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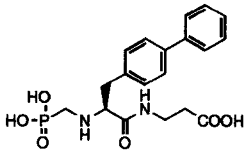
DUAL INHIBITION OF NEUTRAL ENDOPEPTIDASE AND ANGIOTENSIN-CONVERTING ENZYME BY *N*-PHOSPHONOMETHYL AND *N*-CARBOXYALKYL DIPEPTIDES

Stéphane De Lombaert,* Jenny Tan, Lisa J. Stamford, Yumi Sakane, Carol Berry and Rajendra D. Ghai
Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey 07901

Abstract: Structural modifications have been performed on the selective α-amino phosphonic acid NEP inhibitor CGS 24592, to achieve dual ACE/NEP inhibition *in vitro*. (S)-*N*-Phosphonomethyl-valyl-(S)-(4-phenyl)phenylalanine (**19**) is representative of a new type of phosphorus-containing ACE/NEP inhibitors, approaching the *in vitro* potency of the sulfhydryl ACE inhibitor captopril and the NEP inhibitor thiorphan.

Introduction. Inhibition of angiotensin-converting enzyme (ACE), a protease involved in the biosynthesis of the vasoconstrictor octapeptide angiotensin-II, constitutes one of the most successful mechanism-based strategies to manage hypertension.[1] In recent years, inhibitors of neutral endopeptidase 24.11 (NEP) have also attracted considerable attention due to their ability to protect atrial natriuretic peptide (ANP) from enzymatic degradation, thereby potentiating its hypotensive action.[2] We, and others, have been interested in the physiological consequences of the combined inhibition of ACE and NEP, in particular as a potentially beneficial therapy for the treatment of hypertension and congestive heart failure.[3-6] Although ACE and NEP are two distinct zinc-metalloproteases, they share some structural similarities at the active site.[7-9] Thiol-containing compounds have long be favored for dual inhibition because of the strong affinity of sulfur for zinc, which contributes heavily to the overall binding energy. However, such compounds, albeit usually highly potent, often undergo a rapid clearance *in vivo* or an extensive metabolic inactivation.[10-13] Some dicarboxylic acids with dual inhibitory activity have been claimed in recent patents.[14] They typically require additional binding elements to achieve suitable potency *in vitro*, and, therefore, suffer from an increased structural complexity and higher molecular weight as compared to thiol-containing inhibitors.

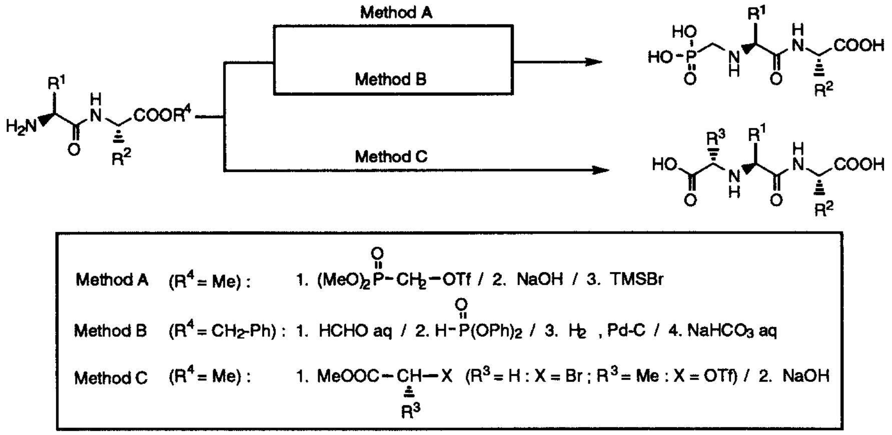
We have recently reported that the α-amino phosphonic acid dipeptide, CGS 24592, is a selective NEP inhibitor which combines high potency and long duration of action,[15] and can be made orally active via a unique prodrug approach.[16,17] If this compound could be altered to display additional ACE inhibitory activity while maintaining its tight binding to NEP and favorable pharmacokinetic profile, an attractive new class of therapeutic agents would be on hand.



CGS 24592

In this Letter, we disclose our preliminary findings on a series of new phosphonomethyl dipeptides capable of inhibiting both ACE and NEP *in vitro* at low nanomolar concentration.

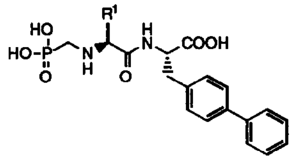
Chemistry. The synthetic methods used in this study basically follow the procedures reported for the preparation of CGS 24592[15] as outlined in the Scheme. With few exceptions (see below), phosphonomethyl dipeptides were prepared according to the standard method A, i.e. phosphonomethylation of the dipeptide methyl esters, followed by saponification, and deprotection of the phosphonic acid with bromotrimethylsilane.[15] The tripeptide homologues **24** and **25** were prepared similarly. To obtain phosphonic acids **22**, **26**, **27**, and **29**, an alternative method B, relying on the mild hydrolysis of diphenyl α-amino phosphonates,[16] was used. Dipeptide benzyl esters were condensed with aqueous formaldehyde, reacted with diphenyl phosphite, and debenzylated by palladium-catalyzed hydrogenation to afford the corresponding diphenyl α-amino phosphonate intermediates.[15] Subsequent bicarbonate-induced hydrolysis gave the desired phosphonic acids.[18] Alkylation of dimethyl (R)-(1-aminoethyl)phosphonate[19] with the triflate of methyl (R)-1-phenyllactate was used to secure the absolute configuration of **23**.[20] Carboxyalkyldipeptides **30**-**32** were readily obtained by alkylation of the dipeptide methyl esters with benzyl α-bromoacetate or the triflate of (R)-methyllactate, followed by saponification (Method C).[21]

