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POTENT NON-PEPTIDIC DUAL INHIBITORS OF ENDOTHELIN-CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE 24.11§

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Abstract: Structural modifications of CGS 26303, a non-peptidic α-aminophosphonate dual ECE/NEP inhibitor lead, were performed to maximize inhibition of recombinant human ECE-1, while maintaining strong NEP inhibition. Specifically, substitution of the α-aminophosphonate moiety with aryl ethyl side-chains led to the discovery of a new class of potent, non-peptidic, dual inhibitors, such as CGS 31447, which blocked ECE-1 and NEP activities with IC_{50}'s of 17 and 5 nM, respectively. © 1997 Elsevier Science Ltd.

Endothelin-1 (ET-1), a highly potent vasoconstrictor and a mitogenic peptide, has been implicated in a remarkable variety of diseases, including several cardio- and cerebrovascular diseases,[1,2] renal failure,[3] and asthma.[4] Consequently, blockade of the ET-1 system has emerged as a promising biological strategy with widespread therapeutic potential. While many of these claims remain to be demonstrated clinically, most of the research activities have focused on antagonizing the two cognate receptors of ET-1.[5] In contrast, much less progress has been achieved on another attractive alternative aimed at preventing ET-1 production. ET-1 is believed to be generated from the post-translational cleavage of the biologically inactive big ET-1 at the Trp^{21}–Val^{22} bond by an endothelin-converting enzyme (ECE).[6] Despite the existence of several isoforms of ECE, gene knockout experiments[7] and data from tissue distribution strongly suggest that ECE-1 is most likely the physiologically relevant protease involved in big ET-1 processing. Recombinant rat, bovine, and human ECE-1 (rhECE-1) have been cloned, expressed, and characterized as highly homologous membrane-bound zinc metalloproteases.[6] In addition, ECE-1 shares structural similarities with neutral endopeptidase 24.11 (NEP), another metalloprotease whose inhibition is also of current therapeutic interest.[8]

Since the discovery that phosphoramidon, a potent dipeptidic NEP inhibitor, is also a modest inhibitor of ECE-1, this compound has served as the major template for designing new ECE inhibitors. However, until now, the most potent ECE-1 inhibitors reported have only shown a 3 to 12-fold increase in inhibitory activity relative to phosphoramidon.[9–16] Recently, we have disclosed that CGS 26303 is a structurally novel non-peptidic dual ECE/NEP inhibitor,[17] which bears little chemical resemblance with phosphoramidon. Pharmacologically, CGS 26303 moderately lowered blood pressure in SHR, a renin-dependent model of hypertension not affected by NEP inhibitors,[17] and prevented cerebral vasospasm after subarachnoid hemorrhage in rabbits, a disease associated with ET-1 overproduction.[18]

Encouraged by these results, we have embarked on a systematic optimization of CGS 26303 with the initial goal of improving on its rhECE-1 inhibitory activity,[19–21] while retaining its strong NEP inhibitor character,[22] a feature expected to be beneficial for the treatment of various cardiovascular disorders.[8]

In this Letter, we present the results of a preliminary study that culminated with the design of highly potent dual inhibitors of rhECE-1 and NEP in vitro. Our optimization of CGS 26303 involved specific modifications of the tetrazole, the biphenylmethyl group, and the zinc binder, as well as some limited substitutions of the α-aminophosphonate residue (Figure 1).

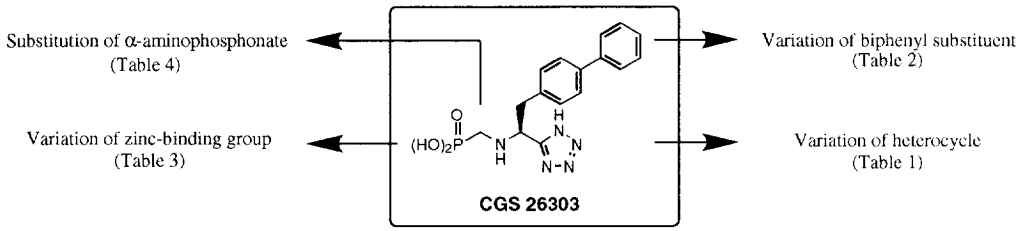
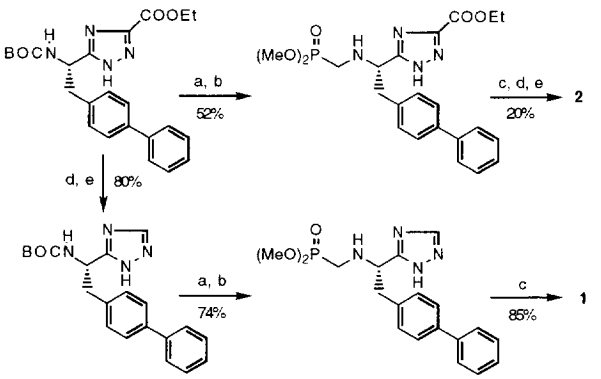


