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N-Formyl Hydroxylamine Containing Dipeptides: Generation of a New Class of Vasopeptidase Inhibitors

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Abstract—Four primary zinc-binding pharmacophores (thiols, carboxylates, phosphorus acids, and hydroxamates) have been utilized in generating inhibitors of zinc metalloproteases such as ACE, NEP, the MMPs, and ECE. Although compounds which inhibit the activity of both ACE and NEP (vasopeptidase inhibitors, VPIs) have been reported which incorporate a thiol, carboxylate, or phosphorus acid pharmacophore, the generation of hydroxamate based vasopeptidase inhibitors has remained elusive. Herein we report the first potent vasopeptidase inhibitors which were generated from the incorporation of conformationally restricted dipeptide mimetics to an *N*-formyl hydroxylamine zinc-binding group. Compounds such as **13c** and **13d** are among the most potent in this series, exhibiting in vitro activity comparable to other classes of inhibitors.

The development of vasopeptidase inhibitors (VPIs: dual inhibitors of angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP)) is an active field of study in cardiovascular research, and several excellent reviews have been published describing this topic in detail.[1–5] Their dual mechanism of action, attenuation of angiotensin II and potentiation of atrial natriuretic peptide and bradykinin, affords the potential to effect greater reductions in blood pressure in a wider patient population as compared to other established antihypertensives. Several vasopeptidase inhibitors are currently in clinical trials for the treatment of hypertension and congestive heart failure and initial reports have demonstrated efficacy in man.[6–9]

Both ACE and NEP are zinc metalloproteases and a historical survey of the field has demonstrated that four primary zinc-binding pharmacophores (thiols, carboxylates, phosphorus acids, and hydroxamates) have been incorporated into the design of potent and selective inhibitors of each enzyme. Additionally, a variety of vasopeptidase inhibitors have been described which contain a thiol, carboxylate, or phosphorus acid zinc-binding pharmacophore. Representative examples include omapatrilat,[10,11] sampatrilat,[12,13] and compound **1**[14] respectively (Fig. 1). Although potent and selective inhibitors of zinc metalloproteases have been described which contain a hydroxamate moiety, the utilization of this pharmacophore in the design of vasopeptidase inhibitors has not yet been reported. In an attempt to find agents within this class, we were intrigued by a report from Roques et al.[15] which depicted the use of *N*-formyl hydroxylamine amino acids (i.e. **2**) as NEP inhibitors (Fig. 2). Although somewhat less potent than the related hydroxamic acids, this functional group served as an attractive yet largely unexplored[16,17] zinc-binding pharmacophore which could be incorporated with conformationally restricted dipeptide surrogates to give compounds of the type **3**.

The requisite acids **4** and **5** were generated following the procedure described by Roques.[15] In the case of the β-benzyloxylamino acid **5**, the compound could be formylated to afford **6** or was resolved via its (1*R*,2*S*)-(−)-ephedrine salt to give **7** as a single enantiomer.[18]

In order to compare the effect of chain length (*n*), acyl substituent (*Y*), and the incorporation of conformationally restricted dipeptide surrogates in this framework, a sub-set of compounds based on Leu-Phe as the dipeptide was initially explored. Leu-Phe was chosen as a representative dipeptide since the related mercaptoacetyl derivative exhibited IC_{50}s of 73 and 54 nM versus ACE and NEP, respectively.[19] Thus, EDAC/HOBT mediated coupling of **4**, **5** or **6** with Leu-Phe(OBn) (**8**) afforded the adducts **9a–c** in good yield (Scheme 2). In the case of **9a** and **9b**, simultaneous removal of the benzyl ether and ester via hydrogenolysis

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afforded the final products **11a** and **11b**, respectively. The related *N*-acetyl **11c** and hydroxy urea **11d** analogues were generated by treatment of **9c** with acetic anhydride or trimethylsilylisocyanate to give **10c** and **10d** respectively, followed by hydrogenolysis.

In vitro inhibition of ACE and NEP for compounds **11a–d** (diastereomeric mixture of isomers at *) are compared in Table 1. Although ~12-fold more selective for ACE than NEP, compound **11b** exhibited reasonable potency against both enzymes. The shortened derivative **11a** was substantially less active versus both enzymes. Paralleling results obtained by Roques in the *N*-acyl hydroxyl amino acid series, the *N*-acetyl **11c** and hydroxy urea **11d** derivatives were poorly active in this series, highlighting the absolute requirement for the *N*-formyl substituent. In previous studies, we demonstrated that the incorporation of suitable conformationally restricted dipeptide surrogates in mercaptoacyl dipeptides leads to a substantial enhancement in in vitro inhibitory active

