Bioorganic & Medicinal Chemistry Letters 20 (2010) 2379–2382

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Thioether acetamides as P3 binding elements for tetrahydropyrido-pyrazole cathepsin S inhibitors

Danielle K. Wiener *, Alice Lee-Dutra, Scott Bembenek, Steven Nguyen, Robin L. Thurmond, Siquan Sun, Lars Karlsson, Cheryl A. Grice, Todd K. Jones, James P. Edwards

Johnson & Johnson Pharmaceutical Research & Development, L.L.C., 3210 Merryfield Row, San Diego, CA 92121, United States

ARTICLE INFO

Article history:
Received 30 October 2009
Revised 15 January 2010
Accepted 20 January 2010
Available online 8 February 2010

ABSTRACT

A series of tetrahydropyrido-pyrazole cathepsin S (CatS) inhibitors with thioether acetamide functional groups were prepared with the goal of improving upon the cellular activity of amidoethylthioethers. This Letter describes altered amide connectivity, in conjunction with changes to other binding elements, resulting in improved potency, as well as increased knowledge of the relationship between this chemotype and human CatS activity.

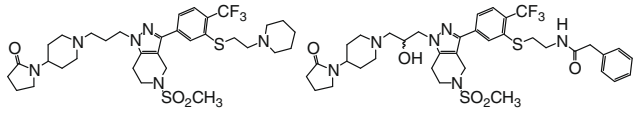
Published by Elsevier Ltd.

Cathepsin S (Cat S) is a cysteine protease that is responsible for the degradation of major histocompatibility complex class II (MHC II)-associated invariant chain (Ii) as a means of facilitating antigen presentation to $CD4^+$ T-cells.[1] Inhibition of CatS may attenuate antigen presentation by impeding the removal of Ii from MHC II molecules, thus regulating immune hyper responsiveness.

Extensive research surrounding tetrahydropyrido-pyrazole core molecules as non-covalent inhibitors of CatS has been reported by our laboratories.[2] Availability of crystal structures enabled the targeting of specific binding pockets of the human CatS enzyme,[2f] leading to the development of various tethered moieties, including the aminoethylthioether **1** (Fig. 1).[2g] Mitigating the basicity of this amine through amide formation (**2**) resulted in a complete loss of cellular activity. To continue this investigation, synthesis of amides with alternate connectivity was pursued.

Nucleophilic displacement of the previously described aryl nitro intermediate **3** with mercaptoethylacetate afforded the ester **4**. Regioselective alkylation of the core pyrazole, followed by acetal hydrolysis and reductive amination, yielded the desired intermediate with 4-piperidinylpyrrolidin-2-one installed as the P5 substituent. Saponification of the ethyl ester provided the carboxylic acid, which could then be coupled with various amines, exemplified by those shown in Table 1. In order to evaluate the activity of amide moieties as P3 binding elements, the P5 substituent and P4 sulfonamide were held constant (Scheme 1).

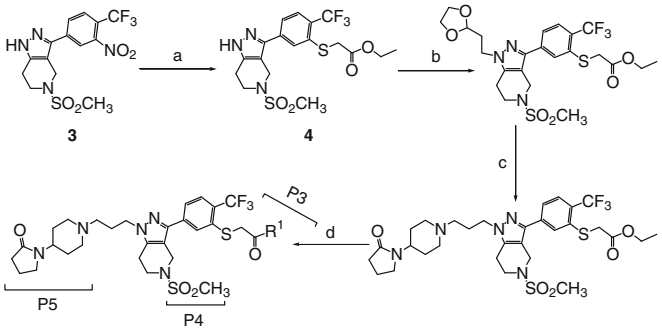
Initial results proved promising, with a variety of amides yielding sub-micromolar enzymatic activity (**5**–**13**). Secondary amides are tolerated, with hCatS IC_{50} = 0.37 and 1.15 μM for compounds **15** and **5**, respectively, while small, cyclic amides generally achieve excellent enzymatic activity between 20 and 210 nM (**7**–**13**). Previously reported amidoethylthioethers, such as compound **2**, are inactive in the JY invariant chain degradation cellular assay.[2g] Reversing the amide connectivity, as seen in the direct comparison of **2** and **15**, results in comparable enzymatic potency (0.13 μM vs



1: hCatS IC_{50} = 0.17 μM
JY Ii IC_{50} = 0.04 μM

2: hCatS IC_{50} = 0.13 μM
JY Ii IC_{50} > 10 μM

Figure 1. Non-covalent CatS inhibitors with thioether substituents.

