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Synthesis and Biological Activity of Novel Potent Endothelin-Converting Enzyme-1 Inhibitors

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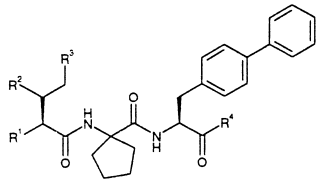
Abstract—Through directed screening of metalloprotease inhibitors, CGS 30084 (**1**) has been identified as a potent endothelin-converting enzyme-1 (ECE-1) inhibitor in vitro ($IC_{50} = 77$ nM). Herein we report the syntheses and biological activities of analogues derived from this lead, based on modifications of the biphenyl moiety. Compound **10**, the thioacetate methyl ester prodrug derivative of compound **6m**, was found to be an orally active and potent inhibitor of ECE-1 activity in rats.

The overproduction of endothelin-1 (ET-1), one of the most potent vasoconstrictive peptides characterized thus far,[1] has been associated with a variety of disorders such as cerebral vasospasm, stroke, asthma, and cardiac and renal failure.[2–5] The discovery of potent inhibitors of ET-1 biosynthesis is thus envisaged as an attractive potential therapeutic approach for the treatment of diseases linked with elevated ET-1 levels.[6] Endothelin-converting enzyme-1 (ECE-1), a zinc metalloprotease, catalyzes the post-translational conversion of big ET-1 to ET-1,[7] thus presenting a logical target for the design of therapeutic agents that regulate the production of ET-1 in vivo.[8,9]

We have previously reported the identification of CGS 30084 (**1**) (Fig. 1, $IC_{50} = 77$ nM) as an attractive starting point for the design of potent ECE-1 inhibitors.[10] As described in our earlier report, various modifications carried out *at the thiol end* of CGS 30084 afforded the highly potent ($IC_{50} = 11$ nM) ECE-1 inhibitor **1a**, and its prodrug **1b** (Fig. 1), a long-acting orally active inhibitor of ECE-1 activity in vivo. The current report describes the modifications carried out *at the biphenyl portion* of CGS 30084 in order to further explore the SAR of ECE-1 inhibition, and to improve the in vivo profile of CGS 30084 by changing its physicochemical properties (*vide infra*). Aside from spatial alternatives to *para*-biphenyl, modifications of *the distal ring* were also explored. In all cases, the thiol end substituents were kept the same (*S*-isopropyl) as CGS 30084.

The target molecules **6** were prepared as previously described,[10] starting from various arylalanines **2** (Scheme 1). The synthesis of target molecules (**6g–v**) containing 4-aryl-substituted phenylalanines presented a significant synthetic challenge. At the time we began our endeavor, only one reliable approach to 4-aryl-substituted phenylalanines existed (Scheme 2), which allowed rapid access to a diverse set of such derivatives.[11] This methodology, which utilizes the Pd-catalyzed cross-coupling reactions of tyrosine triflates (e.g., **7**) with organometallic reagents had a limited scope, however, due to the limited availability of highly functionalized organoboron and organotin reagents from



Compound	R^1	R^2	R^3	R^4
CGS 30084 (**1**)	SH	Me	H	OH
1a	SH	H	Me	OH
1b	SAc	H	Me	OMe

Figure 1. Previously described ECE-1 inhibitors.

