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(4-Piperidinylphenyl)aminoethyl amides as a novel class of non-covalent cathepsin K inhibitors

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Abstract—A series of (4-piperidinylphenyl)aminoethyl amides based on dipeptide anilines were synthesized and tested against cathepsin K, cathepsin L and cathepsin B. These new non-covalent inhibitors exhibited single-digit nM inhibition of the cysteine proteases. Compounds **3** and **7** demonstrated potency in both mouse and human osteoclast resorption assays.

Cathepsin K is a recently discovered cysteine protease that is highly and selectively expressed in bone, osteoclast cells, osteoclast resorbing cartilage, osteoarthritic synovium, and ovary.[1,2] This enzyme is found to efficiently degrade both telopeptide and triple helical regions of type I collagen as well as native type II collagen,[3] which strongly suggests its possible involvement in bone and cartilage breakdown in diseases such as osteoporosis, osteoarthritis, rheumatoid arthritis, hypercalcemia of malignancy, and Paget's disease. Studies of knock-out mouse models and mutations in the human cathepsin K gene have demonstrated its critical role in bone resorption.[4,5] Slowing or blocking the bone resorption process by inhibiting cathepsin K could potentially treat human diseases.

Cysteine protease inhibitors have been studied for the purpose of finding therapeutics for a variety of other diseases.[6,7] However, the majority of these inhibitors form a covalent bond between the thiol, at the active site cysteine, and the reactive functional groups of inhibitors such as aldehydes, ketones, haloketones, diazoketones, epoxides, nitriles, vinylsulfones and ketoamides, which may be a potential liability in toxicity and delivery to the targeted area. Herein, we report a series of (4-piperidinylphenyl)aminoethyl amides as a novel non-covalent class of cathepsin K inhibitors.[8]

Based on inhibitory activities of dipeptidylaldehydes such as Cbz-Leu-Phe-CHO (K_i, 0.020 μM) and Cbz-Leu-Met-CHO (K_i, 0.013 μM) against cathepsin L,[9] we investigated replacement of the aldehyde function with non-reactive chemical moieties. A dipeptidyl derivative **1**, where aniline replaced the aldehyde group, showed an IC_{50} of 1.1 μM against human recombinant cathepsin K and an IC_{50} of 0.230 μM against cathepsin L. Further derivatization of the aniline group furnished more potent cathepsin K inhibitors (Table 1). The 4-piperidinyl aniline derivative **3** had an IC_{50} of 0.010 μM against cathepsin K and 0.002 μM against cathepsin L. To our surprise, potency of **3**, a non-covalent inhibitor against cathepsin L is similar to that of the reactive and covalent inhibitor Cbz-Leu-Phe-CHO (IC_{50}, 0.005 μM).

Potency against cathepsin K was retained when phenylalanine (compound **3**) was replaced for homophenylalanine (compound **8**). For further optimization, we focused our attention on the amino acid component. Subsequent replacement of leucine with other amino acids resulted in discovering not only potent cathepsin K and L inhibitors but also more importantly selective cathepsin K inhibitors. In particular, alanine and proline derivatives **6** and **14** showed greater than 200-fold selectivities over cathepsins L and B. The results are

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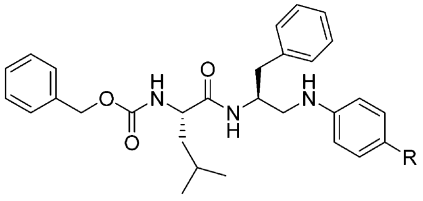
summarized in Table 2. These data support that cathepsin K allows residues with a small side chain for hydrophobic interaction, but cathepsin L prefers residues with a branched or larger side chain.

The synthesis of the compounds **1–14** is straightforward and shown in Scheme 1. Anilines were reacted with *t*-Boc-phenylalanine or *t*-Boc-homophenylalanine under standard amide coupling conditions to give amide **15a–e**. Selective reduction of the amide carbonyl group in **15a–e** with borane provided the corresponding anilines. Exposure of the anilines to acidic conditions furnished **16a–e**, which were then coupled with Cbz-protected amino acids to afford dipeptidyl anilines **1–14**.

