood pressure as in the control period. [f] (RU) = rat uterus employed in the bioassay per corresponding reference.[11] [g] AII-like activity for these peptides were measured by the mmHg of blood pressure increase produced by 1-μg bolus intravenous injection of compound: 7.5 ± 0.6 mm for analogue 8, 10.2 ± 0.8 mm for analogue 9, 8.5 ± 1.0 mm for analogue 11, and 10.0 ± 0.9 mm for analogue 17. [h] Reference 9. [i] Reference 10. [j] Reference 7. [k] Reference 1. [l] Reference 8; the appropriate D-αIle[5] or D-Val[5] analogue has not been reported. [m] Reference 6. [n] Reference 11.

Results and Discussion

D-Amino Acid Substitutions. To study the role of D-amino acid substitution on AII antagonists, a number of analogues of [Sar[1],Ile[8]]-AII (compound **9**) were prepared containing single or double D-amino acid substitutions. These are displayed in Table I, which also displays analogues described in the literature. A comparison of both activities and structures of these literature compounds to ones studied by us is an important aspect of this paper. Substitution of sarcosine into position 1 of AII analogues confers a longer duration of action and stability to membrane-bound aminopeptidases.[11] Substitution of β-D-Asp into position 1 of [Leu[8]]-AII was shown previously[11] to be less effective than sarcosine in position 1. The α-D-Asp analogue was not described. Analogues **11–16** with single D-amino acid substitutions into the interior positions 2–7 display severely reduced antagonist activity. Analogues **19–25** with double D-amino acid substitutions into the interior positions 2–8 also display dramatically reduced antagonist activity.

Although D-His[6] substitution was reported[6] to have deleterious effects on agonist activity ([D-His[6]]-AII, analogue **6**, displayed only 4% of the activity of AII), the authors reported that [D-His[6],D-αIle[8]]-AII (**25**) displayed only slightly lower antagonist activity than [D-αIle[8]]-AII (**18**). Similarly, the same report described an only modest decrease in activity of [Ala[4],D-His[6],Ile[8]]-AII compared to [Ala[4],Ile[8]]-AII. Accordingly, we were suprised to find a marked decrease in antagonist activity in analogue **24**, [Sar[1],D-His[6],D-Val[8]]-AII, which was over 10 times less active than [Sar[1],D-Val[8]]-AII (**17**). Perhaps the D-His[6] substitution is more detrimental in [Sar[1]]-AII analogues than in [Asp[1]]-AII analogues. Clearly, D-amino acid substitutions into the interior positions of [Sar[1],Ile[8]]-AII do not enhance antagonist activity.

D-AA[8] Structure–Activity Relationship. The structure–activity relationship (SAR) of AII analogues bearing D-amino acids in position 8 is displayed in Table II.[13] Most of the D-AA[8]-AII analogues in this study were antagonists; all were devoid of agonist activity in vitro and displayed only residual agonist activity in vivo. Several of these analogues were potent antagonists in vitro or in vivo. Since the relationship of structure to in vivo activity can be influenced by factors other than receptor affinity, this discussion will first examine in vitro activities and consider in vivo results subsequently.

In general, variations of D-AA[8] structure that enhance or reduce antagonist activity (Table II) follow the pattern of variations that alter agonist activity established in the literature for the corresponding L-AA[8] analogues (Table III).[14–21] Later in the discussion a model will be presented

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Table II. Effects of Position 8 D-Amino Acid Substitution on Activities of Angiotensin Analogues

	modification in [Sar[1]]-AII: Sar[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8]		biological activities			
			in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
no.	positions 1–7	position 8	AII-like[c]	pA_2	AII-like[d]	ID_{50}[e]
8		D-Phe[8]	0	9.0	7.5 ± 0.6	12.5 ± 2.3
26		D-Tyr[8]	0	8.25	15.0 ± 1.2	20.0 ± 2.8
27		D-Bph[8]	0	<6.0		
28		D(pCl)-Phe[8]	0	8.7	12.5 ± 1.2	25.0 ± 3.1
29		D-Phg[8]	0	8.0	7.5 ± 0.9	3.5 ± 0.5
30		D-Peg[8]	0	7.35	12.5 ± 0.8	7.5 ± 1.0
17		D-Val[8]	0	7.25	10.0 ± 1.0	50.0 ± 4.2
31		D-Phe-NH_2^8	0	7.5	2.5 ± 0.1	12.5 ± 2.5
32		D-Trp[8]	0	8.9	20.0 ± 2.2	10.0 ± 1.8
			0	7.7 (RU)[f]		
33		D-(2)Nal[8]	0	9.3	5.0 ± 0.2	25.0 ± 3.0
34	Phe[4],	D-Tyr[8]	0	7.0	7.5 ± 0.9	25.0 ± 4.7
35		D-(αMe)Phe[8]	0	9.7	20.0 ± 1.3	10.0 ± 2.3
9		Ile[8]	0	9.1	10.2 ± 0.8	10.0 ± 2.0
			0	8.7 (RU)[f]		
36		Ala[8]	0	8.6	10.0 ± 0.7	15.0 ± 3.0

