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Primary amides as selective inhibitors of cathepsin K

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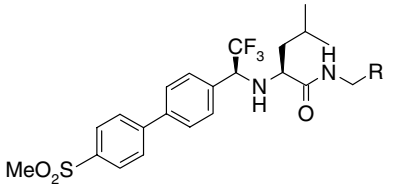
Abstract—The nitrile warhead used in a series of cathepsin K inhibitors can be replaced by a less electrophilic primary amide. The accompanying loss of potency can be partially recovered by introducing a substituent α to the amide. The potency gain resulting from this addition is not achieved with the nitrile derivatives due to a different geometry of the cysteine adduct in the enzyme active site. This study led to the identification of the primary amide **2g**, which is an inhibitory substrate, with an IC_{50} of 10 nM against cathepsin K and excellent selectivity versus the other cathepsins.

Cathepsin K is a lysosomal cysteine protease that is involved in the proteolysis of Type I collagen which is the major organic constituent of bone. Cathepsin K is highly and selectively expressed in osteoclasts, the cells which degrade bone during the continuous cycle of bone degradation and formation.[1,2] Inhibition of cathepsin K represents a potential therapeutic approach for diseases characterized by excessive bone resorption such as osteoporosis.[3]

Many of the reported inhibitors of cathepsin K rely on an electrophilic functionality to interact with the nucleophilic cysteine residue found in the catalytic site of this class of enzymes.[4] Our laboratories have recently reported the preparation and SAR of a series of nitrile-containing compounds that are potent inhibitors of cathepsin K.[5,6] The electrophilic nitrile warhead has been shown by X-ray crystallography to form a covalent adduct with the active site cysteine of the enzyme.[6] This covalent, but reversible, bond is proposed to account for much of the inhibitor's binding energy.

Given the importance of the electrophile to the inhibition, the replacement of the nitrile with a less electrophilic moiety such as an amide will inevitably result in a loss of potency. This is demonstrated by the loss of activity shown in Figure 1 when the nitrile in compound



1a R = CN Cat K IC_{50} = 0.2 nM
2a R = $C(O)NH_2$ Cat K IC_{50} = 238 nM

Figure 1. Potency of nitrile versus amide warhead.

1a (L-873724) is replaced by the less electrophilic primary amide (compound **2a**).

The addition of cysteine to a nitrile warhead results in the formation of a planar sp^2 adduct, a thioimidate (Fig. 2a). Conversely, the cysteine addition to an sp^2 electrophile such as amide gives rise to an sp^3 tetrahedral adduct (Fig. 2b). These hybridizations exert different torsional constraints on the adjacent α-carbon, the attachment point for P1 substitutions, differentially stabilizing the complex for a given P1 moiety. This difference in conformation can lead to an improved enzyme interaction of the P1 substituent of an sp^2 electrophile that is not observed with a nitrile. This is supported by the observation that large effects of P1 substituents have been observed with aldehyde electrophiles,[7] while only small effects have been reported in the case of nitrile inhibitors.[8]

An additional source of binding may be achieved by an amide warhead by the potential formation of an

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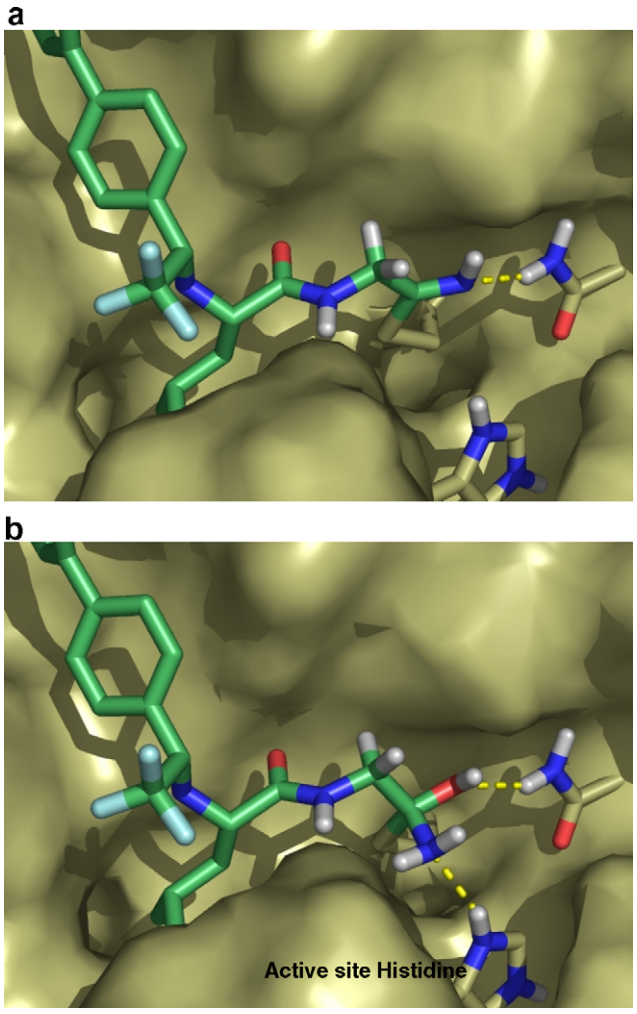