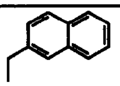
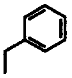
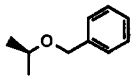
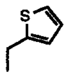
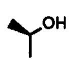
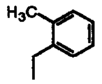
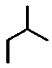
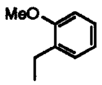
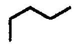
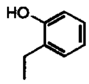
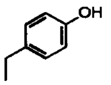
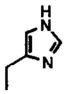
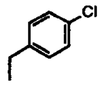
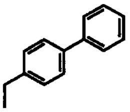
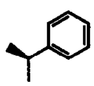
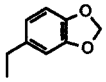


Scheme

Results and Discussion. At the onset of our work, at least two structural features of CGS 24592 were clearly recognized as being responsible for its poor interaction with ACE ($IC_{50} > 1$ μM) : the β-amino acid terminus and the oversized $P_{1'}$ biphenylmethyl group. The former problem was easily solved since the replacement of the β-alanine residue in CGS 24592 by other hydrophobic (S)-α-amino acids, better suited to fit in ACE, was known not to impact significantly on the NEP inhibitory activity.[15] The presence of a $P_{1'}$ biphenylmethyl group posed initially a more challenging dilemma, since this group had been shown to be critical for optimum interaction with NEP. However, considering that both proteases contain a relatively large hydrophobic $S_{2'}$ domain, we envisioned this subsite as a possible host for the lipophilic substituent of new ACE/NEP inhibitors. Thus, the (S)-(4-phenyl)phenylalanine residue was moved to the C-terminus of the phosphonomethyl dipeptides, thereby allowing smaller groups to be introduced in $P_{1'}$ and optimized to conform with both ACE and NEP active sites[22] (Table 1).

Table 1. ACE and NEP Inhibitory Potencies of $P_{1'}$ Modified *N*-Phosphonomethyl Dipeptides[23]

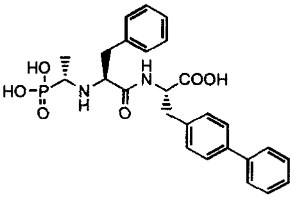


Cpd	R^1	IC_{50} (nM)[a]		Cpd	R^1	IC_{50} (nM)	
		NEP	ACE			NEP	ACE
1[b]	CH_3	1000	660	12		282	53
2		35	117	13		>1000	656
3		73	385	14		>1000	665
4		96	498	15[a]		356	>1000
5		>1000	160	16		37	731
6		>1000	>1000	17		2	50
7		226	>1000	18[b]		>1000	>1000
8		>1000	>1000	19		3	25
9		338	473	20		64	>1000
10		20	414	21		47	138
11		48	147	22		>1000	>1000

[a] racemic (4-phenyl)phenyl alanine was used (1/1 mixture of diastereomers); [b] spirocyclopentyl (cycloleucine)

By showing modest activity in ACE, α-amino phosphonic acid **1** provided a first indication that a (S)-biphenylmethyl group would indeed fit in $S_{2'}$. The lack of NEP activity was attributed to a missing interaction with $S_{1'}$. Early SAR studies have indicated that a $P_{1'}$ benzyl substituent would be generally favorable to both NEP and ACE inhibitors.[8] Among the compounds containing a substituted $P_{1'}$ benzyl group, or a surrogate thereof, **2** showed the most potent dual inhibition. Polar substituents on the aryl ring, as in **5-8**, generally impaired both inhibitory activities. As expected, the biphenylmethyl group in **10** enhanced the activity in NEP, but at the expense of ACE inhibition. Bulky groups, like piperonyl or 2-naphthyl, did not produced the needed increase in potency (e.g. **11** and **12**). Amino acids with alkyl side-chains were subsequently evaluated. The threonine analogues **13** and **14** were poor dual inhibitors. Interestingly, among the various compounds containing a leucine residue, or derivatives thereof (nor-, iso-, cyclo-), the β-branched phosphonomethyl dipeptide **17** displayed the best dual inhibitory activity. A further improvement was achieved with the smaller valine analogue **19**, which turned out to be the most potent dual inhibitor *in vitro* in this study, approaching the activity of the ACE inhibitor captopril (IC_{50} = 10 nM) and comparing favorably with the NEP inhibitor thiorphan (IC_{50}= 5 nM). Sterically more demanding groups, such as cyclohexyl, α-methyl benzyl or tert-butyl, had a detrimental impact on both enzyme activities (e.g. **20** - **22**).