Figure 1

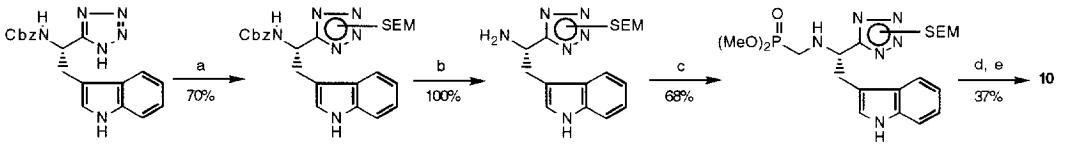
Chemistry. The new α-aminophosphonic acids in Tables 1 and 2 were prepared from the corresponding amines according to methods used previously.[22–24] To obtain the heterocyclic analogs **1** and **2** (Table 1), the carboxylic acid terminus of (S)-4-biphenylalanine was converted into a triazole following literature procedures[25,26] and elaborated further into aminophosphonic acids (Scheme 1).



Reagents: a. HCOOH; b. $(MeO)_2PO\text{-}CH_2\text{-}OSO_2CF_3$, iPr_2NEt; c. Me_3SiBr; d. NaOH; e. HCl.

Scheme 1

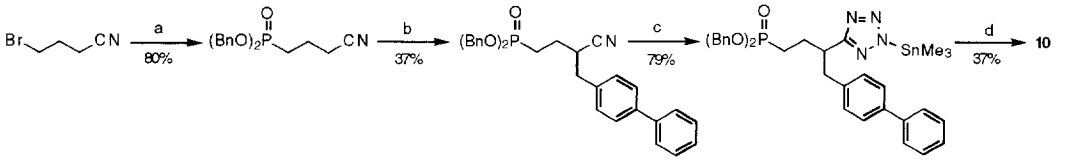
The tetrazole derivatives in Table 2 were synthesized from the corresponding (S)-α-amino acids, as reported for the preparation of CGS 26303,[22] except for compound **9**, which was obtained from the tetrazole analog of tryptophan[27] as depicted in Scheme 2.



Reagents: a. SEMCl; b. H_2, cat. Pd-C; c. $(MeO)_2PO\text{-}CH_2\text{-}OSO_2CF_3$, iPr_2NEt; d. H_2SO_4; e. Me_3SiBr.

Scheme 2

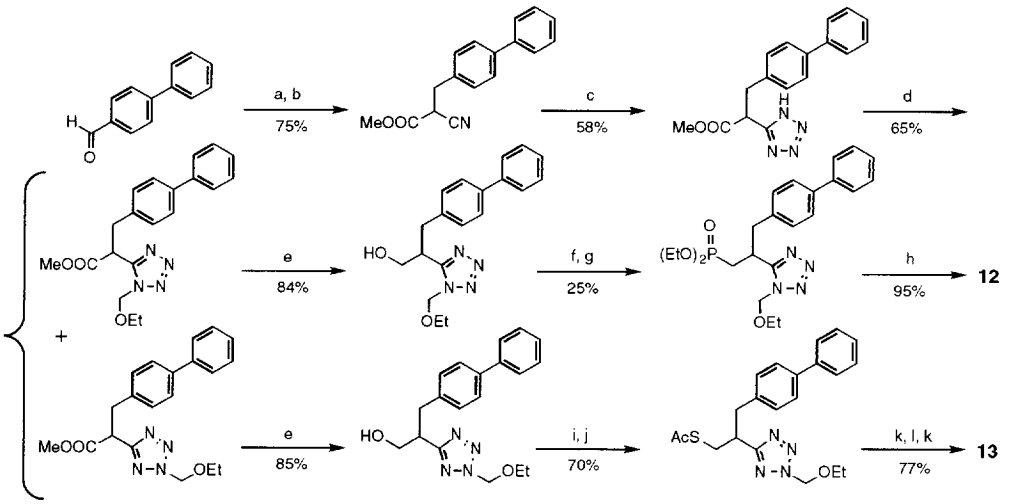
The de-aza analog of CGS 26303 (**10** in Table 3) was synthesized as illustrated in Scheme 3.