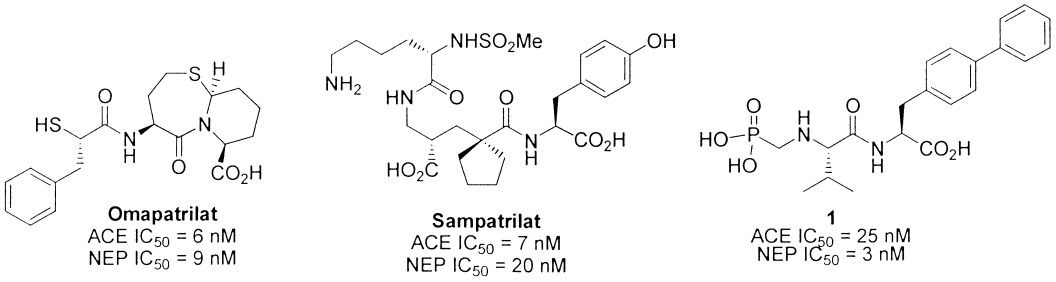


Figure 1.

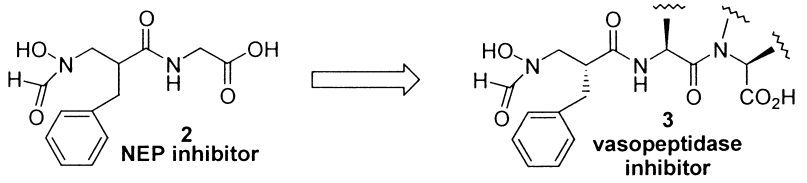
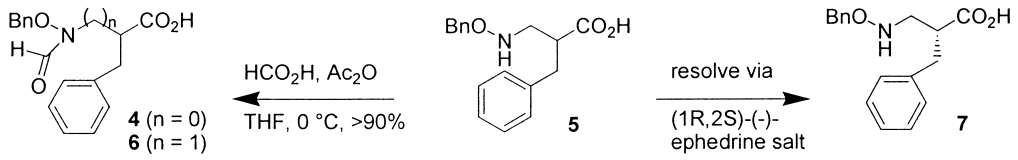
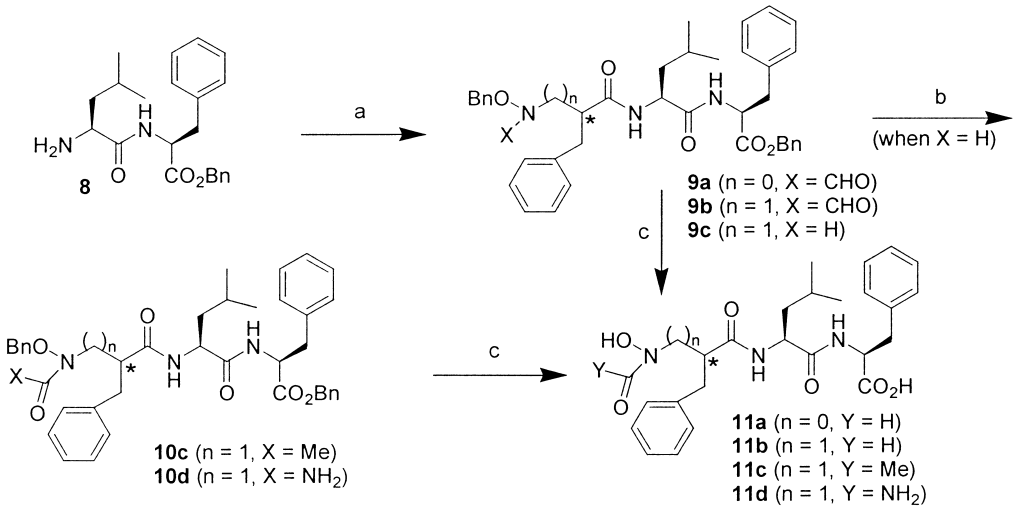


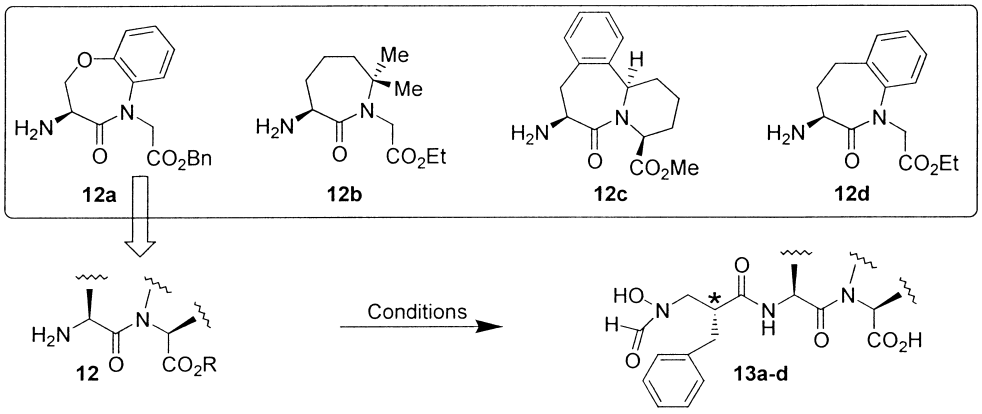
Figure 2.