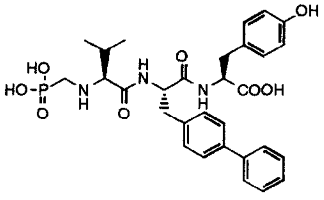
The influence of an additional interaction with the S_1 or $S_{3'}$ subsites was also briefly examined. Relative to **2**, the α-amino phosphonic acid **23**, bearing a P_1 methyl substituent, suffered a loss of NEP activity, but its ability to inhibit ACE was enhanced. Tripeptide homologues of **19** (i.e. **24** and **25**) contain one more C-terminal α-amino acid residue, presumed to interact with the $S_{3'}$ pockets. These compounds remained potent, albeit selective, NEP inhibitors, stressing the importance of maintaining a precise spacing between the zinc-binding group and the C-terminal carboxylic acid for ACE inhibition.[26]



23

IC_{50} (NEP) : 171 nM

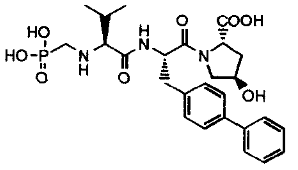
IC_{50} (ACE) : 44 nM



24

IC_{50} (NEP) : 4 nM

IC_{50} (ACE) : >1000 nM



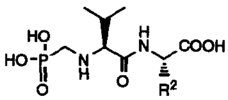
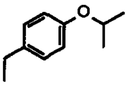
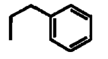
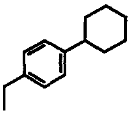
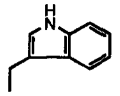
25

IC_{50} (NEP) : 12 nM

IC_{50} (ACE) : >1000 nM

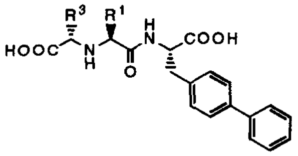
At this point, it remained to evaluate the initial selection of the biphenylmethyl group as the $P_{2'}$ lipophilic substituent of choice in this class of dual inhibitors. Thus, in the most potent compounds, **17** and **19**, the biphenylalanine residue was replaced with other lipophilic α-amino acids (Table 2). In terms of inhibitory activities, none of these compounds, including the hydrogenated derivative **28**, compared favorably with the α-amino phosphonic acids **17** and **19**.

Table 2. ACE and NEP Inhibitory Potencies of $P_{2'}$ Modified *N*-Phosphonomethyl Dipeptides[23]

	Cpd	R^2	IC$_{50}$ (nM)	
			NEP	ACE
	26		9	92
	27		26	100
	28		35	122
	29		9	240

Finally, considering that the α-amino phosphonic acid analogue of the ACE inhibitor enalaprilat has been reported to be substantially less potent than the original carboxyalkyl dipeptide,[27] we have investigated if the replacement of the phosphonyl group in **17** or **19** by a carboxylic acid would result in an increased activity in ACE (Table 3).

Table 3. ACE and NEP Inhibitory Potencies of $P_{1'}$ Modified *N*-Carboxyalkyl Dipeptides[23]

	Cpd	R^3	R^1	IC$_{50}$ (nM)	
				NEP	ACE
	30	H		200	65
	31	CH_3		165	22
	32	CH_3		735	23

The carboxylic acid **30** was clearly less active than its phosphonic acid analogue **17** regarding NEP inhibition. Similarly, the substituted derivatives **31** and **32** essentially retained the ACE activity of their phosphonic acid counterparts, but suffered a substantial loss in NEP activity.

Conclusion. Modifications of the highly potent phosphonomethyl dipeptide NEP inhibitor, CGS 24592, have been performed to take advantage of the large lipophilic $S_{2'}$ subsites of ACE and NEP. In particular, the displacement of the biphenylmethyl substituent from $P_{1'}$ to $P_{2'}$ in conjunction with the structural optimization of the central α-amino acid residue has allowed the design of a new type of potent phosphorus-containing dual inhibitors of ACE and NEP. The most active member, **19**, displays attractive inhibitory potencies *in vitro* and appears therefore suitable for further pharmacological evaluation *in vivo*.

Acknowledgment: We thank Mr. L. Blanchard for the synthesis of compound **22**.

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(18) The rate of the bicarbonate-induced hydrolysis of diphenyl α-amino phosphonate dipeptides was dramatically affected by the steric bulk around the amino group. In contrast to CGS 24592, which was readily produced by the rapid and mild hydrolysis of its diphenylphosphonate, obtention of the valine and, in particular, the tert-leucine analogues **26** and **22** required longer reaction time or higher temperature (22 °C for 2 days and 70 °C, for 22 days, respectively).

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(22) Throughout this study, it will be assumed that the inhibitors display a similar binding mode in both ACE and NEP, an assumption ultimately supported by the SAR study.

(23) NEP activity was measured using the synthetic substrate Glutaryl-Ala-Ala-Phe (ref.15 and 24). ACE activity was determined using the substrate Hippuryl-His-Leu (ref. 25).

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