Reagents: a. $(BnO)_2POH$, NaH; b. Ph-Ph-CH_2-Br, $LiN(SiMe_3)_2$; c. Me_3SnN_3; d. HBr-AcOH

Scheme 3

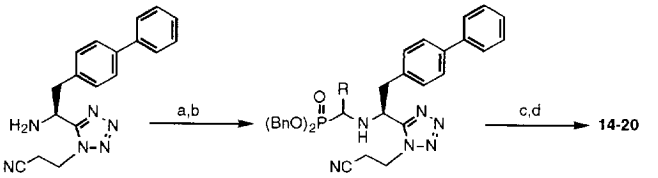
Phosphonamide **11** (Table 3) was prepared by phosphonylation of the corresponding amine by standard methods.[28] Phosphonic acid and thiol analogs **12** and **13** were synthesized as illustrated in Scheme 4.



Reagents: a. MeOOC-CH_2-CN, piperidine, PhCOOH; b. $NaBH_4$; c. NaN_3, NH_4Cl, 110 °C; d. EtO-CH_2-Cl, NEt_3; e. $LiBH_4$; f. I_2, PPh_3, imidazole; g. $P(OEt)_3$; h. Me_3SiBr then MeOH; i. $MeSO_2Cl$, NEt_3; j. AcSK; k. HCl; l. NaOH.

Scheme 4

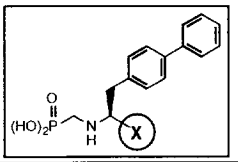
Substituted α-aminophosphonates **14–20** (Table 4) could be prepared under mild conditions, albeit in low yield (25–50%) and without significant stereoselectivity, as two chromatographically separable diastereomers by the method of Rees et al. (Scheme 5).[29,30]



Reagents: a. RCHO, $MgSO_4$; b. $(BnO)_2POH$, Me_3SiCl; c. DBU; d. Me_3SiBr

Scheme 5

Table 1.



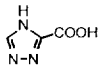
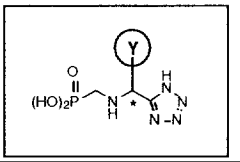
Cpd	X	% inh.
CGS 26303		**79**
1		0
2		14
3	COOH	20
4		0

Table 2.



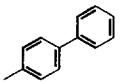
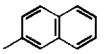
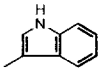
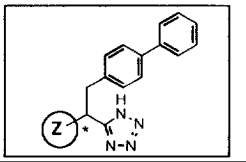
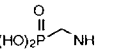
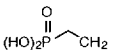
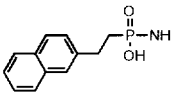
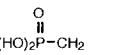
Cpd	Y	*	% inh.
CGS 26303		S	**79**
5		R	0
6		S	0
7		S	0
8		S	0
9		S	10

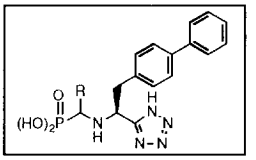
Table 3.

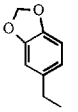
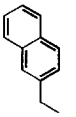


Cpd	Z	*	% inh.
CGS 26303		S	**79**
10		R,S	0
11		S	0
12		R,S	8
13	$HS-CH_2$	R,S	26

% inh: % inhibition of rhECE-1 activity measured at 1 μM concentration of test compound.

Table 4.



Cpd	R	% inh.	rhECE-1 IC_{50} (nM)	NEP IC_{50} (nM)	Cpd	R	% inh.	rhECE-1 IC_{50} (nM)	NEP IC_{50} (nM)
CGS 26303	H	**79**	**410 ± 70**	**0.9 ± 0.1**					
14a		83	280 ± 100	0.74 ± 0.04	**18a**		85	93 ± 13	0.6 ± 0.1
14b		18	5000 ± 1300	17 ± 1	**18b**		76	390 ± 150	2.5 ± 0.3
15a		**95**	**17 ± 3.9**	**4.8 ± 0.4**	**19a**		87	110 ± 26	0.7 ± 0.1
15b		85	160 ± 30	8.8 ± 0.5	**19b**		50	1100 ± 170	ND
16a		91	45 ± 4	6.9 ± 0.2	**20a**		14	> 1000	ND
16b		47	1400 ± 500	ND	**20b**		49	> 1000	ND
17a		93	23 ± 3	1.46 ± 0.49	**Phosphoramidon**		47	1200 ± 70	30 ± 2
17b		22	> 1000	ND	**Thiorphan**		9	> 100 000	4.8 ± 0.2

ND: not determined; IC_{50}'s reported are the average of triplicates ± SEM.

