Bioorganic & Medicinal Chemistry Letters 19 (2009) 4280–4283

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Dipeptidyl nitrile inhibitors of Cathepsin L

Nabil Asaad [a], Paul A. Bethel [a], Michelle D. Coulson [b], Jack E. Dawson [a], Susannah J. Ford [a], Stefan Gerhardt [a], Matthew Grist [a], Gordon A. Hamlin [a], Michael J. James [a], Emma V. Jones [a], Galith I. Karoutchi [a], Peter W. Kenny [a,*], Andrew D. Morley [a,*], Keith Oldham [a], Neil Rankine [a], David Ryan [a], Stuart L. Wells [a], Linda Wood [a], Martin Augustin [c], Stephan Krapp [c], Hannes Simader [c], Stefan Steinbacher [c]

[a] Respiratory & Inflammation Research Area, AstraZeneca, Mereside, Alderley Park, Macclesfield SK10 4TG, UK
[b] Safety Assessment, AstraZeneca, Mereside, Alderley Park, Macclesfield SK10 4TG, UK
[c] Proteros Biostructures, Am Klopferspitz 19, D-82152 Martinsried, Germany

ARTICLE INFO

Article history:
Received 20 April 2009
Revised 20 May 2009
Accepted 21 May 2009
Available online 27 May 2009

Keywords:
Cathepsin
Cathepsin B
Cathepsin K
Cathepsin L
Cathepsin L2
Cathepsin S
Cathepsin V
Cysteine protease
Nitrile
Covalent inhibitor
Selectivity
Protein structure
X-ray crystallography
Structure-based design
Molecular pincer
Conformational lock
Tautomeric lock
Osteoarthritis
Aggrecan
Collagen
Cartilage

ABSTRACT

A series of potent Cathepsin L inhibitors with good selectivity with respect to other cysteine Cathepsins is described and SAR is discussed with reference to the crystal structure of a protein-ligand complex.

Osteoarthritis is a chronic degenerative disease, resulting from loss of articular cartilage and damage to underlying bone, leading to joint instability and pain.[1] The lysosomal cysteine protease[2] Cathepsin L (CatL) is seen as a potential target for intervention in treatment of this disease.[3] The enzyme is secreted into the extracellular matrix and has been shown in vitro that it can degrade proteoglycans, including aggrecan[4] and type II collagen,[5] the major components of articular cartilage.

Selectivity with respect to other Cathepsins is an important consideration. Cathepsins B (CatB) and L2 (CatL2) are key anti-targets in optimization of CatL inhibitors. In mice the combined deficiency of CatB and CatL has been shown to be lethal in the second to fourth week of life.[6] $CatL^{-/-}$ mice show abnormalities in skin and hair differentiation.[7] The human genome encodes a CatL-like cysteine protease (CatL2), which shows a restricted tissue distribution and is not present in the mouse genome. Studies using $CatL^{-/-}$ mice showed that transgenic, keratinocyte-specific expression of human CatL2 largely rescued the skin and hair phenotype, indicating that this protease may play an important role in human skin homeostasis.[8] Both CatL and CatS have roles in MHC class II-mediated antigen processing and presentation and may compensate for

* Corresponding authors. Tel.: +44 1625 514396 (P.W.K.), +44 1509 644772 (A.D.M.).
E-mail addresses: pwk.pub.2008@gmail.com (P.W. Kenny), andy.morley@astrazeneca.com (A.D. Morley).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.071