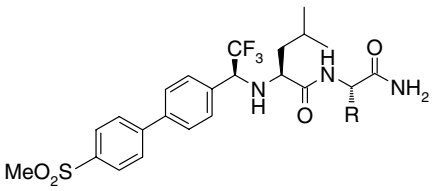
Figure 2. (a) Planar sp^2 adduct resulting from the cysteine addition onto the nitrile warhead of the cathepsin K inhibitor **1a**.[9] (b) Tetrahedral sp^3 adduct resulting from the cysteine addition onto the amide warhead of the cathepsin K inhibitor **2a**.[9]

additional hydrogen bond between the amide nitrogen and the active site histidine of the enzyme (Fig. 2b).

The aim of the current study was to determine if the potency loss observed with the replacement of a nitrile with an amide warhead could be regained by forming a tetrahedral cysteine adduct with beneficial P1 interactions. The effect of P1 substitution in this amide series was evaluated for potency versus cathepsin K and selectivity versus the other cathepsins.

The biological results obtained (Table 1) indicate that P1 substitution in an amide series does indeed increase potency. The smallest possible substitution showed a marginal improvement in potency (compound **2b** vs **2a**). The chain length of the P1 substituent required adjustment according to the appended functionality (i.e., aromatic ring vs heteroatom). When a phenyl ring is attached, the optimal linker was found to be the single methylene of the phenylalaninamide derivative (compound **2g**). Lengthening the chain by preparation of the homophenylalaninamide analog (compound **2h**)

Table 1. Potency of α-substituted amides in purified enzyme assay



Compound	R	hrab Cat K[a] IC_{50}[b] (nM)
2a	H	238 ± 37
2b	Me	174 ± 31
2c	CH_2SMe	66 ± 11
2d	CH_2SO_2Me	109 ± 26
2e	CH_2CH_2SMe	13 ± 2.0
2f	$CH_2CH_2SO_2Me$	151 ± 26
2g	CH_2Ph	10 ± 1.4
2h	CH_2CH_2Ph	273 ± 75

[a] Humanized rabbit enzyme.
[b] IC_{50}s are an average of at least four independent titrations, ± SEM. See Ref. 10 for assay conditions.

resulted in a 27-fold loss of potency. The methylsulfide functionality, being smaller than a phenyl group, required a two-carbon chain to access similar interactions with the S1 pocket of the enzyme's catalytic site. This extra methylene of compound **2e** resulted in a 5-fold improvement of potency over its shorter analog **2c**. Polarity is not well-tolerated in this area of the catalytic site as illustrated by comparing compounds **2d** versus **2c** and compounds **2f** versus **2e**.

To confirm the required stereochemistry introduced at P1, two benzyl analogs were prepared. As seen in Figure 3, the *S* configuration at P1 is optimal, whereas the *R* diastereomer **3** is 600-fold less potent than **2g**. This confirms the expected correlation with the *S* stereochemistry of the natural aminoacids.

Improvement of the intrinsic potency of these amide derivatives resulting from P1 substitution was very important when compared to the corresponding nitrile analogs. As illustrated in Table 2, P1 substitution in the nitrile series (compounds **1a**–**d**) showed no improvement in the ability of these compounds to inhibit the

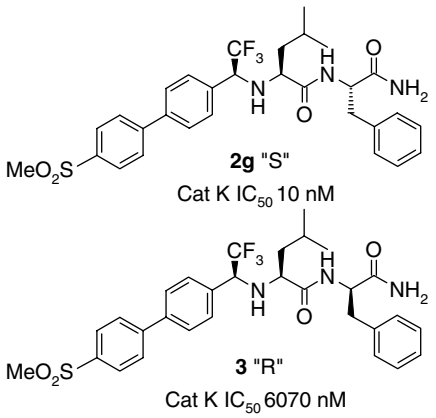
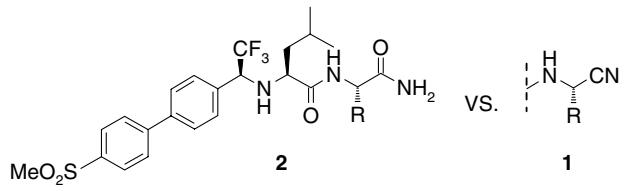


Figure 3. Stereochemistry α to the amide warhead.

