Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2037–2039

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Potent Thiol-Based Inhibitors of Endothelin Converting Enzyme-1

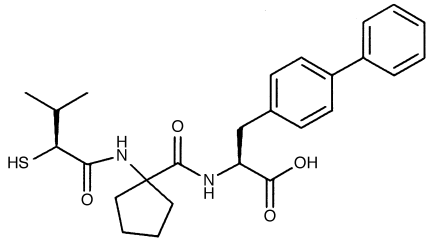
Cynthia A. Fink,* Michael Moskal, Fariborz Firooznia, Denton Hoyer, David Symonsbergen, Dongchu Wei, Ying Qiao, Paula Savage, Michael E. Beil, Angelo J. Trapani and Arco Y. Jeng

Metabolic and Cardiovascular Diseases, Novartis Institute for Biomedical Research, Summit, NJ 07901, USA

Received 25 April 2000; accepted 7 July 2000

Abstract—Through directed screening of compounds prepared as metalloprotease inhibitors a compound, **CGS 30084**, that had potent endothelin converting enzyme-1 (ECE-1) in vitro inhibitory activity ($IC_{50} = 77$ nM) was identified. Herein we report the synthesis and optimization of ECE-1 inhibitory activity of additional analogues from this lead. Compound **3c**, the thioacetate methyl ester derivative of compound **4c**, was found to be a long acting inhibitor of ECE-1 activity in rats after oral administration.

Endothelin-1 (ET-1), a 21-amino acid peptide originally isolated from porcine aortic endothelial cells, is one of the most potent vasoconstrictors known.[1] Abnormally high plasma and tissue levels of ET-1 have been detected in a number of disease states, including hypertension, cerebral vasospasm, asthma, congestive heart failure, and chronic and acute renal failure.[2–5] Modulation of the biological effects of this peptide has been the target of much recent effort in the pharmaceutical community.[6] Two approaches have been investigated to block the biological actions of ET-1: antagonism of the ET-1 G-protein coupled receptors ET_A and ET_B and inhibition of endothelin converting enzyme-1 (ECE-1), a zinc-containing metalloprotease distributed in the vascular endothelium and in brain tissue. ECE-1 is responsible for catalyzing the post-translational conversion of big ET-1, a 38-amino acid precursor to ET-1.[7,8] We chose the latter approach in our efforts to find means to inhibit the detrimental effects of elevated ET-1. A directed screening of historical compounds originally prepared as zinc-metalloprotease inhibitors led to the identification of **CGS 30084**. We then initiated a chemistry program using this compound as our starting point and strived to further improve its ECE-1 inhibitory activity in vitro. In this paper we will describe modifications carried out at the thiol end of the molecule, and the biological activity of the resulting compounds. Modifications made to other portions of **CGS 30084** will be the subject of future publications.

