Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 581–588

BIOORGANIC & MEDICINAL CHEMISTRY

Potent Dipeptidylketone Inhibitors of the Cysteine Protease Cathepsin K

Robert W. Marquis,[a,*] Yu Ru,[a] Dennis S. Yamashita,[a] Hye-Ja Oh,[a] Jack Yen,[a] Scott K. Thompson,[a] Thomas J. Carr,[a] Mark A. Levy,[b,†] Thaddeus A. Tomaszek,[b] Carl F. Ijames,[c] Ward W. Smith,[d] Baoguang Zhao,[d] Cheryl A. Janson,[d] Sherin S. Abdel-Meguid,[d] Karla J. D'Alessio,[e] M. S. McQueney[e] and Daniel F. Veber[a]

[a]*Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA*
[b]*Department of Molecular Recognition, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA*
[c]*Department of Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA*
[d]*Department of Structural Biology, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA*
[e]*Department of Protein Biochemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA*

Received 4 December 1998

Abstract—Cathepsin K (EC 3.4.22.38) is a cysteine protease of the papain superfamily which is selectively expressed within the osteoclast. Several lines of evidence have pointed to the fact that this protease may play an important role in the degradation of the bone matrix. Potent and selective inhibitors of cathepsin K could be important therapeutic agents for the control of excessive bone resorption. Recently a series of peptide aldehydes have been shown to be potent inhibitors of cathepsin K. In an effort to design more selective and metabolically stable inhibitors of cathepsin K, a series of electronically attenuated alkoxymethylketones and thiomethylketones inhibitors have been synthesized. The X-ray co-crystal structure of one of these analogues in complex with cathepsin K shows the inhibitor binding in the primed side of the enzyme active site with a covalent interaction between the active site cysteine 25 and the carbonyl carbon of the inhibitor. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

The maintenance of healthy bone tissue is the result of a balance between the formation of the bone matrix by osteoblasts and the degradation of bone matrix by osteoclasts. Cathepsin K (EC 3.4.22.38), a cysteine protease of the papain superfamily, has been found to be selectively and abundantly expressed within the osteoclast.[1] This abundant and selective expression suggests that this protease may play a crucial role in the degradative phase of the remodeling of the bone matrix and may be an important target for therapeutic intervention for diseases associated with aberrant resorption of the bone matrix. Two lines of evidence have emerged which support the specialized role of cathepsin K in bone homeostasis. First, Gelb and co-workers have shown that mutations in humans of the gene encoding for the synthesis of this protein are associated with pycnodysostosis, a rare autosomal disorder of bone remodeling.[2] Short stature along with dense and brittle bones are among some of the clinical manifestations of this disease. Secondly, through the use of a cathepsin K antisense oligonucleotide, Yamamura and co-workers have determined that the inhibition of m-RNA to protein translation of this enzyme results in the inhibition of osteoclastic pit formation in a dose dependent manner.[3]

In a report from these laboratories, Votta and coworkers have shown peptidyl aldehydes such as **1** and **2** to be potent inhibitors of cathepsin K (Fig. 1).[4] Aldehyde **2** was shown to be an inhibitor of bone loss in the adjuvant-arthritic rat model and to inhibit the increase of calcium levels in the PTH infused thyroparathyroidectomized rat. These data suggest that aldehyde inhibitors of cathepsin K may be effective agents for slowing the rate of osteoclast mediated bone resorption associated with diseases such as osteoporosis. Despite the promising performance of inhibitors such as **2** in the aforementioned in vitro and in vivo models, their therapeutic utility is limited by metabolic liabilities which are commonly associated with aldehyde based inhibitors. As such, it is unlikely that these potent inhibitors

Key words: Cysteine protease; cathepsin K; enzyme inhibitor; alkoxymethylketone; thiomethylketone.
*Corresponding author. Tel.: +1-610-270-6570;
e-mail: robert_w_marquis@sbphrd.com
† Currently at Department of Preclinical Pharmokinetics, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA.