Scheme 1.



Scheme 2. (a) compound **4**, **5** or **6**, EDAC, HOBT, CH_2Cl_2, rt (61–73%); (b) for compound **9d** to **10d**: xs TMSNCO, THF, reflux, (68%); for compound **9c** to **10c**: $Ac_2(O)$, THF, rt, (80%); (c) H_2, 10% Pd/C, MeOH, rt (94–100%).



Scheme 3. Conditions: For **12a**: (a) **4**, EDAS, HOBT, CH_2Cl_2 (84%); (b) H_2, 10% Pd/C, MeOH, rt, then separate diastereomers by HPLC (51%). For **12b–d**: (a) **5**, EDAC, HOBT, CH_2Cl_2 60–72%); (b) HCO_2H, A_2O, THF, 0 °C (77–97%); (c) H_2, 10% Pd/C, MeOH, rt (90–97%); (d) NaOH, H_2O, MeOH, then H_3O^+ (85–94%).

Table 1.

Compound	Stereochemistry at *	ACE (IC_{50}, nM)	NEP (IC_{50}, nM)
omapatrilat	—	6	9
11a	*R,S*	228,000	93,000
11b	*R,S*	106	1270
11c	*R,S*	461,000	214,000
11d	*R,S*	529,000	705,000
13a	*R*	210,000	290
13a′	*R*	93	1.7
13b	*R*	5.4	18
13c	*R*	3.4	1.8
13d	*R*	5.3	1.3

against both enzymes.[20,21] In analogy, a representative sample of mono- and bicyclic dipeptide surrogates (compounds **12a–d**)[22] were incorporated as Leu-Phe replacements in **11b** (Scheme 3). In the case of **12a**, coupling with **6** followed by hydrogenolysis afforded a diastereomeric mixture of **13a** and **13a′** which were separated by preparative HPLC and individually assayed. In the case of **12b–d**, coupling of the enatiomerically pure **7** afforded single diastereomers which were subsequently formylated, debenzylated, and saponified to provide **13b–d**.

Taking into consideration diastereomeric purity, incorporation of the benzofused oxazepine in **13a′** resulted in a 300-fold increase in potency as compared to **11b** against NEP while activity versus ACE was retained. Comparison of **13a′** with its stereoisomer **13a** clearly indicates a preference for the *R* isomer, paralleling observations made in the related mercaptoacyl dipeptide series of VPIs. The most active in this series, compounds **13c** and **13d**, exhibit high potency against both enzymes and are comparable in activity to the most potent VPIs reported to date.

Conclusion

The activity demonstrated by this series of compounds is significant, representing the fourth class of potent vasopeptidase inhibitors disclosed to date. The generally soft and diffuse zinc chelating ability of the *N*-formyl hydroxylamine phamacophore closely parallels that of the related thiols. Unlike carboxylates and phosphorus acids, the *N*-formyl hydroxylamines (like the thiols) exhibit higher pK_a values and their incorporation in charged dipeptides may represent and advantage in the design of VPIs with greater propensity for oral absorption. The evaluation of these compounds in vivo as well as the generation of other hydroxamate based VPIs will be the topic of future disclosures.

Acknowledgement

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18. For the resolution of compound **5** to compound **7**: a solution of acid **5** (2.56 g, 8.9 mmol) in CH_3CN (~20 mL) was treated with (1*R*,2*S*)-(−)-ephedrine (1.52 g) and swirled until homogeneous. Most of the CH_3CN was removed by rotary evaporation and the residue was redissolved in Et_2O (~25 mL) followed by hexane (~16 mL) until the solution became turbid. The solution was seeded and let stand overnight. The resulting precipitate was collected by filtration and washed with 1:1-Et_2O:hexane to afford a white solid (2.10 g, $[\alpha]_D$ −16.4° (c = 0.6, CH_2Cl_2). Repeated crystallization from hot CH_3CN afforded enatiomerically pure material (mp 118 °C, $[\alpha]_D$ −19.7° (c = 0.4, CH_2Cl_2). Absolute configuration of **7** (as shown) was determined by single crystal X-ray analysis of the purified ephedrine salt. The free acid **7** (an oil) was obtained by partitioning the salt between EtOAc and 5% aqueous $KHSO_4$ (pH adjusted to 2.5).
19. The mercaptoacetyl derivative of **11**, HSCH(Bn)CO-Leu-Phe-CO_2H, is readily generated by coupling of (*S*)-α-(acetylthio)-2-benzenepropionic acid with **8** followed by saponification and acidification. Representative experimentals can be found in Robl, J. A. et al. *J. Med. Chem.* **1996**, *39*, 494.
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