[a] Agonist, "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[36] AII antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the AII dose–response curve in vitro at high doses, but not at low doses.[36] The "pA_2" values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[37] [b] Residual agonist "AII-like" activity and antagonist activity, ID_{50}, were measured in vivo in the rat blood pressure assay described by Regoli et al.[38] [c] AII-like activity in vitro is expressed as percent activity relative to AII. [d] AII-like activity is expressed by the mmHg of blood pressure increase produced by a 1-μg bolus intravenous injection of compound. [e] ID_{50} in ng/rat per min (using 250-g rats). [f] (RU) = rat uterus employed in the bioassay per ref 13.

Table III. Effects of Position 8 L-Amino Acid Substitution on Activities of Angiotensin Analogues

	modification in AII: Asp[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8]			biological activities			
				in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
no.	positions 1–7	position 8	ref	AII-like[c]	pA_2	AII-like[c]	ID_{50}[d]
37		Tyr[8]	14	100 (RU)[e]		83	
38		(pNH_2)Phe[8]	15			53	
39		(pBr)Phe[8]	15			50	
40		(MeO)Phe[8]	15			33	
41	Sar[1],	Phg[8]	16	(+)	8.41 (GPI)[f]	(+)	
42	Sar[1],	Peg[8]		(+)[g]	(+)[g]		
43		Val[8]	17			0.5	
			18		8.31 (RU)		
44	Asn[1],Val[5]	Phe[8]-NH_2	19			3.0	
45	Asn[1],Val[5],	Phe[8]-OMe	19			10.0	
46	Sar[1],Phe[4],	Tyr[8]		0	7.5	5.5 ± 0.5	50.0 ± 8.5
47		Trp[8]	20		8.36 (RU)	22.2	
48		(αMe)Phe[8]	21	100 (RU)		80.0	

[a] Agonist, "AII-like" activity, and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[36] AII antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the AII dose–response curve in vitro at high doses but not at low doses.[36] The "pA_2" values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[37] [b] Residual agonist "AII-like" activity and antagonist activity, ID_{50}, were measured in vivo in the rat blood pressure assay described by Regoli et al.[38] [c] AII-like activity in vitro is expressed as percent activity relative to AII. [d] ID_{50} in ng/rat per min (using 250-g rats). [e] (RU) = rat uterus employed in the bioassay per references 14, 18, 20, 21. [f] (GPI) = guinea pig ileum employed in the bioassay per ref 16. [g] Partial agonist at 10 ng/mL.

that distinguishes between L-AA[8] peptides which stimulate the target cell and D-AA[8] peptides which do not. First, the L-AA[8] SAR will be compared with the D-AA[8] SAR.

Para-substituted aromatic L-amino acids in position 8 of AII gave rise to analogues **37–40** (Table III) with significantly lower agonist activities.[14,15] Similarly, the antagonist analogues **26–28** (Table II) containing para-substituted aromatic D-amino acids in position 8 display significantly lower antagonist activities in vitro.

As with the L-AA[8] series, the orientation of the phenyl ring in the D-AA[8] series is important. Removal of the β-methylene group from L-Phe gives an amino acid L-Phg with a phenyl side chain that clearly cannot adopt the same side-chain orientations as Phe. The corresponding L-Phg analogue **41** displays only residual agonist activity and proved to be an antagonist.[16] D-Phg[8] substitution for D-Phe[8] is similarly deleterious, since the D-Phg[8] analogue **29** is a weaker antagonist than the D-Phe[8] analogue **8** in vitro. The presence of a second methylene group between the C^{α}-carbon and the phenyl group gives an amino acid Peg with a phenyl side chain that also cannot adopt the same side-chain orientations as Phe. [Sar[1], Peg[8]]-AII (**42**) displays potent partial agonist activity at 10 ng/mL, hindering quantitative measurement of either activity, but the effect of Peg is clearly detrimental to agonist activity. Similarly,

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the D-Peg^8 analogue **30** displays lower in vitro antagonist activity than analogues **8** or **29**, but unlike the L-Peg^8 analogue, this analogue is a potent antagonist with only residual agonist activity.

Aliphatic L-amino acids, e.g., L-valine, in position 8 gave rise to analogues (e.g., [Val^8]-AII, **43**) with very little agonist activity, but significant antagonist activity.[17,18] Similarly, analogues bearing aliphatic D-amino acids, as in the D-Val^8 analogue **17**, are weaker antagonists than analogues bearing aromatic D-amino acids.

Replacement of the C-terminal carboxylate group in AII with nonionic groups, e.g., $CONH_2$ in analogue **44**, dramatically lowered agonist activity.[19] Similarly, [Sar^1,D-Phe^8]-AII-NH_2 (**31**) is a poorer antagonist in vitro than [Sar^1,D-Phe^8]-AII (**8**).

