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Synthesis and Biological Activity of Potent Heterocyclic Thiol-Based Inhibitors of Endothelin-Converting Enzyme-1

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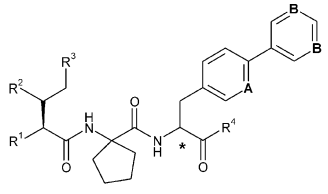
Abstract—Directed screening of metalloprotease inhibitors identified CGS 30084 (**1**) as a potent inhibitor of endothelin-converting enzyme-1 (ECE-1) in vitro ($IC_{50} = 77$ nM). Herein we report the syntheses and biological activities of analogues containing modified biphenyl moieties, bearing heterocyclic proximal rings. Compound **20**, the thioacetate ethyl ester prodrug derivative of compound **19a**, was found to be an orally active and potent inhibitor of ECE-1 activity in rats.

Potent inhibitors of endothelin-1 (ET-1) production are considered to be attractive potential therapeutic agents for the treatment of disorders such as cerebral vasospasm, stroke, asthma, and cardiac and renal failure,[1–4] which are linked with elevated ET-1 levels.[5] Endothelin-converting enzyme-1 (ECE-1) catalyzes the post-translational conversion of big ET-1 to ET-1,[6] thus presenting a logical target for the design of therapeutic agents that regulate the production of ET-1 in vivo.[7,8]

In a prior communication,[9] we reported the identification of CGS 30084 (**1**) (Fig. 1, $IC_{50} = 77$ nM) as a starting lead structure. Modifications carried out at *the thiol end* of CGS 30084 led to the discovery of compound **2**, which displayed higher potency ($IC_{50} = 11$ nM) as an ECE-1 inhibitor. More importantly, the corresponding prodrug (**3**) was found to be a long-acting orally available inhibitor of ECE-1 activity in vivo. We then turned our attention to modifications of *the biphenyl portion* of CGS 30084, initially focusing on variations at the *distal aromatic ring*.[10] These efforts resulted in the identification of compound **4** ($IC_{50} = 120$ nM for the DL mixture), and its prodrug **5**, which also possessed a superior in vivo profile compared to CGS 30084 and its prodrug.[10] The present report describes our continued efforts in further exploring the SAR of the biphenyl moiety, focusing on compounds containing *a heteroaryl proximal aromatic ring*.

We had previously noted that the introduction of 2-substituted distal aromatic rings, which lead to an increased twisting angle between the two aryl moieties, significantly reduced (ca. 5-fold)[10] the in vitro potency of ECE-1 inhibitors. We hoped that the introduction of a nitrogen atom in place of a proximal CH group in compounds such as **6a** would impart a higher degree of co-planarity between the two rings, thus improving potency. The in vitro activity of **6a** ($IC_{50} = 77 \pm 5$ nM for the DL mixture) provided some support for our hypothesis.



Compd	*	A	B	R^1	R^2	R^3	R^4
CGS 30084 (**1**)	L	CH	CH	SH	Me	H	OH
2	L	CH	CH	SH	H	Me	OH
3	L	CH	CH	SAc	H	Me	OMe
4	DL	CH	N	SH	Me	H	OH
5	DL	CH	N	SAc	Me	H	OMe
6a	DL	N	CH	SH	Me	H	OH

Figure 1. Various derivatives of CGS 30084.

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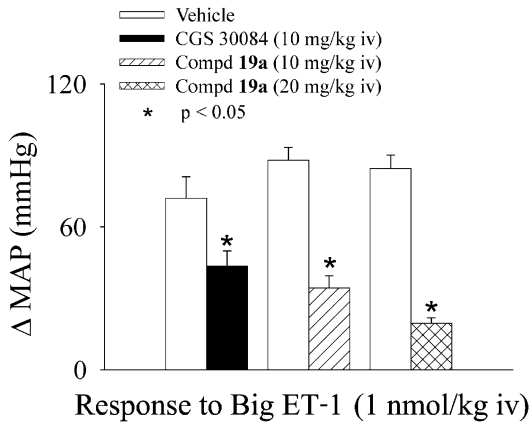
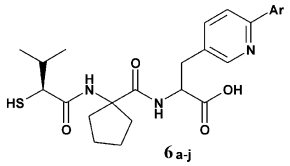


Figure 2. Comparison of the inhibition of the big ET-1 pressor response by compounds **19a** and CGS 30084 (iv in anesthetized rats), at 15 min after dosing.

