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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3891–3895
Bioorganic & Medicinal Chemistry Letters

Ketoheterocycle-based inhibitors of cathepsin K: A novel entry into the synthesis of peptidic ketoheterocycles

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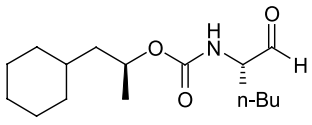
Received 5 May 2005; revised 23 May 2005; accepted 25 May 2005
Available online 1 July 2005

Abstract—Ketoheterocyclic inhibitors of cathepsin K have been disclosed. SAR of potency enhancing P^2–P^3 groups coupled with ketoheterocyclic warheads to provide cathepsin K inhibitors have been described. In addition, a novel route to access α-ketothiazoles using a key thioamide functionality has been disclosed. The mild method employed allows for the presence of diverse functional groups, such as amide and carbamate functionalities, commonly found in protease inhibitors that have peptidomimetic scaffolds. This new method should provide a quick entry into functionally diverse protease inhibitors.

Bone remodeling is a dynamic process wherein bone resorption by osteoclasts is balanced by bone rebuilding by osteoblasts. A shift in this equilibrium favoring bone resorption results in deterioration of the skeletal microarchitecture, resulting in osteoporosis.[1] Bone resorption involves dissolution of the mineral portion in a highly acidic environment, followed by proteolytic degradation of the organic matrix. Cathepsin K, a cysteine protease highly expressed in osteoclasts, has been shown to be the primary proteolytic enzyme that is responsible for this process.[2] Several lines of evidence including suppression of osteoclast bone resorption by a cathepsin K antisense oligonucleotide,[3] osteopetrotic phenotypes of inactivating mutations in the gene expressing cathepsin K,[4–6] and bone mass in mice deficient in cathepsin K point to the importance of this enzyme in the development of osteoporosis.[7,8] There have been several reports that cover the field of cathepsin K inhibitors.[9–11]

In the design of cathepsin K inhibitors, a reversible cathepsin K inhibitor was desired. In addition to aldehyde,[12,13] cyanamide,[14] and ketoamide-based inhibitors,[15–20] efforts from these laboratories were also focused on using ketoheterocyclic-based inhibitors for inhibition of cathepsin K. The use of ketoheterocycles as reversible inhibitors of serine proteases is well-documented in the literature.[21,22] Work disclosing the use of ketoheterocycles, as inhibitors of cysteine protease, has also been described.[23,24] Efforts in these laboratories were directed toward using cysteine protease recognition motifs designed in these laboratories, such as P^1/P^2 groups in aldehyde-based inhibitor **1** (IC_{50} = 2.7 nM).[12,13] The stability of ketoheterocyclics as an alternate warhead over aldehyde-based inhibitors made this approach attractive.[13] The present work also describes a new synthetic route into peptidic ketoheterocyclics using a key thioamide functionality as an handle to access various heterocyclic moieties from the same precursor. This new method is exemplified by the synthesis of ketothiazoles.



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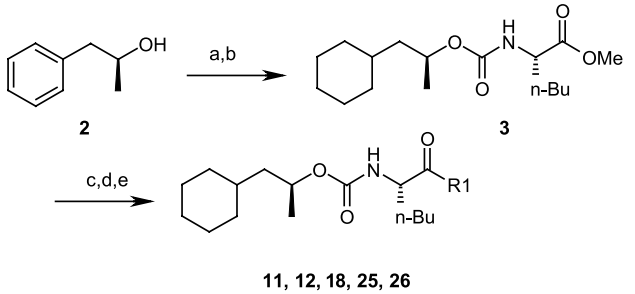
doi:10.1016/j.bmcl.2005.05.091