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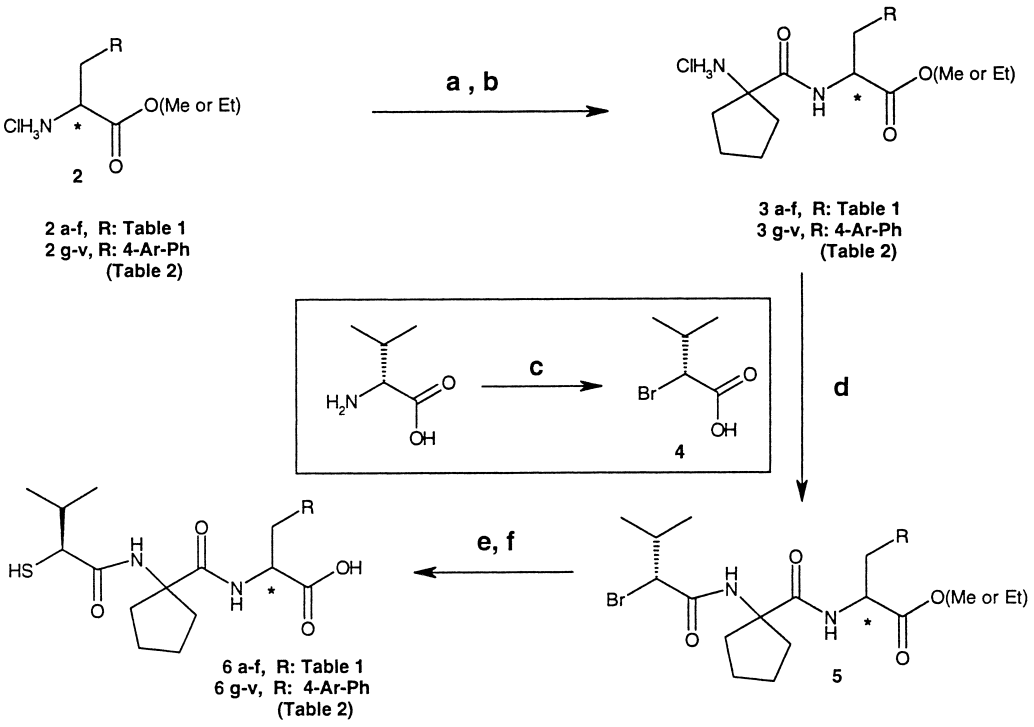
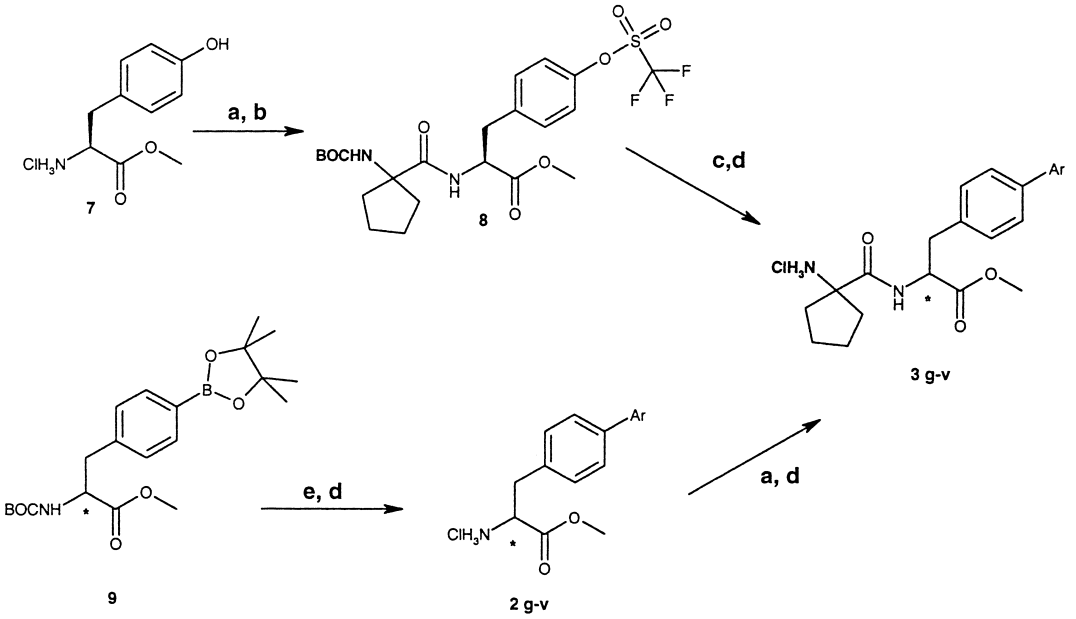
commercial sources at the time. We have developed an alternative more versatile procedure for the synthesis of 4-aryl-phenylalanines **2g–v**, employing the Pd-catalyzed cross-coupling reaction of the boronophenylalanine reagent **9** with aryl halides or triflates (Scheme 2).[12,13] In this manner, we were able to incorporate a large number of diverse 4-aryl-phenylalanines into our target molecules.

The compounds thus prepared were tested for their ability to inhibit ECE-1 activity in vitro. The experimental details for the assays utilized have been reported previously.[14] The results are summarized in Tables 1 and 2.

Table 1 summarizes our results in exploring spatial alternatives for the *para*-biphenyl moiety in CGS 30084.



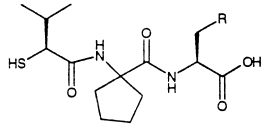
Scheme 1. (a) BOCNH-cycloleucine (EDCI, HOBt) or (DCC, HOAt), Et_3N, CH_2Cl_2, rt; (b) HCl (gas), CH_2Cl_2; (c) 48% HBr, KBr, $NaNO_2$; (d) **4** (EDCI, HOBt) or (DCC, HOAt), Et_3N, CH_2Cl_2, rt; (e) AcSK, THF; (f) 1 N NaOH, MeOH. * Stereochemistry at the chiral center bearing the biphenyl.



