ORGANIC LETTERS

2002
Vol. 4, No. 12
2047−2050

Synthesis and Biological Activity of Hydroxamic Acid-Derived Vasopeptidase Inhibitor Analogues

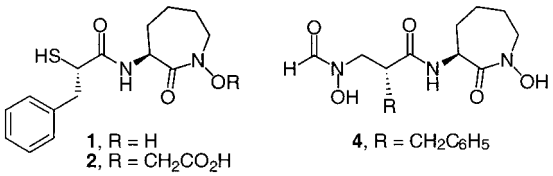
Andrew J. Walz and Marvin J. Miller*

Department of Chemistry and Biochemistry, 251 Nieuwland Science Hall, University of Notre Dame, Notre Dame, Indiana 46556

marvin.j.miller.2@nd.edu

Received March 19, 2002

ABSTRACT



Syntheses of novel hydroxamic acid-derived azepinones containing pendant mercaptoacyl groups or formyl hydroxamates are described. These new analogues of therapeutically important ACE and NEP inhibitors include unprecedented changes at the previously assumed essential acid component.

Inhibition of the zinc-containing metalloproteases, angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP), is of considerable interest for the development of therapeutically useful antihypertensive agents.[1−10] ACE, through the formation of angiotensin II (AII) from angiotensin I (AI) and the release of aldosterone, increases blood pressure. Atrial natriuretic peptide (ANP) is a vasodilator and a substrate for the protease NEP. Thus, inhibition of both ACE and NEP by vasopeptidase inhibitors serves to synergistically decrease the production of the vasoconstrictor AII and increase the lifetime of the vasodilator ANP. Recently, scientists at Bristol-Myers Squibb (BMS) published a report on the synthesis and biological activity of substituted azepinones in mercaptoacyl dipeptides (Figure 1).[9] These compounds, with geminal and spirocyclic substitutions at R and R′, demonstrated good to excellent ACE and NEP inhibition. The proposed structural properties of these molecules necessary for activity are also shown in Figure 1. Another paper reported that replacement of the thio (mercapto) group with an *N*-formyl hydroxylamine provided additional potent peptide-based vasopeptidase inhibitors.[10]

(1) Delaney, N. G.; Barrish, J. C.; Neubeck, R.; Natarajan, S.; Cohen, M.; Rovnyak, G. C.; Huber, G.; Murugesan, N.; Girotra, R.; Sieber-McMaster, E.; Robl, J. A.; Asaad, M. M.; Cheung, H. S.; Bird, J. E.; Waldron, T.; Petrillo, E. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1783−1788.

(2) Robl, J. A.; Simpkins, L. M.; Stevenson, J.; Sun, C.-Q.; Murugesan, N.; Barrish, J. C.; Asaad, M. M.; Bird, J. E.; Schaeffer, T. R.; Trippodo, N. C.; Petrillo, E. W.; Karanewsky, D. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1789−1794.

(3) Robl, J. A.; Simpkins, L. M.; Sulsky, R.; Sieber-McMaster, E.; Stevenson, J.; Kelly, Y. F.; Sun, C.-Q.; Misra, R. N.; Ryono, D. E.; Asaad, M. M.; Bird, J. E.; Trippodo, N. C.; Karanewsky, D. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1795−1800.

(4) Robl, J. A.; Sun, C.-Q.; Simpkins, L. M.; Ryono, D. E.; Barrish, J. C.; Karanewsky, D. S.; Asaad, M. M.; Schaeffer, T. R.; Trippodo, N. C. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2055−2060.

(5) Das, J.; Robl, J. A.; Reid, J. A.; Sun, C.-Q.; Misra, R. N.; Brown, B. R.; Ryono, D. E.; Asaad, M. M.; Bird, J. E.; Trippodo, N. C.; Petrillo, E. W.; Karanewsky, D. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2193−2198.

(6) Slusarchyk, W. A.; Robl, J. A.; Taunk, P. C.; Asaad, M. M.; Bird, J. E.; Dimarco, J.; Pan, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 753−758.