Table 1. Inhibition of dipeptidyl anilines against recombinant human cathepsins

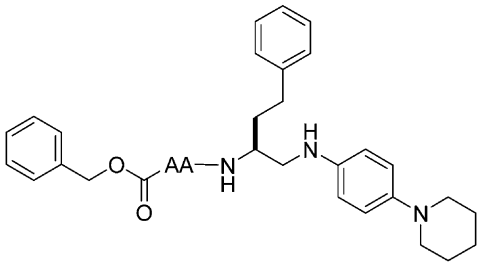


Compd	R	Cat K (IC_{50}, μM)[a]	Cat L (IC_{50}, μM)[b]
1	H	1.100	0.230
2	Benzene	0.706	0.032
3	Piperidine	0.010	0.002
4	Morpholine	0.015	0.002

[a] Recombinant human cathepsins K (3.3 nM) was used in a fluorescence assay using 20 mM Cbz-Phe-Arg-AFC as substrate in 0.45 M NaOAc (pH 5.2), 4.8 mM EDTA, 0.1% Triton X-100, 8 mM DTT.
[b] Recombinant human cathepsins L (1.2 nM): was used in a fluorescence assay using 20 mM Cbz-Phe-Arg-AFC as substrate in 0.45 M NaOAc (pH 5.2), 4.0 mM EDTA, 0.1% Triton X-100, 8mM DTT.

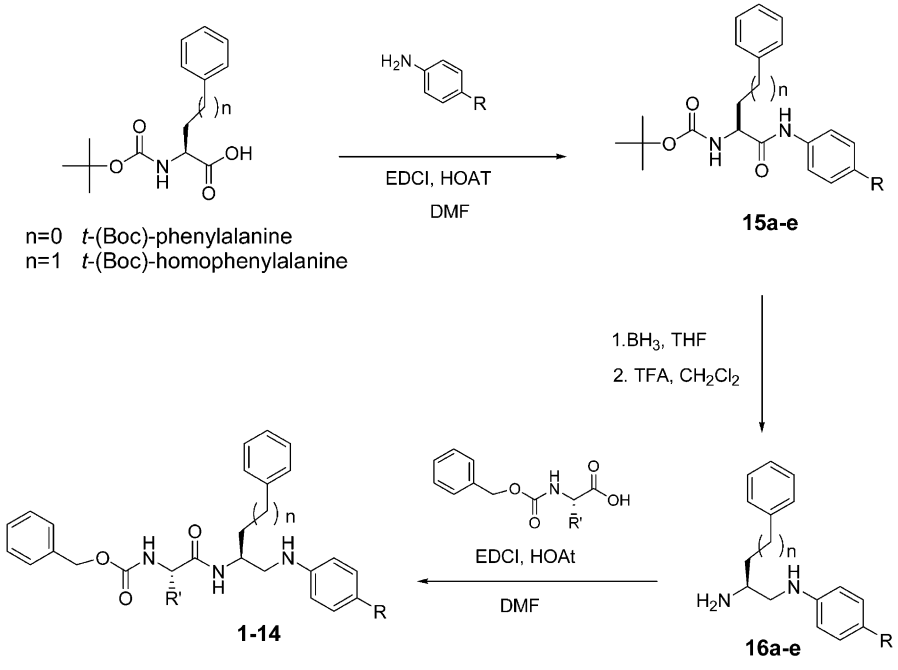
To address the ability of these compounds to inhibit bone resorption, **3** and **7** were evaluated in murine[10] and human osteoclast resorption assays.[11] These compounds showed inhibitory activities in both mouse (**3**, **7**: 1 μM, 0.330 μM) and human (**3**, **7**: 1 μM, 0.560 μM) bone resorption assays, and IC_{50} values were determined by

Table 2. Inhibition of dipeptidyl 4-piperidylanilines against cathepsins



Compd	AA (amino acid)	Cat K (IC_{50}, μM)[a]	Cat L (IC_{50}, μM)[b]	Cat B (IC_{50}, μM)[c]
5	Gly	>10.00	>10.00	>10.00
6	Ala	0.009	4.110	2.094
7	Val	0.004	0.001	0.007
8	Leu	0.005	0.002	0.658
9	Met	0.003	0.036	0.012
10	Phe	0.003	0.001	0.167
11	Tyr	0.008	0.001	0.106
12	HPhe	0.014	0.009	9.664
13	Trp	0.048	0.001	3.362
14	Pro	0.012	7.844	>10.00

[a] Recombinant human cathepsins K (3.3 nM) was used in a fluorescence assay using 20 mM Cbz-Phe-Arg-AFC as substrate in 0.45 M NaOAc (pH 5.2), 4.8 mM EDTA, 0.1% Triton X-100, 8 mM DTT.
[b] Recombinant human cathepsins L (1.2 nM): was used in a fluorescence assay using 20 mM Cbz-Phe-Arg-AFC as substrate in 0.45 M NaOAc (pH 5.2), 4.0 mM EDTA, 0.1% Triton X-100, 8 mM DTT.
[c] Recombinant human cathepsins B was used in a fluorescence assay using 20 mM Cbz-Phe-Arg-AFC as substrate in 0.45 M NaOAc (pH 5.2), 4.0 mM EDTA, 0.1% Triton X-100, 8 mM DTT.



Scheme 1.