Substitution of the bulky aromatic amino acid tryptophan in position 8 of AII resulted in a weaker but partial agonist analogue **47**.[27] The corresponding D-Trp^8 analogue [Sar^1,D-Trp^8]-AII (**32**), recently reported by Matsoukas et al.,[13] was found to be a weaker antagonist in the rat uterus than [Sar^1,Ile^8]-AII. In the rabbit aorta, however, analogue **32** behaves differently. It is almost as potent in the rabbit aorta as [Sar^1,Ile^8]-AII. Analogue **33**, containing the bulky D-(2-naphthyl)alanine, is actually more potent in vitro than **8** or **9**. Thus, the parallelism followed so far for both [L-AA^8]- and [D-AA^8]-AII analogues does not seem to follow for bulky aromatic side-chain groups. Side-chain lipophilicity may be an important factor in D-AA^8 antagonist affinity.

One of the very first antagonist analogues of AII to be described in the literature was [Phe^4,Tyr^8]-AII.[22] It displayed only weak antagonist activity. We prepared the Sar^1 analogue [Sar^1,Phe^4,Tyr^8]-AII and found that this analogue (**46**) proves to be a weak antagonist in vitro as did the D-Tyr analogue **34**, [Sar^1,Phe^4,D-Tyr^8]-AII. Undoubtedly, both the lack of the important phenolic group in position 4[1,2] and the presence of a para-substituted aromatic ring in position 8 combine to give a weaker antagonist than [Sar^1,D-Phe^8]-AII (**8**).

Replacement of the α proton of Phe with a methyl group gives (αMe)Phe, which prefers to adopt α-helical conformations in peptides.[23] The analogue (**48**) of AII bearing L-(αMe)Phe^8 was shown to be a pure agonist, equipotent with L-Phe^8-AII.[21] The analogue [Sar^1,D-(αMe)Phe^8]-AII (**35**) is an antagonist that is dramatically more potent in vitro than [Sar^1,D-Phe^8]-AII (**8**). This result suggests that the bioactive conformations of both AII and [Sar^1,D-Phe^8]-AII about position 8 prefer the same type of conformations, although in this C-terminal position we can only specify Φ angle preferences (60° or −60°).[23]

The in vivo bioassay results are more difficult to interpret. Most of the in vivo potencies correlate with in vitro affinities, with the noteworthy exceptions of analogues **29–31**. These analogues are considerably more potent in vivo than their in vitro affinities would suggest. Peptides **29** and **30** are actually more potent in vivo than either [Sar^1,Ile^8]-AII (**9**) or [Sar^1,D-Phe^8]-AII (**8**). Greater resistance to carboxypeptidase-like degradation is suggested by these results, although an attendant increase in duration of action was not observed. We have not ruled out the possibility that these analogues are more selective for some tissue receptor bed other than the aorta, but as yet we have not been able to provide evidence for such selectivity.

Analogue **31** is noteworthy in its reduced partial agonist activity compared to [Sar^1,Ala^8]-AII (**36**) (Table II). Most AII antagonists elevate rat blood pressure by 10 mmHg or more upon bolus injection of 1 μg of antagonist. While analogue **31** is not completely devoid of partial agonist activity, it represents interesting progress over other angiotensin antagonists.

The effects of similar L-AA^8 and D-AA^8 modifications on in vitro activities are summarized in Table IV. In general, the structure and orientation of the aromatic side chain, as well as the presence of a free carboxylate group,

Table IV. Effects of Structural Modifications on Activities of L-AA^8 and D-AA^8-AII Analogues

binding group	analogue modification	effects on activity: (agonist) L-AA^8-AII	effects on activity: (antagonist) D-AA^8-AII
side chain position 8	para-substituted aromatic side chain	lower	lower
	Phg	lower	lower
	Peg	lower	lower
	aliphatic side chain	lower	lower
	bulky aromatic side chain	lower	lower/greater
C-terminal carboxylate	carboxamide	lower	lower
rest of molecule	position 4 OH removal	lower	lower
	position 6 D-His	lower	lower

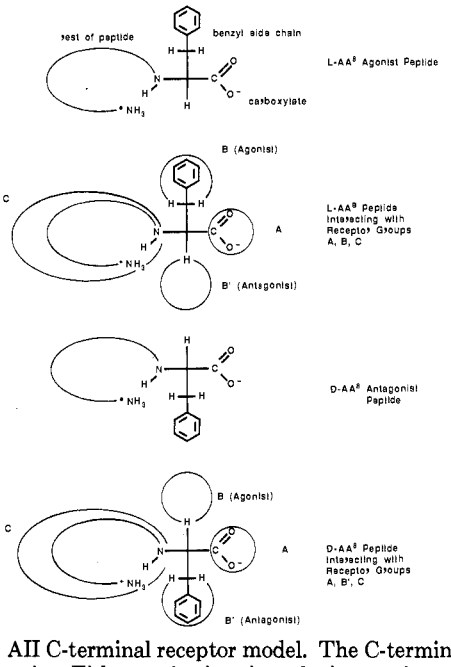

Figure 1. AII C-terminal receptor model. The C-terminal amino acid is drawn in a Fisher projection since the interaction of peptide with receptor is viewed directly from above. The relative size of the rest of the peptide is diminished to save space.