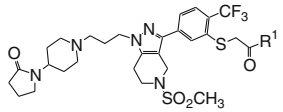


Scheme 1. Reagents and conditions: (a) $HSCH_2CO_2CH_2CH_3$, K_2CO_3, DMF, 70 °C (90%); (b) 2-(2-bromoethyl)-1,3-dioxolane, Cs_2CO_3, DMF (95%); (c) (i) 1 N HCl (aq), acetone, 55 °C; (ii) 1-piperidine-4-yl-pyrrolidin-2-one, acetic acid, $NaBH(OAc)_3$, CH_2Cl_2 (29%, two steps); (d) (i) LiOH, 3:1:1 THF/H_2O/EtOH (42%); (ii) HOBt, EDCI, HR^1, DMF (40–60%).

* Corresponding author. Tel.: +1 858 320 3384; fax: +1 858 450 2089.
E-mail address: dwiener@its.jnj.com (D.K. Wiener).

0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.01.103

Table 1
Results of thioether amide screen

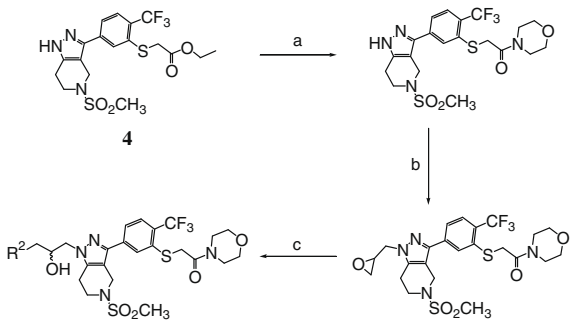


Compd	R^1	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a] (μM)
5	$NHCH_3$	1.15	ND
6	$N(CH_3)_2$	0.52	ND
7	Pyrrolidine	0.06	1.97
8	Piperidine	0.07	3.92
9	Morpholine	0.02	0.45
10	2-Methyl-pyrrolidine	0.10	~6
11	Pyrrolidin-3(*RS*)-ol	0.09	0.52
12	4-Methyl-piperidine	0.21	~8
13	Piperidine-4-yl-methanol	0.12	0.34
14	*N*-Methyl-piperazine	0.74	0.11
15	*N*-Benzylamine	0.37	~10

[a] CatS IC_{50} and JY li degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously[2b]; ND = not determined.

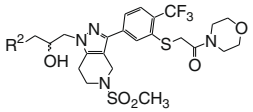
0.37 μM, respectively). Further, both the original amidoethylthioether (**2**) and the reverse linkage thioether acetamide (**15**) are inactive in the cellular assay. Continued manipulation of the amide substitution, however, offered various patterns with significantly improved cellular potency. Consider the pyrrolidine (**7**) and piperidine (**8**) amides: the addition of hydroxyl group substitution significantly improves cellular activity (**11**, **13**), while the addition of methyl substituents to the same ring systems results in a loss of cellular activity (**10**, **12**). Further, the incorporation of a second heteroatom within the amide ring improves cellular activity (**9**, **14**), revealing a very promising lead in morpholine amide **9**. Interestingly, the addition of a basic amine, *N*-methylpiperazine amide **14**, results in enhanced potency in the cellular assay versus enzymatic inhibition. This difference may correspond to previously reported findings in which lysosomotropism triggers the accumulation of cathepsin inhibitors in cells.[2f,g,3] These data support the hypothesis that within this series of thioethers removal of amine basicity in the P3 binding element may eliminate this effect.