Discussion. Results from Tables 1–3 indicate that all attempts at altering the core structure of CGS 26303 resulted in a drastic loss of ECE-1 inhibitory activity. The simple replacement of the tetrazole by the less acidic triazole (e.g., **1**) suppressed all ECE-1 inhibitory activity. More surprisingly, re-installing a C-terminal carboxylic acid, as in **2–4**, only produced weak ECE-1 inhibitors, underlining the important role of the tetrazole ring in the binding to the enzyme (Table 1). The biphenylmethyl group also emerged as a critical feature of CGS 26303, since alternative aromatic substituents were detrimental to the inhibitory activity (Table 2). Furthermore, the (R) enantiomer of CGS 26303 (**5**) was also inactive. Variations of the aminomethyl phosphonate group were not met with more success (Table 3). The de-aza analog of CGS 26303 (**10**) lacked ECE-1 inhibitory activity. In addition, despite a previous observation that lipophilic phosphonamide analogs of phosphoramidon displayed modest inhibitory activity,[12,28] surprisingly, **11** did not inhibit ECE-1. The phosphonic acid **12** was also essentially devoid of activity. More recently, thiols combining some structural features of both thiorphan and phosphoramidon have been reported to be ECE inhibitors, albeit not more potent than phosphoramidon.[31] The thiol analog **13**, which borrows from the structures of thiorphan and CGS 26303, was also a weaker ECE-1 inhibitor than phosphoramidon.

Substitution of the α-aminophosphonate moiety was investigated next as a means to increase the binding affinity of these inhibitors towards ECE-1 (Table 4).[9,13] Introduction of a phenethyl group, as in **14a**, provided the first evidence that more potent derivatives of CGS 26303 could indeed be obtained. Naphthyl-, quinolyl-, and benzodioxolanyl-containing analogs were especially potent ECE-1 inhibitors (**15–19**). This suggests that these groups fit in a wide and essentially lipophilic pocket of ECE-1, adjacent to the narrower and deep hydrophobic subsite that accomodates the 4-biphenyl substituent of the inhibitor. Shortening the ethylidene spacer to a methylene reduced the inhibitory activity (**20**). In all cases, the ECE-1 inhibitory activity resided mostly in one diastereomer whose absolute configuration remains to be determined. Overall, the SAR in this series parallels that of the less potent phosphonoalkyl dipeptides.[9] Interestingly, all compounds active in the rhECE-1 assay were also very potent NEP inhibitors, but followed distinct SAR's. At this point, it has still to be established whether these dual inhibitors adopt a similar binding mode in ECE-1 and NEP. Taken together, our results reinforce the notion that ECE-1 is substantially more discriminating than NEP.

Conclusions. In the course of the optimization of CGS 26303, it was observed that all attempted alterations to its core structure resulted in a dramatic reduction of inhibitory potency against ECE-1. However, the substitution of the α-aminophosphonate moiety with arylethyl substituents produced inhibitors of recombinant human ECE-1 that were not only the most potent known to date, but also retained potent NEP inhibitory activity. In particular, the aminophosphonic acid **15a** (**CGS 31447**), with ECE-1 and NEP inhibitory potencies 70- and 6-fold, respectively, superior to those of phosphoramidon, is representative of a new class of highly potent, non-peptidic dual ECE/NEP inhibitors.

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19. The determination of ECE inhibition in vitro was performed as described previously (ref 20), except that rhECE-1 expressed in CHO cells (ref 21) and human bigET-1 were used in the current study. In this assay, the IC_{50} for CGS 26303 was 410 nM, while a value of 1.1 μM had been determined with partially purified porcine ECE (ref 17). In both cases, CGS 26303 was about 3 times more potent than phosphoramidon as an ECE-1 inhibitor. The assessment of NEP inhibition was performed as described previously (ref 23).
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