The ketoheterocycles **11**–**18** were accessed starting from the alcohol **2**. Rhodium-catalyzed hydrogenation of the phenyl moiety, followed by treatment with phosgene, afforded the chloroformate, and subsequent addition of the amino ester provided carbamate **3**. Hydrolysis of the ester to the acid, followed by amidation and subsequent treatment with aryl/heteroaryl lithiums, afforded the target compounds **11**, **12**, **18**, **25**, and **26** (see Scheme 1). Synthesis of oxadiazole- and thiazole-substituted ketoheterocycles required the synthesis of the key nitrile intermediate **6**. The nitrile was synthesized from the BocNle-OMe wherein, hydrolysis of the ester, followed by subsequent reduction of the in situ generated anhydride with sodium borohydride, afforded the desired alcohol. Oxidation of the alcohol with sulfur trioxide pyridine complex and subsequent treatment of the aldehyde **5** with the silyl cyanide gave nitrile **6**. Treatment of the nitrile **6** with hydroxylamine in the presence of sodium acetate, followed by subsequent reaction with acetic anhydride, afforded the silyl protected oxadiazole. Removal of the silyl-protecting group with fluoride, followed by oxidation of the alcohol, afforded oxadiazole **17**.

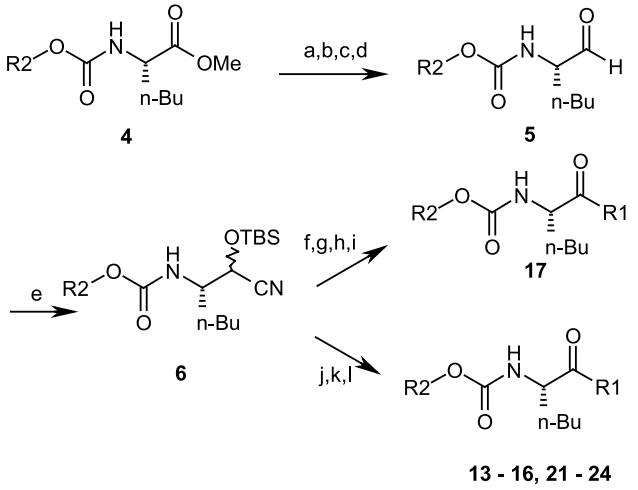
The ketothiazoles were synthesized from the same nitrile intermediate **6** by treatment with diethyldithiophosphate in refluxing tetrahydrofuran to afford the thioamide, which on reaction with various α-haloketones yielded the protected thiazoles. Cleavage of the silyl ether and the amine protecting group with trifluoroacetic acid afforded the amino alcohol **7**. The amino alcohol was coupled to various chloroformates to afford the carbamates. Oxidation of the secondary alcohol with sodium hypochlorite gave the desired ketothiazoles **13**–**16**, **21**–**24** (see Scheme 2).

The ketothiazole containing amide functionality was synthesized, as shown in Scheme 3 from **7**. The amino alcohol **7** was coupled to a commercially available acid to afford the amide, which was then oxidized to afford the desired ketothiazole **27**.

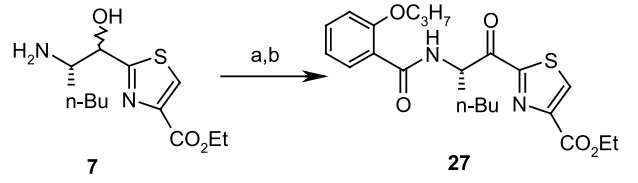
The dipeptide containing ketothiazoles were synthesized, as shown in Scheme 4. Coupling of the amino alcohol **8** with Cbz-Leu, followed by oxidation and silylcyanohydrin formation, gave the nitrile **9**. Conversion of the nitrile **9** to a thioamide with diethyldithiophosphate, followed by treatment with ethyl α-bromopyruvate, afforded the silyl-protected thiazole. Deprotection of the alcohol, followed by oxidation, gave the final product **10**.



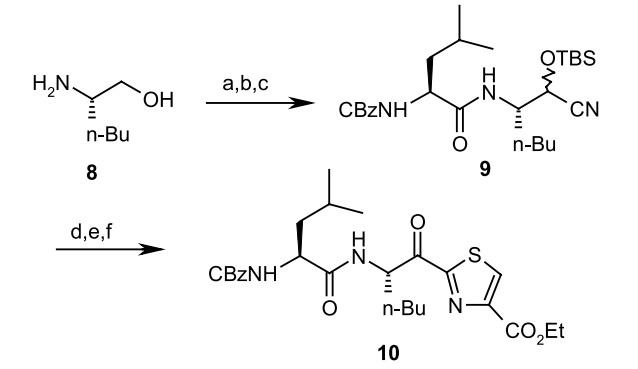
Scheme 1. Reagents and conditions: (a) H_2, $RhCl_3 \cdot H_2O$, Aliquat 336, 90%; (b) 1.93 M $COCl_2$ in PhMe, THF, 0 °C to rt; Nle-OMe, $^iPr_2NEt$; (c) LiOH, THF, 87%; (d) HN(MeO)(Me), EDC, HOBT, DCM, 70%; (e) aryl/heteroaryl lithiums, THF, −78 °C, 30–64%.



