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Discovery and Parallel Synthesis of a New Class of Cathepsin K Inhibitors

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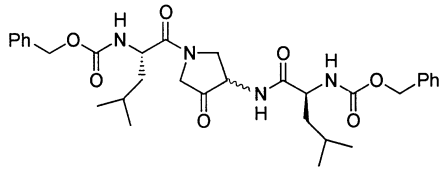
Abstract—Peptidomimetic aminomethyl ketones have been identified as a new class of cathepsin K inhibitors. Traditional and high-speed parallel synthesis techniques were applied to investigate this series. Structure–activity relationships were established, and certain analogues were characterized with IC_{50} values in the range 200–500 nM.

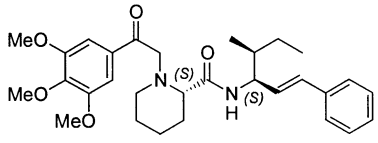
Cathepsin K (EC 3.4.22.38), a cysteine protease that is predominantly expressed in osteoclasts,[1] has been implicated in bone resorption through the use of selective peptide inhibitors,[2] antisense oligonucleotides,[3] and the existence of a human genetic disorder, pycnodysostosis.[4] These observations provide a rationale for the use of cathepsin K inhibitors as a treatment for diseases characterized by excessive bone loss, such as osteoporosis.[5] Very recently, the first indication that inhibition of cathepsin K leads to a reduction in bone resorption in primates was achieved, by administration of the inhibitor SB-357114 (12 mg/kg sc qd).[6]

A variety of compound classes have been developed as cysteine protease inhibitors.[7] Recently reported inhibitors of cathepsin K include peptide-based α,α′-diacylamino ketones (e.g., **1**),[8] alkoxymethyl ketones,[9] cyanamides,[10] and pyridoxal propionate derivatives.[11]



1: $K_i = 2$ nM

High-throughput screening of the Bayer compound collection provided the micromolar hit **2**.[12] The presence of an electrophilic carbonyl group (aminomethyl ketone moiety) in this molecule suggested the possibility for reversible formation of a covalent tetrahedral adduct with the active-site Cys-25, similar to the case with other ketone inhibitors such as **1**.[8,9] In this report, we describe our investigation of analogues of **2**, synthesized by both traditional and high-speed parallel synthesis methodologies.



2

Our synthetic efforts focused primarily on variation of the aryl ketone group, the α-amino acid fragment, and the aralkyl amine group in **2**. Using traditional synthetic techniques, several analogues of **2** were prepared as summarized in Scheme 1. This route involves the formation of an *N*-Boc-α-amino aldehyde via the corresponding *N,O*-dimethyl hydroxamate, followed by conversion to the desired allyl amine, coupling to an α-amino acid core fragment, and *N*-alkylation by an α-halomethyl ketone. An alternative for the olefin-forming reaction involved the use of benzyl triphenyl-phosphonium bromide (*t*BuOK, THF). To enable the synthesis of greater numbers of derivatives, an efficient parallel synthesis method was required. For synthetic flexibility and to minimize the amount of time expended on method development, we embarked on a solution-phase synthesis which made extensive use of polymer-supported reagents (Scheme 2).[13]

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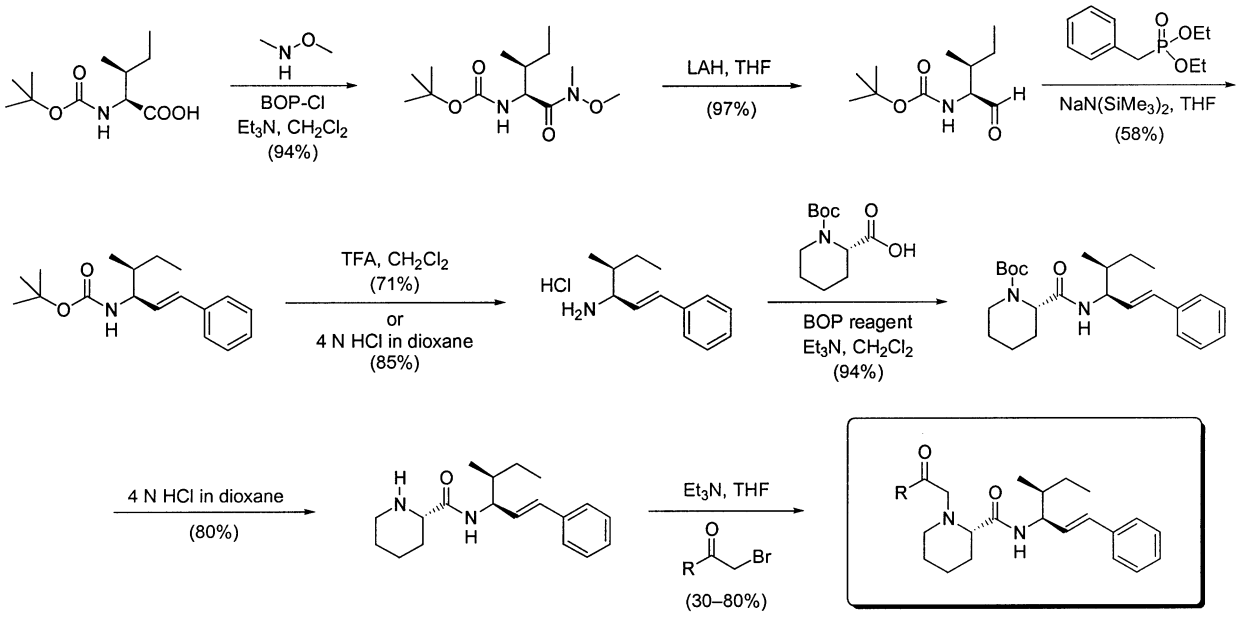
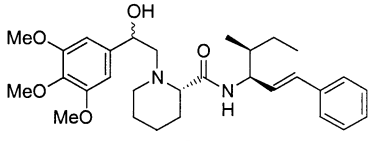


