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DESIGN AND SYNTHESIS OF DIAMINOPYRROLIDINONE INHIBITORS OF HUMAN OSTEOCLAST CATHEPSIN K

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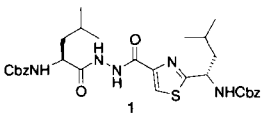
Abstract: The structure-based design and synthesis of lactam-constrained azapeptide inhibitors of human cathepsin K are described. Enhanced stability to proteolytic cleavage over acyclic analogues is discussed.

The process of bone resorption is carried out by osteoclasts, which are multinuclear cells of hematopoietic lineage. Upon attachment to the bone surface, an enclosed extracellular compartment is formed that is acidified by proton pumps and into which the osteoclast secretes proteolytic enzymes. The low pH of the compartment dissolves hydroxyapatite crystals at the bone surface, while the proteolytic enzymes digest the protein matrix. Several published studies have demonstrated that inhibitors of cysteine proteases (e.g., leupeptin and Z-Phe-Ala-CHN_2) are effective at inhibiting osteoclast-mediated bone resorption while serine protease inhibitors were ineffective indicating an essential role for a cysteine proteases in bone resorption.[1–4] The abundant, selective expression of one cysteine protease, cathepsin K, in osteoclasts strongly suggests that this enzyme is essential for bone resorption.[5,6] Thus, selective inhibition of cathepsin K may provide an effective treatment for diseases of excessive bone loss such as osteoporosis, Paget's disease, hypercalcemia of malignancy, and metabolic bone disease.



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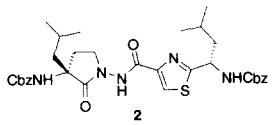

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Reports from these laboratories[7] described the rational design and synthesis of a series of diacylhydrazines containing a thiazole amide bond isostere that are potent and selective inhibitors of human cathepsin K, exemplified by structure **1**. Although potent inhibition was achieved (for **1**; K_i = 10 nM[7a]), one concern with such inhibitors was their potential to act as substrates for cathepsin K through proteolytic cleavage at either or both hydrazide carbonyl groups. Since the carbonyl group adjacent to the thiazole is required for formation of a covalent enzyme–inhibitor complex, structural modifications that prevent nucleophilic attack at this carbonyl would be undesirable. The alternative pathway for proteolytic processing can be blocked, however, by hindering the approach of nucleophiles to the second hydrazide carbonyl group. The X-ray crystal of **1** bound to human cathepsin K[7a] (Figure 1) suggests that five-membered ring formation between the leucine derived

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α-carbon onto the nearest hydrazide nitrogen atom should be well tolerated, and the diaminopyrrolidinone **2** should therefore be a potent inhibitor of human cathepsin K with enhanced stability to proteolytic degradation.[8,9]

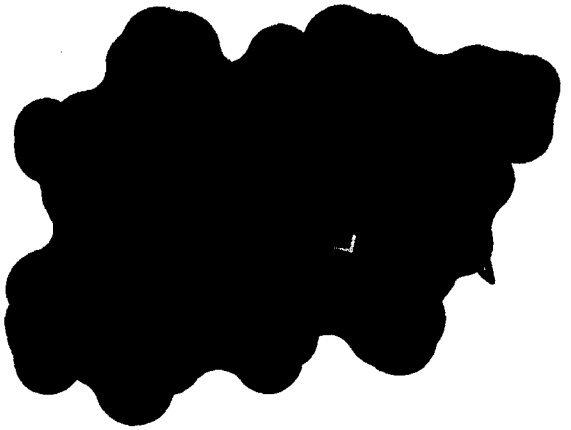
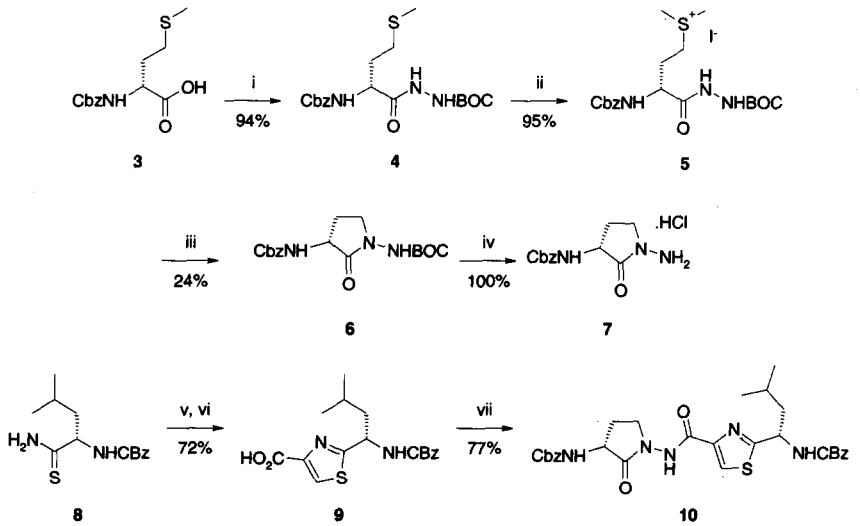


