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Synthesis and evaluation of heteroarylalanine diacids as potent and selective neutral endopeptidase inhibitors

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ABSTRACT

Heteroarylalanine derivatives **4** were designed as potential inhibitors of neutral endopeptidase (NEP EC 3.4.24.11). Selectivity over other zinc metalloproteinases was explored through occupation of the S2′ subsite within NEP. Structural optimisation led to the identification of 5-phenyl oxazole **4f**, a potent and selective NEP inhibitor. A crystal structure of the inhibitor bound complex is reported.

Neutral endopeptidase (NEP, EC 3.4.24.11) is a zinc metalloproteinase involved in the degradation of atrial natriuretic peptide (ANP), a 28 amino acid vasoactive peptide that exhibits diuretic, natriuretic and vasodilatory activities.[1] Potentiation of ANP through NEP inhibition may be of clinical benefit for the treatment of hypertension and congestive heart failure. Candoxatril (previously disclosed by these laboratories) is the indanyl ester prodrug of the selective NEP inhibitor Candoxatrilat **1**. Candoxatril is limited by its relatively high dose and short half-life in man.[2] The identification of a potent and selective inhibitor of NEP (over Angiotensin Converting Enzyme (ACE) and other related zinc metalloproteinases) with improved pharmacokinetics was sought.

Dual inhibition of ACE and NEP as a strategy for treating hypertension has been extensively investigated, including contributions from these laboratories through the discovery of Sampatrilat **2**.[3] Given that both targets are related zinc metalloproteinases, dual enzyme inhibition can be achieved within a single pharmacophore possessing a modified peptide backbone linked through to a zinc ion chelator (carboxylic acid) (Table 1). Selectivity for either ACE or NEP is highly influenced by the nature of the additional functionality present in the S2′ subsite. Selective NEP inhibitors do not always require an α-amino acid at the C-terminus (e.g., Candoxatrilat **1**, Fig. 1), however both ACE and NEP can tolerate biarylalanine substituents as disclosed by Zambon in their dual ACE/NEP inhibitor **3** (Fig. 1).[4] Exploration of the S2′ subsite of NEP within the Candoxatrilat template may enable identification of a selective NEP inhibitor with enhanced properties. This letter explores the lipophilic binding pocket at S2′ with conformationally restricted heteroarylalanines in place of the tyrosine in Sampatrilat and the biphenyl group of compound **3**. The preferred natural amino acid stereochemistry was employed, enabling the design and synthesis of all analogues to be derived from L-aspartic acid.

Oxazoles **7** were synthesised by peptide coupling of the appropriately substituted aminoethanol with N-BocAsp-OBn **5**, to give the corresponding amides **6**. Oxidation of the alcohol with Dess–Martin periodinane and cyclisation using either iodine and triphenylphosphine[5] or dibromotetrachloroethane and triphenylphosphine,[6] followed by final N-Boc deprotection with trifluoroacetic acid (TFA) furnished the desired amino esters **7a–c** expressing the heteroaryl alanine (Scheme 1).

1,2,4-oxadiazole **9** was obtained by acylating N-hydroxybenzamidine with **5** to afford acylamidoxime **8**, followed by cyclisation as a melt. TFA deprotection furnished the desired amino ester intermediate as a second class of heterocyclic variants (Scheme 1).

Isomeric oxazoles **11a–c** were synthesised via N-CbzAspOEt **10**, which was obtained according to literature precedent from Cbz-aspartic acid anhydride.[7] Amide bond formation with the appropriate aminoketone[8] followed by $POCl_3$ cyclisation produced the desired heterocycles. N-Cbz deprotection with HBr/AcOH furnished the amino esters (Scheme 2).