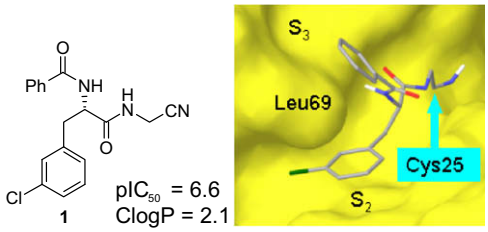


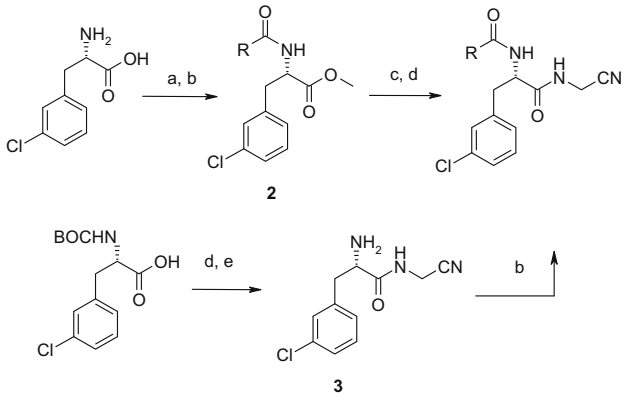
Figure 1. Potential binding mode of the adduct of **1** with Cathepsin L showing molecular surface of protein and locations of key residues. The covalent bond is formed between the nitrile carbon atom and the sulfur atom of the catalytic cysteine (Cys25; hidden by surface).

each other, suggesting that selectivity with respect to CatS is desirable in CatL inhibitors.[9]

Directed screening led to the identification of **1** as an inhibitor of CatL with pIC_{50}[10] of 6.6. This compound is >10-fold selective with respect to CatB and CatK but shows no selectivity with respect to CatS or CatL2. Close structural analogues of **1** have been described previously as CatL inhibitors and show similar selectivities with respect to CatK and CatS.[11] The binding of **1** to CatL was modeled (Fig. 1) with a covalent bond between nitrile carbon and active site cysteine sulfur.[12,13] The 3-chlorophenyl substituent occupies the S_2 pocket which is deeper in CatL than in CatK. The phenyl ring of the capping group lies in the shallow S_3 pocket, making contact with Leu69, although the interaction appears suboptimal. The corresponding residues in other Cathepsins are tyrosine (CatK, CatB) or phenylalanine (CatL2, CatS). Leu69 also makes contact with the chlorophenyl ring and is held in what can be termed a 'molecular pincer'. The carbonyl oxygen of Gly68 accepts a hydrogen bond from the benzamide donor.

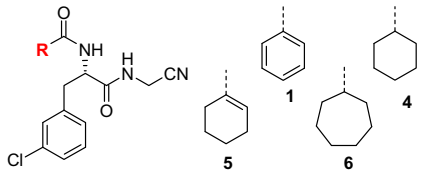
Synthesis of compounds is summarised in Scheme 1. Esterification of the commercial 3-chlorophenylalanine followed by standard amide coupling chemistry furnished **2**. Ester hydrolysis followed by concentration of the reaction mixture and subsequent amide coupling of the crude lithium salts with aminoacetonitrile provided good yields of the target compounds. The alternative, of introducing diversity in the final step by acylating **3**, resulted in low yields and challenging purification.

Structure activity relationships (SARs) for CatL inhibitors are presented in Tables 1–5. Replacement of phenyl in **1** with the more lipophilic cyclohexyl (**4**) leads to a 0.7 drop in pIC_{50} (Table 1). This may reflect differences in dihedral angle preference for the bonds linking substituent and carbonyl groups. The larger and more



Scheme 1. Reagents: (a) 10 M HCl/MeOH; (b) Acid, HATU, DIPEA, DMF; (c) LiOH, MeOH/H_2O/THF; (d) H_2NCH_2CN, HATU, DIPEA, DMF; (e) Formic acid.

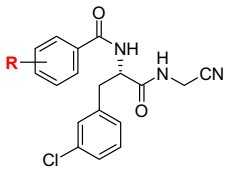
Table 1
SAR for aromatic and cycloalkyl substituents



Compd	ClogP[a]	CatL pIC_{50}[b]	CatL2 pIC_{50}[b]	CatS pIC_{50}[b]	CatK pIC_{50}[b]	CatB pIC_{50}[b]
1	2.1	6.6	6.1	6.9	5.5	5.3
4	2.5	5.9	5.2	5.6	<5	<5
5	2.3	6.3	6.4	5.7	<5	<5
6	3.0	6.5	5.2	5.6	5.3	<5

[a] See Ref. 14.
[b] Values are means of at least three experiments; see Refs. 10,15–17.