Scheme 2. (a) BOCNH-cycloleucine, HOBt, EDCI, Et_3N, CH_2Cl_2, rt; (b) Tf_2O, pyridine; (c) $ArSnMe_3$, $PdCl_2(dppf)$, LiCl, dioxane, reflux, or $ArB(OH)_2$, $PdCl_2(dppf)$, DME, K_3PO_4, reflux; (d) HCl (g), CH_2Cl_2; (e) Ar-X (X = Cl, Br, I, OTf), $PdCl_2(dppf)$, K_3PO_4, DME, reflux. *Stereochemistry at the chiral center bearing the biphenyl.

The size and orientation of the biphenyl substituent appear to be very important for ECE-1 inhibitory activity. Neither *m*-biphenyl nor *o*-biphenyl showed inhibitory activity, suggesting a 'narrow' binding pocket for the biphenyl group. Even a slightly larger distal ring (**6f**) proved detrimental for ECE-1 inhibitory activity. It appears, however, that some minimal 'size' is required at this position in order to achieve potent ECE-1 inhibition, as the monophenyl derivative **6a** is virtually inactive. The naphthyl derivatives, especially **6e**, offer a possible replacement for the biphenyl group, without significant loss of ECE-1 inhibitory activity.

Table 1. Spatial alternatives for the *p*-biphenyl group in CGS 30084

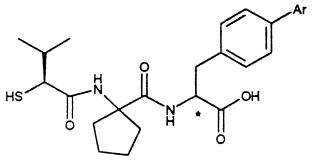


6a-f

Compds	ECE-1 Inhibition IC_{50} (nM)[a]	R
CGS 30084	77[b]	*p*-Biphenyl
6a	26%	Ph
6b	39%	*m*-Biphenyl
6c	0%	*o*-Biphenyl
6d	320 (±80)	1-Naphthyl
6e	180 (±3)	2-Naphthyl
6f	490 (±40)	4-Cyclohexyl-Ph

[a]Standard deviation is given in parentheses.
[b]Obtained from ref 10 (% = % inhibition of ECE-1 activity at 1 μM).

Table 2. Modifications at the distal ring of the biphenylalanine moiety in CGS 30084



6g-v

Compds	ECE-1 Inhibition IC_{50} (nM)[a]	* Stereochemistry	Ar
CGS 30084	77[b]	L	Ph
6g	18%	D	Ph
6h	130 (±20)	L	3-Thienyl
6i	300 (±60)	L	2-Furyl
6j	220 (±40)	DL	Pyridin-2-yl
6k	150 (±10)	DL	Pyridin-3-yl
6l	22%	DL	Pyridin-4-yl
6m	120 (±20)	DL	Pyrimidin-3,5-yl
6n	990 (±10)	DL	3,5-Dimethoxy-Ph
6o	350 (±30)	DL	2,3-Dimethoxy-Ph
6p	810 (±180)	L	4-Cl-Ph
6q	49%	L	4-Methoxy-Ph
6r	54%	L	4-CF_3-Ph
6s	650 (±130)	L	4-Fluoro-Ph
6t	370 (±160)	L	2-Methoxy-Ph
6u	650 (±200)	L	3-CF_3-Ph
6v	560 (±40)	L	3-Cl,4-F-Ph

[a]Standard deviation is given in parentheses.
[b]Obtained from ref 10 (% = % inhibition of ECE-1 activity at 1 μM).

Table 2 summarizes the results of our investigation of the SAR of *the distal ring* of the biphenyl moiety in CGS 30084. The absolute stereochemistry of the chiral center bearing the biphenyl moiety is very important for ECE-1 inhibitory activity. While the compound bearing the L-biphenylalanine stereoisomer (CGS 30084) is a potent ECE-1 inhibitor, the compound bearing D-biphenylalanine (**6g**) has virtually no activity towards ECE-1.[15] The distal phenyl ring can be replaced with a thiophene, without significant loss of ECE-1 inhibitory activity (**6h** vs CGS 30084). Furthermore, the distal ring of the biphenylalanine moiety tolerates basic or non-basic heteroatoms at the 2- and 3-position (**6h–k** and **6m**), while the presence of a nitrogen at the 4-position of the distal ring (**6l**) significantly reduces the ECE-1 inhibitory activity. It appears that the presence of a heteroatom is slightly better tolerated at the 3-position compared to the 2-position (see **6k** and **6m** vs **6j**). Electron-withdrawing groups at the 4-position of the distal ring appear to have a detrimental effect towards ECE-1 inhibitory potency (see **6r** and **6s** vs CGS 30084). In addition, there appears to be a 'size limit' to the substituents allowed at the 4-position of the distal ring, with the inhibitory activity decreasing from 4-fluoro (**6s**) to 4-chloro (**6p**) to 4-trifluoromethyl (**6r**) to 4-methoxy (**6q**) derivatives. Substitutions at the 2-position of the distal ring are preferred to those at the 3-position (e.g. **6o** and **6t** vs **6n**). In general, none of the various electron-donating or electron-withdrawing substitutions on the distal ring provided a significant improvement of in vitro ECE-1 inhibitory activity over CGS 30084.