Reasoning that the incorporation of heteroatoms in **4** was the key contributor to its more favorable pharmacokinetic profile,[10] we also hoped to discover ECE-1 inhibitors with improved in vivo activity when replacing the proximal phenyl ring with a pyridine moiety. Indeed, analogues of **6a** showed a significantly greater inhibition of the big ET-1 pressor response[11] upon iv administration in anesthetized rats when compared with CGS 30084 (Fig. 2).

The target molecules **6a–j** were prepared originally as diastereomeric mixtures, using the synthetic approach previously described,[9] starting from various aryl-substituted pyridyl alanines **9a–j** (Scheme 1). These starting amino acids were in turn synthesized via the alkylation of the protected glycine derivative **7** with bromide **8** (accessible through bromination of 2-bromo-5-methylpyridine), followed by Suzuki or Stille cross-coupling reactions with the appropriate arylboronic acids or aryl stannanes, respectively.

Table 1. Modifications at the distal ring of thiols bearing a proximal pyridine ring



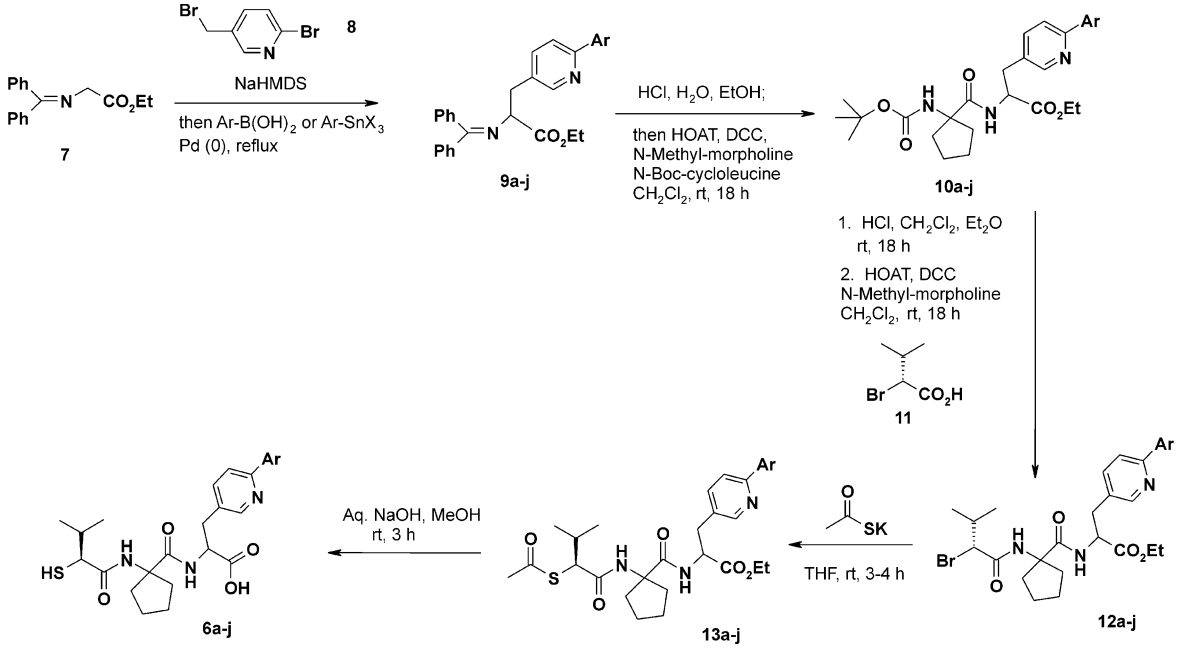
6a-j

Compd	Ar	ECE-1 IC_{50} (nM)[a]
6a	Ph	77±5
6b	2-Furyl	43±7
6c	3-Furyl	39±10
6d	2-Thienyl	49±13
6e	3-Thienyl	30±5
6f	Pyridin-3-yl	41±2
6g	2-Methoxy-Ph	700±150
6h	3-Amino-Ph	64±4
6i	3-Nitro-Ph	98±17
6j	3-Acetamido-Ph	130±18

[a]Values are mean±SEM.