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are important position 8 structural requirements for activity. AII analogues solely bearing modifications to the Phe[8] carboxyl groups have not displayed antagonist activity,[19,24] while AII analogues solely bearing modifications to the Phe[8] side chain often display antagonist activity. Thus, it is the structure and orientation of the side chain in Phe[8] analogues, and *not* the carboxyl group, that governs whether a Phe[8] analogue is an agonist or antagonist.

Model Distinguishing L-AA[8] Agonists from D-AA[8] Antagonists. In this series of analogues, simple transposition of the Phe[8] side chain with the α hydrogen atom from L-Phe[8] to D-Phe[8] is responsible for loss of receptor stimulation. The situation is depicted with the hypothetical model in Figure 1 to emphasize the point. This transposition does not appear to alter binding requirements, since the structure–activity relationships of both L-AA[8] and D-AA[8] series are roughly the same. Both L- and D types of analogues appear to be interacting with the same types of receptor elements. In the antagonist series, however, increased side-chain lipohilicity and/or steric bulk may be an important factor. Support for this model would come from receptor binding studies, which are under way in our laboratories.

In the hypothetical model in Figure 1, cellular stimulation results only after complete stereospecific interaction of a receptor side B with a benzyl side chain in position 8 of the peptide and stereospecific interactions of other receptor sites with other functionality in AII. Side chains in L-AA[8] that lack proper orientation or are to bulky for complete interaction with site B, such as phenyl, phenethyl, and indolylmethyl, or that undergo only partial interactions with site B, such as aliphatic side chains, would bind to site B, but the event would not culminate in cellular stimulation. Such peptides would be antagonists to angiotensin II. The results in Table III show that maximal agonist activity is achieved if the L-AA[8] side chain is a benzyl group, while phenyl, phenethyl, indolymethyl, and aliphatic side chains give rise to L-AA[8] analogues that act as antagonists. Thus, the hypothetical model in Figure 1 is consistent with the decreased agonist activity in the corresponding AII analogues. The model suggests that there is an alternate site B′ for the D-AA[8] side chain with side-chain preferences that are similar to site B.

The receptor model of Figure 1 does not indicate the three-dimensional orientation of the receptor binding groups relative to the peptide. A certain degree of flexibility among receptor groups would be expected. Thus, this model does not account for cooperative interactions between hormone and receptor. One might postulate, furthermore, that there could be more angiotensin antagonist conformations that interact with the receptor without cellular stimulation than agonist conformations that interact with the receptor to effect cellular stimulation. Further explorations of the interactions of AII agonists and antagonists with the AII receptor will be described in subsequent papers.

Experimental Procedures

tert-Butyloxycarbonyl amino acids and peptide reagents were obtained from Bachem Fine Chemicals, Inc., Protein Research Foundation, or Chemical Dynamics Corp. and were used without further purification. Boc-D-Phg,[25] Boc-Peg,[26] and Boc-D-Peg[26] were prepared by standard procedures from commercially available amino acids. Syntheses of Boc-D-Bph and Boc-D-(αMe)Phe are described below. Thin-layer chromatography (TLC) was performed on Brinkman precoated silica gel plates (SIL-G-25). The spots were visualized by ninhydrin or Pauly reagent.