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PII: S0968-0896(99)00011-5

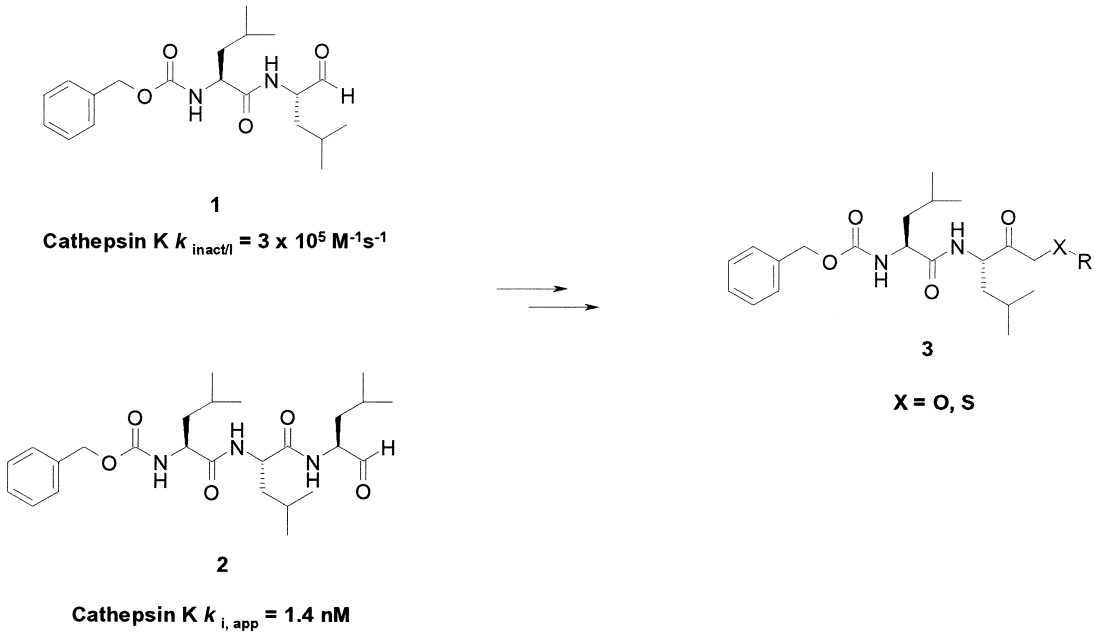


Figure 1. The evolution of the alkoxymethylketone and thiomethylketone cathepsin K inhibitors **3** from the aldehyde inhibitors **1** and **2**.

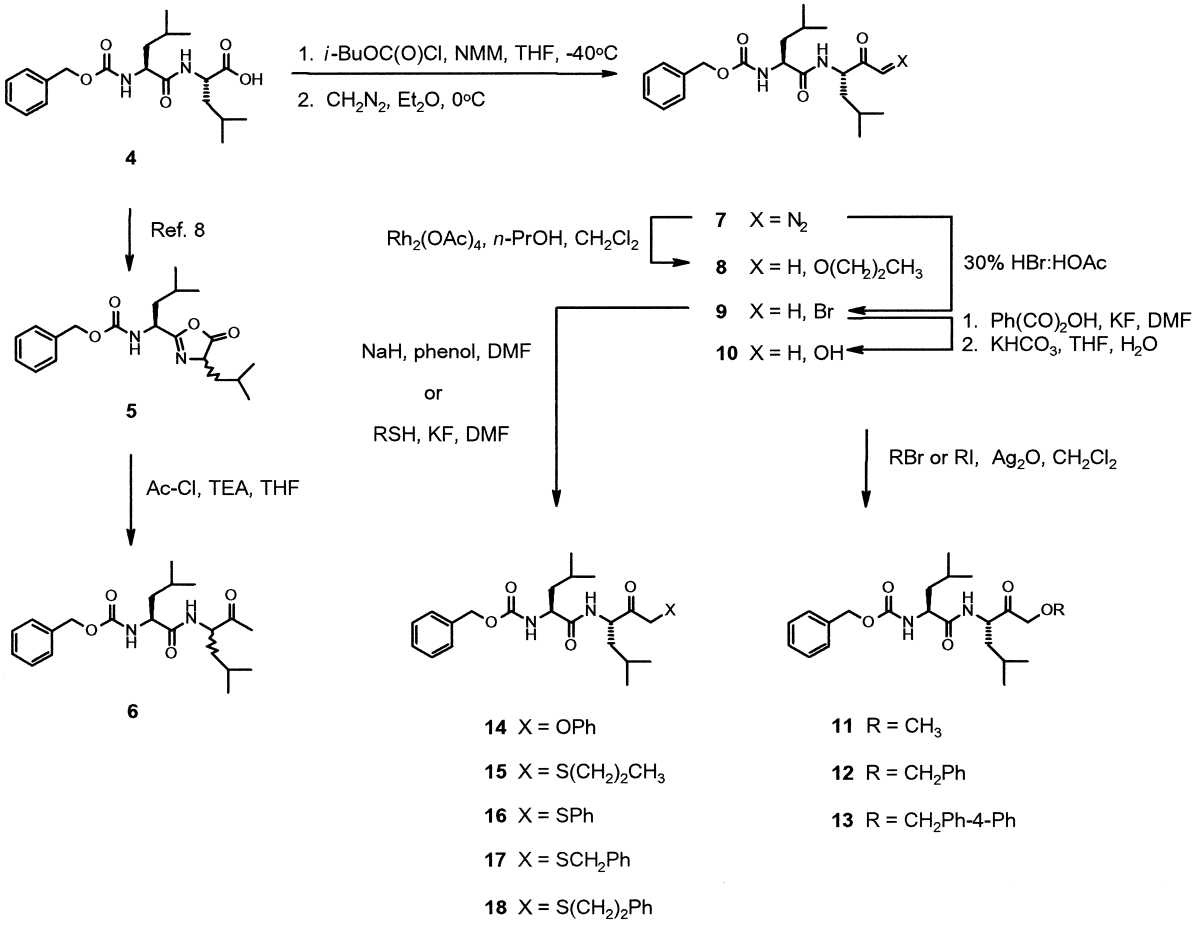
could serve as viable therapeutic agents in a disease target which would require chronic administration. In an effort to circumvent these liabilities a series of α-heteroatom substituted ketone dipeptides which would serve to attenuate the reactive nature of the aldehyde carbonyl moiety were synthesized (**3**, Fig. 1). Similar approaches have been employed for the design of potent inhibitors of the serine protease thrombin[5] and for the cysteine protease ICE.[6] In this paper the synthesis and SAR of a series of potent and selective α-heteroatom ketone dipeptide inhibitors of the cysteine protease cathepsin K which are based on the dipeptidyl aldehyde derivative **1** are detailed.

