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Targeting ACE and ECE with dual acting inhibitors

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Abstract—A series of urea analogues related to SA6817 and a GSK phosphonic acid with reported ACE inhibitory activity were prepared and tested for dual ACE and ECE activities. Although excellent ACE and NEP inhibition was achieved, only modest ECE inhibition was observed with one analogue.

Cardiovascular disease manifests itself in a number of clinically relevant ways, affecting a large segment of the adult population regardless of geographic location.[1] The market for a variety of drugs prescribed for two of the more prevalent indications, namely hypertension[2] and hypercholesteremia,[3] accounts for several billion dollars annually. In spite of the availability of highly effective drugs to lower blood pressure and control levels of cholesterol in humans, the quest for newer entities with improved therapeutic profiles and overall better tolerance is an on-going objective.[4] Inhibition of angiotensin converting enzyme (ACE) has been a highly successful program in the pharmaceutical industry resulting in a number of effective antihypertensive drugs.[5] Elegant studies relying on structure-based design have laid a strong foundation for early pioneering efforts in the development of Captopril[6] and several of its variants.[7] Another enzyme that has been identified in relation to hypertension is endothelin converting enzyme (ECE),[8,14a] which cleaves big-endothelin into endothelin, a highly vasoconstricting peptide. The absence of X-ray crystallographic information on ECE has hampered progress in the development of inhibitors with clinical potential, although the literature is abound with synthetic compounds showing promising inhibitory activity.[9]

A third enzyme, neutral endopeptidase (NEP),[10] is also a potential target in the field of hypertension. However the clinical development of dual ACE–NEP inhibitors as well as triple ACE–ECE–NEP inhibitors has been the subject of many failures, mainly due to unwanted NEP-inhibition related side-effects such as angiodema. Thus, the overall benefits of developing NEP inhibitors are not as evident as with ACE, ECE, or renin[11] which is well known for its hypertensive activity in humans.

The development of a dual acting inhibitor toward ACE and ECE and selective versus NEP presents a veritable challenge. Toward this end we selected two known inhibitors exemplified by SA6817[12] **1** and **2** (GSK)[9c,13] (Fig. 1) which are structurally unrelated except for the presence of Zn binding groups, a hallmark of small molecule inhibitors of metalloproteases.[14] They formed the basis of our choice toward the synthesis of hybrid structures with potential dual inhibitory activity against ACE and ECE. Herein we report the results of our efforts toward this goal.

Our main focus was to prepare a series of substituted urea congeners of SA6817 **1** in which we systematically varied the hydrophobic substituent R_1, and probed the nature of the amino acid residue R_2 (Fig. 1).

The readily available L-isoserine **4** was transformed in three steps into the *N*,*O*-protected ester 5 (Scheme 1). Removal of the *N*-Cbz group by hydrogenolysis afforded the corresponding amine which was subjected to

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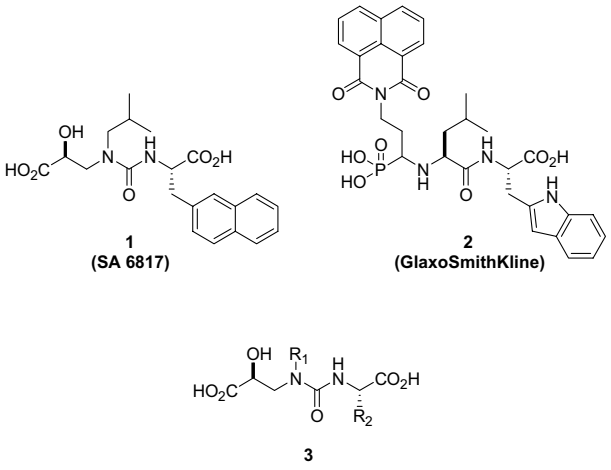
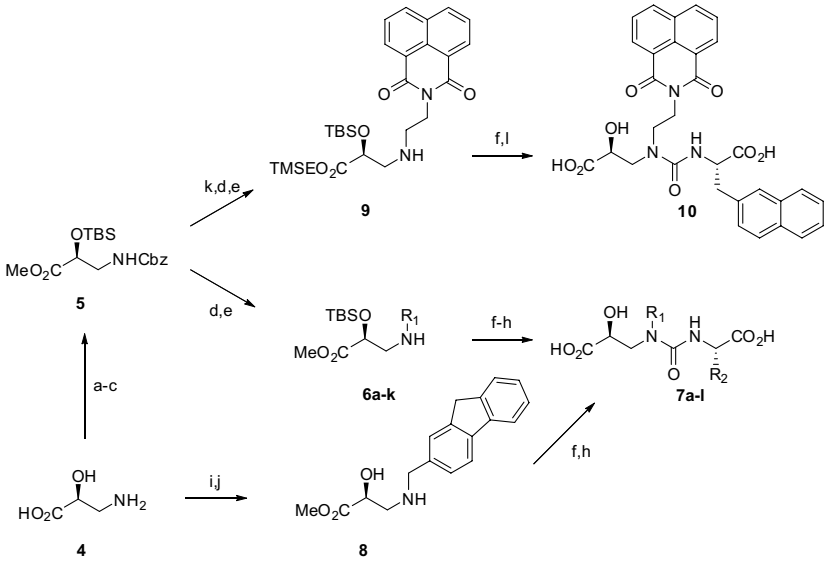


