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DIPEPTIDE GLYCOLS: A NEW CLASS OF RENIN INHIBITORS

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SUMMARY: The discovery of a new class of novel renin inhibitors consisting of protected dipeptide amides derived from aminoglycols (Formula I) prompted a study of structure-activity in vitro and efficacy in vivo. Thus, Boc-L-Phe-N-[(1S,2R)-1-benzyl-(2,3-dihydroxy)propyl]-L-leucinamide (1) and the corresponding histidinamide (2) inhibit human renin in vitro (IC50: 8.7 x 10-6 M and 2.6 x 10-6 M, respectively). Compound 1 has a slight inhibitory effect on pepsin and compound 2 does not inhibit pepsin at all (at 10-4M); these compounds are inactive against rat renin. Compound 1 is efficacious in lowering plasma renin activity in the Rhesus monkey (i.v.). Results indicate that this new class of low molecular weight inhibitors is specific for human renin and thus constitutes a new source of drug candidates.

The search for an orally active inhibitor of human renin (EC 3.4.99.19) for the treatment of hypertension has intensified greatly in recent years (1), but despite tremendous interest, such an inhibitor remains elusive. Nevertheless, the commercial and clinical success of inhibitors of angiotensin I converting enzyme (EC 3.4.15.1) has fueled interest in renin inhibition as an alternative approach that should in principle be equally successful. Renin catalyzes the hydrolytic release of angiotensin I from the N-terminus of angiotensinogen; this process is the rate-limiting step in the formation of pressor substance angiotensin II. Renin inhibitors are thus potentially important modulators of plasma renin activity and pathologically high blood pressure.

Design and Selection of Inhibitors. In seeking new chemical candidates for clinical trial, we focused on compounds that

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satisfied the following requirements: 1) the substance must exhibit a high degree of in vitro specificity for renin vs pepsin, insuring that an oral dose will not become bound to gastric pepsin, and to decrease the likelihood of inhibition of other acid proteases; 2) the inhibitor should be of low molecular weight (<600) to increase the probability of gastrointestinal absorption; 3) it should be metabolically stable, possessing at most one peptide bond and should be free of reactive electrophilic functionality such as aldehyde; 4) the in vitro potency should be equal to or greater than that of pepstatin; 5) the substance must lower plasma renin activity in a primate model. In this Communication we wish to report a new class of renin inhibitors that satisfy the above requirements: N-terminal protected dipeptide amides derived from aminoglycols (Formula I).

METHODS AND MATERIALS:

In vitro inhibition assays for human renin, rat renin, and porcine pepsin were performed using known methods (2,3,4) as modified recently (5). A monkey model for assaying the effect of test compound on plasma renin activity (PRA) was developed: Rhesus monkeys (6/group) weighing 6.3-9.9 kg were treated with Lasix (furosemide) at 2 mg/kg, both in the a.m. and p.m. on Day 1. On Day 2, test compound at 10 mg/kg was administered intravenously. Plasma samples (K-EDTA) were taken at intervals as indicated in Table 2; PRA was then determined by a known method (6).

Details of the dipeptide glycol synthesis will be published elsewhere. Compounds **1** through **9** were prepared by amidation of a protected dipeptide or amino acid with an appropriate aminoglycol (see Table 1 and Figure 1 for structures). All amino acids are of the L configuration.

RESULTS:

Table 1 summarizes the in vitro inhibition data for the dipeptide glycols and related compounds. The new glycol inhibitors have the general formula:

$$\text{N-protected-Dipeptide-NHCH}(CH_2Ph)\overset{*}{C}\text{H}([CH_2]_n\text{-OH})\text{OH} \qquad \text{(I)}$$

To demonstrate in vivo efficacy, compound **1** was administered i.v. to Rhesus monkeys. Table 2 shows the PRA for both the pepstatin treated and compound **1** groups. For both groups, PRA is

Table 1. In Vitro Inhibition[a]

no.	structure	Human Renin %	Human Renin IC_{50}	Pepsin %	Pepsin IC_{50}	Rat Renin %
1	Boc-Phe-Leu-V	87	8.7	67	50	0
2	Boc-Phe-His-V	90	2.6	4	>1000	0
3	Boc-Nal(1)-Leu-V	84	15.4	63		0
4	tBuAc-Phe-Leu-V	84	14.0	59		0
5	Boc-Phe-Lys(Cbz)-V	70	24.0			
6	Boc-Phe-Leu-W	25	>100			0
7	Boc-Phe-Leu-X	70	63	59		0
8	Boc-Phe-Phe-Y	34	>100	4		
9	Boc-Phe-Leu-Z	8	>100			
10	Boc-Phe-Phe-Phe-ol	22	>100			
11	Boc-Phe-Leu-Leu-al	98	2.2	46	100	50
	Pepstatin	86	13.6	100	0.34	60.3

[a]percent (%) figures indicate percent inhibition at 10^{-4} M. IC_{50}'s are in micromolar units. Compounds 1-9 are the formal result of linking a protected dipeptide acid via an amide bond to an appropriate aminoglycol. V= (1S,2R)-1-benzyl-(2,3-dihydroxypropyl)amino; W= (1S,2S)-1-benzyl-(2,3-dihydroxypropyl)amino; X= (1S,2S)-1-benzyl-(2,4-dihydroxybutyl)amino; Y= (1S,2S)-2-hydroxy-1-hydroxymethyl-(2-phenylethyl)amino; Z= (1S,2R)-1-benzyl-(2,4-dihydroxybutyl)amino. Nal(1) denotes 3-(1'-naphthyl)-L-alaninyl.

