Vol. 160, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1989 Pages 1-5

A New Class of Orally Active Glycol Renin Inhibitors Containing Phenyllactic acid at P_3

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Received February 15, 1989

Summary: We prepared a new series of renin inhibitors based on dipeptide glycols, replacing the P4-P3 subsites with an O-(N-morpholinocarbonyl)-3-L-phenyllactic acid residue. This modification proved bioisosteric with Boc-L-phenylalanine, giving rise to highly potent human renin inhibitors (1-5 nM), e.g., SC-46944 (IC_{50} = 5 nM). Moreover, this change produced compounds that are orally efficacious in reducing plasma renin activity in salt-depleted marmosets.

Orally effective inhibitors of human renin as agents for the treatment of essential hypertension and congestive heart failure are being enthusiastically pursued (1,2,3,4). An effective approach to the development of orally active compounds has centered on truncated analogues of the natural substrate, angiotensinogen, incorporating non-hydrolyzable scissile dipeptide analogues (1). The discovery of renin inhibitory dipeptide glycols (5,6), typified by SC-46507 in figure 1, provided the impetus for the design and development of highly potent, low molecular weight glycol-based inhibitors (7) that are active *in vivo* when administered intravenously. However, even the most potent of these substances, SC-46507, was inefficacious when administered *orally* to monkeys. We now wish to report that oral activity can be achieved by converting the dipeptide glycols to a new series of **non-peptides** (figure 2), formed by bioisosteric substitution of the Boc-Phe residue with O-(N-morpholinocarbonyl)-3-L-phenyllactyl; this is a formal replacement of the amino acid NH with oxygen of lactic acid.

Methods and Materials

The glycols were prepared by coupling O-(N-morpholinocarbonyl)-3-L-phenyllactic acid to an appropriate amino acid-aminoglycol conjugate using the procedure of Benoiton (8). The compounds were characterized by NMR spectra, combustion analysis, melting point and HPLC data. The synthetic experimental details will be described elsewhere.

The human renin inhibition test has been previously described in detail (7). Human renin was obtained from the National Institute for Biological Standards, London. In a total volume of 0.25mL 100 mM Tris-acetate buffer at pH 7.4, 25 x 10^{-6} Goldblatt units of renin, 0.05mL of plasma from human volunteers taking oral contraceptives, 6.0 mM sodium EDTA, 2.4 mM phenylmethyl sulfonyl fluoride, 1.5 mM 8-hydroxyquinoline, 0.4 mg/mL BSA, and 0.024 mg/mL

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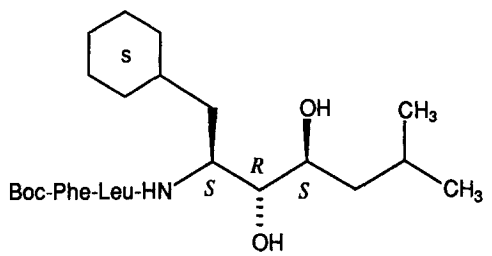
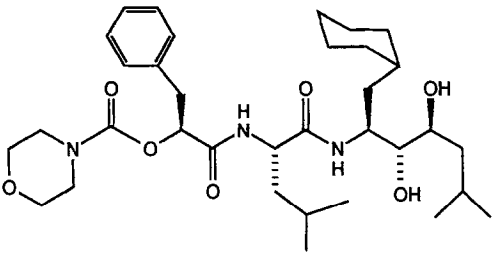
0006-291X/89 $1.50



(1) $IC_{50} = 5 \times 10^{-9}$ M (Human Renin)



(2) $IC_{50} = 5 \times 10^{-9}$ M (Human Renin)

Figure 1. Potent dipeptide glycol renin inhibitor SC-46507.

Figure 2. SC-46944, an orally active renin inhibitor containing a 3-phenyllactic acid residue at P_3.

