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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4741–4744
Bioorganic & Medicinal Chemistry Letters

Trifluoroethylamines as amide isosteres in inhibitors of cathepsin K

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Received 13 June 2005; revised 15 July 2005; accepted 25 July 2005
Available online 9 September 2005

Abstract—The P2-P3 amide of dipeptide cathepsin K inhibitors can be replaced by the metabolically stable trifluoroethylamine group. The non-basic nature of the nitrogen allows the important hydrogen bond to Gly66 to be made. The resulting compounds are 10- to 20-fold more potent than the corresponding amide derivatives. Compound **8** is a 5 pM inhibitor of human cathepsin K with >10,000-fold selectivity over other cathepsins.

Cathepsin K (Cat K) is a lysosomal cysteine protease thought to be the principal enzyme responsible for degradation of type I collagen in osteoclastic bone resorption. Thus, an inhibitor of Cat K may be effective as an anti-resorptive for treatment of osteoporosis. A number of Cat K inhibitors have been previously reported, many of which contain multiple amide bonds.

Replacement of amide bonds with appropriate isosteres is a classic approach in medicinal chemistry. Many amide replacements are known which retain the geometry of the amide bond or maintain the hydrogen bond-accepting properties of the amide.[1] However, there are a few functional groups, which are capable of preserving the hydrogen bond-donating properties of the amide. Sulfonamides, anilines, secondary alcohols, hydrazines, and certain heterocycles constitute this list. The problem of identifying an NH amide replacement can be recast as how to minimize the basicity of an NH donor so that a NH_2^+ moiety is not formed in the biological milieu. Such charged groups are poorly tolerated deep in the active site of a protein where binding interactions cannot compensate for the energetic cost of desolvation. In this paper, we describe that a trifluoromethyl group can replace the carbonyl of an amide and generate a metabolically stable, non-basic amine that maintains the excellent hydrogen bond of an amide.

Replacement of this functional group has been previously explored by Zanda and coworkers[2] who have used it to replace both a glycine amide bond and a malonamide of a partially modified retropeptide.[3] These authors succinctly note that the trifluoroethylamino group features 'low NH basicity, a $CH(CF_3)$—NH—CH backbone angle close to 120°, [and] a C—CF_3 bond substantially isopolar with the C=O'. A report on the use of a CH(CN)—NH group to replace an amide bond in CCK-B has also been presented.[4]

Much of the published literature on Cat K inhibitors describe peptide-based molecules. Dipeptide nitrile inhibitors have been reported with good pharmacokinetics in preclinical species and anti-resorptive activity in animal models of osteoporosis.[5] Nonetheless, we felt that the overall properties of these inhibitors could be improved by decreasing their peptidic nature.

X-ray crystallography of the irreversible dipeptide inhibitor **1** bound to Cat K shows that the P1-P2 amide forms hydrogen bonds to the protein with both its NH and carbonyl components (Fig. 1). In contrast, the P2-P3 amide bond forms only one hydrogen bond to Gly66

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doi:10.1016/j.bmcl.2005.07.071

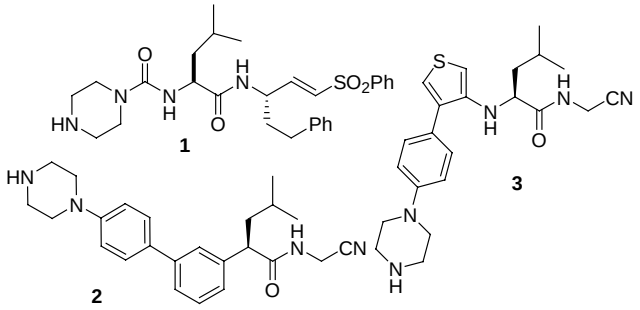


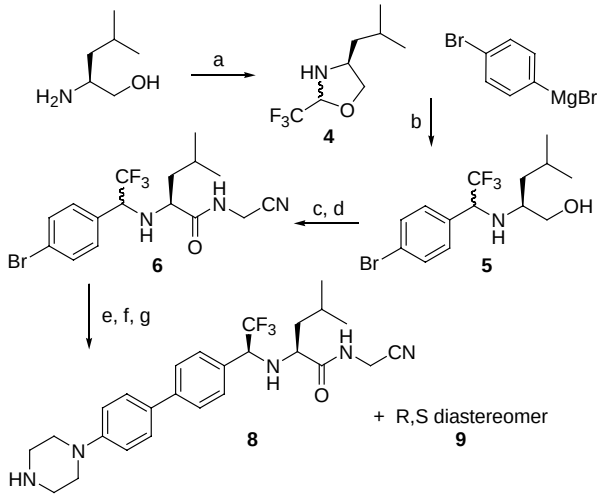
Figure 1. Structures of peptidomimetic cathepsin K inhibitors.