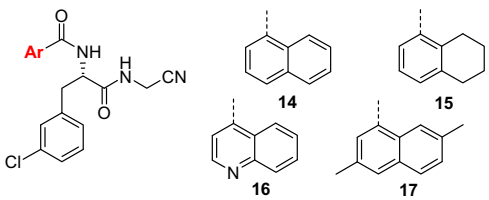
Table 2
SAR for phenyl substitution



Compd	R	ClogP[a]	CatL pIC_{50}[b]	CatL2 pIC_{50}[b]	CatS pIC_{50}[b]	CatK pIC_{50}[b]	CatB pIC_{50}[b]
1	H	2.1	6.6	6.1	6.9	5.5	5.3
7	4-Me	2.6	6.6	6.0	6.2	5.9	5.6
8	3-Me	2.6	6.6	6.1	6.4	5.2	5.3
9	2-Me	2.3	6.1	5.3	5.8	5.3	<5
10	3-Cl	3.0	7.1	6.7	6.7	5.2	5.4
11	2,5-diMe	2.8	7.1	5.8	5.9	5.2	5.3
12	2,3-diMe	2.7	6.5	5.4	5.7	<5	<5
13	3,5-diMe	3.1	7.3	6.7	6.5	5.3	5.1

[a] See Ref. 14.
[b] Values are means of at least three experiments; see Refs. 10,15–17.

Table 3
SAR for bicyclic substitiuents

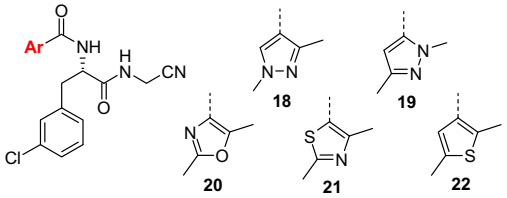


Compd	ClogP[a]	CatL pIC_{50}[b]	CatL2 pIC_{50}[b]	CatS pIC_{50}[b]	CatK pIC_{50}[b]	CatB pIC_{50}[b]
14	3.3	6.6	5.4	5.5	<5	5
15	3.7	6.4	<5	<5	<5	<5
16	2.7	7.0	5.5	5.5	<5	<5
17	4.3	7.7	6.0	6.0	<5	<5

[a] See Ref. 14.
[b] Values are means of at least three experiments; see Refs. 10,15–17.

lipophilic cycloheptyl analogue (**6**), is nearly as active as **1** and shows improved selectivity with respect to CatL2.

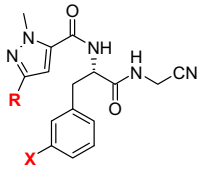
Table 4
SAR for heteroaryl substituents



Compd	ClogP[a]	CatL pIC_{50}[b]	CatL2 pIC_{50}[b]	CatS pIC_{50}[b]	CatK pIC_{50}[b]	CatB pIC_{50}[b]
18	0.6	5.9	5.9	5.9	5.6	<5
19	0.9	6.3	5.9	6.1	5.2	<5
20	0.7	6.2	5.6	6.1	5.0	<5
21	1.9	6.4	6.6	6.7	5.9	5.0
22	2.6	7.1	6.3	6.1	5.3	5.7

[a] See Ref. 14.
[b] Values are means of at least three experiments; see Refs. 10,15–17.