Results and Discussion

Synthesis and cathepsin K inhibition data of dipeptidyl alkoxymethyl and thiomethyl ketone inhibitors

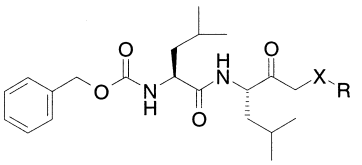
Initial SAR studies focused on determining the effects of the incorporation of both a ketone and various heteroatom ketones into the Z-Leu-Leu template of the aldeyde inhibitor **1**. The methyl ketone **6** was prepared via Dakin–West[7] reaction as shown in Scheme 1. Conversion of Cbz-Leu-Leu-OH (**4**) to the known oxazolinone **5**[8] followed by acylation with acetyl chloride, rearrangement and decarboxylation gave the methyl ketone analogue **6** as a mixture of diastereomers. The synthesis of a series of alkoxy and thiomethyl dipeptidyl ketones is also detailed in Scheme 1. Following the literature procedure,[5] Cbz-Leu-Leu-OH (**4**) was converted to the α-diazoketone **7** by formation of the mixed anhydride with *iso*-butylchloroformate followed by trapping with diazomethane. Conversion of diazoketone **7** to the *n*-propyl ether derivative **8** was effected with rhodium acetate and *n*-propyl alcohol.[9] Alternatively, treatment of **7** with 30% HBr in acetic acid at 0 °C provided the bromomethyl ketone **9**[10] which was converted to the hydroxymethyl ketone **10** by reaction with benzoylformic acid followed by saponification of the intermediate formate ester (not shown) with aqueous potassium hydrogen carbonate. Conversion of **10** to the methoxymethyl ketone **11** was accomplished by etherification with silver(I) oxide and methyl iodide in refluxing dichloromethane. Similarly, etherification of **10** with benzyl iodide or 4-(bromomethyl)biphenyl provided inhibitors **12** and **13**. The phenoxymethyl ketone analogue **14** was synthesized by reaction of **9** with sodium phenoxide in DMF. The thiomethyl ketones **15**, **16**, **17** and **18** were synthesized by treatment of **9** with the appropriate sulphide and KF in DMF at room temperature.

Cathepsin K inhibition data for the ketone inhibitors are shown in Table 1.[11] The Z-leucinyl-leucine methyl ketone derivative **6** inhibited cathepsin K with a $K_{i,app} = 500$ nM as a mixture of diastereomers. This loss in activity relative to the more reactive aldehyde derivative **3** was probably due to the reduced electrophilicity of ketones compared to that of aldehydes. Increasing the reactivity of the ketone inhibitors via the incorporation of an α-methoxy group gave analogue **11** which was significantly more active than the methyl ketone derivative **6**. This increased inhibition may be a reflection of the electron withdrawing nature of oxygen which would serve to increase the reactivity of the carbonyl carbon toward addition by the reactive site cysteine 25 of cathepsin K over that of the ketone analogue **6**. In a publication from these laboratories,[12] the X-ray co-crystal structure of inhibitor **11** with papain revealed that this inhibitor binds in a non-covalent manner with the phenyl moiety of the carbonylbenzyloxy group binding in the S3′ pocket of the enzyme.[13] This preference for primed side inhibitor binding was also seen in the X-ray co-crystal structure of the weakly time dependent *n*-propoxymethyl ketone inhibitor **8**



Scheme 1. Synthesis of alkoxymethyl and thiomethylketone inhibitors of cathepsin K.