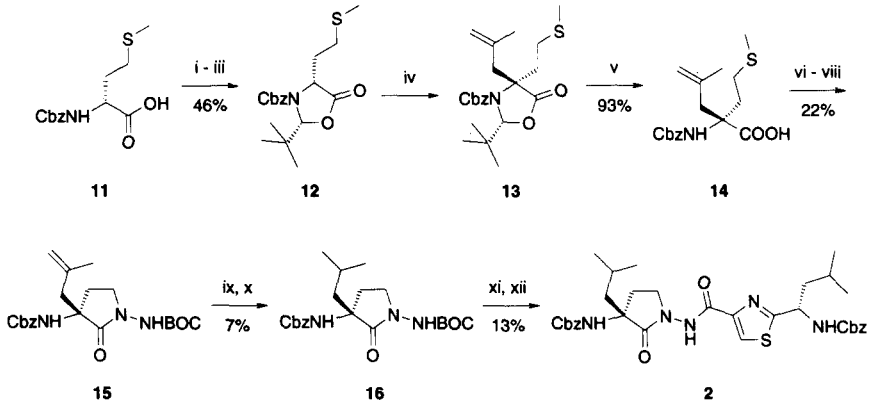
Figure 1. Modeled[10] structure of diaminopyrrolidinone **2** (red) superimposed upon X-ray crystal structure of hydrazide **1** bound to cathepsin K whose solvent-accessible active site is represented in blue.

Compound **10**, the analog of **2** lacking the P_2 isobutyl side chain, was first prepared as outlined in Scheme 1 following procedures analogous those described by Freidinger et al.[8] Benzyloxycarbonyl-D-methionine **3** was condensed with *tert*-butylcarbazate under standard coupling conditions to afford compound **4**. Treatment of **4** with methyl iodide gave sulfonium salt **5**, which cyclized upon treatment with base to afford (3*R*)-3-benzyloxycarbonylamino-1-*tert*-butoxycarbonylaminopyrrolidin-2-one **6** in 24% yield. Despite the moderate yield obtained, this sequence was readily scaleable to provide multi-gram quantities of intermediate **6**. Removal of the *tert*-butyl protecting group with hydrochloric acid gave the amine hydrochloride **7** in quantitative yield. Thiazole acid **9** was prepared in high yield from L-leucinethioamide **8**[11] by condensation with ethyl bromopyruvate followed by trifluoroacetic anhydride promoted cyclization then basic hydrolysis of the ethyl ester. Acid **9** was then coupled with amine **7** to give diaminopyrrolidinone **10**[12] in high yield.



Scheme 1. (i) $BOCNHNH_2$, DMF, EDC, HOBt; (ii) MeI; (iii) NaH, CH_2Cl_2, DMF; (iv) HCl, 1,4-dioxane; (v) $EtO_2CC(O)CH_2Br$, acetone then $(CF_3CO)_2O$, pyridine, CH_2Cl_2; (vi) LiOH, THF, H_2O; (vii) **7**, EDC.HCl, HOBt, NMM, DMF.