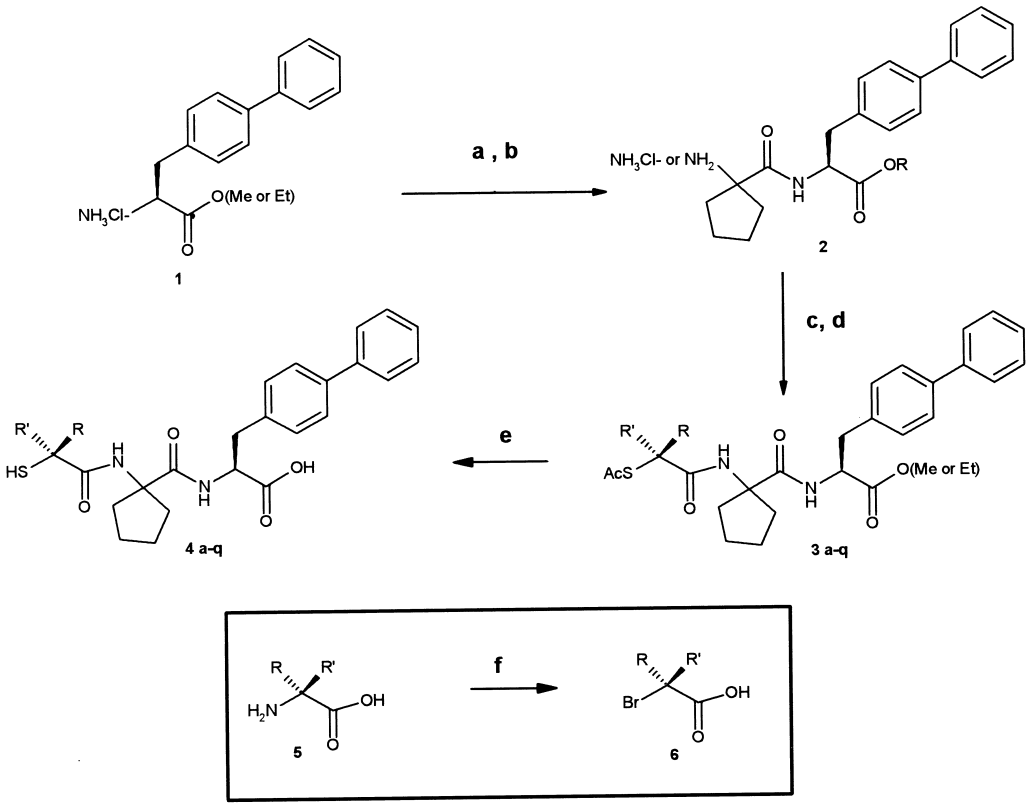


CGS 30084
IC_{50}(ECE) = 77 nM

The target molecules were prepared as outlined in Scheme 1. Treatment of 4-phenyl-phenyl alanine ester with *N*-BOC or *N*-Cbz-protected cycloleucine, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), and 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) followed by removal of the *N*-BOC or *N*-Cbz groups afforded the amine hydrochlorides or free bases **2**, respectively. Subsequent treatment of these amines (**2**) with a variety of α-bromo acids (**6**), EDCI, and HOAt provided the α-bromo dipeptides.[9] Treatment then with the potassium salt of thio acetic acid afforded the thioacetates **3**. The esters were hydrolyzed by treatment with 1 N sodium hydroxide or lithium hydroxide in degassed methanol to give the free thiol acids **4**. The required α-bromoacids could be prepared in good yield from the appropriate

*Corresponding author. Tel.: +1-908-277-7532; fax: +1-908-277-2405; e-mail: cynthia.fink@pharma.novartis.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00403-0



Scheme 1. (a) EDCI, HOBt or HOAt, Et_3N, CH_2Cl_2, CbzNH-cycloleucine or BOCNH-cycloleucine, rt (>95%); (b) HCl (gas) or 10% Pd/C (95–99%); (c) EDCI, HOAt, Et_3N, **6**, CH_2Cl_2 (41–93%); (d) AcSK (20–90%); (e) 1 N LiOH or NaOH, methanol (65–98%); (f) 48% HBr, KBr, $NaNO_2$ (56–92%).

amino acids via diazotization with sodium nitrite and treatment with hydrobromic acid and potassium bromide. The free thiol dipeptides prepared are shown in Table 1. These compounds were tested for their ability to inhibit human ECE-1 activity in vitro. The experimental details for the assay have been reported previously.[10] As can be seen from Table 1, the isopropyl group of **CGS 30084** can be replaced with other *n*-alkyl and branched alkyl groups leading to compounds with similar or improved ECE-1 inhibitory activity (e.g., compounds **4c** and **4g**). The simple methylene compound (**4a**) had very little ECE-1 inhibitory activity. It appears that the (*S*)-stereochemistry at the center bearing the thiol group is preferred for ECE-1 inhibitory activity in vitro (compare, for example, compounds **4c** and **4d**). The introduction of a heteroatom in the alkyl chain, as can be seen in comparing compounds **4c** and **4m**, does not diminish ECE-1 inhibition. The free hydroxyl analogue of **4m**, compound **4l**, was however less potent in vitro. The only aryl containing analogue synthesized, compound **4p**, was less active than many of the alkyl and branched alkyl analogues prepared. We selected one of the most potent derivatives, compound

Table 1. In vitro activity for the α-thiol derivatives

Compound	R	R′	ECE IC_{50}, (nM)[a]
CGS 30084	-$CH(Me)_2$	H	77[b]
4a	H	H	26%
4b	-Me	H	62%
4c	-Propyl	H	11 (0)
4d	H	-Propyl	240 (30)
4e	-Butyl	H	54 (8)
4f	H	-Butyl	170 (40)
4g	-$CH_2CH(Me)_2$	H	19 (3)
4h	H	-$CH_2CH(Me)_2$	210 (20)
4i	-CH(Me)(Et), (*R*)	H	97 (20)
4j	-CH(Me)(Et), (*S*)	H	120 (15)
4k	H	-CH(Me)(Et), (*R*)	410 (80)
4l	-CH_2OH	H	200 (20)
4m	-CH_2OMe	H	11 (0.9)
4n	-CH(OMe)Me, (*S*)	H	140 (20)
4o	-$(CH_2)_2SMe$	H	40 (5)
4p	-CH_2Ph	H	280 (85)
4q	-Me	-Me	510 (100)

[a]Standard deviation is given in parentheses (% = % inhibition of activity at 1 μM).
[b]Only $n = 1$ for this IC_{50} measurement.