Table 1. Cathepsin K inhibiton data for alkoxymethyl and thiomethylketone inhibitors



Compound	XR	$K_{i,app}$ (nM)	$k_{inact/I}$ ($M^{-1}s^{-1}$)
6*	H	500	—
8	$O(CH_2)_2CH_3$	—	4100
11	OCH_3	60	—
12	OCH_2Ph	600	—
13	OCH_2Ph-4-Ph	22	—
14	OPh	IC_{50} = 3.7 nM	
15	$S(CH_2)_2CH_3$	—	22,000
16	SPh	—	4500
17	SCH_2Ph	—	6500
18	$S(CH_2)_2Ph$	—	1650

*Analogue **6** was prepared as a mixture of diastereomers at the α-ketone center (see ref 8).

with cathepsin K (see Figure 2). The X-ray co-crystal structure suggests that inhibitor **8** forms a covalent bond with cysteine 25 of cathepsin K. This observation contrasts the noncovalent binding of the methoxymethyl ketone **11** seen in the papain co-crystal structure. The *n*-propyl group binds on the unprimed side of the active site barely penetrating the P2 pocket. The phenyl group of inhibitor **8** is seen bound in the P3′ pocket where it forms a π-stacking interaction with Trp184.

The phenoxymethyl ketone analogue **14** was a potent inhibitor with a $K_{i,app}$ = 8.0 nM. In addition to reversible addition of the active site cysteine to the ketone carbonyl of inhibitor **14**, one can envision an irreversible alkylation process analogous to that seen by Krantz and co-workers for the peptidyl (acyloxy)methyl ketones.[14] The phenoxide moiety of inhibitor **14** would be expected to be a poorer leaving group than the benzoate of the Krantz inhibitors therefore the reaction would be expected to be slower. That this does indeed occur has been confirmed by mass spectroscopic analysis. Upon incubation of the enzyme with a fivefold excess of inhibitor **14** for 2 h at pH 6 the mass spectrum showed equal amounts of unmodified enzyme and a mass corresponding to enzyme + inhibitor with the loss of phenol (374.4 Da). The ability of the phenoxymethyl ketone **14** to alkylate the enzyme was removed by the incorporation of a methylene unit which gave weakly active benzyl ether **12**, which under the time course of the kinetic determination did not show any time dependence. Interestingly, the 4-phenyl benzyl ether derivative **13** was a potent inhibitor with a $K_{i,app}$ = 22 nM. If one assumes that inhibitor **13** binds to cathepsin K in a manner analogous to compound **8** then it is difficult to

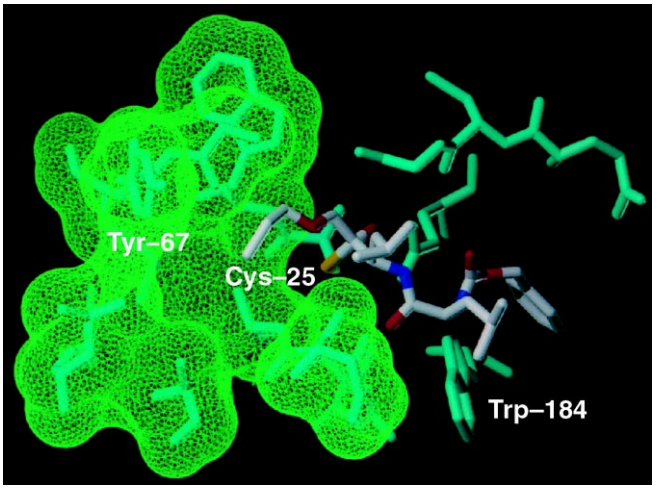


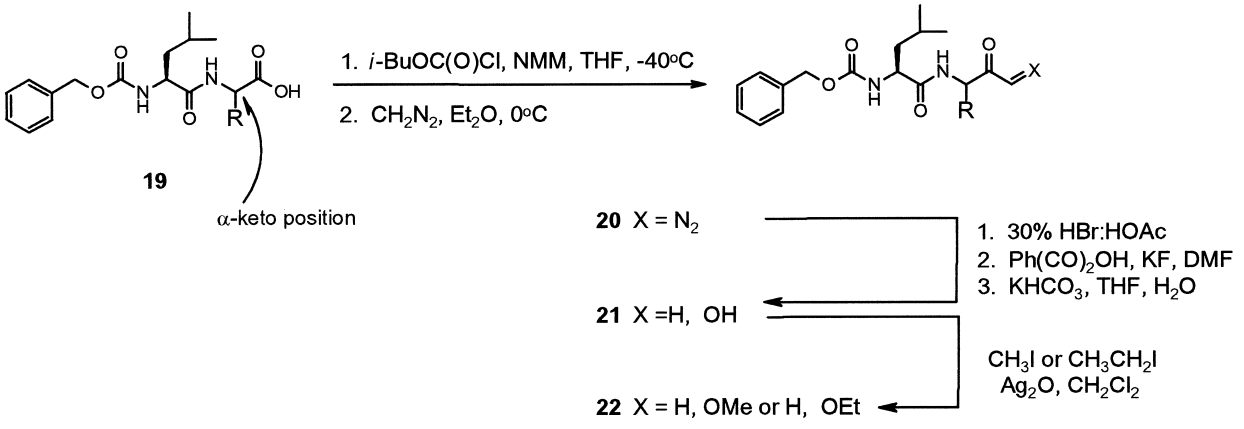
Figure 2. View of the X-ray co-crystal structure of inhibitor **8** (white) bound in the active site of cathepsin K (blue). Cysteine 25 (yellow) is attached covalently to the carbonyl group of the inhibitor forming a hemi-thioketal. The *n*-propyl ether slightly penetrates the P2 pocket of the enzyme. The phenyl group of the Cbz is bound in the P3′ pocket forming a π-stacking interaction with Trp184.