Figure 1.

reductive amination with a variety of aldehydes to afford intermediates **6a**–**k**. The formation of dialkylated products was minimized by addition of Et_3N. Conversion of the secondary amines to the intended ureas was accomplished in the presence of carbonyldiimidazole and an amino acid ester. Removal of the TBS protective group and hydrolysis with LiOH gave a series of analogues **7a**–**k**. Alternatively, L-isoserine was converted to the *N*-alkyl analogue **8**, then coupled to the amino acid as the methyl ester, followed by hydrolysis to afford the analogue **7l**. Final products were purified by preparative HPLC and isolated as the free dicarboxylic acids. To access a 'hybrid' analogue in which the original *iso*butyl group was replaced by *N*-alkyl naphthylimide residues, we adopted an alternative sequence. The ester group of choice was trimethylsilylethyl rather than methyl. Thus, **5** was transesterified, the product converted to the secondary amine, and the latter was treated with appropriate naphthylimide *N*-alkanals under reductive amination conditions to give the adduct exemplified by the *N*-ethyl analogue **9**. Activation of the amine as the isocyanate and coupling with L-naphthylalanine followed by deprotection gave **10** as a prototype of this class.

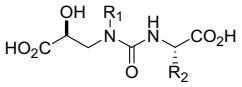
The phosphonic acid hybrid analogues of SA 6817 were prepared in a standard fashion from aminoalkyl phosphonic acid esters **11** and **12** (Scheme 2). Installation of the *N-iso*butyl group by reductive amination led to **13** and **14**, which were converted to the phosphonic acid analogues **15** and **16**.

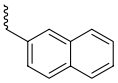
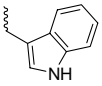
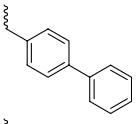
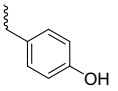
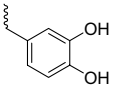
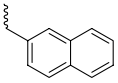
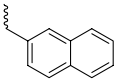
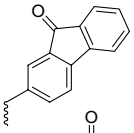
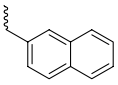
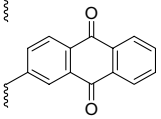
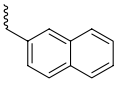
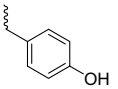
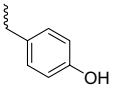
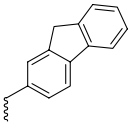
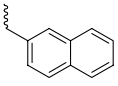
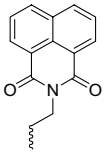
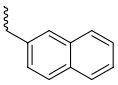
Preliminary enzyme inhibitory studies identified a number of individual hits toward one or more of the three target enzymes.[15] Of these, we selected those that exhibited measurable IC_{50} nM inhibition against at least ACE, ECE, or NEP and combinations thereof. For purposes of comparison, we list the compounds grouped according to the R_1 and R_2 variations in Table 1. The known SA 6817 **1** showed potent ACE and NEP inhibitory activities at IC_{50} 52 and 4 nM, respectively, while it was barely active as an ECE inhibitor with an IC_{50} of 2.5 μM (Table 1, entry 1). Variation of the R_2 group as in analogues **7a**–**e** maintained NEP activity at the expense of ACE with the exception of **7c** and **7e**, but the minimal activity against ECE was lost. Next we varied the R_1, *N*-alkyl substituent in the naphthylalanine series (Table 1, entries 8–11 and 14–15, compounds **7f**–**i**, **7l**, and **10**). Increasing the bulk of R_1 led to good ACE inhibition while eradicating NEP as exemplified by compounds **7h**–**i** and **7l**. Surprisingly the naphthylimide analogue **10** restored some ECE activity at the expense of ACE (Table 1, entry 15).