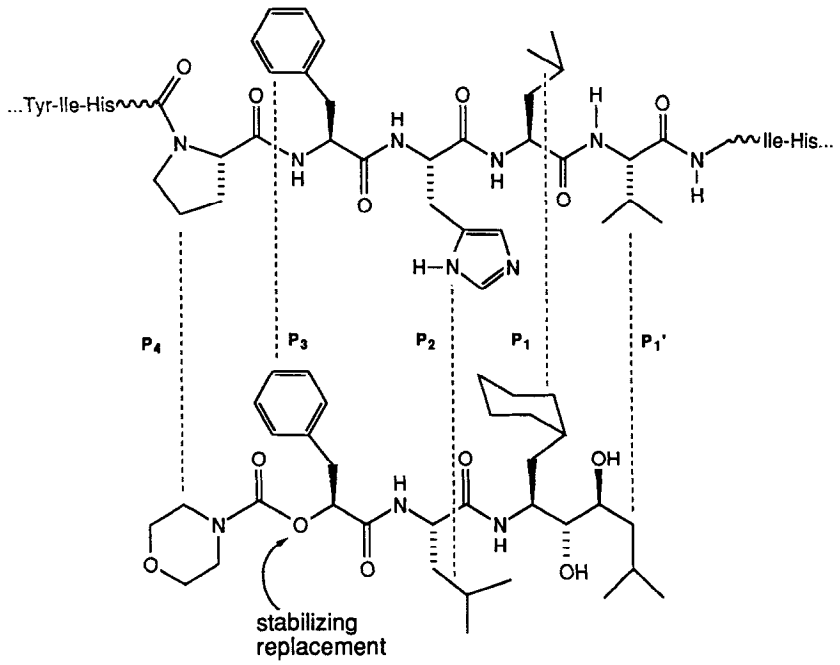
neomycin sulfate were incubated for two hours at 37°C in the presence or absence of renin inhibitors. The produced angiotensin I was determined by radioimmunoassay (New England Nuclear kit). Test compounds to be assayed were dissolved in DMSO and diluted with 100mM Tris-acetate buffer at pH 7.4 containing 0.5% BSA to the appropriate concentration. The final concentration of organic solvent in the reaction mixture was less than 1%. Control incubations at 37°C were used to correct for effects of organic solvent on renin activity.

Oral activity in marmosets is determined as follows. Common marmosets weighing 250-350g are placed on a low sodium diet (< 0.05% Na) for 7-14 days. Previous work had established that this mild sodium depletion raises plasma renin activity (PRA) to ca. 30 ngAI/mL/hr. The animals are anesthetized with isoflurane and a catheter placed in the femoral artery. The animal is restrained in a tube which protects the femoral catheter and is allowed to recover from the anesthesia for 2 hours. Dosing with test compound (dissolved in polyethylene glycol 400) is done intragastrically. Blood is then sampled before and 30, 60, 120 minutes after compound administration; PRA is determined at pH6 as described previously (7).

Results and Discussion

The rationale behind the preparation of modified glycol-based inhibitors was to introduce a key structural modification to enhance proteolytic stability while maintaining necessary correspondence to the natural substrate (figure 3). Stability to degrading enzymes such as chymotrypsin is an issue that Plattner (3) has addressed by showing that the Phe-His (P_3-P_2) peptide bond of renin inhibitors is cleaved *in vitro* by chymotrypsin. Taking chymotrypsin as a model of the *in vivo* degrading enzymes, we postulated, on the basis of crystallographic work of Matthews (9), that elimination of the dipeptide glycol phenylalanine NH (at P_3), which presumably forms an hydrogen bond with Ser214 of chymotrypsin, would result in enhanced enzymic stability. Thus a necessary condition for oral activity would be satisfied. Replacement of the NH in SC-46507 with O, giving lactic acid-containing glycols, successfully resulted in high affinity non-peptides (Table 1). The *in vitro* potency is comparable to the most potent dipeptide glycol SC-46507 (IC_{50} = 5 nM). *These results show that the morpholinoyl phenyllactyl group is bioisosteric with Boc-Phe.* Substituting a histidine residue at P_2, as occurs in the natural substrate, to give SC-47563 (IC_{50} = 1 nM), results in one of the most potent glycol-based inhibitors we have studied. A further modification to increase enzymic stability was the inversion of configuration at the α-carbon of the L-phenyllactic acid residue to give SC-48272; this D-lactic acid derivative exhibits sharply reduced (140 fold) potency toward human renin, suggesting that the P_4 and P_3 subsites of this series are not interchangeable in the human renin active site.

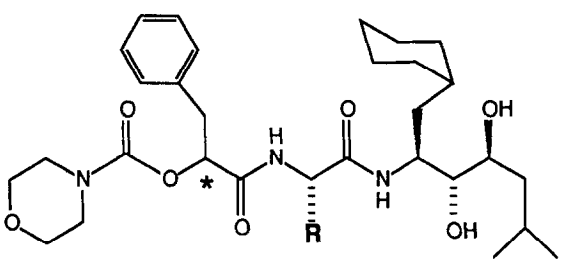
Human Angiotensinogen



SC-46944

Figure 3. The correspondence between SC-46944 and human angiotensinogen.