with the NH, while the carbonyl projects into solution.[6] This implies that the P2-P3 amide bond might be replaced with an isostere without dramatic loss of potency. Our first attempt at such a replacement was to introduce a phenyl ring in place of the amide.[7] The resulting compound **2** had modest potency but excellent pharmacokinetics, justifying further pursuit of amide replacements. The potency of these compounds could be improved by using an aminothiophene group as in compound **3** to take advantage of the hydrogen bond acceptor at Gly66.[8] Other methods of introducing the H-bond (alcohols, sulfonamides) were attempted without success.

In the context of developing the piperazine SAR of the aminothiophene derivatives, we found that an *N*-trifluoroethyl group resulted in a dramatic decrease in the basicity of the piperazine. It became quickly apparent that this could be a method for replacing an amide carbonyl, while maintaining a neutral nitrogen H-bond.

The prototype molecule **6** was prepared by condensation of trifluoroacetaldehyde with leucinol to form oxazoline **4** as a 3:1 mixture of diastereomers.[9] This oxazoline was added to three equiv of 4-bromophenyl magnesium bromide to give trifluoroethylamino alcohol **5** as a 1:1 mixture of diastereomers. More recently, an efficient diastereoselective method for the synthesis of this type of molecule has appeared.[10] Alcohol **5** was oxidized to the carboxylic acid using periodic acid catalyzed by chromic acid.[11] Coupling of the aminoacetonitrile was carried out using conventional methods to give **6** (Scheme 1). Trifluoroethylamine **6** behaved as a neutral molecule on TLC ($R_f = 0.40$, 40% EtOAc/1% HOAc/hexanes) and could not be extracted from ethyl acetate with dilute acid. Titration against Cat K showed that **6** was 5-fold *more* potent than the corresponding dipeptide derivative **7**, even as a 1:1 mixture of diastereomers (Table 1).

Previous work in other series had shown that a phenylpiperazine provides optimal binding in S3.[7] This P3 fragment was added to **6** using a Suzuki coupling, as previously described.[8] The resulting compound was separated by HPLC into its pure diastereomers **8** and **9**. Compound **8** has a Cat K IC_{50} of $\leqslant 5$ pM, the most potent compound ever obtained in this program. Only an upper bound for this IC_{50} could be determined due to limitations of the assay.[12] Diastereomer **9** was 1000-fold less potent in this assay with poor selectivity over Cat L.



Scheme 1. Reagents and conditions: (a) trifluoroacetaldehyde ethyl hemiacetal, benzene, Dean-Stark, 18 h; (b) THF, 0 °C, 1 h; (c) H_5IO_6 (3 equiv), CrO_3, CH_3CN, 2 h; (d) PyBOP, aminoacetonitrile · HCl, Et_3N, DMF, 14 h; (e) BocPipPhB$(OH)_2$, $PdCl_2$dppf, Na_2CO_3, DMF, 90 °C, 6 h; (f) MsOH, THF, 14 h; (g) HPLC separation.

Table 1. Potency of inhibitors in purified enzyme assays

Compound	IC_{50} (nM)[a]			
	Cat K[b]	Cat B[c]	Cat L[c]	Cat S[c]
2	11	3950	3725	2010
3	1.0	123	352	102
6	1.3	3353	34	121
7	7.3	2477	84	145
8	⩽0.005[d]	1111	47	451
9	4.6	>10,000	68	902
10	0.015[d]	344	26	120
12	0.6	1050	3903	>10,000
13	5.0	>10,000	>10,000	>10,000
14	0.4	614	2274	>10,000

[a] IC_{50}s are an average of at least two independent titrations. See Ref 15 for assay conditions.
[b] Humanized rabbit enzyme.
[c] Human enzyme.
[d] Low enzyme conditions—see Ref 12.