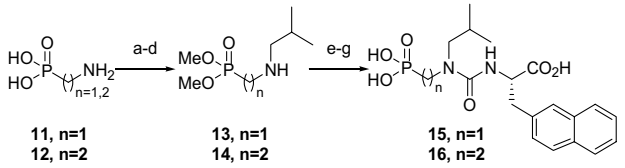
Scheme 1. Typical synthesis of inhibitors. Reagents and conditions: (a) 1 M NaOH, CbzCl, 0 °C to rt, 5 h; (b) $TMSCHN_2$, MeOH, 0 °C; (c) TBSCl, ImH, DMAP, DCM, rt, 70% (3 steps); (d) H_2, Pd/C, MeOH, rt, 2 h, quantitative; (e) $MgSO_4$, DCM, aldehyde, Et_3N, $NaHB(OAc)_3$, 5 h, rt, 50–90%; (f) amino acid, CDI, ImH, THF, rt then reflux; (g) TBAF, AcOH (1:1), THF, rt, 0.5 h; (h) LiOH 1 M, MeOH, 0 °C to rt; (i) fluorene-2-carboxaldehyde, KOH, H_2O/EtOH (1:1, v/v), $NaBH_4$; (j) $SOCl_2$, MeOH; (k) TMSEtOH, $Ti(Oi\text{-}Pr)_4$, 80 °C, 75%; (l) TFA/H_2O (9:1, v/v), 0 °C to rt, 3 h.

Table 1. IC_{50} values for compounds **1**, **7a**–**l**, and **10**



Entry	Compounds	R_1	R_2	IC_{50} (nM)		
				ACE	ECE	NEP
1	**1**			52	2500	4
2	**7a**			103	16,500	8
4	**7b**			110	—[a]	9
5	**7c**			35	3490	10
6	**7d**			60	—	6
7	**7e**			23	—	19
8	**7f**			191	—	5
9	**7g**			—	—	36
10	**7h**			39	—	—
11	**7i**			40	—	—
12	**7j**			—	—	90
13	**7k**			—	—	1540
14	**7l**			150	—	19,000
15	**10**			1980	600	650

[a] IC_{50} were not calculated, but enzymatic inhibitions at 0.1 and 10 μM were obtained (see Supporting Information).



Scheme 2. Synthesis of phosphonic acid analogues. Reagents and conditions: (a) NaOH 2 N, $NaHCO_3$, Na_2CO_3, CbzCl, 0 °C to rt; (b) $TMSCHN_2$, MeOH, 0 °C to rt, 1 h; (c) H_2, Pd/C, MeOH, rt, 2 h, quantitative; (d) $MgSO_4$, *iso*-butyraldehyde, $NaHB(OAc)_3$, DCM, 1 h; (e) naphthylalanine methyl ester, CDI, ImH, THF, rt then reflux; (f) $LiOH{\cdot}H_2O$, $THF:H_2O$:MeOH, 0 °C, 2 h, quantitative; (g) TMSI, DCM, 0 °C to rt, 2 h then CH_3CN, H_2O, 4 °C, 16 h.

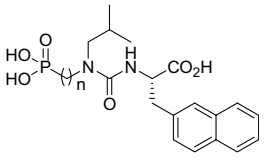
Lengthening the alkyl chain by one and two methylene groups resulted in loss of activity against the three enzymes.[15] Variation of R_1 and R_2 as in **7j** and **7k** (Table 1, entries 12 and 13) resulted in loss of ACE and ECE activity, but maintained NEP. The 15-fold improvement in going from an *N*-cyclohexyl to *N*-cyclopentyl is inexplicably odd.

Compound **15**, the phosphonate deshydroxy analogue of SA 6817 **1**, was a potent inhibitor of ACE and NEP. Although the homologue **16** maintained an excellent NEP activity, the ACE activity was lost. Neither analogue was active against ECE (Table 2). A total of 43 analogues with R_1 and R_2 variations were also prepared and tested against the three enzymes at concentrations of 0.1 and 10 μM. Disappointingly none exhibited activities at these concentrations.[15] In spite of the diversity in the nature of R_1 and R_2, it was not possible to find any logical SAR within each series.

Seemingly small variations resulted in extensive erosion of inhibitory activity. The aromatic tricyclic R_1 series eliminated NEP activity which was encouraging with regard to ACE/NEP selectivity. However, ECE activity was also lost. The most promising analogue **10** with IC_{50} 600 nM against ECE could not be further optimized, since lengthening the alkyl tether of the naphthylimide unit was detrimental to all activities.[15]

In conclusion, we have prepared a series of analogues related to SA 6817 **1** as well as hybrid analogues in which the R_1 and R_2 groups were derived from SA 6817 **1** and the GSK inhibitor **2**. Potent inhibitors of ACE and NEP were found among these analogues, but our desire to achieve dual acting inhibitors against ACE and ECE in particular was not realized.

Table 2. IC_{50} values for compounds **15** and **16**



Entry	Compounds	*n*	IC_{50} (nM)		
			ACE	ECE	NEP
1	**15**	1	29	—	2
2	**16**	2	—	—	6

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.013.

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15. See Supporting Information.