rationalize this increase in potency since no obvious binding interaction is available for the 4-phenyl group compared to analogue **12**. Alternatively, if inhibitor **13** has bound with the Cbz-Leu moiety on the unprimed side of the active site, then it would be possible for the biphenyl group to access both the S_2' and S_3' subsites and engage in a π–π interaction with Trp184. This type of interaction could account for the increased affinity of analogue **13** over that of benzyl ether **12**. The thiomethylketone inhibitors **15–18** displayed weak, time dependent inactivation of the enzyme. This weak time dependent activity may be a reflection of the decreased electrophilicity of the ketone carbonyl of these analogues upon substitution of the less electronegative sulfur for oxygen. The weak time dependent inhibition of the thiomethylketone inhibitors may also reflect the better leaving group capability of sulfur over oxygen. It is interesting to note that the sulfur analogue **15** is approximately five times more potent than its oxygen counterpart **8**. This increased activity may be a result of the longer C–S bond which would allow enhanced penetration of the *n*-propyl moiety into the P2 pocket. Other direct comparisons (**12** versus **17** and **14** versus **16**) are at best tenuous because time-independent inhibition has changed to time-dependent inhibition and no structural data to indicate the direction of binding for these particular inhibitors is available.

Synthesis and cathepsin K inhibition data of P1 modified dipeptidyl alkoxymethyl ketone inhibitors

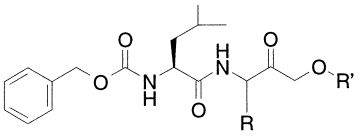
Prior to the availability of crystallography data, we sought to further improve the activity of the dipeptidyl α-alkoxyketones using inhibitor **11** as a basis for further SAR studies. Initially the effects of substitution at the then perceived P1 amino acid α to the keto group of the Z-Leu-Leu-alkoxymethylketone template was studied. The synthesis of these α modified keto compounds parallels that of derivative **11** and is outlined below in Scheme 2. The appropriate dipeptide acid **19** was converted to the diazomethyl ketone **20** by formation of the mixed anhydride followed by reaction with diazomethane. Treatment of the diazomethyl ketones with 30% HBr followed by displacement with benzoylformic acid and subsequent saponification of the formate ester provided the hydroxymethyl ketones **21**. Etherification of **21** was effected with either methyl or ethyl iodide and silver(I)-oxide. This five step sequence provided the P1 modified dipeptidyl alkoxymethyl ketones **23–30** (Table 2).

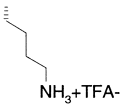
Inhibition data for the α-modified dipeptidyl alkoxymethylketones are shown in Table 2. Compound **23**, in which the chiral center formed by the α-isobutyl substitution of **11** has been inverted, resulted in a ninefold decrease in activity. Incorporation of glutamic acid gave a weakly time dependent inhibitor **24**. Alternatively, substitution with the basic lysine residue gave the potent inhibitor **25**. Incorporation of phenylalanine, norleucine, serine and glycine gave analogues **26**, **27**, **28** and **29** respectively. These modifications were not well tolerated. Substitution of the leucine of α-methoxy ketone **11** with L-alanine gave the active analogue **30**. Difficulties arise in the interpretation of this data because two distinct binding modes for various alkoxy ketone inhibitors bound to both papain and cathepsin K have been observed (covalent and non-covalent primed side binding). Based on our experience with peptide aldehydes we feel that it is reasonable to expect that a binding mode



Scheme 2. Sythesis of P2 modified alkoxymethylketone inhibitors.

Table 2. Cathepsin K inhibition data for P1 modified alkoxy ketone inhibitors



Compound	R	R′	$K_{i,app}$ (nM)	$k_{inact/I}$ ($M^{-1}\,s^{-1}$)
11		CH_3	60	—
23		CH_3	4800	—
24		CH_2CH_3	—	850
25		CH_3	110	—
26		CH_3	1200	—
27		CH_3	490	—
28		CH_3	790	—
29		CH_3	1300	—
30		CH_3	150	—

with the Cbz-Leu-Leu on the unprimed side of the active site cysteine may occur for some analogues even though we have not yet detected this mode of binding by X-ray analysis.