Scheme 1. Synthesis of analogues of **2**.

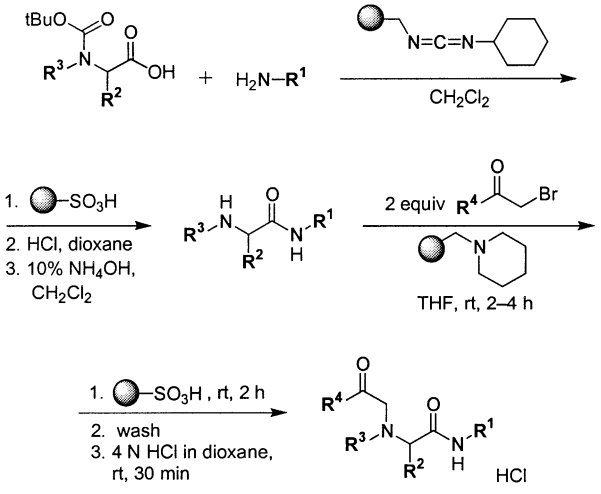
In this method, amines (both custom-prepared and commercially available) were coupled to *N*-Boc-protected amino acids through the use of a polymer-supported carbodiimide.[14] After treatment with polymer-bound sulfonic acid to scavenge any unreacted amine from the product, the *N*-Boc protecting group was removed (HCl/dioxane). The amine hydrochloride product was free-based (10% NH_4OH/CH_2Cl_2), and then alkylated by treatment with two equivalents of α-halomethyl ketone in the presence of polymer-supported piperidine. A 'catch-and-release' purification strategy[13c] via polymer-supported sulfonic acid was used to isolate the products in generally high purity.

In the cathepsin K inhibition assay,[15] Lineweaver–Burk analysis established that the lead **2** behaved as a competitive inhibitor, with $IC_{50} = 1.2$ μM, and $K_i = 0.79$ μM. Furthermore, inhibition by **2** was observed to be non-time dependent, consistent with a reversible inhibition mechanism. The reduced-ketone analogue **3** (mixture of diastereomers, 8% inhibition at 1 μM) was significantly less potent than the lead **2**. This supports the proposed formation of a covalent tetrahedral adduct via addition of the active-site Cys-25 thiol to the electrophilic carbonyl moiety in **2**, consistent with the observations for related inhibitors.[8,9]



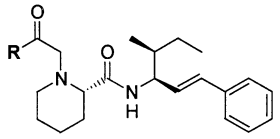
3

Variation of the aryl ketone fragment provided analogues with a range of inhibitory activities (Table 1). Replacement of the 3,4,5-trimethoxyphenyl fragment with less bulky, but electron-rich or lipophilic aryl groups afforded more potent inhibitors. In particular,



Scheme 2. Parallel synthesis of analogues of **2**.

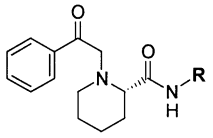
Table 1. Variation of the aryl ketone fragment



Compd	R	Cathepsin K % inh. (1 μM)	Cathepsin K IC_{50} (μM)
2	3,4,5-$(MeO)_3$Ph		1.2
4	Ph		0.23
5	4-Cl-Ph		0.28
6	4-MeO-Ph		0.41
7	2,6-$(MeO)_2$Ph		0.70
8	3,4-(OCH_2O)Ph		0.59
9	4-NO_2-Ph	28	
10	3-NO_2-Ph	11	
11	4-CN-Ph	16	
12	4-AcNH-Ph	42	
13	Et	22	
14	Me	3	

analogues **4**, **5**, and **6** were found to be significantly more effective as cathepsin K inhibitors than the lead **2**. In contrast, incorporation of electron-deficient or hydrophilic aryl groups at this position, as exemplified by **9**–**12**, gave a substantial loss in potency. Likewise, replacement of the aryl group with a small aliphatic fragment gave relatively ineffective compounds (**13** and **14**). Hence, activity does not correlate with electrophilicity of the ketone moiety, but the presence and electron density of an aryl ring is important. This suggests that lipophilic and/or π–π interactions of this fragment with the protein subsite contribute more to binding energy than the ability of the ketone to form a hemithioketal adduct.