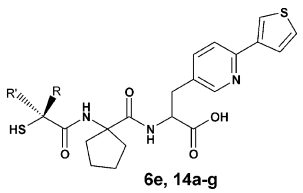
The compounds thus prepared were tested for their ability to inhibit ECE-1 activity in vitro. The experimental details for the assays utilized have been previously described.[11] The results are summarized in Table 1.

Table 1 shows the SAR for ECE-1 inhibition by thiol derivatives in which the biphenyl ring of CGS 30084 is replaced with a proximal pyridine ring, and various distal aromatic moieties. As expected, all unsubstituted distal aromatic rings (**6a–f**) led to improved potency,[12] while 2-substitution of the distal ring (**6g**) resulted in



Scheme 1. Preparation of ECE-1 inhibitors containing proximal pyridine rings.

Table 2. Modifications at the thiol end of compound **6e**



6e, 14a-g

Compd	R	R′	ECE-1 IC_{50} (nM)[a]
6e	*i*-Pr	H	30±5
14a	*i*-Bu	H	9.8±1.1
14b	*n*-Pr	H	27±2.3
14c	H	*n*-Pr	82±14
14d	*n*-Bu	H	24±2.3
14e	H	*n*-Bu	56±6.8
14f	$MeS(CH_2)_2$	H	55±13
14g	Cyclohexyl-CH_2	H	23±5.8

[a]Values are mean±SEM.

significant loss of ECE-1 inhibitory activity. Electronic effects on the distal ring do not appear to play a significant role in potency, although there is a slight preference noted for electron-donating groups (**6h**) over electron-withdrawing substituents (**6i–j**).

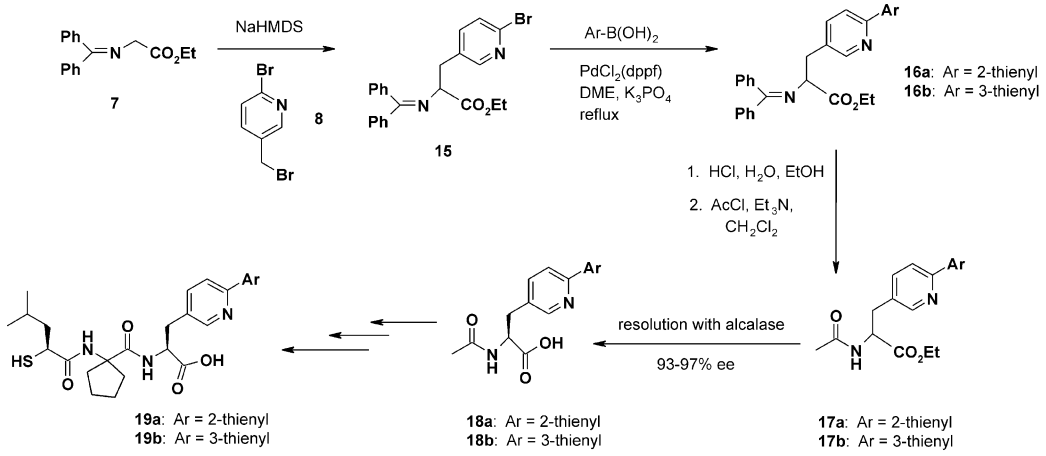
Encouraged by these results, we chose to further investigate derivatives of **6e**, and focused on the substituents at the carbon bearing the thiol group. The results of these investigations are summarized in Table 2. As previously noted,[9] there appears to be a slight preference for the (*S*)-stereochemistry at the chiral center bearing the thiol functionality (see **14b** and **14c**). A variety of alkyl chains, both linear and branched, are tolerated, with the isobutyl group providing the highest in vitro potency (**14a**). The presence of a heteroatom in the alkyl chain (**14f**) does not appear to have a detrimental effect on the in vitro ECE-1 inhibitory activity.

Finally, we chose to synthesize the chiral (L-pyridylalanine) derivatives of **14a** and its 2-thienyl analogue, in order to evaluate their biological activity in vivo. The chiral inhibitors **19a–b** were prepared as shown in Scheme 2. The protected glycine precursors **16a–b** were synthesized via alkylation of **7** followed by Suzuki cross-coupling with 2- or 3-thiopheneboronic acid. Deprotection of the imines under acidic conditions, followed by acetylation, led to the formation of the key derivatives **17a–b**, which were then resolved using alcalase, according to previously described procedures.[13] The resulting *N*-acetyl L-amino acids **18a–b** were deprotected, esterified, and then converted to the desired inhibitors **19a–b** as described in Scheme 1.