Table 1
In Vitro Human Renin Inhibition



Searle no.	R	* configuration	IC_{50} (nM)
SC-47563	CH_2-imidazole	L	1
SC-46944	i-Bu	L	5
SC-47557	CH_2-imidazole(Ts)	L	78
SC-48272	i-Bu	D	700

Table 2

Percent Change from Pretreatment Plasma Renin Activity in Sodium-Depleted Marmosets 20mg/kg, i.g.

Searle no.	Pretreatment PRA ng AI/mL/hr	PRA change (%) 30min	60min	120min	n
SC-47557	65	-100	-99	-93	1
SC-46944	38	-95	-95		5
SC-47563	62	-74	-92	-91	4

These non-peptides were tested for their ability to lower plasma renin activity (PRA) in salt-depleted marmosets @ 20 mg/kg, i.g. (Table 2). The morpholinoyl-phenyllactyl glycols (MPG's) reduce PRA by greater than 90% over a 60-120 minute period. In contrast, SC-46507, the most potent *(in vitro)* dipeptide glycol, fails to produce any measureable PRA reduction @ 20 mg/kg

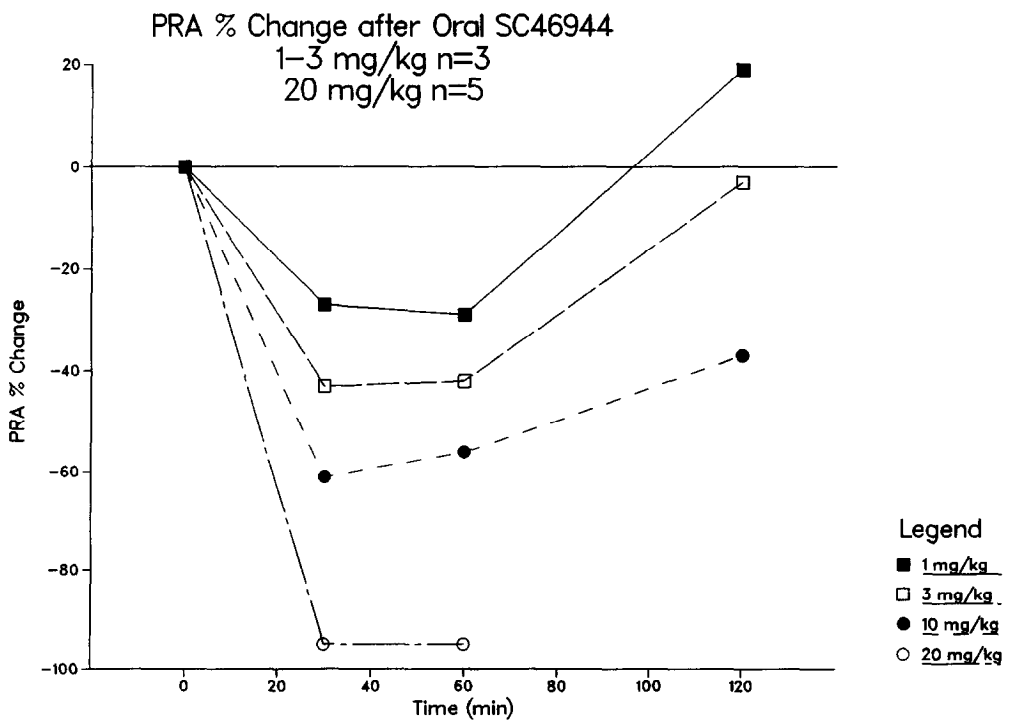


Figure 4. The plasma renin lowering effect of SC-46944 in salt-depleted marmosets upon oral administration at doses of 1, 3, 10, 20 mg/kg. The assay for PRA is determined at pH 6.

when given orally. The oral efficacy of the non-peptide MPG series lends support to the idea of enhanced *in vivo* enzymic stability of this class of renin inhibitors. An oral dose-response study was conducted on SC-46944, showing that this substance reduces PRA by 50% with an oral dose of *ca.* 6 mg/kg (figure 4). Thus the objective of converting the potent dipeptide glycol renin inhibitor series into analogues exhibiting oral activity has been accomplished by the replacement of the P_3 NH with an oxygen atom. The stage is set for further pharmacological evaluation of these new compounds as anti-hypertensive medicaments.

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