Cathepsin K, B and L selectivities

Selectivity data for four of the more potent analogues versus cathepsins K, B and L is shown in Table 3.[15] The overall selectivity profile of these analogues for cathepsin K is quite good with the exception of analogue **13** which is also a potent inhibitor of cathepsin L. The evaluation of specificity profiles is obviously problematic owing to the potential for different binding modes. For example, inhibitor **13** may bind in one mode to cathepsin K and in an alternate mode to cathepsin L.

Conclusions

In this paper we have detailed a series of alkoxymethylketones which are potent inhibitors of the cysteine protease cathepsin K. Relative to the previously described aldehyde inhibitors, the incorporation of substituted alkoxymethyl and thiomethyl groups provided a series of analogues with reduced potency versus cathepsin K. Substitution in the position α to the ketone of the inhibitors has revealed a lack of specific recognition at this site as seen in the activities of the alanine and lysine derivatives **30** and **25** respectively. This lack of recognition may be due to the ability of these, and possibly all of the inhibitors described in this paper, to bind in different modes within the active site of cathepsin K. In this series of ketone inhibitors, these multiple binding modes make the interpretation of SAR data difficult for all analogues for which there is no X-ray co-crystal structure data available.

Experimental

Crystallization of the complex of cathepsin K with inhibitor 8. Crystals of mature activated cathepsin K complexed with the inhibitor grew from a protein solution

Table 3. Selectivity of alkoxymethyl ketone inhibitors versus cathepsins K, B and L

Compound	Cat K $K_{i,app}$ (nM)	Cat B $K_{i,app}$ (nM)	Cat L $K_{i,app}$ (nM)
11	60	> 1000	513
13	22	1310	63
25	110	2700	1700
31	55	$K_{obs/I} = 57$	$K_{obs/I} = 135$

of 10 mg/mL mixed with equal volume of a precipitant solution of 30% PEG 8000 in 0.1 M Na citrate, pH 4.5 containing 0.2 M Li_2SO_4. Crystals of the complex are tetragonal, space group $P4_32_12$, with cell constants of a = 57.6 Å, and c = 131.2 Å. The crystals contain one molecule in the asymmetric unit. X-ray diffraction data were measured from a single crystal using a Siemens 2-D position-sensitive detector on a Siemens rotating anode generator operating at 5 KW. The structure was determined at 2.5 Å resolution by molecular replacement as implemented in X-PLOR. The structure determination was carried out using X-PLOR with a model consisting of all protein atoms from the previously determined cathepsin K-E64 structure.[16] The model was refined by rigid-body refinement using X-PLOR, and the resulting phases were used to calculate Fourier maps with coefficients $|F_o\text{-}F_c|$ and $|2F_o\text{-}F_c|$, into which the atomic model of the inhibitor was built using the molecular graphics program FRODO. Conventional positional refinement was used to refine the structure during model building. Several cycles of map fitting and refinement were carried out to a final R_c of 0.245.

Incubation experiments with inhibitor 14 and cathepsin K. Cathepsin K, 27 uM in MES buffer at pH 6.0 was incubated with inhibitor **14** by adding 6.5 nmol of **14** in 20% DMSO:water to 1.3 nmol protein so that the final protein concentration was 13.5 uM and the DMSO concentration was 10%. After vortexing, the solution was allowed to stand at room temperature for 2 h before being loaded onto a peptide trap cartridge (Michrom), washed with 40 uL A, and back-eluted onto a 1×40 mm Aquasil C18 reverse phase HPLC column (Keystone) for LC/MS analysis with a gradient from 5% to 95% B in 10 min at a flow rate of 60 uL/min. Solvent A was 0.02% TFA:water, and B was 0.018% TFA:90% MeCN:10% water. The mass spectrometer (Sciex API III) was scanned from m/z 800 to 1800 once every 3.95 s.

Experimental procedures

Nuclear magnetic resonance spectra were recorded at either 250 or 400 MHz using, respectively, a Bruker AM 250 or Bruker AC 400 spectrometer. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. Mass spectra were taken on PE Sciex API III or Micromass platform API-only instruments, using electrospray (ES) ionization techniques. Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Flash chromatography was carried out on E. Merck Kieselgel 60 (230–400 mesh) silica gel.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-5-methyl-2-hexanone (6). Acetyl chloride (43 μL, 0.61 mmol) was added dropwise to a solution of 1-(4-isobutyl-5-oxo-4,5-dihydro-oxazol-2-yl)-3-methyl-butyl-carbamic acid benzyl ester[8] (**5**) (200 mg, 0.56 mmol) and triethylamine (155 μL, 1.11 mmol) in THF (1.0 mL) at rt and was stirred overnight. The reaction mixture was diluted with EtOAc (20 mL) and extracted with H_2O (5 mL); the combined organics were dried with magnesium sulfate, filtered, then concentrated in vacuo. The crude product was then dissolved in 1:1 AcOH:pyridine (1.0 mL) and was heated to 110 °C for 1 h. The reaction mixture was cooled to rt, diluted with EtOAc (50 mL) and extracted with 1N HCl (3×20 mL); the combined organics were dried with magnesium sulfate, filtered, concentrated in vacuo and chromatographed (silica gel, 1–5% MeOH:CH_2Cl_2) to yield the title compounds as a mixture of diastereomers of unknown relative configurations (44 mg, 22%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35–7.27 (m, 5H), 6.55–6.45 (m, 1H), 5.12 (s, 2H), 4.27–4.17 (m, 1H), 4.20–4.06 (m, 1H), 2.18 (s, 3H), 1.70–1.35 (m, 6H), 0.99–0.94 (m, 12H).