Table 5
3-Substituted pyrazole SAR analogues



Compd	R	X	ClogP[a]	CatL pIC_{50}[b]	CatL2 pIC_{50}[b]	CatS pIC_{50}[b]	CatK pIC_{50}[b]	CatB pIC_{50}[b]
19	Me	Cl	0.9	6.3	5.9	6.1	5.2	<5
23	Et	Cl	1.4	7.2	6.3	6.1	5.3	5.1
24	*i*Pr	Cl	1.8	7.1	6.3	6.1	5.7	5.3
25	*t*Bu	Cl	2.2	7.9	6.7	6.0	5.5	5.2
26	*t*Bu	Me	2.0	7.9	6.4	6.1	5.6	5.4

[a] See Ref. 14.
[b] Values are means of at least three experiments; see Refs. 10,15–17.

Substitution of the phenyl ring of **1** with a single chloro or methyl group led to increases in CatL pIC_{50} of no more than 0.5 (Table 2). The most interesting profile is that shown by **11** in which the phenyl ring is substituted with methyl groups at the 2 and 5 positions. This compound is more than 10-fold selective against both CatL2 and CatS. Contact between the 5-methyl substituent and protein surface restricts the torsional space for the carbonyl–phenyl bond in the bound state and effectively forces the 2-methyl into contact with Leu69. The equivalents of Leu69 in CatL2 and CatS are more sterically demanding aromatic amino acids and the dimethyl-substitutions are deleterious for anti-target potency, especially CatS.

The SAR of 2,3-disubstituted phenyl rings was explored further using bicyclic aromatic systems (Table 3). Compounds **14** and **15** are equipotent with **12** although **15** shows a better selectivity profile. Aza-substitution of **14** at C4 actually results in a small increase in potency, which reflects likely exposure of this position to solvent in the bound state. The 2,3 and 2,5 substitution patterns of **11** and **12** are brought together in **17**. This compound is more potent than analogues **11–14** and shows better than 50-fold selectivity with respect to the other Cathepsins.

The improvements in potency and selectivity of **17** relative to **1** were achieved at the cost of increasing lipophilicity (Clog*P*) by >2 units. The results presented in Table 4 show that substituting five-membered heteroaromatic rings (e.g., **19** and **20**) for phenyl can lead to significant reductions in lipophilicity with minimal loss of potency. Analogues of **19** have been described as Cathepsin inhibitors in the patent literature.[18]

Increasing the size of the substituent at pyrazole C3 in **19** leads to an increase in CatL potency (Table 5). The CatL pIC_{50} value of **25** exceeds that of **1** by 1.6 units. CatL2 ($\Delta pIC_{50} = 0.5$) and CatS ($\Delta pIC_{50} = -0.1$) are much less sensitive to this structural change with the result of an improved selectivity profile relative to **1**. Compound **25** is as least as selective with respect to CatB and CatS as previously described CatL inhibitors.[3]

A weak correlation ($\sigma = 0.41$) was observed between CatL pIC_{50} and Clog*P* (Fig. 2) for these inhibitors. The correlations of CatL2 and CatS inhibition with lipophilicity are much weaker, which reflects the presence of CatL selective inhibitors in the data set. We believe that Figure 2, in which selectivity is overlaid onto potency-lipophilicity relationships, represents a useful and general approach to summarizing results from selectivity assays.

The crystal structure[19–21] (Fig. 3) of **26** bound to CatL provides a rationale for the SAR observed for **19**, **23**, **24** and **25**. The 1-methyl group of the pyrazole makes some contact with Leu69 and appears to force the pyrazole ring out of co-planarity with the amide, probably functioning as a conformational lock.[22,23] Less obviously, the 1-methyl group may also function as a tautomeric lock because