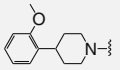
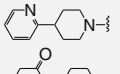
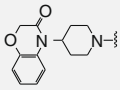
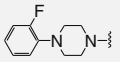
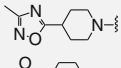
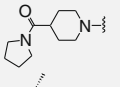
Utilizing the encouraging potency of the morpholine thioether acetamide **9**, evaluation of the overall binding motif through alteration of the P5 element proceeded. In order to readily probe this region of the molecule, a modified synthesis was established in which the mercaptoethyl acetate intermediate **4** was converted directly to the morpholine amide, by way of microwave heating (Scheme 2). Alkylation with epichlorohydrin yielded 20:1 pyrazole N-atom regioselectivity with the morpholine amide intermediate. Surprisingly, this transformation was not regioselective in the presence of mercaptoethyl acetate, though good selectivity was observed with the amide moiety elaborated. Access to an intermediate with the morpholine amide in place allowed for more facile analog synthesis through ring-opening of the epoxide using various amine P5 substituents (Table 2).



Scheme 2. Reagents and conditions: (a) Morpholine, 220 °C, microwave 30 min (80%); (b) epichlorohydrin, Cs_2CO_3, DMF (75%); (c) HR^2, EtOH, 80 °C (35–85%).

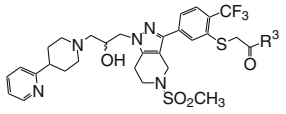
Table 2
Varying P5 substitutents with morpholino amide P3 binding element



Compd	R^2	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a] (μM)
16		0.01	0.19
17		0.01	0.38
18		0.01	0.91
19		0.01	3.13
20		0.02	3.46
21		0.04	0.28
22		0.30	~10

[a] CatS IC_{50} and JY li degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously[2b]; all compounds tested as racemic mixtures.

Table 3
Varied thioether-linked amides with preferred P5 substituent

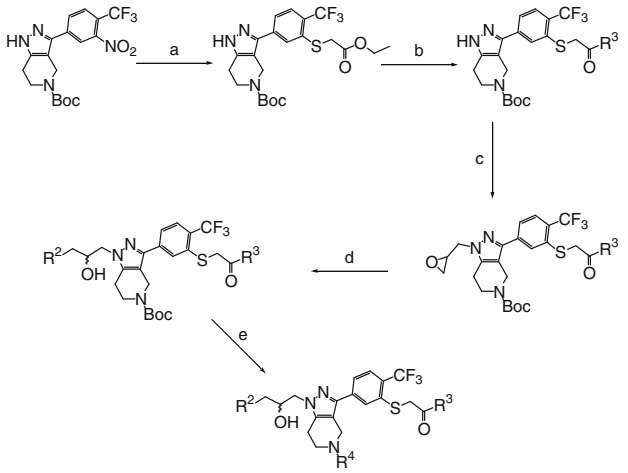


Compd	R^3	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a] (μM)
17	Morpholine	0.01	0.38
23	Pyrrolidine	0.001	0.47
24	Pyrrolidin-3(*R*)-ol	0.007	0.03
25	Pyrrolidin-3(*S*)-ol	0.007	0.03
26	Piperidine	0.01	1.54

[a] See Table 2 for details.

While changes to the P5 region result in enzymatic potency comparable to the parent morpholine amide **9**, the cellular potency varies widely. The 4-aryl-piperidine P5 elements (**16**, **17**) proved most effective in this investigation; and disappointingly, truncating the P5 region of the molecule results in a complete loss of cellular potency (**22**). Direct comparison of molecules with and without the hydroxyl group in the linker between the core and the P5 binding element display no significant difference in activity (data not shown).

Incorporation of an aryl-piperidine P5 element to thioether amides of interest from Table 1 yielded excellent results (Table 3), with pyrrolidinol analogs **24** and **25** reaching single digit nanomolar activity in the hCatS enzymatic assay. Pyrrolidine amide analogs such as **23** consistently maintain cellular potency despite the lack of additional solubilizing features such as oxygen-containing heterocycles or hydroxyl-substitution, which may improve cell permeability.