Scheme 2. Reagents and conditions: (a) LiOH, THF, 96%; (b) ^{i}PrOCOCl, THF, NMM; (c) $NaBH_4$, THF; (d) $SO_3 \cdot Py$, DCM; (e) TBSCN, DCM, 18-crown-6, KCN, 84%; (f) NH_2OH, AcONa; (g) Ac_2O, toluene, reflux, 65%; (h) TBAF, THF, 87%; (i) TEMPO, KBr, NaOCl, DCM/H_2O, 72%; (j) diethyldithiophosphate, THF, reflux, 78%; α-haloketones, NMM, CH_3CN, 69%; chloroformate, $^iPr_2NEt$, THF; (k) TFA, DCM, 72%; (l) TEMPO, KBr, NaOCl, DCM/H_2O, 56%.



Scheme 3. Reagents and conditions: (a) 4 N HCl in Dioxane 99%; subst. benzoic acids, EDC, HOBT, NMM, 86%; (b) TEMPO, KBr, NaOCl, DCM/H_2O, 53%.



Scheme 4. Reagents and conditions: (a) EDC, HOBT, NMM, Cbz-Leu, 89%; (b) DMSO, $(COCl)_2$, DCM, −65 °C, 78%; (c) TBSCN, DCM, 18-crown-6, KCN, 81%; (d) diethyldithiophosphate, THF, reflux, 72%; (e) ethylbromopyruvate, CH_3CN, 68%; (f) TBAF, THF; TEMPO, KBr, NaOCl, DCM/H_2O, 50%.

Aryl and heteroaryl groups directly attached to a ketone increase the electrophilicity of the carbonyl carbon to attack by active site cysteine nucleophiles to form hemithioketals. In addition to activating the ketone, these moieties can form binding interactions with the active site trough and/or S′ subsites. Since there were no known ketoheterocyclic inhibitors of cathepsin K disclosed at the time of this work, a model of compound **11**, docked into the active site, formed the basis for SAR developed in the present work (Fig. 1). This binding mode was found to be consistent with the X-ray structures that have been recently reported for other ketoheterocyclic inhibitors of cathepsin K.[24] The α-keto moiety of compound **11** and the active site thiol of ^{25}Cys of the enzyme form a covalent hemithioketal intermediate, which is consistent with previously reported structures. The hydroxyl of the hemithioketal group occupies the oxy-anion hole and is stabilized by hydrogen bonds to the side chain of ^{19}Gln and the backbone NH of ^{25}Cys, similar interactions are seen in published aldehyde and ketone cathepsin K structures. In addition, two more hydrogen bonds to the peptide backbone of the enzyme are suggested from the model that stabilize the inhibitor in the active site; the carbamate carbonyl accepts a hydrogen bond from the NH of ^{66}Gly, while the carbamate NH donates a hydrogen bond to the backbone carbonyl of ^{161}Asn. Further, the S^1 wall formed from ^{23}Gly, ^{24}Ser, ^{64}Gly, and ^{65}Gly provide van der Waals interactions with one face of the P^1 *n*-butyl group of the inhibitor. The methyl group lies in the deep lipophilic S^2 pocket composed of ^{67}Tyr, ^{68}Met, ^{134}Ala, ^{163}Ala, and ^{209}Leu. The cyclohexyl moiety does not extend into the S^3 pocket composed primarily of ^{61}Asp and ^{67}Tyr (shared with S^2), but forms a van der Waals interaction with one face of the phenolic ring of ^{67}Tyr. It was envisaged that heterocycles might accept a hydrogen bond from the indole NH of ^{184}Trp or possibly form a π-stacking interaction with the indole ring. As shown in Table 1, a number of different ketoheterocycles containing the P^2/P^3 piece from aldehyde **1** were prepared and tested for cathepsin K inhibition. The naked thiazole **11** was found to be a moderate inhibitor of cathepsin K (IC_{50} = 3100 nM). Addition of a 4-phen-