A direct comparison of **8** to the corresponding dipeptide **10** (not shown) has demonstrated that both potency and selectivity were increased by replacing the amide bond by a trifluoroethylamine moiety (Table 1).

To determine the stereochemistry of the active diastereomer, we turned to X-ray crystallography. The diastereomer of intermediate **5**, which led to the active compounds, was derivatized as an imidazole carboxylic ester **11**. A single crystal X-ray structure revealed that the CF_3 center was in the *S* configuration (Fig. 2).[13]

In the dipeptide nitrile series of inhibitors, the optimal P2 substituent had been found to be a 1,1-cyclohexyl group, as exemplified by **12** (L-006235/CRA-013783).[5] When this P2 group was used to replace leucine in the trifluoroethylamine compounds, the resulting compound **13** (Fig. 3) was found to be 1000-fold less active

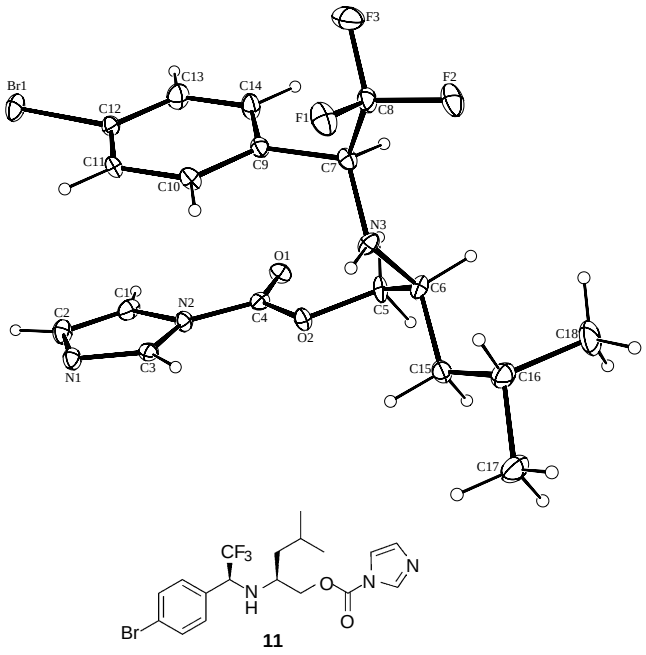


Figure 2. ORTEP representation of **11**. Non-hydrogen atoms are represented by ellipsoids corresponding to 30% probability. Hydrogen atoms have been drawn at an arbitrary size.

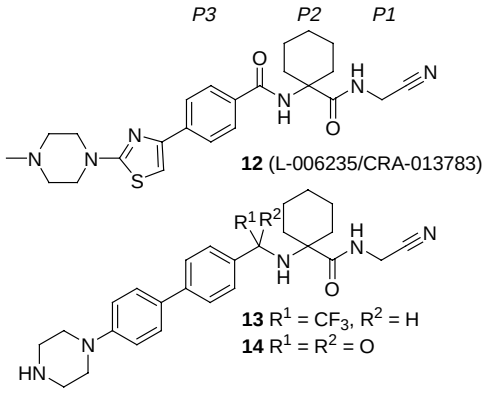
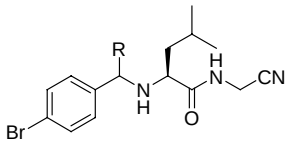


Figure 3. Optimized dipeptide inhibitor L-006235/CRA-013783 and P2-cyclohexyl derivatives **13** and **14**.

than the corresponding amide derivative **14**. Molecular modeling analysis indicated that a steric clash between the CF_3 group and the cyclohexyl ring prevents the molecule from adopting the conformation required for binding. This suggests that only a single substituent will be tolerated at P2 when a CF_3 group replaces the amide carbonyl.