Table 2. The Effect of Dipeptide Glycol 1 and Pepstatin on Plasma Renin Activity of Lasix Treated Rhesus Monkeys

Time	Glycol 1 (n=6)	Pepstatin (n=6)
Pre-Lasix -24 hr	12.2 ± 2.9	13.9 ± 2.5[b]
Post-Lasix 0 hr	47.2 ± 7.9	53.1 ± 6.1
2 min	8.4 ± 2.6	13.0 ± 0.2
5 min	15.1 ± 3.5	22.7 ± 9.2
15 min	23.5 ± 4.6	44.7 ± 5.4

[b]mean ± S.E.

increased by Lasix and is returned to pre-Lasix values at 2 min after treatment. The PRA for the group treated with **1** was still diminished to 50% of the initial value after 15 min, while the PRA of the pepstatin group had returned to post-Lasix levels within 15 min.

DISCUSSION:

Recent work (7) on aldehydic renin inhibitors (8) postulated that a C-terminal geminal glycol (i.e. the aldehyde hydrate) might be the pharmacophore, functioning as a transition-state mimic. Because the parent aldehyde moiety is likely to be unstable under physiological conditions, we decided to explore the in vitro and in vivo inhibitory properties of the more stable homologous glycols: 1,2 and 1,3 diols, i.e. substances where n= 1,2 respectively, in Formula **I**; this would test our hypothesis that diol may be bioisosteric with formyl.

As can be seen from Table 1, this approach was successful in generating new primate renin inhibitor leads, especially when n=1: Compounds **1** and **2** (Figure 1) with IC_{50}'s of 8.7 and 2.6 micromolar respectively are more potent inhibitors than the standard, pepstatin; compounds **3**, **4**, and **5** possessing naphthylalaninyl, tert-butylacetyl, and Cbz-lysinyl substitutions respectively, are comparable in potency to pepstatin. The one-carbon homologue **7**

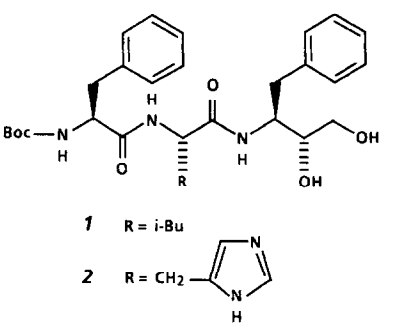

Figure 1. Most Potent Dipeptide Glycols.

(n=2) was also active (IC_{50} 6.3 x 10^{-5} M), although less potent than pepstatin. The orientation of the hydroxyls is probably important for activity because phenylserinol derivative **8** is only weakly active. That the diol moiety is necessary for activity is clear because the monohydric alcohol **10** is inactive.

The stereochemistry at the asymmetric carbon bearing the secondary hydroxyl (C* in Formula I) is critical for activity: for example, the epimer of **1** (n=1), i.e. compound **6**, is inactive, and the epimer of **7** (n=2), i.e. compound **9**, is also inactive. The configuration at this center in the active series (i.e. those compounds derived from **V** and **X**) **is the same** as that at C-3 in (S,S)-statine (9), the active principle of pepstatin. Quite possibly, **1** and **2** occupy much the same space as the competitive inhibitor pepstatin in the active-site of renin. The superior potency of histidine-containing **2** could be linked to the fact that there is a closer sequence analogy between inhibitor **2** and the natural substrate: angiotensinogen is positioned during the enzymatic reaction with the sequence ---His^{9}-Leu^{10}-Val^{11}--- at the active-site. It is possible that glycols **1** and **2** are acting as both transition state and collected-substrate inhibitors (10).

A remarkable feature of these inhibitory diols is their specificity for **human renin** versus rat renin or pepsin. Table 3 illustrates the specificity of **1** and **2** for renin versus pepsin compared with other inhibitors. Compound **2** exhibits a greater than 385:1 preference for inhibiting human renin over pepsin; glycol **1** shows a corresponding 6:1 preference. Interestingly, these substances also show species specificity: Compounds **1** and **2** do not inhibit rat renin, whereas aldehyde **11**, the postulated (7) lower homologue (n=0 in I) is active. It is remarkable that mere dipeptides such as **1** and **2** contain sufficient information to render them specific for human renin; such specificity for renin over pepsin has not been obtained even for a large heptapeptide containing statine (11).

Table 3. Specificity of Renin Inhibitors

Renin Inhibitor	SPECIFICITY (IC_{50} for pepsin / IC_{50} for renin)	Reference
Pepstatin	0.025	
Iva-His-Pro-Phe-His-Sta-Ile-Phe-NH_2	23	11
1 Boc-Phe-Leu-**V**	6	
2 Boc-Phe-His-**V**	>385	

As part of the protocol for selecting an agent for clinical trial, we tested diol **1** for PRA lowering activity in the monkey (Table 2). Compound **1** proved more efficacious than the standard pepstatin, exhibiting greater duration of action. This is consistent with our hypothesis that a minimum of peptide bonds in prospective inhibitors is desirable for in vivo utility.

CONCLUSION:

The novel dipeptide glycols of Formula **I**, typified by **1** and **2**, represent an important new class of renin inhibitors. These compounds are the lowest molecular weight non-aldehydic renin inhibitors known, possess only one peptide bond, and contain an attractively stable and convenient-to-handle 1,2 diol pharmacophore. Because of their demonstated in vitro activity, they could function as probes for SAR studies of S^1,S^2, and S^3 renin subsites (7); their in vivo activity makes them an attractive theme for the design of new therapeutics. In addition, the 1,2-diol unit was shown to be bioisosteric with formyl, thus constituting an addition to the armamentarium of the medicinal chemist.

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