Scheme 3. Reagents and conditions: (a) $HSCH_2CO_2CH_2CH_3$, K_2CO_3, DMF, 70 °C (67%); (b) HR^3, 170 °C microwave, 90 min (41–48%); (c) epichlorohydrin, Cs_2CO_3, DMF (77–84%); (d) HR^2, EtOH; (e) (i) 4 N HCl/dioxane, CH_2Cl_2; (ii) Dowex 550A resin; (iii) HOR^4, HATU, HOAt, DIEA, DMF, 70 °C; or ClR^4, CH_2Cl_2; or HR^4, $NaBH(OAc)_3$, MeOH (11–31% three steps).

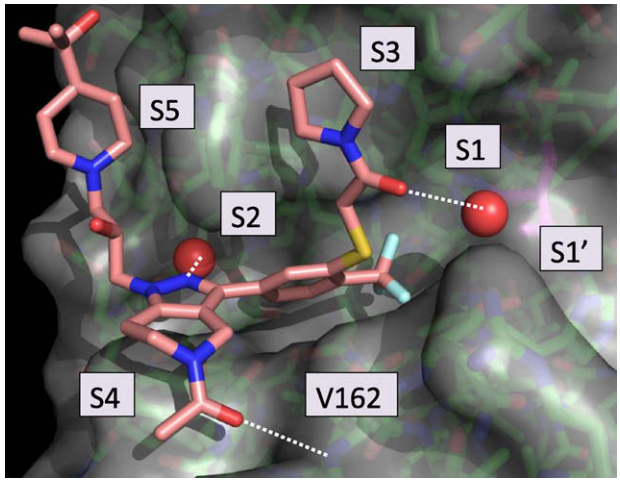


Figure 2. Crystal structure of **36** bound to a Cys25Ser mutant of cathepsin S. Compound conformation is shown as an average of two enantiomers. PDB deposition: 3KWN.

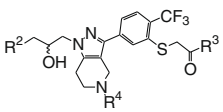
With optimized P3 and P5 substituents we sought to evaluate the impact of the P4 element, which had been held constant as a methyl sulfonamide. The synthetic sequence of Scheme 2 was altered to accommodate the use of a Boc-protected tetrahydropyrido-pyrazole core (Scheme 3). Lowering the temperature of amide formation limited premature Boc-removal; however the temperature of 170 °C used to form the desired amides excluded the use of enantiopure pyrrolidinol reagents due to potential epimerization of the stereogenic center. Analysis of the P4 region was therefore conducted using pyrrolidino and morpholino amides. After Boc-removal under acidic conditions, treatment with Dowex basic resin allowed for direct manipulation of the amino intermediate to yield the structures described in Table 4.

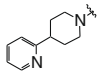
This data set depicts encouraging results for this series. Not only did acetamide and substituted acetamide P4 elements maintain potency in both the enzymatic and cellular assays (**27**, **31**, **32**), but deletion of the P4 substituent altogether was well tolerated (**29**, **34**). Again, pyrrolidino amides impart cellular activity where morpholino amides fail (**29** vs **28**, **34** vs **33**). Additionally, smaller P5 binding elements maintain activity when coupled with acetamide P4 functionality (**35**, **36**). This finding is particularly interesting in light of previously reported crystal structures[2f] depicting P4 substituent interactions locking the tetrahydropyrido-pyrazole core into the S2 pocket of the enzyme.

An X-ray crystal structure of **36** was obtained (Fig. 2). The resulting data not only confirmed binding of the thioether acetamide in the S3 pocket of the CatS enzyme, but also revealed a potential hydrogen bond interaction between the carbonyl of the amide and a water molecule in the S1 pocket. This additional binding interaction may account for the improved enzymatic potency of thioether acetamides when compared to previously reported aminoethylthioethers.[2g,h] It may also explain the ability of this series of compounds to function without substitution in the P4 region of the molecule—the new interaction may serve the function of fixing the tetrahydropyrido-pyrazole into the S2 pocket.

In conclusion, thioether acetamides were discovered as potent, non-covalent CatS inhibitors within the tetrahydropyrido-pyrazole series. Access to a beneficial hydrogen bond between the carbonyl of the amide moiety and a conserved water in the binding pocket allowed the removal of P4 binding elements while maintaining potency.