The role of the CF_3 group was explored in a brief SAR study (Table 2). No substitution (**15**) or a simple methyl substituent at this position (**16**) resulted in a dramatic loss of activity. Pentafluoroethyl (**17**) gave similar potency to CF_3. Aromatic substituents were generally quite potent, as illustrated by the phenyl sulfone substituent (**18**). With the exception of the hydrogen and methyl cases, these amine derivatives tended to have neutral properties, as determined by their non-polar behavior on TLC analysis. This behavior was qualitatively

Table 2. Comparison of CF_3 replacements



Compound	IC_{50} (nM)			
	R	Cat K[a]	R_f[b]	pK_a[c]
6	CF_3	0.9	0.40	1.3
15	H	802	0.05	6.7
16	CH_3	988	0.03	6.7
17	CF_2CF_3	2.4	0.42	1.8
18	4-$MeSO_2Ph$	2.5	0.38	4.6
19	CN	30	0.67	0.7

[a] Humanized rabbit enzyme.
[b] TLC on SiO_2 plates with 40% EtOAc/1% HOAc/hexanes.
[c] Calculated using ACD/pK_a.

captured by the pK_a of the amine, as calculated by ACD/pK_a.[14] Most interestingly, a nitrile substituent (**19**), which also gave a non-basic compound, showed a 30-fold loss in activity, implying that the substituent effect was more complex than simply neutralizing the amine.

Compound **6** has been modeled into a Cat K crystal structure (Fig. 4). This model shows that the CF_3 group does not form any lipophilic interactions with the enzyme, but rather is directed away from the active site into water. The hydrogen bond closely mimics the hydrogen bond of the amide, but due to the sp^3 hybridization of the nitrogen, it is oriented better toward the Gly66 carbonyl, potentially forming a stronger hydrogen bond. The aromatic substituent is situated over the glycine shelf in essentially the identical orientation as the aromatic group of the dipeptide inhibitors.

The sum of the above data allows us to propose a rationale for the increased potency of the trifluoroethylamine,

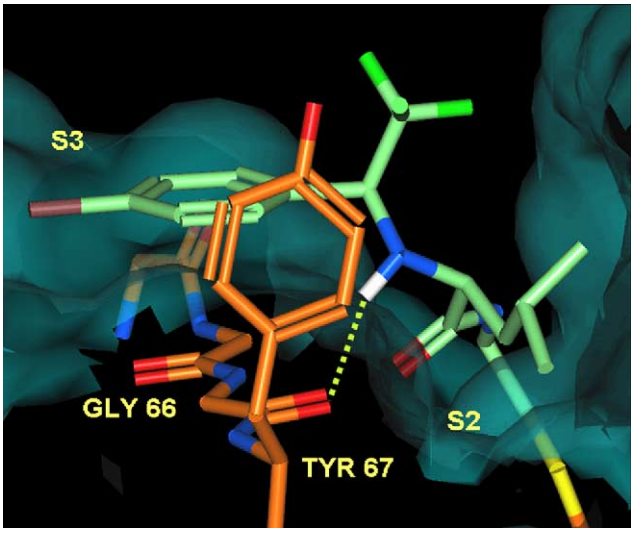


Figure 4. Compound **6** modeled into the active site of human Cat K (pdb entry 1MEM) showing the interaction of the P2-P3 trifluoroethylamine moiety.

relative to the amide. The sp^2 atoms of the amide impose a suboptimal conformational restraint on the aromatic ring and the hydrogen bond. In contrast, the CF_3 group attached to an sp^3 carbon orients itself perpendicular to the aromatic ring and stabilizes the aromatic ring in its bioactive conformation. The sp^3 hybridized nitrogen allows the simultaneous formation of an optimal hydrogen bond. In the case of compound **19**, the nitrile substituent is considerably smaller than the CF_3 group resulting in a low barrier to rotation for the aromatic ring. As long as a substituent in this position is large enough to enforce the conformational preference of the aromatic ring, and can prevent protonation of the amine group at physiological pH, a potent compound will be obtained.

This replacement of an amide with a trifluoroethylamine leads to advantages other than potency and selectivity. In the dipeptide nitriles, while the P2-P3 amide bond was found to be metabolically stable, the P1-P2 amide bond can be hydrolyzed to give the carboxylic acid. This was especially apparent in dipeptide inhibitors containing leucine in P2. Replacing the leucine with cyclohexyl effectively blocked this route of metabolism. When the P2-P3 amide is replaced by the phenyl ring of compound **2**, this P1-P2 amide hydrolysis is not observed. Similarly, in the case of trifluoroethylamine derivatives described, we found no evidence of a P1-P2 amide cleavage, even though these compounds contain leucine in P2.