(7) Robl, J. A.; Cimarusti, M. P.; Simpkins, L. M.; Brown, B.; Ryono, D. E.; Bird, J. E.; Asaad, M. M.; Schaeffer, T. R.; Trippodo, N. C. *J. Med. Chem.* **1996**, *39*, 494−502.

(8) Robl, J. A.; Sun, C.-Q.; Stevenson, J.; Ryono, D. E.; Simpkins, L. M.; Cimarusti, M. P.; Dejneka, T.; Slusarchyk, W. A.; Chao, S.; Stratton, L.; Misra, R. N.; Bednarz, M. S.; Asaad, M. M.; Cheung, H. S.; Abboa-Offei, B. E.; Smith, P. L.; Mathers, P. D.; Fox, M.; Schaeffer, T. R.; Seymour, A. A.; Trippodo, N. C. *J. Med. Chem.* **1997**, *40*, 1570−1577.

(9) Robl, J. A.; Sulsky, R. A.; Sieber-McMaster, E.; Ryono, D. E.; Cimarusti, M. P.; Simpkins, L. M.; Karanewsky, D. S.; Chao, S.; Asaad, M. M.; Seymour, A. A.; Fox, M.; Smith, P. L.; Trippodo, N. C. *J. Med. Chem.* **1999**, *42*, 305−311.

(10) Robl, J. A.; Simpkins, L. A.; Asaad, M. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 257−260.

10.1021/ol025896m CCC: $22.00
Published on Web 05/14/2002

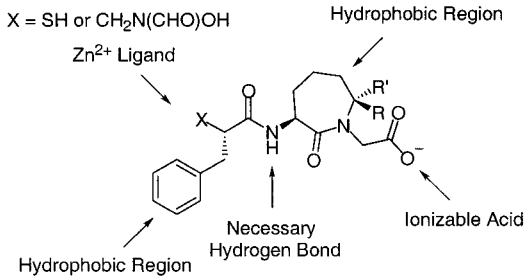


Figure 1. Vasopeptidase inhibitors developed by Bristol-Myers Squibb.

Despite considerable structure−activity relationship (SAR) studies that defined apparently essential features shown in the generalized structure in Figure 1, the literature reports of these vasopeptidase inhibitors did not present any data pertaining to structural variation of the ionizable acid functionality. During our studies related to the synthesis and biological activity of mycobactins (mycobacterial iron-sequestering agents) and analogues,[11] we noted the similarity of the terminal cyclolysine (cobactin component) to the synthetic BMS vasopeptidase inhibitors. This resemblance and earlier work in our labs related to the syntheses of novel *N*-hydroxy-based β-lactam antibiotics (oxamazins)[12] as well as the related development of monobactams (i.e., aztreonam) and monosulfactams at BMS[13] suggested that incorporation of the iron-binding, lysine-derived cyclic hydroxamic acid (cobactin) substructure of the mycobactins into the vasopeptidase inhibitor framework might allow preparation of analogues with novel and variable ionizable groups (Figure 2). Analogues **1** and **2** would provide acidic functionalities with altered pK_a values relative to the inhibitors shown in Figure 1. The cobactin-related component of analogue **1** also contains a metal-binding, O-unsubstituted cyclic hydroxamic acid that could provide interesting additional metal coordination favorable toward inhibition of the target metalloproteases. Furthermore, target **3** would be derived from a recently synthesized cobactin analog,[11] while compound **4** incorporates the N-terminal formyl hydroxamic acid developed by workers at BMS and shown in Figure 1.

A previously reported synthesis of the desired L-lysine-derived cyclic hydroxamic acid (*N*-hydroxy-3-amino-azepin-2-one, the iron binding portion of cobactin) from these laboratories involved the oxidation of *Z*-L-lysine with dimethyldioxirane (DMD) in acetone followed by hydroxylamine formation. Cyclization of the intermediate hydroxylamine was accomplished with a 5-fold excess of DCC,