Table 2. Variation of the 3-phenyl-3-propenyl fragment



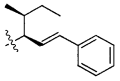
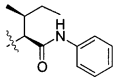
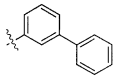
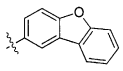
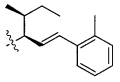
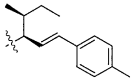
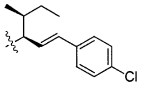
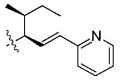
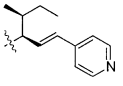
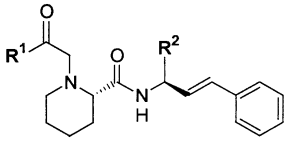
Compd	R	Cathepsin K % inh. (1 μM)	Cathepsin K IC_{50} (μM)
4			0.23
15		8	
16		2	
17		<1	
18			0.59
19			0.74
20			1.4
21		26	
22		8	

Table 3. Variation of the 3-phenyl-3-propenyl fragment

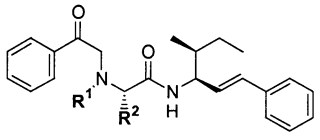


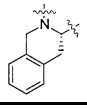
Compd	R^1	R^2	Cathepsin K % inh. (1 μM)	Cathepsin K IC_{50} (μM)
2	3,4,5-$(MeO)_3$Ph	*s*-Bu		1.2
23	3,4,5-$(MeO)_3$Ph	*iso*-Bu		1.5
24	3,4,5-$(MeO)_3$Ph	CH_2Ph	20	
4	Ph	*s*-Bu		0.23
25	Ph	*iso*-Bu		0.84
26	Ph	*iso*-Pr		0.78

Simple replacements and potential mimics (in particular, **15**) for the *E*-3-phenyl-3-propenyl *N*-substituent gave significantly less potent inhibitors (Table 2). Methyl substitution on the phenyl group in this fragment was generally well tolerated, whereas 4-chlorophenyl and pyridyl groups gave a loss in activity. Variation of the side-chain group on this fragment also gave somewhat decreased potency (Table 3). Finally, exploration of the central amino acid fragment demonstrated a strict requirement for the piperidine ring system found in **2** and **4** (Table 4). For example, contraction to a five-membered ring (**27**) or replacement with a tetrahydro-isoquinoline (**29**) gave substantially weaker inhibitors.[16]

In conclusion, an integrated exploratory medicinal and combinatorial chemistry approach was directed to efficiently investigate the amino ketone **2** as a lead inhibitor of cathepsin K. A series of analogues was prepared by both traditional and high-speed parallel synthesis techniques. The most potent analogues reached potencies in the 200–500 nM range, and solid structure–activity relationships were established. This structure class represents a novel and useful template for cathepsin K inhibitor design, and holds promise for further optimization.

Table 4. Variation of the amino acid fragment



Compd		Cathepsin K % inh. (1 μM)	Cathepsin K IC_{50}, (μM)
4			0.23
27[a]		15	
28		43	
29		29	

[a]3,4,5-$(MeO)_3PhCOCH_2$ derivative (analogue of **2**).

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15. All data reported herein reflect samples having high purity (>95%) and confirmed identity (LC/UV/MS); IC_{50} values reflect samples with further characterization (^{1}H NMR, MS). The cathepsin K assay conditions (pH 5.5, 100 mM sodium acetate, 5 mM EDTA, 20 mM cysteine) were the same as described elsewhere.[2] Test compounds in 50% DMSO were added in a dosed out, duplicate manner spanning a 2 log range (2.5% final DMSO concentration). Cleavage of an added substrate, benzyloxycarbonyl-Leu-Arg-7-amido-4-methyl-coumarin HCl (3 μM, 1/2 K_m), was initiated by adding recombinantly expressed cathepsin K at a concentration of 31 ng/mL. This 100 μL reaction was incubated with shaking for 45 min at room temperature before fluorescence (excitation/emission 360/460 nm) was measured with a Perkin–Elmer CytoFluor II plate reader. As a control measure, several α-bromomethyl ketones, as used in the synthesis of analogues of **2**, were tested in the cathepsin K assay; no inhibition was observed in these experiments at up to 5 μM concentration.
16. The backbone chiral carbons of **2** and other analogues in this article have *(S,S)* chirality. Other stereoisomers investigated were found to be significantly less effective inhibitors.