Table 2. Effect of P1 substitution on the amides versus the nitriles



R	Amides	hrab Cat K[a] IC_{50}[b] (nM)	Nitriles	hrab Cat K[a] IC_{50}[b] (nM)
H	**2a**	238 ± 37	**1a**	0.2 ± 0.04
Me	**2b**	174 ± 31	**1b**	0.9 ± 0.05
CH_2CH_2SMe	**2e**	13 ± 2.0	**1c**	0.2 ± 0.01
CH_2Ph	**2g**	10 ± 1.4	**1d**	0.6 ± 0.07

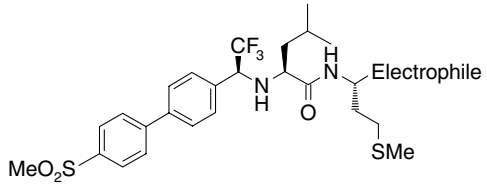
[a] Humanized rabbit enzyme.
[b] IC_{50}s are an average of at least four independent titrations, ±SEM (except compound **1c** where $n = 2$). See Ref. 10 for assay conditions.

enzyme, while the same substitution in the amide series gave rise to more than 20-fold improvement in potency.

Other electrophilic functionalities, which would also form a tetrahedral cysteine adduct with the enzyme, were evaluated. As seen in Table 3, the primary amide **2e** remains the most active analog despite the fact that it is not the most electrophilic. For example the methyl ketone **6** and the ester **7**, which are more electrophilic than the amide **2e**, do not display improved potency. This is consistent with the possibility for the amide to form hydrogen bonding interaction with the catalytic histidine as mentioned earlier (Fig. 2a).

Any substitution on the amide nitrogen resulted in a decrease in potency (compounds **4**, **5**, **9**, and **10**). The acid analog (compound **8**), which is potentially the hydrolysis product of the amide, remains relatively potent (IC_{50} = 120 nM) considering the poor electrophilic character of this warhead. This reflects the strong affinity of the biphenyl-trifluoroethylamine backbone of this class of compounds for the S2 and S3 pockets of the catalytic site of cathepsin K.

Table 3. Effect of modifying the electrophilic warhead



Compound	Electrophile	hrab Cat K[a] IC_{50}[b] (nM)
2e	$CONH_2$	13 ± 2.0
4	CONHMe	67 ± 15
5	$CONMe_2$	1045 ± 160
6	COMe	41 ± 8.3
7	CO_2Me	27 ± 7.7
8	CO_2H	120 ± 22
9	CONHBn	87 ± 16
10	$CONHCH_2CF_3$	277 ± 44
11	$CONHSO_2Me$	238 ± 18

[a] Humanized rabbit enzyme.
[b] IC_{50}s are an average of at least four independent titrations, ±SEM (except compound **6** where $n = 2$). See Ref. 10 for assay conditions.

On closer examination, it was found that these amides are in fact slowly processed substrates for the enzyme. Incubation of amide **2a** with cathepsin K resulted in the formation of the carboxylic acid **12** as observed by reverse phase HPLC analysis of the incubation media (Fig. 4).

Kinetic constants were determined for three amide inhibitors in competition experiment with the synthetic substrate Z-Leu-Arg-AMC (Table 4).[11]

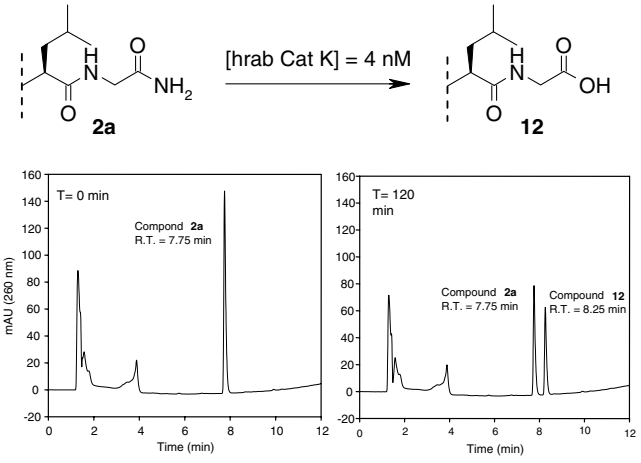
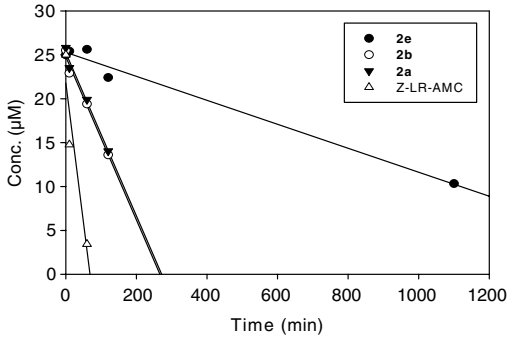