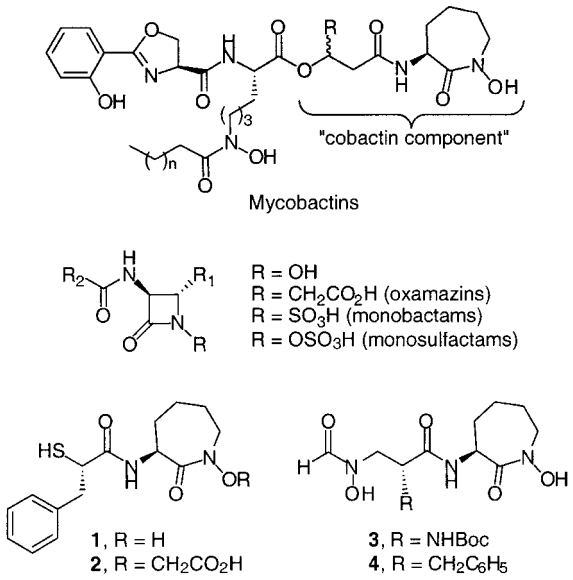
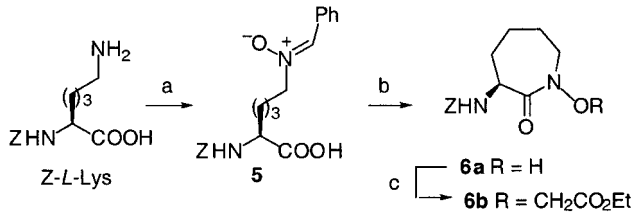


Figure 2. Mycobactins, monocyclic β-lactams, and proposed hydroxamic acid-containing analogues of vasopeptidase inhibitors.

DMAP, and DMAP(HCl) followed by protection of the hydroxamic acid with TBDPSCl.[14] Subsequently, Kropp reported a direct, oxone-mediated, microwave-assisted amine to hydroxylamine oxidation, including the oxidation of the ϵ-amino group of α-protected lysines.[15]

An alternative procedure that generates stable, storable nitrone intermediates is shown in Scheme 1.

Scheme 1[a]



[a] Reagents and conditions: (a) (1) PhCHO, KOH, MS, MeOH, rt, 16 h; (2) *m*-CPBA, MeOH, from 0 °C to room temperature, 4 h; (3) TFA, CH_2Cl_2, rt, 1 h; (4) PhCHO, EtOAc, from 0 °C to room temperature, 66% overall. (b) (1) NH_2OH(HCl), MeOH, 65 °C, 20 min; (2) EDC, HOAt, $NaHCO_3$, CH_3CN, DMF, rt, 48 h, (55%). (c) Ethyl bromoacetate, K_2CO_3, THF, H_2O, rt, 24 h, 97%.

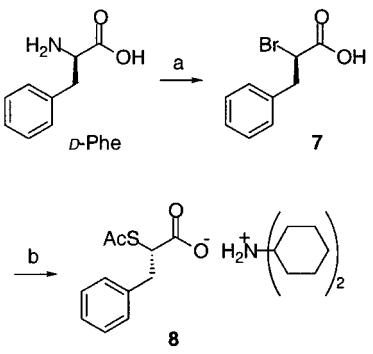
Thus, using chemistry recently employed in our group,[16] α-Cbz-L-lysine was treated with benzaldehyde under basic conditions to provide the corresponding ϵ-imine that was immediately used in subsequent reactions. Oxidation of the imine, with dry *m*-CPBA was followed by TFA-promoted isomerization of the intermediate oxaziridine to nitrone **5**.

(11) Vergne, A. F.; Walz, A. J.; Miller, M. J. *Nat. Prod. Rep.* **2000**, *17*, 99−116.
(12) (a) Woulfe, S. R.; Miller, M. J. *Tetrahedron Lett.* **1984**, *25*, 3293−3296. (b) Woulfe, S. R.; Miller, M. J. *J. Med. Chem.* **1985**, *28*, 1447−1453. (c) Boyd, D. B.; Eigenbrot, C.; Indelicato, J. M.; Miller, M. J.; Pasini, C. E.; Woulfe, S. R. *J. Med. Chem.* **1987**, *30*, 528 and references therein. (d) Miller, M. J. *Acc. Chem. Res.* **1986**, *19*, 49−56.
(13) (a) Slusarchyk, W. A.; Dejneka, T.; Gordon, E. M.; Weaver, E. R.; Koster, W. H. *Heterocycles* **1984**, *21*, 191. (b) Cimarusti, C. M.; Sykes, R. B. *Med. Res. Rev.* **1984**, *4*, 1.
(14) Hu, J.; Miller, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3462−3470.
(15) Fields, J. D.; Kropp, P. J. *J. Org. Chem.* **2000**, *65*, 5937−5941.
(16) Lin, Y.-M.; Miller, M. J. *J. Org. Chem.* **1999**, *64*, 7451−7458.