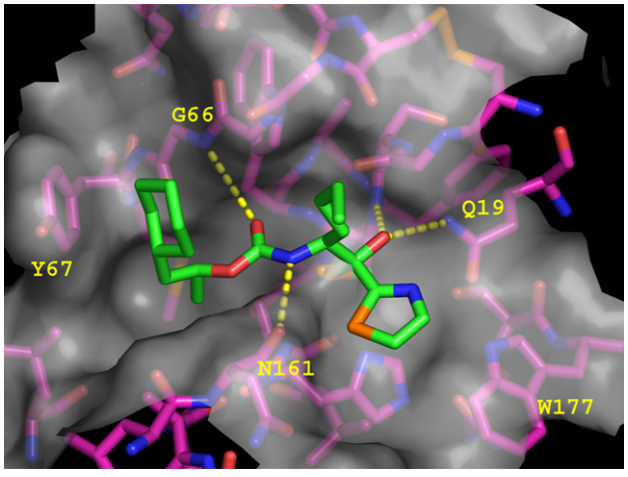
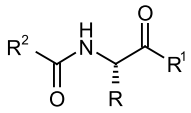
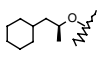
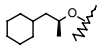
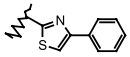
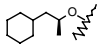
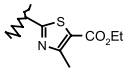
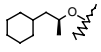
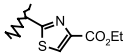
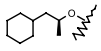
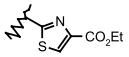
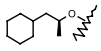
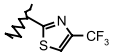
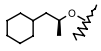
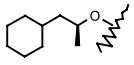
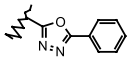
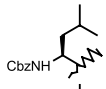
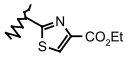
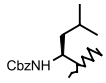
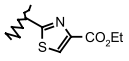
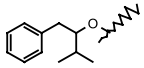
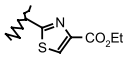
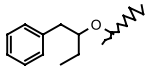
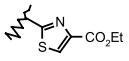
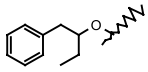
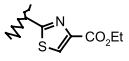
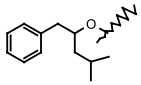
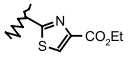
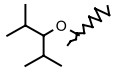
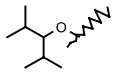
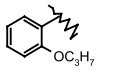
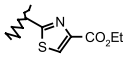


Figure 1. Active site of model structure **11** complexed with cathepsin K. The cathepsin K carbons are colored magenta, with the inhibitor **11** carbons colored cyan. The semitransparent white surface represents the molecular surface and possible hydrogen bonds are represented as yellow dashed lines. This figure was generated using PYMOL version 0.97 (Delano Scientific, www.pymaol.org).

Table 1. Inhibition of human cathepsin K by ketoheterocycles



Compound	R^2	R	R^1	IC_{50} (nM)[a]
11		*n*-Bu		3,100
12		*n*-Bu		7,100
13		*n*-Bu		10%[b]
14		*n*-Bu		230
15		H		0%[b]
16		*n*-Bu		13,000
17		nBu		8,100
18		*n*-Bu		4,000
19		*n*-Bu		13,000
20		H		20%[b]
21		*n*-Bu		28%[b]
22		*n*-Bu		>13,000
23		H		0%[b]
24		*n*-Bu		31%[b]
25		*n*-Bu		2%[b]
26		*n*-Bu		1,900
27				>13,000

[a] Inhibition of recombinant human cathepsin K activity in a fluorescence assay using 10 μM Cbz-Phe-Arg-AMC as a substrate in 100 mM NaOAc, 10 mM DTT, and 120 mM NaCl, pH 5.5. The IC_{50} values are means of two or three inhibition assays, individual data points in each experiment were within a 3-fold range of each other.
[b] Inhibition at 12.6 μM.

yl group as in analog **12**, that could potentially form a favorable hydrophobic interaction with ^{184}Trp, resulted in no additional gain in potency. Incorporation of electron-withdrawing groups to increase the electrophilicity of the carbonyl group was then studied. Addition of an ester to the 5-position of the thiazole, analog **13**, resulted in a decrease in inhibitory activity. In contrast, the 4-substituted thiazole **14** (IC_{50} = 230 nM) gave a 10-fold boost in potency.