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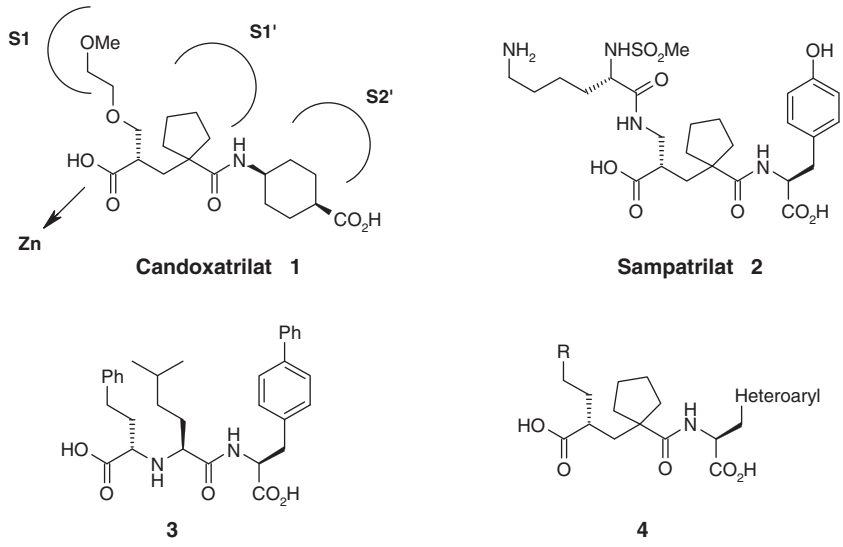
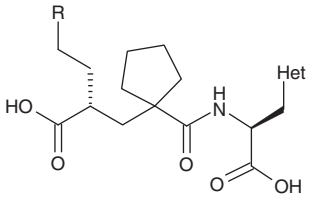
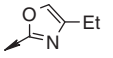
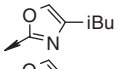
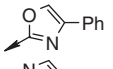
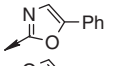
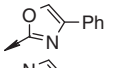
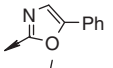
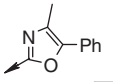
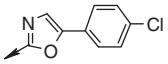
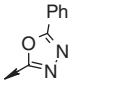
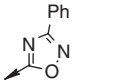


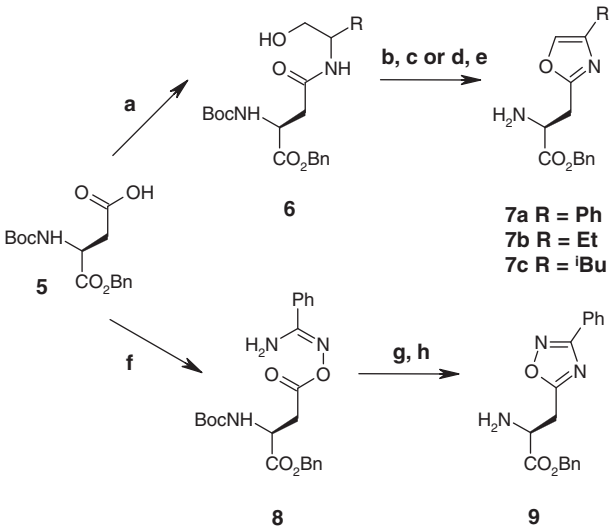
Figure 1.

Table 1
NEP and ACE activity of **1**–**4**



Compound	R	Het	NEP IC_{50} (nM)	ACE IC_{50} (nM)
1	—	—	7.8	Inactive
2	—	—	0.6[a]	3.4
3	—	—	28[b]	1.5[b]
4a	CH_3		22	130
4b	CH_3		20	75
4c	CH_3		7	25
4d	CH_3		0.6[a]	82
4e	CH_3O		4.8	130
4f	CH_3O		0.3[a]	270
4g	CH_3O		5	210
4h	CH_3O		0.3[a]	195
4i	CH_3O		6	>3 μM
4j	CH_3O		1.9	58

[a] Where IC_{50} was <1.5 nM, lower enzyme concentrations were used to determine the true IC_{50}, below the tight binding limit of the standard assay.
[b] Literature values.