Experimentally, it was found that treatment of the reaction mixture with additional benzaldehyde at this stage improved the yield of the nitrone, perhaps by reacting with small amounts of prematurely released hydroxylamine. Conversion of the nitrone to the hydroxylamine by an exchange reaction with hydroxylamine hydrochloride was followed by EDC/HOAt-mediated cyclization to hydroxamic acid **6a**. Similar to our earlier syntheses of oxamazins,[12] treatment of **6a** with ethyl bromoacetate provided **6b** in excellent yield.

With the syntheses of the desired cyclolysine components **6a**,**b** complete, attention was focused on the preparation and incorporation of the N-terminal residues responsible for metal binding. The synthesis of dicyclohexylamine salt of (*S*)-2-(acetylthio)benzenepropanoic acid is shown in Scheme 2.

Scheme 2[a]



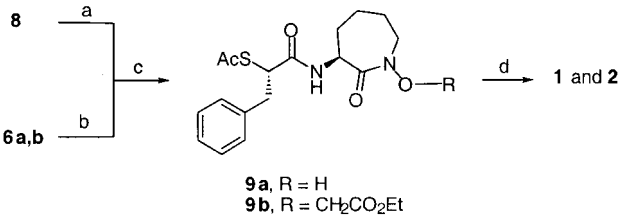
[a] Reagents and conditions: (a) KBr, 48% HBr, $NaNO_2$, H_2O, from −13 °C to room temperature, 24 h, 85%. (b) (1) CsSAc, DMF, rt, 24 h; (2) dicyclohexylamine, Et_2O, rt, 48 h, 65%.

The literature reports the synthesis of this salt[9] and the free acid[17] without experimental detail. Workers at BMS reported the use of Kellogg's methodology for the synthesis of optically active thiols.[18]

The requisite acid **8** was synthesized by adaptation of the separate procedures of Petit and Maimind for conversion of α-amino acids to α-bromoacids with retention of configuration.[19] Treatment of D-phenylalanine with sodium nitrite in a HBr/KBr solution provided bromo acid **7** in good yield. Treatment of this acid with 1.5 equiv of the cesium salt of thioacetic acid in DMF, followed by addition of dicyclohexylamine after workup, provided acid salt **8** in moderate yield.

The coupling of the carboxylates of amino acid derivatives to amines in the presence of unprotected hydroxamic acids has been previously demonstrated by our group.[20] The same conditions (EDC/HOAt/DMF) proved to be successful in the coupling of the free amine of **6a** with the liberated acid of **8** to give protected analogue **9a** (Scheme 3). The free amine

Scheme 3[a]

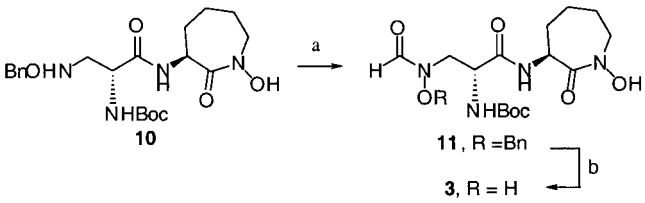


[a] Reagents and conditions: (a) 5% $KHSO_4$, EtOAc. (b) H_2, 10% Pd−carbon, MeOH, room temperature and pressure. (c) EDC, HOAt, DMF, rt, 3 h, 85% from **6a**, 75% from **6b**. (d) NaOH, MeOH, from 0 °C to room temperature, 6−12h, **1** (87%), **2** (87%).

of alkylated hydroxamic acid **6b** was also successfully coupled to the (*S*)-2-(acetylthio)benzenepropanoic acid under the same conditions to provide protected analogue **9b**. Final deprotection of **9a**,**b** was accomplished with NaOH in methanol under an inert atmosphere to generate analogues **1** and **2**.