With a suitable synthetic route to 1,3-diaminopyrrolidin-2-ones in hand, attention was then turned to the synthesis of compound **2** (Scheme 2). The starting point was oxazolidinone **12**, which was straightforwardly prepared from Cbz-D-methionine **11** via literature methods.[13] The potassium enolate of **12** was readily generated at –78 °C using KHMDS and then quenched with methallyl bromide to cleanly afford **13** as a single diastereomer. Without further purification, alkaline hydrolysis then gave α,α-disubstituted amino acid **14** in excellent overall yield. Via a similar protocol to that outlined in Scheme 1, **14** was converted into the 1,3-diaminopyrrolidinone intermediate **15** in three steps. In this example the pivotal heterocyclization forming the pyrrolidinone ring proceeded in 25% yield on a multi-gram scale. Hydrogenolysis of the CBz group was accompanied by double bond reduction and the resulting primary amine was reprotected to give benzyl carbamate **16**.[14] Deprotection of the BOC group and acylation with acid **9** finally afforded diaminopyrrolidinone **2**.[15]



Scheme 2. (i) NaOH, EtOH; (ii) trimethylacetaldehyde, hexanes; (iii) CbzCl, CH_2Cl_2; (iv) KHMDS, methallyl bromide, THF; (v) NaOH, MeOH; (vi) NH_2NHBOC, EDC, HOBt, DMF; (vii) MeI; (viii) NaH, CH_2Cl_2, DMF; (ix) H_2, Pd-C, EtOH; (x) CbzCl, Et_3N, 1,4-dioxane; (xi) HCl, 1,4-dioxane; (xii) **9**, EDC.HCl, HOBt, NMM, DMF.

Diaminopyrrolidinone **2** is a reasonably potent inhibitor of human osteoclast cathepsin K (K_i = 33 nM)[16] being only about threefold less potent than its acyclic analogue **1** (K_i = 10 nM).[17] Removal of the isobutyl side chain, compound **10**, results in a drop in inhibitory potency (K_i = 330 nM) owing to the loss of interaction between the P_2 isobutyl group and the hydrophobic S_2 pocket of cathepsin K.

Preliminary investigations into the proteolytic stability of the diaminopyrrolidinones prepared in this letter have shown that these compounds are less susceptible to degradation by human cathepsin K than acyclic analogues. For example, diacylhydrazine **1** loses all ability to inhibit the processing of a fluorescent substrate (Z-Phe-Arg-AMC)[7a] by cathepsin K after a 1 h pre-incubation time the protease. In contrast diaminopyrrolidinone **2** retains almost complete inhibitory potency after the same pre-incubation period. A more detailed analysis of the proteolytic degradation and metabolic fate of the diaminopyrrolidinone class of cathepsin K inhibitors is under investigation and will be reported in due course.

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12. Data for **10**: $C_{29}H_{33}N_5O_6S.½H_2O$ requires: %C, 59.2, %H, 5.8, %N, 11.9; found %C, 59.1, %H, 5.8, %N, 11.5.
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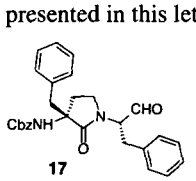
14. The conversion in the reprotection step was low resulting in the poor isolated yield of **16**. Acylation of the intermediate primary amine with aryl carboxylic acids typically progresses in >80% yield (studies will be published elsewhere).
15. Data for **2**: $C_{33}H_{41}N_5O_6S$ requires: %C, 62.3; %H, 6.5; %N, 11.0; found %C, 62.0; %H, 6.5; %N, 10.8.
16. Thereby providing good evidence that the stereochemistry at the quaternary center is as predicted.
17. Recently Roush et al. (*Bioorg. Med .Chem.* **1998**, *6*, 2477) investigated the inhibition of various cysteine proteases by conformationally-contrained peptidomimetics such as the lactam-constrained analogue of Cbz-Phe-Phe-CHO aldehyde shown below (**17**). Their results showed that inclusion of the pyrrolidinone ring can reduce dramatically the inhibition of certain cysteine proteases (e.g., cruzain) presumably due to unfavourable steric interactions while it's presence is tolerated for other proteases, in this case the *Leishmania major* protease (which likely have more relaxed steric requirements at the position where the pyrrolidinone is introduced). The results presented in this letter fall within the latter category.



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