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-bromo-5-methyl-2-hexanone (9). 1-Methyl-3-nitro-1-nitrosoguanidine (5.9 g) in ether (200 mL) was cooled to 0 °C. 40% potassium hydroxide is added slowly and the diazomethane was allowed to collect in the ether solution for 30 min at 0 °C. Cbz-L-leucinyl-L-leucine (4.0 gm, 10.6 mmol) was stirred in tetrahydrofuran at −40 °C. *N*-Methyl morpholine (1.07 g, 10.6 mmol, 1.16 mL) and isobutyl chloroformate (1.45 g, 10.6 mmol, 1.38 mL) are added. The mixture was stirred at −40 °C for 15 min and then filtered into a cold flask to remove precipitated salts. To the filtered solution was added an excess of the previously prepared diazomethane solution and the mixture was allowed to stand at 0 °C for 16 h. An excess of 30% HBr in acetic acid was added at 0 °C and the reaction was allowed to stir until complete consumption of the α-diazoketone was observed (TLC analysis). The solution was then washed successively with 1.0 N citric acid, saturated aqueous sodium bicarbonate (carefully), and brine. The solution was dried over sodium sulfate, filtered, and evaporated to give the **9** as a clear solid (4.10 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 6.51 (d, 1H), 5.15 (d, 1H), 5.10 (s, 2H), 4.78 (m, 1H), 4.20 (m, 1H), 4.04 (dd, 2H), 1.63 (m, 6H), 0.93 (m, 12H). IR (thin film): 1716, 1700, 1670 cm^{-1}.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-(1-propoxy)-5-methyl-2-hexanone (8). (3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-diazo-5-methyl-2-hexanone (**7**) (150 mg, 0.37 mmol) was dissolved in 1-propanol (2.5 mL) and rhodium acetate (2 mg) was added and the reaction was stirred at rt for 2 h. The reaction mixture was concentrated and chromatographed (silica gel, 20% EtOAc:hexanes) to yield the *n*-propoxyketone **8** as a white solid (59 mg, 37%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4–7.15 (m, 5H), 6.4 (d, 1H), 5.5–4.0 (m, 13H), 5.01 (s, 2H), 3.4 (m, 2H), 1.6 (m, 6H), 0.9 (m, 12H) MS(ES) $M + H^+ = 435$, $M + NH_4^+ = 452$, $2M + H^+ = 869.6$.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-hydroxy-5-methyl-2-hexanone (10). The bromomethylketone **9**