The synthesis of formylated hydroxylamine analogue **3** began with the formylation of the cobactin analogue **10** with CDI/formic acid to give **11**.[21] After purification of **11**, hydrogenolytic removal of the benzyl protecting group gave the free formyl hydroxamate **3** in excellent overall yield from **10**[22] (Scheme 4).

Scheme 4[a]

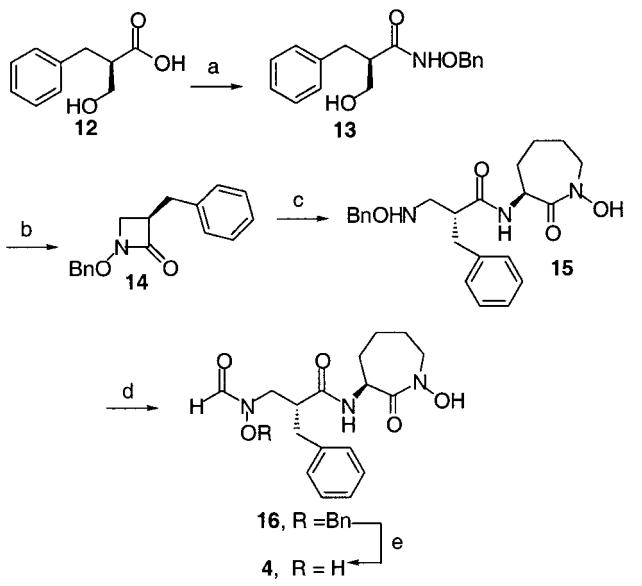


[a] Reagents and conditions: (a) CDI, HCOOH, THF, 0 °C, 30 min, and then **10**, 0 °C, 87%. (b) H_2, 10% Pd−carbon, MeOH, room temperature and pressure, 97%.

Preparation of the *β*-*N*-hydroxy amino acid-containing analogue **4** involved the synthesis of known benzylated hydroxamic acid **13** and *β*-lactam **14**.[23] The desired (*S*)-enantiomer of acid **12** was obtained through precedented alkylation of BOMCl with the enolate of a dihydrocinnamic acid-derived chiral oxazolidinone amide.[22,24,25] Subsequent reaction of the known chiral acid **12** with $BnONH_2$ in the presence of EDC led to the benzylated hydroxamic acid **13** in excellent yield and was followed by cyclization to give

(17) Spaltenstein, A.; Leban, J. J.; Furfine, E. S. *Tetrahedron Lett.* **1993**, *34*, 1457−1460.
(18) Strijtveen, B.; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664−3671.
(19) (a) Petit, Y.; Larcheveque, M. *Org. Synth.* **1997**, *75*, 37−43. (b) Maimind, V. I.; Ermolaev, K. M.; Shemyakin, M. M. *Zh. Obshch. Chim.* **1956**, *26*, 2313.
(20) Xu, Y.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 4314−4322.
(21) Krishnamurty, H. G.; Prasad, J. S. *Tetrahedron Lett.* **1977**, 3071−3072.
(22) Prepared from the corresponding serine-derived *β*-lactam by methods similar to those previously described (ref 20).
(23) Jin, Y.; Kim, D. H. *Synlett* **1998**, 1189−1190.
(24) Crawforth, J. M.; Rawlings, B. J. *Tetrahedron Lett.* **1995**, *35*, 6345−6346.
(25) Nakano, M.; Atsuumi, S.; Koike, Y.; Tanaka, S.; Funabashi, H.; Hashimoto, J.; Ohkubo, M.; Morishima, H. *Chem. Lett.* **1990**, 505−508.