Replacement of the 4-substituted ester with an electronegative trifluoromethyl substituent as in analog **16** resulted in a loss of potency. Thus, the enhanced activity of the ester **14** may be partly attributed to its ability to accept a hydrogen bond, possibly through a bridging water molecule versus the enhanced electrophilicity of the carbonyl thiol reactive group. Alternately, the trifluoromethyl group may not be accommodated by the enzyme active site. Analog **15** lacks norleucine-derived P^1 substituent. In the aldehyde series, 100-fold decrease in inhibitory activity was seen when the P^1 moiety was changed from *n*-butyl to hydrogen.[12] The loss of conformational bias combined with the loss of S^1 subsite van der Waals interactions also led to a loss of activity in the ketoheteroaryl series (analogs **15**, **20**, and **23**). Replacement of the thiazole with oxadiazoles, as in examples **17** and **18**, resulted in analogs that were as potent as the thiazoles. As in the thiazole **12**, no additional gain in potency was observed by having a phenyl substituent (**18**).

To increase the diversity of these inhibitors, various aldehyde inhibitor-derived P^1, P^2 and P^3 groups were used, keeping the ketoheterocycle group constant, as in examples **19**–**24**, **27**. The dipeptide, as well as the other P^1, P^2, and P^3 groups, were all found to be less active against cathepsin K. Since the di-*iso*-propyl moiety was known to enhance potency against cathepsin K,[10,16] a few ketoaryl/heteroaryl analogs were also made, as in examples **25** and **26**. Interestingly, the phenyl analog **29** was found to be inactive, whereas the thiophene isostere **26** was a low micromolar inhibitor of cathepsin K.

The most active thiazole analog **14** was screened for selectivity against a select set of cysteine proteases, as shown in Table 2. Analog **14** was found to be over 40-fold selective over cathepsins L, V, H, and B.

Table 2. Inhibitory potencies (IC_{50}) versus human cathepsins K, L, V, S, and B

Compound	K (IC_{50} nM)	L/K[a]	V/K[b]	S/K[c]	B/K[d]
14	230	>40	>40	>40	>40

[a] Inhibition of recombinant human cathepsin L activity in a fluorescence assay using 5 μM Cbz-Phe-Arg-AMC as a substrate in 100 mM NaOAc, 10 mM DTT, and 120 mM NaCl, pH 5.5.

[b] Inhibition of recombinant human cathepsin V activity in a fluorescence assay using 2 μM Cbz-Phe-Arg-AMC as a substrate in 100 mM NaOAc, 10 mM DTT, and 120 mM NaCl, pH 5.5.

[c] Inhibition of recombinant human cathepsin S activity in a fluorescence assay using 10 μM Cbz-Val-Val-Arg-AMC as a substrate in 100 mM NaOAc, 10 mM DTT, and 120 mM NaCl, pH 5.5.

[d] Inhibition of recombinant human cathepsin B activity in a fluorescence assay using 10 μM Cbz-Phe-Arg-AMC as a substrate in 100 mM NaOAc, 10 mM DTT, and 120 mM NaCl, pH 5.5. The IC_{50} values are means of two or three inhibition assays, individual data points in each experiment within a 2-fold range of each other.

In conclusion, the present work describes the use of a ketoheterocyclic warhead for the inhibition of cysteine protease, cathepsin K. In addition to the use of standard protocols for their synthesis, a novel route using the thioamide functionality as a handle to get into ketothiazoles has been described. Access to other heterocyclic groups from the thioamide moiety using known methods (e.g., S-alkylation, followed by subsequent displacement with appropriate synthons) can be easily envisaged. The use of this mild method allows for the presence of diverse functional groups, such as amide and carbamate functionalities, commonly found in protease inhibitors that have peptidomimetic scaffolds. In addition, this method allows for incorporation of peptidomimetic scaffolds extending into the S′ region of proteases.

Acknowledgments

The authors thank Randy Rutkowski, Robert Johnson, and Peter Kitrinos for analytical support.

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