Even though we were not able to significantly improve the in vitro potency of CGS 30084, we were intrigued by compounds **6k** and **6m** (DL mixtures), which provided alternatives with virtually equal potency,[15] and possibly different pharmacokinetic properties, due to different solubility profiles.[16] We therefore selected compound **6m** and its thioacetate methyl ester prodrug **10** (Figs. 2 and 3)[10] for additional in vivo studies. First, compound **6m** was evaluated in comparison with CGS 30084 for its ability to inhibit the pressor response produced by big ET-1 in anesthetized rats (Fig. 2),[14] measured as the change in mean arterial pressure produced by big ET-1 (1 nmol/kg) administered iv. Upon iv administration at 10 and 20 mg/kg, compound **6m** inhibited the big ET-1 pressor response by 56 and 73%, respectively, after 15 min. Under similar conditions, CGS 30084 (administered iv at 10 mg/kg) was only able to inhibit the big

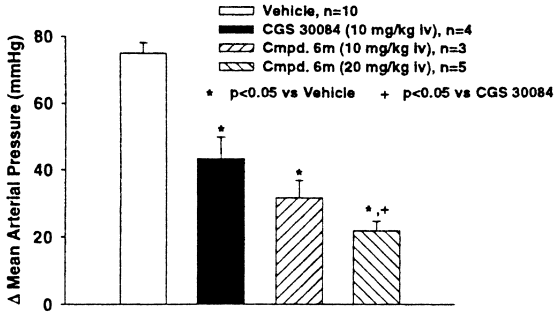


Figure 2. Comparison of the inhibition of big ET-1 pressor response by compounds **6m** and CGS 30084 (iv in anesthetized rats).

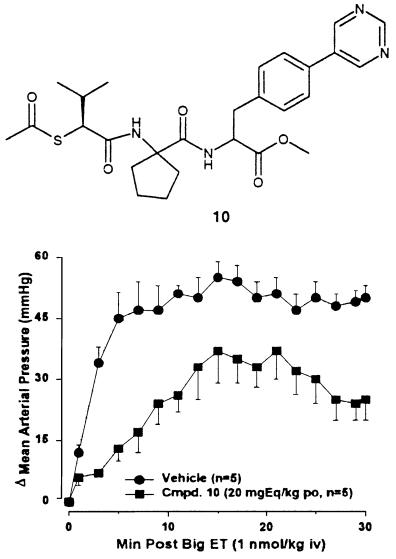


Figure 3. Inhibition of big ET-1 pressor response by compound **10**, prodrug of compound **6m** (po in conscious rats).

ET-1 pressor response by 40%. These results clearly indicate a superior in vivo profile for **6m** compared to CGS 30084, especially considering that **6m** contains 50% inactive D-arylalanine stereoisomer.

We next evaluated compound **10**, the thioacetate methyl ester prodrug of **6m**. Conscious rats were dosed with either vehicle or compound **10** and 2 h later challenged with big ET-1 at 1 nmol/kg iv. Figure 3 shows the change in mean arterial pressure produced by big ET-1 for 30 min following its administration. Upon po (20 mg Eq/kg) administration, compound **10** inhibited the big ET-1 pressor response[14] by 45% at 2 h post dosing.

In contrast, the corresponding prodrug of CGS 30084 was inactive under similar conditions after 30 min. In conclusion, through exploration of the biphenyl region of CGS 30084, we have discovered compound **10**, a potent, orally active inhibitor of ECE-1 activity in vivo.

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13. Due to cost issues, only DL-mixtures of the boronophenylalanine derivative **9** were prepared at first, and used for the synthesis of the target compounds. It was envisaged that all interesting compounds would be synthesized in optically pure form at a later date, if necessary.
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15. Similar results were observed with D-enantiomers of several other biphenylalanine derivatives (data not shown).
16. Compound **6m** was 10 times more soluble than CGS 30084 (0.02 vs 0.002 mg/mL) at pH 1.0. At pH 6.8 CGS 30084 was more soluble (ca. 0.2 vs 0.05 mg/mL) than **6m**.