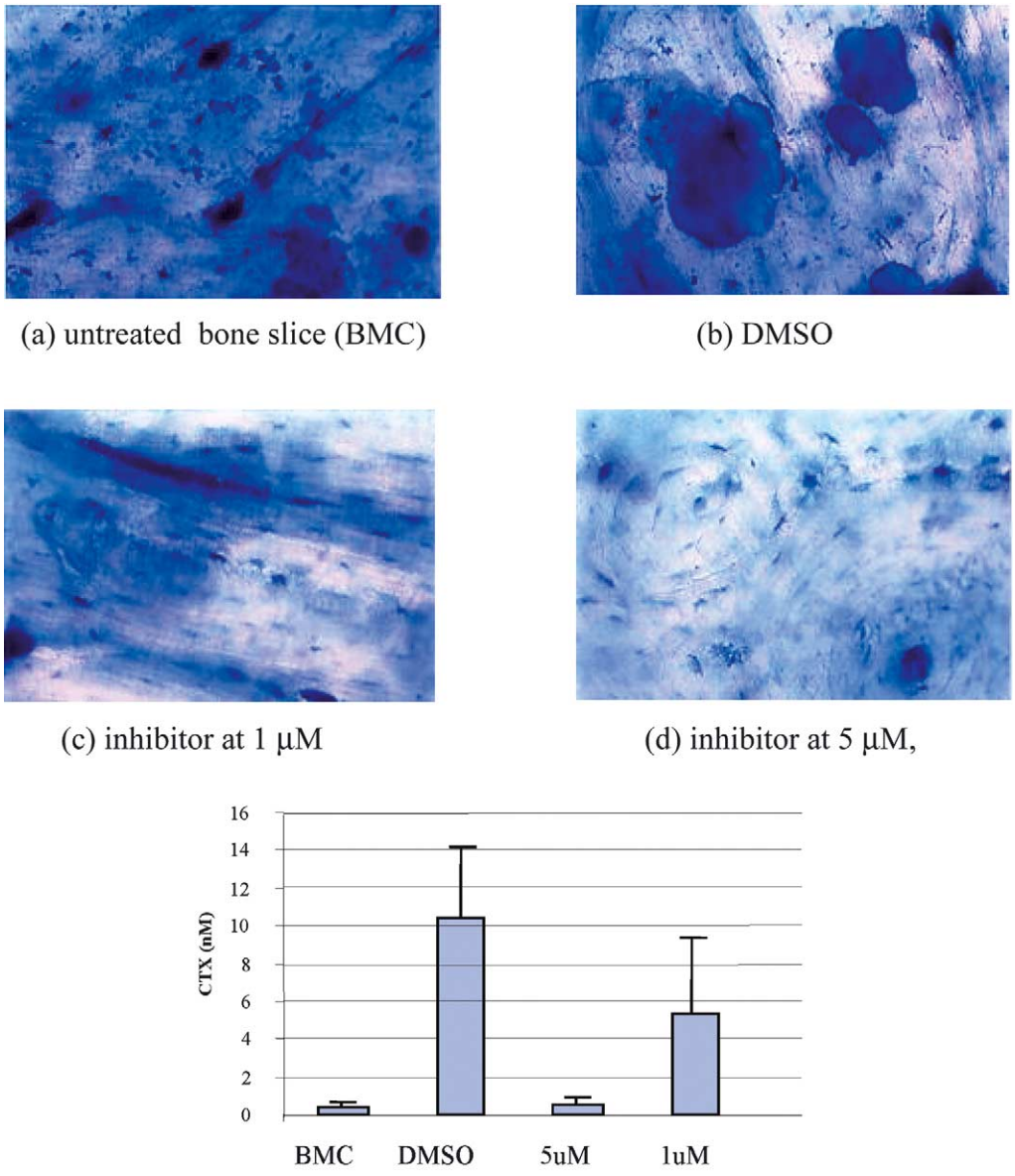


Figure 1. Effect of cathepsin K inhibitor **3** on mouse bone resorption assay: (a) untreated bone slice (BMC); (b) DMSO; (c) inhibitor at 1 μM; (d) inhibitor at 5 μM. Toluidine blue stained bone slices show inhibition of resorption pit formation (×10 magnification).

reading the C-terminal telopeptides (CTX) of type I collagen released. As shown in Figure 1, compound **3** partially inhibited pit formation at 1 μM and completely inhibited at 5 μM.

With potent and selective cathepsin K inhibitors in hand, we investigated their kinetic profiles. Simple Lineweaver–Burk analyses of **3** using Cbz-Phe-Arg-AFC as a substrate showed it to be a competitive inhibitor. In addition, reversibility of these inhibitors was demonstrated by the recovery of activity of inhibited enzyme after dialysis and dilution.

In conclusion, we described novel, non-covalent, competitive, and selective cathepsin K inhibitors and demonstrated that (4-piperidinylphenyl)aminoethyl amides exhibit potency in a bone resorption assay.

Acknowledgements

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