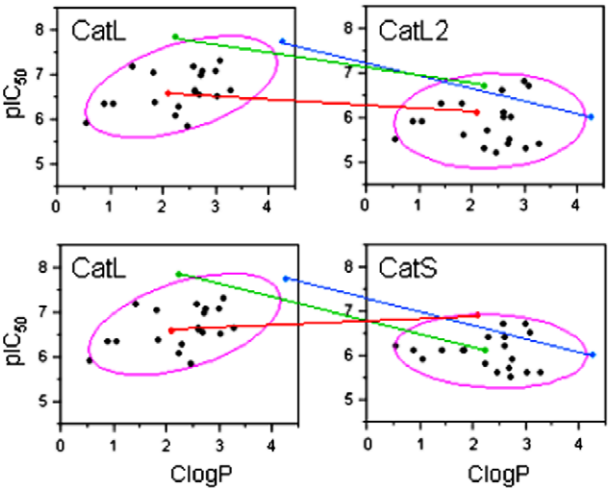


Figure 2. Relationships between pIC50 and Clog*P* for Cathepsins L, L2 and S showing comparisons for **1** (red), **17** (blue) and **25** (green). Ellipses correspond to probability of 90%.

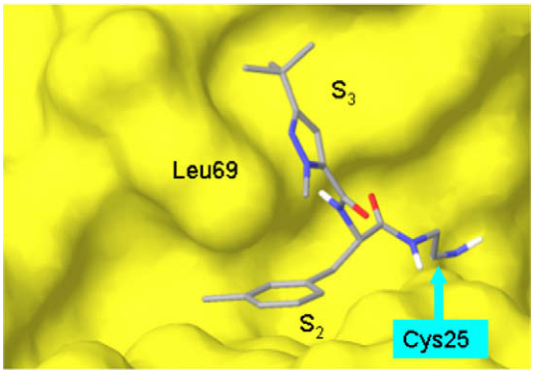


Figure 3. Crystal structure of **26** bound to Cathepsin L showing molecular surface of the protein and locations of key residues. The covalent bond is formed between the nitrile carbon atom and the sulfur atom of the catalytic cysteine (Cys25; hidden by surface).

the more stable tautomer[24] of the des-methyl analog is likely to be the one with the imino nitrogen next to the amide linker.[25]

All three methyl groups of the *t*-butyl substituent make contact with the protein. The similarity of the pIC_{50} values for **23** (7.2) and **24** (7.1) suggests that these three methyl groups do not contribute equally to potency. One rationale for the observed trend in potency is that the contribution to binding of one of the methyl groups is primarily conformational.

In conclusion, we have discussed selectivity requirements for safe Cathepsin L inhibitors and shown how variation of the S_3 substituent can be used to modulate potency and selectivity.

Acknowledgments

We thank Lyn Rosenbrier Ribeiro and Helen Sawney for sharing assay development expertise.