Figure 4. Enzymatic hydrolysis of the amide **2a** as observed by reverse phase HPLC.

Table 4. Kinetics of amide compounds as enzyme substrates



Compound	R	k_{cat}[a] (s^{-1})	K_m[b] (nM)	k_{cat}/K_m (s^{-1} nM^{-1})
2a	H	23.2	119	0.19
2b	Me	23.2	87	0.26
2e	CH_2CH_2SMe	3.25	6	0.54
Z-Leu-Arg-AMC		79	2000[c]	0.04

[a] All compounds (25 μM) were added to 4 nM of humanized rabbit cathepsin K and incubated at room temperature.
[b] K_m of the compound was estimated from its IC_{50} value with the following relationship: $IC_{50} = K_m\ (1 + S/K_s)$ using Z-Leu-Arg as substrate.
[c] K_m was measured in a conventional kinetic experiment.

Table 5. Selectivity of the amide compounds versus the other cathepsins

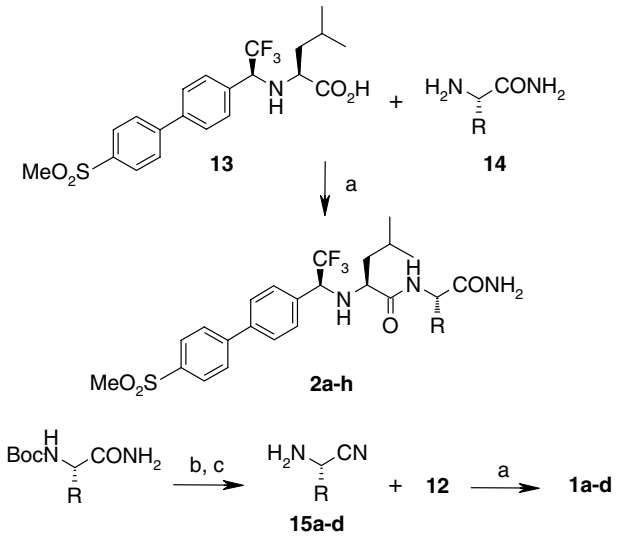
Compound	R	hrab Cat K[a] IC_{50}[c] (nM)	hCat B[b] IC_{50}[c] (nM)	hCat L[b] IC_{50}[c] (nM)	hCat S[b] IC_{50}[c] (nM)
2a	H	238	>100,000	63,060	41,082
2e	CH_2CH_2SMe	13	>100,000	55,728	76,938
2g	CH_2Ph	10	>100,000	17,043	66,656

[a] Humanized rabbit enzyme.
[b] Human enzyme.
[c] IC_{50}s are an average of at least two independent titrations. See Ref. 10 for assay conditions.

The value of the catalytic constant k_{cat} of the amide compound toward the enzyme appears to be related to the size of the substitution α to the amide. At the same time, the affinity of these compounds (estimated K_m) for the active site is improved by the P1 substitution. These combined effects result in the compounds docking tightly in the active site; their hydrolysis rates are very slow when compared to the synthetic substrate, leading these compounds to behave like enzyme inhibitors.

Introducing P1 substitution also has the potential to improve selectivity over other cathepsins. Selected examples of primary amide compounds are listed in Table 5 with inhibition activity against cathepsins B, L, and S. The unsubstituted amide **2a** displays a 170-fold selectivity for cathepsin K versus cathepsin S. The most potent cathepsin K inhibitors **2e** and **2g** both display excellent selectivity with **2e** being slightly superior with regard to its cathepsin L selectivity (4300-fold vs 1700-fold for **2g**). The substitution in P1 improves potency against cathepsin K while having little effect in cathepsin B, L, and S.