Table 4
The effects of varying P4 substitution





A

B

Compd	R^2	R^3	R^4	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a] (μM)
27	A	Morpholine	$C(O)CH_2OH$	0.01	0.16
28	A	Morpholine	H	0.11	1.68
29	A	Pyrrolidine	H	0.06	0.06
30	A	Pyrrolidine	CH_2CH_3	0.25	ND
31	B	Morpholine	$C(O)CH_2OH$	0.01	0.48
32	B	Morpholine	$C(O)CH_3$	0.02	0.90
33	B	Morpholine	H	0.19	1.85
34	B	Pyrrolidine	H	0.06	0.12
35		Pyrrolidine	$C(O)CH_3$	0.18	0.08
36		Pyrrolidine	$C(O)CH_3$	0.15	0.11

[a] See Table 2 for details.

References and notes

1. (a) Gupta, S.; Kumar Singh, R.; Dastidar, S.; Ray, A. *Exp. Opin. Ther. Targets* **2008**, *12*, 291; (b) Villandangos, J. A.; Bryant, R. A. R.; Deussing, J.; Driessen, C.; Lennon-Dumenil, A.-M.; Riese, R. J.; Roth, W.; Saftig, P.; Shi, G.-P.; Chapman, H. A.; Peters, C.; Ploegh, H. L. *Immunol. Rev.* **1999**, *172*, 109; (c) Nakagawa, T. Y.; Rudensky, A. Y. *Immunol. Rev.* **1999**, *172*, 121; (d) Shi, G.-P.; Villadangos, J. A.; Dranoff, G.; Small, C.; Gu, L.; Haley, K. J.; Riese, R.; Ploegh, H. L.; Chapman, H. A. *Immunity* **1999**, *10*, 197.
2. (a) Thurmond, R. L.; Beavers, M. P.; Cai, H.; Meduna, S. P.; Gustin, D. J.; Sun, S.; Almond, H. J.; Karlsson, L.; Edwards, J. P. *J. Med. Chem.* **2004**, *47*, 4799; (b) Thurmond, R. L.; Sun, S.; Sehon, C. A.; Baker, S. M.; Cai, H.; Gu, Y.; Jiang, W.; Riley, J. P.; Williams, K. N.; Edwards, J. P.; Karlsson, L. *J. Pharm. Exp. Ther.* **2004**, *308*, 268; (c) Gustin, D. J.; Sehon, C. A.; Wei, J.; Cai, H.; Meduna, S. P.; Khatuya, H.; Sun, S.; Gu, Y.; Jiang, W.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1687; (d) Grice, C. A.; Tays, K.; Khatuya, H.; Gustin, D. J.; Butler, C. R.; Wei, J.; Sehon, C. A.; Sun, S.; Gu, Y.; Jiang, W.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2209; (e) Wei, J.; Pio, B. A.; Cai, H.; Meduna, S. P.; Sun, S.; Gu, Y.; Jiang, W.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5525; (f) Ameriks, M. A.; Axe, F. U.; Bembenek, S. D.; Edwards, J. P.; Gu, Y.; Karlsson, L.; Randal, M.; Sun, S.; Thurmond, R. L.; Zhu, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 21; (g) Lee-Dutra, A.; Wiener, D. K.; Arienti, K. L.; Liu, J.; Neelakandha, M.; Ameriks, M. K.; Axe, F. U.; Gebauer, D.; Desai, P. J.; Nguyen, S.; Randal, M.; Thurmond, R. L.; Sun, S.; Karlsson, L.; Edwards, J. P.; Jones, T. K.; Grice, C. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2370; (h) Wiener, J. J. M.; Wickboldt, A. T.; Wiener, D. K.; Lee-Dutra, A.; Edwards, J. P.; Karlsson, L.; Nguyen, S.; Sun, S.; Jones, T. K.; Grice, C. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2375.
3. Lysosomotropic effects were reported by Merck researchers during their studies on cathepsin K inhibitors: Falgueyret, J.-P.; Desmarais, S.; Oballa, R.; Black, W. C.; Cromlish, W.; Khougaz, K.; Lamontagne, S.; Masse, F.; Riendeau, D.; Toulmond, S.; Percival, M. D. *J. Med. Chem.* **2005**, *48*, 7535.