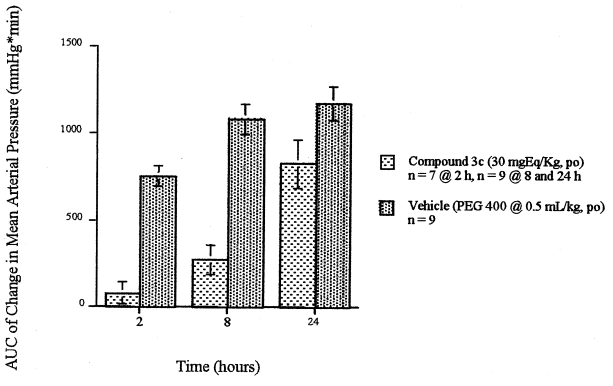
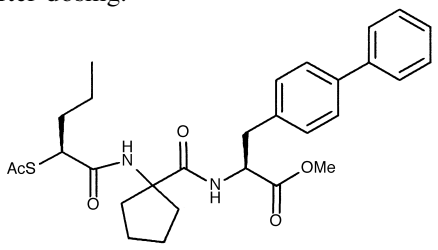


Figure 1. Inhibition of big ET-1 pressor response by compound **3c** in conscious rats. 30 mgEq/kg dose of **3c** is the molar equivalent of a 30 mg/kg dose of compound **4c**.

4c, for additional in vivo studies. The methyl ester, thioacetyl prodrug of this compound, analogue **3c**, was evaluated in vivo in rats for its ability to inhibit the pressor response produced by big ET-1 administration.[11] The results are expressed in Fig. 1 as the area under the curve for the change in mean arterial pressure produced by big ET-1 (0.3 nmol/kg, iv) injected at 2, 8, and 24 h. As can be seen in Fig. 1, compound **3c** is a long-acting orally active inhibitor of ECE-1 activity in vivo. It inhibits big ET-1 pressor response by 75% at 8 h and 30% at 24 h after dosing.



3c

The further evaluation of these inhibitors in vivo, as well as the synthesis and activity of other thiol containing ECE-1 inhibitors will be presented in future disclosures.

Acknowledgements

The authors wish to thank Dr. Stephane De Lombaert for his helpful discussions and constant encouragement throughout this work and the Novartis Analytical Chemistry Staff (Summit) for providing the physicochemical data on the compounds presented.

References and Notes

1. Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Yazaki, Y.; Goto, K.; Masaki, T. *Nature* **1988**, *332*, 411.
2. Cheng, X. M.; Nikam, S. S.; Doherty, A. M. *Curr. Med. Chem.* **1994**, *1*, 271.
3. Goto, K.; Hama, H.; Kasuya, Y. *Jpn. J. Pharmacol.* **1996**, *72*, 261.
4. Miyauchi, T.; Masaki, T. *Annu. Rev. Physiol.* **1999**, *61*, 391.
5. Battistini, B.; Dussault, P. *Pulm. Pharmacol. Ther.* **1998**, *11*, 79.
6. Jeng, A. Y.; De Lombaert, S. *Curr. Pharm. Des.* **1997**, *3*, 541 and references cited therein.
7. Telemaque, S.; Emoto, N.; Dewit, D.; Yanagisawa, M. *J. Cardiovasc. Pharmacol.* **1998**, *31*, S548.
8. Turner, A. J.; Murphy, L. *J. Biochem. Pharmacol.* **1996**, *51*, 91.
9. Triethylamine was omitted as the base when the free base amines (**2**) were used in the coupling reaction to provide **3**.
10. Wallace, E. M.; Moliterni, J. A.; Moskal, M. A.; Neubert, A. D.; Marcopoulos, N.; Stamford, L. B.; Trapani, A. J.; Savage, P.; Chou, M.; Jeng, A. Y. *J. Med. Chem.* **1998**, *41*, 1513.
11. From previous experience in profiling thiol-containing metalloprotease inhibitors, the ester thioacetate prodrugs in general gave better in vivo results as compared to the carboxylic acid thiols; see Fink, C. A.; Carlson, J. E.; McTaggart, P. A.; Qiao, Y.; Webb, R.; Chatelain, R.; Jeng, A. Y.; Trapani, A. J. *J. Med. Chem.* **1996**, *39*, 3158.