In conclusion, we have found that trifluoroethylamine is an excellent surrogate for the P2 amide bond in the inhibitors of Cat K. Not only does this functional group enhance potency and selectivity over other cathepsins, but the resulting compounds are stable to P1-P2 amide bond cleavage that is observed in analogous dipeptide inhibitors. The fully elaborated inhibitor **8** is a $\sim$5 pM inhibitor of Cat K and is >10,000-fold selective over other cathepsins.

References and notes

1. Venkatesan, N.; Kim, B. H. *Curr. Med. Chem.* **2002**, *9*, 2243; Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699.
2. Volonterio, A.; Bellosta, S.; Bravin, F.; Bellucci, M. C.; Bruché, L.; Colombo, G.; Malpezzi, L.; Mazzini, S.; Meille, S. V.; Meli, M.; Ramírez de Arellano, C.; Zanda, M. *Chem. Eur. J.* **2003**, *9*, 4510.
3. Molteni, M.; Volonterio, A.; Zanda, M. *Org. Lett.* **2003**, *5*, 3887; Volonterio, A.; Bravo, P.; Zanda, M. *Org. Lett.* **2000**, *2*, 1827.
4. Herrero, S.; Suárez-Gea, M. L.; González-Muñiz, R.; García-López, M. T.; Herranz, R.; Ballaz, S.; Barber, A.; Fortuño, A.; Del Río, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 855.
5. Palmer, J. T.; Bryant, C.; Wang, D. -X.; Davis, D. E.; Setti, E. L.; Rydzewski, R. M.; Venkatraman, S.; Tian, Z.-Q.; Burrill, L. C.; Mendonca, R. V.; Springman, E.; McCarter, J.; Chung, T.; Cheung, H.; McGrath, M.; Somoza, J.; Enriquez, P.; Yu, Z. W.; Strickley, R. M.; Liu, L.; Venuti, M. C.; Percival, M. D.; Falgueyret, J.-P.; Prasit, P.; Oballa, R.; Riendeau, D.; Young, R. N.; Wesolowski, G.; Rodan, S. B.; Johnson, C.; Kimmel, D. B.; Rodan, G. *J. Med. Chem.* **2005** in press.
6. McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Bromme, D. *Nat. Struct. Biol.* **1997**, *4*, 105–109; McGrath, M. E.; Sprengeler, P. A.; Hill, C. M.; Martichonok, V.; Cheung, H.; Somoza, J. R.; Palmer, J. T.; Janc, J. W. *Biochemistry* **2003**, *42*, 15018.
7. 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.; Mendoca, R.; Palmer, J. T. *J. Med. Chem.* **2003**, *46*, 3709.
8. Robichaud, J.; Bayly, C.; Oballa, R.; Prasit, P.; Mellon, C.; Falgueyret, J.-P.; Percival, M. D.; Wesolowski, G.; Rodan, S. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4291.
9. Ishii, A.; Higashiyama, K.; Mikami, K. *Synlett* **1997**, 1381.
10. Gosselin, F.; Roy, A.; O'Shea, P. D.; Chen, C.; Volante, R. P. *Org. Lett.* **2004**, *6*, 641; Gosselin, F.; O'Shea, P. D.; Roy, S.; Chen, C.; Reamer, R. A.; Chen, C.; Volante, R. P. *Org. Lett.* **2005**, *7*, 355.
11. Zhao, M.; Li, J.; Song, Z.; Desmond, D.; Tschaen, D. M.; Grabowski, E. J. J.; Reider, P. J. *Tetrahedron Lett.* **1998**, *39*, 5323.
12. Inhibitory potencies were determined in a version of the published Cat K assay in which the enzyme concentration was reduced to 10 pM—the lowest enzyme concentration at which activity could be reliably measured. At this concentration, IC_{50}s below 5 pM cannot be determined.
13. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 274083 for **11**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [e-mail: *deposit@ccdc.cam.ac.uk*].
14. Advanced Chemistry Development, Inc., Toronto, Canada; v6.00.
15. 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.