These amide inhibitors can be prepared in one step via a peptide coupling of the acid precursor **13**[12,13] and an aminoamide **14** (Scheme 1). Subsequently, these amides can be dehydrated using trifluoroacetic anhydride in pyridine to give access to the nitrile analogs (compounds **1a–d**). However, this approach gave low yields of nitriles and many degradation products were observed, probably due to the presence of more than one amide bond. An alternative route to the nitrile inhibitors involved the preparation of substituted aminoacetonitrile precursors (**15a–d**) and performing the peptide coupling reaction at the end of the sequence as in the amide series. Analysis of the resulting products by HPLC confirmed the stereochemical integrity of the substituent α to the nitrile.



Scheme 1. Reagents and conditions: (a) HATU, Et_3N, DMF, 18 h, rt, 75–95% yield; (b) TFAA, pyridine, 30 min, rt, 70–75% yield; (c) methane sulfonic acid, THF, 18 h, rt, 60–75% yield.

In this novel amide series of cathepsin K inhibitors, introduction of the appropriate P1 substituent resulted in a partial recovery of the potency loss encountered when a nitrile warhead is replaced by a primary amide. These compounds were shown to be competitive substrates for cathepsin K, although they are turned over at such a slow rate that they behave like inhibitors. This series of compounds also showed excellent selectivity against the cathepsins B, L, and S.

References and notes

1. Troen, B. R. *Drug News Perspect.* **2004**, *17*, 19.
2. Lecaille, F.; Kaleta, J.; Brömme, D. *Chem. Rev.* **2002**, *102*, 4459.
3. Yasuda, Y.; Kaleta, J.; Brömme, D. *Adv. Drug Deliv. Rev.* **2005**, *57*, 973.
4. Grabowska, U. B.; Chambers, T. J.; Shiroo, M. *Curr. Opin. Drug Discov. Devel.* **2005**, *8*(5), 619.
5. Black, W. C.; Bayly, C. I.; Davis, D. E.; Desmarais, S.; Falgueyret, J. P.; Léger, S.; Li, C. S.; Massé, F.; McKay, D. J.; Palmer, J. T.; Percival, M. D.; Robichaud, J.; Tsou, N.; Zamboni, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4741.
6. Li, C. S.; Deschênes, D.; Desmarais, S.; Falgueyret, J. P.; Gauthier, J. Y.; Kimmel, D. B.; Léger, S.; Massé, F.; McGrath, M.; McKay, D. J.; Percival, M. D.; Riendeau, D.; Rodan, S. B.; Thérien, M.; Truong, V. L.; Wesolowski, G.; Zamboni, R.; Black, W. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1985.
7. Catalano, J. J.; Deaton, D. N.; Furfine, E. S.; Hassell, A. M.; McFadyen, R. B.; Miller, A. B.; Miller, L. R.; Shewchuk, L. M.; Willard, D. H., Jr.; Wright, L. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 275.
8. Altmann, E.; Aichholz, R.; Betschart, C.; Buhl, T.; Green, J.; Lattmann, R.; Missbach, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2549.
9. The bound complex coordinates shown are obtained from molecular dynamic simulations. Starting with the PDB entry 1MEM for the Cat-k structure, a 700 ps trajectory was performed on the covalently bound complex in TIP3P water with periodic boundary conditions. The image is generated by averaging coordinate snapshots of the last 100 ps.
10. Falgueyret, J. P.; Black, W. C.; Cromlish, W.; Desmarais, S.; Lamontagne, S.; Mellon, C.; Riendeau, D.; Rodan, S.; Tawa, P.; Wesolowski, G.; Bass, K. E.; Venkatraman, S.; Percival, M. D. *Anal. Biochem.* **2004**, *335*, 218.
11. Robichaud, J.; Oballa, R.; Prasit, P.; Falgueyret, J. P.; Percival, M. D.; Wesolowski, G.; Rodan, S. B.; Kimmel,

D.; Johnson, C.; Bryant, C.; Venkatraman, S.; Setti, E.; Mendonca, R.; Palmer, J. T. *J. Med. Chem.* **2003**, *46*, 3709.

12. Bayly, C. I.; Black, C.; Léger, S.; Li, C. S.; McKay, D.; Mellon, C.; Gauthier, J. Y.; Lau, C.; Therien, M.; Truong, V.-L.; Green, M. J.; Hirschbein, B. L.; Janc, J. W.; Palmer, J. T.; Baskaran, C. WO 075836A2, 2003.
13. Roy, A.; Gosselin, F.; O'Shea, P. D.; Chen, C. Y. *J. Org. Chem* **2006**, *71*, 4320.