(1.3 g, 2.9 mmol) and benzoylformic acid (0.515 g, 3.5 mmol) were stirred in dimethylformamide (10 mL) and treated with potassium fluoride (0.25 g, 4.4 mmol). The mixture was stirred for 16 h at room temperature and partitioned between ethyl acetate and water. The organic layer was dried over sodium sulfate, filtered, and evaporated to give a residue which was chromatographed (silica gel, 25% ethyl acetate in hexane) to give the formate ester as a white solid (1.04 g). The formate ester (1.02 g, 1.9 mmol) was stirred vigorously in a mixture of tetrahydrofuran (100 mL) and 1.0 M potassium bicarbonate (100 mL) at room temperature for 18 h. The layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, dried over sodium sulfate, filtered, and evaporated to give **10** as a white solid (0.768 g). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35–7.31 (m, 5H), 6.74 (d, J = 7.6 Hz, 1H), 5.35 (d, J = 7.6 Hz, 1H), 5.10 (s, 2H), 4.7–4.6 (m, 1H), 4.36 (s, 2H), 4.25–4.18 (m, 1H), 3.2 (br s, 1H), 1.7–1.43 (m, 6H), 0.93 (d, J = 6.0 Hz, 3H), 0.91 (2d, J = 6.0 Hz, 6H), 0.90 (d, J = 6.0 Hz, 3H); IR (thin film): 3307, 1716, 1699, 1652 cm^{-1}; MS(ES) $M+H^+$ = 393.2, $2M+H^+$ = 785.4.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-methoxy-5-methyl-2-hexanone (11). Methyl iodide (0.32 mL, 5.1 mmol) was added to a solution of the α-hydroxyketone **10** (0.4 g, 1.0 mmol) and silver(I) oxide (0.46 g, 2.0 mmol) in methylene chloride (30 mL), and the reaction mixture was refluxed for 7 h. Additional methyl iodide (1.0 mL, 15.5 mmol) was added and the reaction mixture was stirred at rt over 2 days. The reaction mixture was filtered, concentrated in vacuo, then chromatographed (silica gel, 25% EtOAc:hexane) to give the title compound as a white solid (0.17 g, 42%): ^{1}H NMR ($CDCl_3$, 400 MHz): ^{1}H NMR ($CDCl_3$, 360 MHz): δ 7.35–7.24 (m, 5H), 6.35–6.33 (d, 1H, J = 7.74 Hz), 5.06 (s, 2H), 4.81–4.75 (m, 1H), 4.16 (br s, 1H), 4.12 (AB, J_{AB} = 10.3, $\Delta\delta_{AB}$ = 0.097, 2H), 3.40 (s, 3H), 1.60–1.30 (m, 6H), 0.92 (d, 9H, J = 6.2 Hz), 0.90 (d, 3H, J = 6.47 Hz); IR (KBr): 3301, 1731, 1694, 1646 cm^{-1}; MS(ES) $M+H^+$ = 407.5, M + Na = 429.5, $2M+H^+$. Anal. calcd for $C_{22}H_{34}N_2O_5$: C, 65.00; H, 8.43; N, 6.89. Found: C, 65.09; H, 8.61; N, 6.93.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-benzoyloxy-5-methyl-2-hexanone (12). The benzyl ether analogue **12** was synthesized in the same manner as that described for the synthesis of **11** except substituting benzyliodide for methyliodide. Analogue **12** was prepared as a white solid (0.17 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (m, 5H), 6.60 (d, 1H), 5.23 (d, 1H), 5.01 (s, 2H), 4.79 (m, 1H), 4.51 (d, 2H), 4.08 (m, 3H), 1.53 (m, 6H), 0.82 (m, 12H); MS(ES) $M+H^+$ = 483.2, $M+NH_4^+$ = 500.2, $2M+H^+$ = 965.4.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-5-methyl-1-phenoxy-2-hexanone (14). Phenol (0.138 gm, 1.5 mmol) and sodium hydride (0.036 gm, 1.5 mmol) were stirred in dimethylformamide at −20 °C under an argon atmosphere for 15 min and bromomethylketone **9** (0.66 g, 1.5 mmol) was added. The reaction was stirred at −20 °C for 30 min and it was warmed to room temperature and stirred for an additional 2 h. The mixture was neutralized with 0.3M potassium bisulfate and the solvent removed in vacuo. The residue obtained was partitioned between ethyl acetate and water and the organic extracts were washed with brine. The solution was dried over magnesium sulfate, filtered, and evaporated to give a residue which was chromatographed (silica gel, 25% ethyl acetate in hexane) to give **13** as a white solid (0.48 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 7H), 6.92 (t, 1H), 6.80 (d, 2H), 6.42 (d, 1H), 5.13 (d, 1H), 4.98 (s, 2H), 4.91 (m, 1H), 4.60 (d, 2H), 4.12 (m, 1H), 1.58 (m, 4H), 1.39 (m, 2H), 0.84 (m, 12H); MS(ES) $M+H^+$ = 469.2, $2M+H^+$ = 937.6.

(3*S*)-3-[(*N*-Benzyloxycarbonyl)-L-leucinyl]amino-1-(phenylthio)-5-methyl-2-hexanone (16). Thiophenol (21 mg, 20 μL, 0.190 mmol) was added to a solution of **9** (70 mg, 0.154 mmol) in DMF (0.5 mL) at rt. Then solid potassium fluoride (10 mg, 0.172 mmol) was added, and the reaction mixture was stirred overnight. Concentration of the reaction mixture in vacuo followed by column chromatography (silica gel, 25% EtOAc:hexane) of the residue gave the title compound as a white solid (52 mg, 67%): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38–7.22 (m, 10H), 6.55 (br d, J = 7.7 Hz, 1H), 5.24 (br d, J = 8.2 Hz, 1H), 5.12 (s, 2H), 4.85–4.78 (m, 1H), 4.22–4.15 (m, 1H), 3.78 (s, 2H), 1.70–1.40 (m, 6H), 0.95 (d, J = 6.4 Hz, 6H), 0.90 (d, J = 6.4 Hz, 6H). MS(ES) $M+H^+$ = 485.5, $M+Na^+$ = 507.5, $2M+Na^+$ = 991.7.

Acknowledgements

The authors would like to acknowledge Dr. Michael L. Moore for his critical review of this manuscript.

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