References and notes

1. Goldring, M. B.; Goldring, S. R.. *J. Cell Physiol.* **2007**, *213*, 626.
2. Vasiljeva, O.; Reinheckel, T.; Peters, C.; Turk, D.; Turk, V.; Turk, B. *Curr. Pharm. Des.* **2007**, *13*, 387.
3. Marquis, R. W.; James, I.; Zeng, J.; Trout, R. E. L.; Thompson, S.; Rahman, A.; Yamashita, D. S.; Xie, R.; Ru, Y.; Gress, C. J.; Blake, S.; Lark, M. A.; Hwang, S.-M.; Tomaszek, T.; Offen, P.; Head, M. S.; Cummings, M. D.; Veber, D. F. *J. Med. Chem.* **2005**, *48*, 6870.
4. Nguyen, Q.; Mort, J. S.; Roughley, P. J. *Biochem. J.* **1990**, *266*, 569.
5. Maciewicz, R. A.; Wotton, S. F.; Etherington, D. J.; Duance, V. C. *FEBS Lett.* **1990**, *269*, 189.
6. Felbor, U.; Kessler, B.; Mothes, W.; Ploegh, H. L.; Goebel, H. H.; Bronson, R. T.; Olsen, B. R. *Proc. Natl. Acad. Sci.* **2002**, *99*, 7883.
7. Reinheckel, T.; Hagemann, S.; Dollwet-Mack, S.; Martinez, E.; Lohmüller, T.; Zlatkovic, G.; Tobin, D. J.; Maas-Szabowski, N.; Peters, C. *J. Cell Sci.* **2005**, *118*, 3387.
8. Hagemann, S.; Günther, T.; Dennemarker, J.; Lohmüller, T.; Brömme, D.; Schüle, R.; Peters, C.; Reinheckel, T. *Eur. J. Cell Biol.* **2004**, *83*, 775.
9. Nakagawa, T. Y.; Rudensky, A. Y. *Immunol. Rev.* **1999**, *172*, 121.
10. $pIC_{50} = -\log(IC_{50}/M)$.
11. Lser, R.; Schilling, K.; Dimmig, E.; Gtschow, M. *J. Med. Chem.* **2005**, *48*, 7688.
12. Guncar, G.; Pungercic, G.; Klemencic, I.; Turk, V.; Turk, D. *EMBO J.* **1999**, *18*, 793.
13. The model of the binding mode for **1** was generated from the RSB (home.rcsb.org) Protein Data Bank (www.rcsb.org/pdb/home/home.do) entry with reference code 1ICF (see Ref. 10) using the Maestro molecular modelling program. The structure was energy-minimized using MacroModel (OPLS 2005 force field; water solvent model). Both Maestro and MacroModel were licensed from Schrödinger, LLC (www.schrodinger.com).
14. ClogP, Version 4.3, BioByte (www.biobyte.com).
15. Values of pIC_{50} for inhibition of Cathepsins L, L2, S, K and B were determined from dose dependent inhibition of cleavage of fluorogenic, AMC-tagged, peptide substrates. See also Refs. 16,17.
16. Hulkower, K. I.; Butler, C. C.; Linebaugh, B. E.; Klaus, J. L.; Keppler, D.; Giranda, V. L.; Sloane, B. F. *Eur. J. Biochem.* **2000**, *267*, 4165.
17. Werle, B.; Staib, A.; Jülke, B.; Ebert, W.; Zladoidsky, P.; Sekernik, A.; Kos, J.; Speiss, E. *Biol. Chem.* **1999**, *380*, 1109.
18. Altmann, E.; Betschart, C.; Gohda, K.; Horiuchi, M.; Lattmann, R.; Missbach, M.; Sakaki, J.; Takai, M.; Teno, N.; Cowen, S. D.; Greenspan, P. D.; McQuire, L. W.; Tommasi, R. A.; Van Duzer, J. H. **1999**, WO 9924460.
19. Diffraction data for compound **26** were collected at 100 K on beamline PX at the Swiss Light Source (SLS, Villigen, Switzerland). The structure was solved by molecular replacement and refined to a final resolution of 1.27 Å and R-factor of 11.6% using the CCP4 (Ref. 19) and Coot software (Ref. 20) packages. This structure has been deposited in the RSB (home.rcsb.org) Protein Data Bank (www.rcsb.org/pdb/home/home.do) with reference code 3HHA.
20. Collaborative Computatonal Project, Number 4; (http://www.ccp4.ac.uk) *Acta Crystallogr., Sect. D* **1994**, *50*, 760.
21. Coot: http://www.ysbl.york.ac.uk/~emsley/coot.
22. Black, E.; Breed, J.; Breeze, A. L.; Embrey, K.; Garcia, R.; Gero, T. W.; Godfrey, L.; Kenny, P. W.; Morley, A. D.; Minshull, C. A.; Pannifer, A. D.; Read, J.; Rees, A.; Russell, D. J.; Toader, D.; Tucker, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2503.
23. Boström, J.; Grant, J. A. Exploiting Ligand Conformations in Drug Design. In *Drug Properties: Measurement and Computation*; Mannhold, R., Kubinyi, H., Folkers, G., Eds.; Wiley-VCH, 2007; p 183.
24. Elguero, J.; Marzin, C.; Katritzky, A. R.; Linda, P. *The Tautomerism of Heterocycles*; Academic Press: New York, 1976.
25. Claramunt, R. M.; García, M. A.; López, C.; Trofimenko, S.; Yap, G. P. A.; Alkorta, I.; Elguero, J. *Magn. Reson. Chem.* **2005**, *43*, 89.