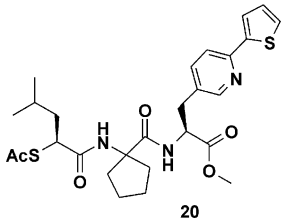
Compound **19a** ($IC_{50}=9.5\pm2.1$ nM) showed a superior in vivo profile (Fig. 2) when compared with **19b** (data not shown). In anesthetized rats, intravenous, bolus injection of **19a** as its HCl salt at 10 mg/kg inhibited the big ET-1 (1 nmol/kg, iv)-induced pressor response by 61% at 15 min after dosing (Fig. 2). Under similar conditions, CGS 30084 (administered iv at 10 mg/kg) was only able to inhibit the big ET-1 pressor response by 40%. The in vivo effect of **19a** is dose-dependent, as a dose of 20 mg/kg blocked the big ET-1-induced increase in mean arterial pressure (MAP) by 77% at 15 min after dosing (Fig. 2).

We next evaluated **20**, the thioacetate methyl ester prodrug of **19a**. Conscious rats were dosed with either vehicle or **20** and 2 h later challenged with big ET-1 at 0.3 nmol/kg iv. The ECE-1 inhibitory effect of **20** is shown in Figure 3 as the area under the curve (AUC) of the change in MAP produced during the first 30 min following the intravenous, bolus injection of big ET-1. Upon oral administration at doses between 3 and 30 mg/kg, **20** inhibited the big ET-1-induced pressor response by 65–90% at 2 h post-dosing. There were no significant differences in the degree of inhibition produced by the 3 doses of **20**.

Most importantly, **20** showed significantly long duration of action when inhibiting the effects of ECE-1 in vivo (Fig. 4). At 0.5, 2, 4, and 8 h after an oral dose of **20** at 3 mg/kg, the pressor effects of big ET-1 were blocked by 90, 82, 70, and 34%, respectively ($p<0.05$ at all time points). However, no significant effect was observed at 24 h after dosing.



Scheme 2. Synthesis of chiral heterocyclic ECE-1 inhibitors.



20

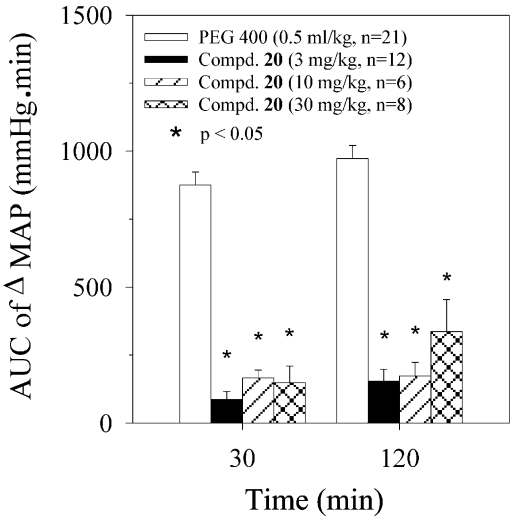


Figure 3. Inhibition of big ET-1 pressor response by **20**, prodrug of **19a** (po in conscious rats).

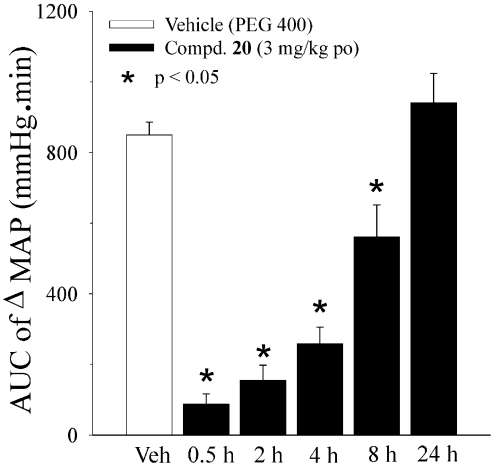


Figure 4. Duration of action for ECE-1 inhibition by **20**.

In conclusion, through variations at the proximal and distal rings of the biphenyl region of CGS 30084, we were able to identify **20**, a potent, long acting, and orally available inhibitor of ECE-1 activity in vivo.

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