Boc-D-*p*-(Dihydroxyboryl)phenylalanine (Boc-D-Bph). An aqueous solution of D-(dihydroxyboryl)phenylalanine ethyl ester (D-Bph-OEt) was obtained as a filtrate from exhaustive chymotryptic resolution of D,L-Bph-OEt[27] (24 mmol) from which L-Bph was removed by filtration. The pH of the solution was adjusted to 13 with 1 N NaOH. The solution was allowed to stand until starting ethyl ester was no longer detectable by TLC. The solution was neutralized with 1 N HCl and lyophilized to a granular powder. The powder was dissolved in 50% aqueous acetone (70 mL). Boc-ON (6.5 g, 26 mmol) and Et_3N (4.9 mL, 36 mmol) were added to the solution. The solution was allowed to stir overnight until starting material could no longer be detected by TLC ($CHCl_3$–MeOH–AcOH, 85:10:5). The volume of the mixture was reduced to ~35 mL in vacuo. The solution was washed with ethyl acetate (2 × 30 mL). The ethyl acetate layers were combined and washed with 10% Na_2CO_3 (2 × 10 mL). The carbonate solutions were combined and acidified with stirring to pH 2 with slow addition of 3 N HCl. The aqueous solutions were washed with EtOAc (2 × 30 mL). The ethyl acetate layers were combined and dried in vacuo to an off-white powder (6 g, 84%). Crude product was applied to a 5 × 100 cm flash chromatography column containing 230–400-mesh silica gel in a solution of $CHCl_3$–MeOH–AcOH, 85:2:2. The column was eluted with a volume (750 mL) of the solvent mixture, followed by a solution of $CHCl_3$–MeOH–AcOH, 85:10:5 (750 mL). The latter eluant was dried in vacuo to an oil. The oil was dissolved in water and lyophilized to a white powder (2.3 g, 32%): TLC ($CHCl_3$–MeOH–AcOH, 85:10:5), R_f 0.47; ^{1}H NMR (CD_3OD) δ 7.5 (dd, 4 H), 4.4 (m, 1 H), 3.1 (m, 2 H), 1.4 (s, 9 H); $[\alpha]^{25}_{589}$ −15.23 (c 0.65, MeOH). Anal. Calcd for $C_{14}H_{20}BNO_6$: C, 54.39; H, 6.52; N, 4.53; B, 3.50. Found: C, 54.37; H, 6.52; N, 4.70; B, 3.60.

Boc-D-α-methylphenylalanine Dicyclohexylamine Salt (Boc-D-(αMe)Phe·DCHA). Triethylamine (0.3 mL, 2.15 mmol) was added to a solution of D-(αMe)Phe[28] (0.25 g, 1.4 mmol) and Boc-ON (0.4 g, 1.5 mmol) in 50% aqueous acetone (15 mL). After 2 days TLC ($CHCl_3$–MeOH–AcOH, 95:4:1) showed less than 2% starting amino acid. The volume of solution was reduced to half in vacuo. Aqueous 10% Na_2CO_3 (15 mL) was added and the mixture was washed with ethyl acetate (2 × 30 mL). The aqueous solution was acidified to pH 2 with 3.0 N HCl. The solution was washed twice with EtOAc (30 mL each). The EtOAc layers were washed twice with H_2O (10 mL), combined, and rotary evaporated to an oil (0.38 g, 98%): TLC ($CHCl_3$–MeOH–AcOH, 95:4:1) R_f 0.43; ^{1}H NMR ($CDCl_3$) δ 9.20 (s, 1 H), 7.20 (s, 5 H), 3.26 (s, 2 H), 1.52 (s, 3 H), 1.48 (s, 9 H).

Dicyclohexylamine (0.27 g, 1.5 mmol) was added to a solution of the crude Boc-D-(αMe)Phe in ether (80 mL). The mixture was cooled to 0 °C and kept cold overnight. A white crystalline solid was filtered from solution and washed with ether. The solid was recrystallized from MeOH–ether to give pure Boc-D-(αMe)-Phe·DCHA (0.30 g, 47%): mp 229–230.5 °C; TLC ($CHCl_3$–MeOH–AcOH, 95:4:1) R_f 0.52; $[\alpha]^{25}_D$ −59.28 (c 0.921, MeOH). Anal. Calcd for $C_{27}H_{44}N_2O_4$: C, 70.43; H, 9.57; N, 6.09. Found: C, 70.42; H, 9.39; N, 6.09.

Peptide Synthesis and Purification. All peptides were prepared by the solid-phase method on Beckman 990-B peptide synthesizers.[29,30] The C-terminal residue was esterified to a chloromethylated copolymer of styrene and 2% divinylbenzene (Bio-Rad) via a cesium salt procedure.[31] The degree of substitution was determined by amino acid analysis of a hydrolysate obtained by treating the amino acid-resin with HCl–PrOH (1:1) at 120 °C for 3 h.[32] Routine deprotection of Boc-amino protecting groups was accomplished with 30% TFA in CH_2Cl_2 and neutralization with 10% TEA in CH_2Cl_2. Coupling of each amino acid was performed with a 2.5 molar excess of (*tert*-butyloxycarbonyl)amino acid and DCC in CH_2Cl_2 with completeness of reaction monitored by the ninhydrin test.[33] Side chain protecting groups were as follows: Arg, tosyl; Tyr, α-Br-Z; His, Tosyl.