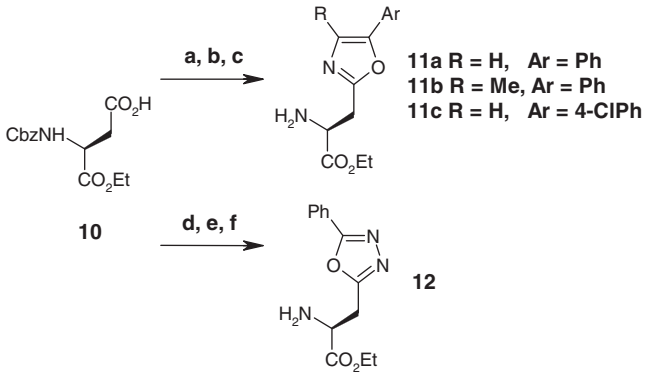


Scheme 1. Reagents and conditions: (a) $H_2NCH(R)CH_2OH$, WSCDI, HOBt, NMM, DCM, 54–85%; (b) Dess–Martin periodinane, DCM, 66–73%; (c) I_2, PPh_3, NEt_3, THF, 25%; (d) $Br_2C_2Cl_4$, PPh_3, DBU, 2,6-di-tbutylpyridine, 30–69%; (e) TFA, DCM, 95–100%; (f) N-hydroxybenzamidine, WSCDI, HOBt, NMM, DCM, 30%; (g) Melt, 114 °C, 88%; (h) TFA/DCM, quant.

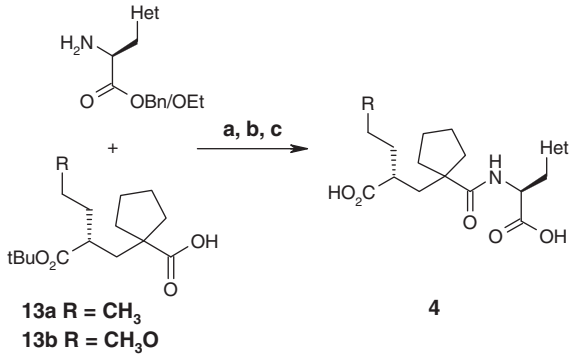
A similar protocol was employed to obtain the isomeric 1,3,4-oxadiazole **12**. Benzoyl hydrazine was reacted with the acid chloride of **10** in good yield, followed by cyclisation in the presence of chlorodimethylimidazolinium tetrafluoroborate.[9] N-Cbz deprotection under palladium catalysed hydrogenolysis furnished the desired amino ester (Scheme 2).

Amide bond formation of the synthesised amino esters with carboxylic acids **13a** and **b**[10] followed by a two step deprotection strategy (TFA removal of the ^{t}Bu ester followed by base hydrolysis of the ethyl ester) completed the synthesis of the novel heterocyclic diacids **4** (Scheme 3). These compounds were tested for their ability to inhibit both ACE and NEP activity in vitro. The experimental details for the pharmacological assays have been previously described.[11] The results are summarised in Table 1.

Table 1 shows the structure–activity relationship for the conformationally restricted heteroarylalanine analogues **4**. Within the 4-substituted oxazoles **4a–c**, we found that increasing lipophil-



Scheme 2. Reagents and conditions: (a) $SOCl_2$, NEt_3, DMF, $H_2NCH(R)C{=}OAr$, 50–77%; (b) $POCl_3$, 100 °C, toluene, 50–73%; (c) HBr AcOH 65–100%; (d) (i) $(COCl)_2$, DMF, DCM, (ii) Py, DCM, benzoyl hydrazine, 62%; (e) (i) 2-chloro-1,3dimethyl imidazolinium tetrafluoroborate, NEt_3, DCM; (ii) Toluene, 80 °C, 33%; (f) 10% Pd/C, EtOH, 50%.