Scheme 5[a]



[a] Reagents and conditions: (a) $BnONH_2$(HCl), EDC, DMAP, H_2O, DMF, rt, 16 h, 80%. (b) PPh_3, CCl_4, TEA, CH_3CN, rt, 16 h, 83%. (c) (1) 4 N HCl, 1,4-dioxane, 60 °C, 1 h; (2) deprotected **6a**, EDC, HOAt, DMAP, DMF, rt, 48 h, 33%. (d) CDI, HCOOH, THF, 0 °C, 30 min, and then **14**, 0 °C, 16 h, 90%. (e) H_2, 10% Pd−carbon, MeOH, room temperature and pressure, 97%.

β-lactam **14** (Scheme 5).[26] An initial attempt at the synthesis of the penultimate protected hydroxylamine **15** utilized a cyanide anion β-lactam activation for the formation of the amide bond. Thus, treatment of the amine derived from hydrogenolysis of **6a** and β-lactam **14** with TMSCN/TBAF[27] led to the desired coupled product **15** but as a mixture of diastereomers that were chromatographically inseparable. This loss of optical integrity was surprising and encouraged us to first hydrolyze β-lactam **14**[22] and couple the resulting free carboxylic acid to the amine of the cobactin component again obtained by hydrogenolysis of **6a**, a process we had previously used successfully in the synthesis of cobactin analogues.[19] The reaction proceeded in moderate overall yield to provide **15** with no evidence for the presence of any undesired diastereomer in the ^1H and ^{13}C NMR spectra. Formylation of **15** provided protected analogue **16** uneventfully. Hydrogenolytic removal of the benzyl protecting group gave analogue **4** in good yield.

(26) Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. F. *J. Am. Chem. Soc.* **1980**, *102*, 7026−7032.

(27) Soli, E. D.; Manoso, A. S.; Patterson, M. C.; DeShong, P.; Favor, D. A.; Hirschmann, R.; Smith, A. B., III. *J. Org. Chem.* **1999**, *64*, 3171−3177.

Compounds **1**−**4** were subjected to primary ACE and NEP inhibition assays at PanLabs of Taiwan (MDS Pharma Services) with the results summarized in Table 1. These assays give the percent inhibition of the target enzymes upon treatment with the potential inhibitors at a concentration of 10 μM. Under these assay conditions, compound **1** inhibited 63% of ACE and 93% of NEP. Compound **2** inhibited 76% of ACE and 95% of NEP. Not surprisingly based on a previous SAR study at BMS, **3** only poorly inhibited ACE (5%) and NEP (12%), but **4**, with the apparently key aryl substituent, was remarkably selective with barely detectable ACE inhibition (5%) but outstanding inhibition of NEP (99%). Thus, it appears that attachment of the acid functionality to the hydroxamic acid group of the cyclolysine component of ACE/NEP inhibitors is not necessary for activity.

Table 1. ACE/NEP Inhibitory Activity of Compounds **1**−**4**

compound	enzyme	concentration	% inhibition
1	ACE (rabbit)	10 μM	63%
1	NEP (human)	10 μM	93%
2	ACE (rabbit)	10 μM	76%
2	NEP (human)	10 μM	95%
3	ACE (rabbit)	10 μM	5%
3	NEP (human)	10 μM	12%
4	ACE (rabbit)	10 μM	5%
4	NEP (human)	10 μM	99%

Four new vasopeptidase inhibitors **1**−**4** were synthesized and found to have variable but promising and/or selective ACE and NEP inhibition. The therapeutic importance of ACE/NEP inhibition indicates that these new compounds may have significant utility.

Acknowledgment. We gratefully acknowledge support for the mycobactin research from the NIH that led to the consideration of the syntheses of the novel vasopeptidase inhibitors described. We acknowledge Don Schifferl and Dr. Jaroslav Zajicek for NMR assistance and Dr. Bill Boggess and Nonka Sevova for mass spectrometry. Special thanks are extended to Maureen Metcalf for her assistance with this manuscript.

Supporting Information Available: Experimental procedures and characterization data for products **1**, **2**, **4**, **5**, **6a**, **6b**, **7**, **8**, **9a**, **9b**, **11**, and **13**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL025896M