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Table V. Peptide Analytical Data[a]

	amino acid analysis[b]								TLC, R_f			HPLC		
	1	2	3	4	5	6	7	8	A	B	C	solvent (% CH_3CN)	K'	% purity
8	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Phe	0.19	0.55	0.72	23	8.6	96
	(+)	(1.01)	(1.01)	(1.02)	(0.96)	(1.02)	(0.99)	(1.02)						
9	Sar	Arg	Val	Tyr	Ile*	His	Pro	Ile*	0.19	0.67	0.75	20	2.80	>98
	(+)	(0.99)	(0.97)	(0.97)	(1.01)	(1.07)	(0.99)	(1.01)						
11	Sar	D-Arg	Val	Tyr	Ile*	His	Pro	Ile*	0.22	0.48	0.34	20	2.73	>98
	(+)	(1.00)	(1.01)	(71.02)	(0.97)	(1.02)	(1.01)	(0.97)						
12	Sar	Arg	D-Val	Tyr	Ile*	His	Pro	Ile*	0.22	0.50	0.46	20	2.87	>98
	(+)	(1.01)	(1.03)	(1.02)	(0.97)	(1.01)	(1.00)	(0.97)						
13	Sar	Arg	Val	D-Tyr	Ile*	His	Pro	Ile*	0.26	0.64	0.56	20	5.97	94
	(+)	(1.01)	(1.03)	(1.02)	(0.95)	(1.03)	(1.02)	(0.95)						
14	Sar	Arg	Val*	Tyr	D-Val*	His	Pro	Ile	0.13	0.51	0.62	20	2.18	>98
	(+)	(0.99)	(1.00)	(0.99)	(1.00)	(1.00)	(1.01)	(1.01)						
15	Sar	Arg	Val	Tyr	Ile*	D-His	Pro	Ile*	0.27	0.81	0.76	20	3.11	>98
	(+)	(1.01)	(1.00)	(1.01)	(0.99)	(1.03)	(0.98)	(0.99)						
17	Sar	Arg	Val*	Tyr	Ile	His	Pro	D-Val*	0.28	0.71	0.78	20	2.24	96
	(+)	(1.01)	(1.00)	(1.00)	(0.98)	(1.03)	(0.99)	(1.00)						
19	Sar	D-Arg	Val	D-Tyr	Ile*	His	Pro	Ile*	0.18	0.49	0.51	20	4.21	>98
	(+)	(1.01)	(1.03)	(0.99)	(0.98)	(0.98)	(1.01)	(0.98)						
20	Sar	Arg	D-Val*	Tyr	D-Val*	His	Pro	Ile	0.23	0.53	0.69	15	2.97	>98
	(+)	(0.99)	(1.01)	(1.00)	(1.01)	(1.01)	(0.99)	(1.00)						
21	Sar	Arg	Val	D-Tyr	Ile*	D-His	Pro	Ile*	0.28	0.45	0.78	20	4.42	>98
	(+)	(1.02)	(1.02)	(1.02)	(0.98)	(1.00)	(1.00)	(0.98)						
22	Sar	Arg	Val*	Tyr	D-Val*	His	D-Ala	Ile	0.26	0.55	0.62	20	2.57	>98
	(+)	(1.03)	(0.99)	(1.03)	(0.99)	(1.06)	(0.97)	(0.98)						
23	Sar	Arg	Val	Tyr	Ile	D-His	Pro	D-Phe	0.15	0.16	0.57	20	2.69	>98
	(+)	(0.99)	(1.02)	(1.02)	(0.97)	(0.97)	(1.02)	(1.01)						
24	Sar	Arg	Val*	Tyr	Ile	D-His	Pro	D-Val*	0.14	0.36	0.46	20	2.21	>98
	(+)	(0.99)	(1.02)	(1.00)	(0.99)	(0.98)	(0.99)	(1.02)						
26	Sar	Arg	Val	Tyr*	Ile	His	Pro	D-Tyr*	0.14	0.44	0.48	15	8.00	>98
	(+)	(1.03)	(1.02)	(0.99)	(0.98)	(1.01)	(0.99)	(0.99)						
27	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Bph	0.43	0.76	0.68	20	3.50	>98
	(+)	(1.00)	(1.00)	(1.06)	(1.00)	(0.97)	(1.01)	(0.96)						
28	Sar	Arg	Val	Tyr	Ile	His	Pro	D-(pCl)Phe	0.19	0.55	0.55	20	4.20	>98
	(+)	(1.01)	(1.00)	(1.01)	(0.96)	(0.98)	(1.03)	(+)						
29	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Phg	0.31	0.47	0.56	17	3.92	>97
	(+)	(1.06)	(1.04)	(0.92)	(0.98)	(0.97)	(1.03)	(+)						
30	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Peg	0.19	0.56	0.49	25	4.35	>95
	(+)	(1.00)	(1.01)	(1.01)	(0.98)	(1.02)	(1.01)	(+)						
31	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Phe-NH_2	0.13	0.64	0.72	25	2.81	>98
	(+)	(1.01)	(0.99)	(0.99)	(0.97)	(1.01)	(1.04)	(1.00)						
32	Sar	Arg	Val	Tyr	Ile	His	Pro	D-Trp	0.30	0.50	0.61	20	4.5	90
	(+)	(1.02)	(1.03)	(1.02)	(0.94)	(1.0)	(0.97)	(0.80)						
33	Sar	Arg	Val	Tyr	Ile	His	Pro	D-(2)Nal	0.28	0.67	0.52	29	4.39	>97
	(+)	(1.00)	(1.00)	(1.01)	(0.97)	(1.02)	(1.00)	(1.00)						
34	Sar	Arg	Val	Phe	Ile	His	Pro	D-Tyr	0.03	0.08	0.53	35	10.9	85
	(+)	(1.05)	(1.03)	(1.03)	(0.95)	(0.98)	(1.04)	(0.92)						
35	Sar	Arg	Val	Tyr	Ile	His	Pro	D-(αMe)Phe	0.36	0.54	0.69	20	4.60	>98
	(+)	(1.04)	(1.05)	0.96)	(0.95)	(0.98)	(1.02)	(+)						
36	Sar	Arg	Val	Tyr	Ile	His	Pro	Ala	0.06	0.29		10	5.86	>98
	(+)	(1.04)	(1.00)	(1.04)	(0.99)	(1.01)	(0.94)	(0.97)						
46	Sar	Arg	Val	Phe	Ile	His	Pro	Tyr	0.19	0.39	0.58	20	2.79	91
	(+)	(1.05)	(1.03)	(1.03)	(0.95)	(0.97)	(1.05)	(0.92)						
42	Sar	Arg	Val	Tyr	Ile	His	Pro	Peg	0.18	0.48	0.54	25	2.6	89
	(+)	(1.06)	(1.02)	(1.03)	(0.90)	(0.93)	(1.07)	(+)						