Scheme 3. Reagents and conditions: (a) WSCDI, HOBt, NMM, DCM, 50–92%; (b) TFA, DCM, 85–98%; (c) NaOH, dioxan, H_2O, 53–96%.

icity through to the 4-phenyloxazole **4c** increased NEP inhibition; however selectivity over ACE was modest. Co-crystallisation of **4c** with human NEP[12] shows the binding mode of the P2′ phenyl oxazole in the deep, lipophilic S2′ pocket (Fig. 2). Key interactions include the left hand acid (as drawn) zinc coordination, and hydrogen bonding between the inhibitor amide group and Arg 717 and Asn 542. The natural amino acid stereochemistry enables the right-hand side acid to interact with key Arg 110 and 102 residues in the active site, orientating the phenyl oxazole deep into the S2′ cavity. The subsite is fully occupied and replacement of the pendant phenyl in P2′ for a less lipophilic alkyl substituent such as ethyl and isobutyl (**4a**, **4b**) only served to lower NEP activity.

The isomeric 5-phenyloxazole **4d** not only exhibited increased potency against NEP, but also a decreased ACE inhibition thus providing encouraging selectivity. Changing the P1 group from an *n*-propyl to a more polar methoxyethyl at the left hand acid (as seen in compound pairs **4c**–**4e** and **4d**–**4f**) decreased ACE inhibition further such that **4f** showed around 1000-fold selectivity over ACE. Introduction of a second substituent onto the oxazole, such as with 4-methyl-5-phenyl-oxazole **4g**, gave a slight reduction in activity, whilst simple *para*- chlorination **4h** of the phenyl group gave similar levels of activity to the parent **4f**. The two isomeric oxadiazoles **4i** and **4j** offered no pharmacology advantage over the oxazole systems.

Compound **4f** is 25-fold more potent against NEP than Candoxatrilat **1**, and significantly more selective over ACE than **2** or **3**. Interactions within the S2′ lipophilic pocket are well optimised

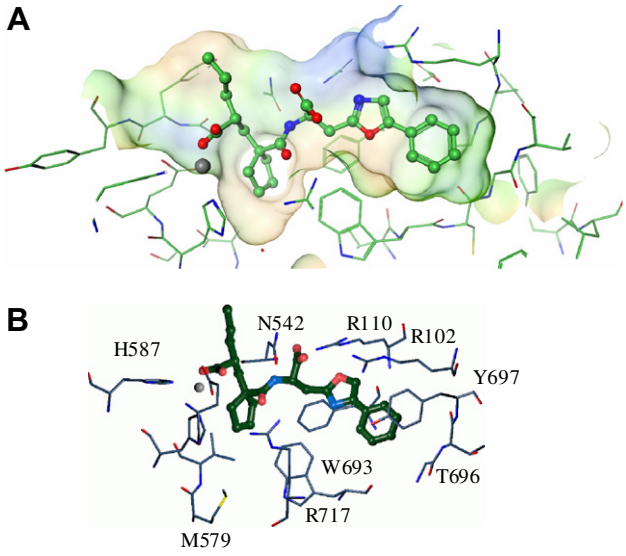


Figure 2. (A) Crystal. Structure of **4c** complex with human NEP. A surface representation illustrating the 5-phenyl oxazole packing within the S2′ pocket. The surface is coloured according to hydrophobicity with brown indicating the most hydrophobic areas of the binding site. Zn is represented as a grey sphere. (B) The binding site with key residues highlighted.

for NEP. The i.v. pharmacokinetics of compound **4f** compare favourably with those of candoxatrilat.[2] Compound **4f** has an i.v. clearance of 4.8 ml/min/kg and a half-life of 2.1 h in rat. Oral bioavailablity for **4f** was found to be low (1.7%) in a seperate rat study.

In conclusion, this letter demonstrates that selective NEP inhibition can be achieved with heteroarylalanine derivatives **4**. Natural amino acid stereochemistry for the novel heterocyclic substituents at P2′ in combination with a methoxyethyl S1 substituent enables phenyl oxazoles to exhibit potent and selective NEP inhibition. Compound **4f** has a useful pharmacokinetic half-life in rat.

Acknowledgments

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