[a] See text for details of analytical procedures. [b] Amino acid analysis expressed in molar ratios of the D,L amino acids in the peptides. (+) = amino acid present in roughly 1 molar equiv (in cases where quantitation is difficult). * = amino acid present in two positions. Value expressed is one half the experimental value.

In most cases coupling was complete after 2 h. If the ninhydrin test remained positive, a recoupling cycle was performed. After the last coupling and deprotection, the peptide was cleaved from resin by treatment with anhydrous HF containing 50% (v/v) anisole at 0 °C for 60 min. After vacuum evaporation of HF, the resin was rinsed with Et_2O to remove anisole and then rinsed with glacial HOAc and filtered. The filtrate was diluted with water and lyophilized to a powder of crude peptide material.

The crude peptides were purified to homogeneity either by (a) partitioning through 200 transfers of counter-current distribution in *n*-BuOH–HOAc–H_2O (4:1:5), (b) partition chromatography[34] on Sephadex G-15 in *n*-BuOH–HOAc–H_2O (4:1:5), or (c) reversed-phase semipreparative HPLC[35] on a Whatman C^{18} column using the appropriate solvent mixture of CH_3CN–0.1 N NH_4OAc, pH 4. The volumes of chromatographic fractions containing pure peptide were reduced by partial rotary evaporation and dried to powders by lyophilization to constant weight.

Homogeneity of each peptide was determined by the following methods: (a) amino acid analysis of 72 h acid hydrolysate (6 N HCl, 110 °C) performed on a Beckman Model 120C analyzer; (b)

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analytical TLC on silica gel plates with solvent systems (A) *n*-BuOH-AcOH-H_2O (4:1:5), (B) *n*-BuOH-AcOH-H_2O-EtOAc (1:1:1:1), (C) *n*-BuOH-AcOH-H_2O-pyridine (15:3:12:10), visualizing spots with Pauly reagent;[33] (c) analytical reversed-phase HPLC on C_{18}-silica gel column using the appropriate CH_3CN–0.1 N NH_4OAc (pH 4) mixture, following elution by UV (250-nm detection).

Analytical data for all peptide are listed in Table V.

Registry No. **1**, 51833-71-7; **2**, 67037-14-3; **3**, 3438-23-1; **4**, 34305-50-5; **5**, 57667-99-9; **6**, 49707-73-5; **7**, 111821-38-6; **8**, 111821-39-7; **9**, 37827-06-8; **10**, 51887-63-9; **11**, 111771-38-1; **12**, 111771-39-2; **13**, 101713-05-7; **14**, 111821-40-0; **15**, 111821-41-1; **16**, 111821-42-2; **17**, 111821-43-3; **18**, 49707-74-6; **19**, 111771-40-5; **20**, 111771-41-6; **21**, 111771-42-7; **22**, 111771-43-8; **23**, 111821-44-4; **24**, 111821-45-5; **25**, 49707-72-4; **26**, 111771-44-9; **27**, 111771-45-0; **28**, 111771-46-1; **29**, 111821-46-6; **30**, 111771-47-2; **31**, 111771-48-3; **32**, 95841-12-6; **33**, 111771-49-4; **34**, 111771-50-7; **35**, 111771-51-8; **36**, 38027-95-1; **37**, 25061-67-0; **38**, 90937-06-7; **39**, 111771-52-9; **40**, 25061-71-6; **41**, 53935-04-9; **42**, 111821-47-7; **43**, 35492-37-6; **44**, 6663-62-3; **45**, 47917-11-3; **46**, 111821-48-8; **47**, 111771-53-0; **48**, 111771-54-1; AII, 11128-99-7; H-DL-Bph-OEt, 111771-55-2; H-D-Bph-OEt, 111771-56-3; H-D-Bph-OH, 111821-49-9; BOC-D-Bph-OH, 111771-57-4; H-D-(αMe)Phe-OH, 17350-84-4; BOC-D-(αMe)Phe-OH, 111771-58-5; BOC-D-(αMe)Phe-OH·DCHA, 111771-59-6.

2,4-Diamino-6,7-dimethoxyquinazolines. 4. 2-[4-(Substituted oxyethoxy)piperidino] Derivatives as α_1-Adrenoceptor Antagonists and Antihypertensive Agents

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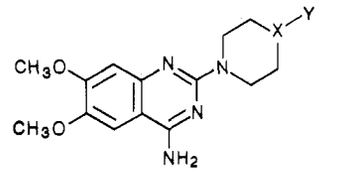
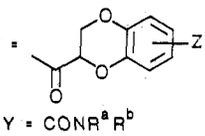
A series of 4-amino-6,7-dimethoxy-2-[4-(substituted oxyethoxy)piperidino]quinazoline derivatives (**2**) was synthesized and evaluated for α-adrenoceptor affinity and antihypertensive activity. Most compounds showed binding affinities within the nanomolar range for α_1-receptors, although **25** and **26** showed enhanced potency (K_i, ca. 1.5×10^{-10} M), equivalent to that of prazosin. Series **2** also displaced [^{3}H]clonidine from α_2-adrenoceptors, but at relatively high doses of 10^{-6} M, and selectivity for α_1 sites still predominated. In a rabbit pulmonary artery preparation, **12**, **16**, and **25** were potent antagonists of the α_1-mediated, postjunctional vasoconstrictor activity of norepinephrine with no effect at the prejunctional α_2 sites which modulate transmitter release. Physicochemical measurements gave a pK_a of 7.63 ± 0.10 for **12**, and N-1 protonation will be favored (60%) at physiological pH to provide the α_1-adrenoceptor pharmacophore, **28**. Antihypertensive activity of series **2** was evaluated following oral administration to spontaneously hypertensive rats, and blood pressure was measured after 1 and 6 h. Compounds **12**, **13**, **16**, **23**, and **37** displayed moderate efficacy and duration of action in lowering blood pressure, but the plasma half-life (ca. 2 h) of **16** in dogs was not compatible with potential once-daily administration in humans.

In previous papers, the synthesis and biological activities of two series of 2-[4-(1,4-benzodioxan-2-ylcarbonyl)-piperazin-1-yl]- and 2-[4-[(substituted amino)carbonyl]-piperidino]quinazoline derivatives **1a** and **1b** were reported.[1,2] In these studies, the roles of the relatively rigid



1a, X = N, Y =

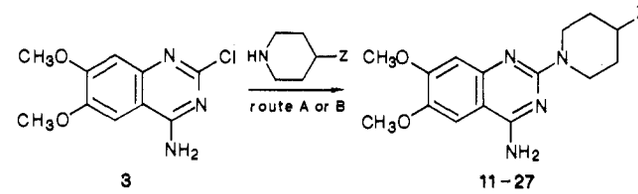


b, X = CH, Y = $CONR^aR^b$

c, X = N, Y = heteroaryl

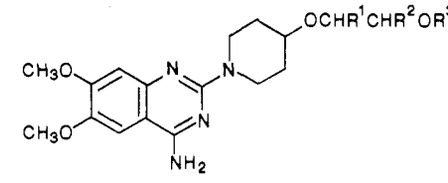
(**1a**) and flexible (**1b**) carboxamide moieties were compared with respect to effects on α_1-adrenoceptor affinity and antihypertensive activity. Subsequently, it was also demonstrated that the carbonyl function present in **1a** and **1b** could be replaced by an appropriately substituted heteroaromatic π system (**1c**) with no adverse effects on in vitro or in vivo activity.[3] These structure–activity relationship (SAR) studies suggested that, although the quinazoline 2-substituents play an important role in modulating α_1-adrenoceptor affinity and antihypertensive activity, more

Scheme I



3 **11–27**

marked structural variations than those already reported might also be tolerated. In this paper, the carbonyl or heteroaromatic π systems common to the previously disclosed series are replaced by an ethylenedioxy function (**2**),



2

and further substitution of the alkyl chain is explored for effects on in vitro receptor affinity and in vivo antihypertensive activity.

Chemistry. All of the compounds for pharmacological testing were prepared by condensation of 4-amino-2-chloro-6,7-dimethoxyquinazoline (**3**) with an appropriate 4-alkoxypiperidine derivative in butanol under reflux (Scheme I).[4] In route A, chromatographic purification of **13–15**, **22**, and **24–27** was required whereas in route B,

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