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Discovery and SAR of novel pyrazole-based thioethers as cathepsin S inhibitors: Part 1

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ABSTRACT

A series of pyrazole-based thioethers were prepared and found to be potent cathepsin S inhibitors. A crystal structure of **13** suggests that the thioether moiety may bind to the S3 pocket of the enzyme. Additional optimization led to the discovery of aminoethylthioethers with improved enzymatic activity and submicromolar cellular potency.

Cathepsin S (CatS) is a cysteine protease of the papain family that is involved in the presentation of antigens to the cell surface of certain antigen-presenting cells (APCs) for recognition by $CD4^+$ T-cells. The main target of the proteolytic activity of CatS is the invariant chain (Ii), a chaperone molecule for major histocompatibility complex class II molecules (MHC II).[1] Inhibition of CatS activity slows the removal of Ii and attenuates antigen presentation to $CD4^+$ T-cells, resulting in immunosuppression with specificity for these T-cells.

We have previously reported our efforts to identify novel noncovalent inhibitors of CatS based on a tetrahydropyrido-pyrazole heterocycle (Fig. 1).[2] Much of this work focused on the SAR of substituent variations of the 'headgroup' as well as the P4 group, as exemplified by compounds **1** and **2**. More recently, we became interested in exploring substitution that would access S1 binding, ultimately discovering novel arylalkynes, such as compound **3**, that bind to this region of the enzyme.[2f] Concurrent efforts to explore alternative binding elements led to the preparation of analogs containing aryl thioethers as a replacement for these arylalkynes.

Preliminary investigation of such thioethers was conducted using aryl iodide intermediates **4** and **5**, which were synthesized as previously reported.[2f] Metal-mediated coupling with commercially available thiols afforded the desired thioether products (Scheme 1).[3]

As seen in Table 1, these initial compounds exhibited promising activity. Compounds **6** and **7** share similarly moderate enzymatic activity, indicating that the elongation of the methylene tether is not detrimental to activity. Replacement of the phenyl group with a methyl group (**8**) appears to decrease activity, whereas the incorporation of a phenyl ether (**9**) leads to submicromolar inhibition. Exchanging the *para*-chloro moiety with a trifluoromethyl group maintains activity (**9** vs **10**). However, substituting the morpholine headgroup with 1-piperidin-4-yl-pyrrolidin-2-one led to threefold improved activity for compound **11**.

Re-design of the synthesis route enabled more facile access to thioether variations through aromatic substitution rather than palladium-mediated coupling (Scheme 2). In this instance, initial reaction with mercaptoethanol was followed by alkylation with 2-(2-bromoethyl)-1,3-dioxolane. Unmasking the aldehyde under acidic conditions and subsequent reductive amination provided hydroxyethylthioether **12**, which was found to be equipotent to ether analog **11**.

A crystal structure was obtained for a close analog of alcohol **12** (compound **13**, Fig. 2).[4] Notably, the hydroxyethylthioether of **13** points toward the S3 region of the active site rather than toward

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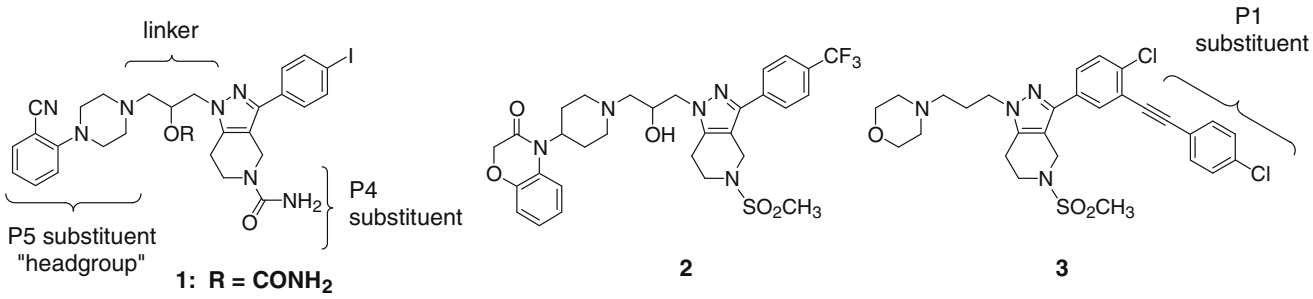
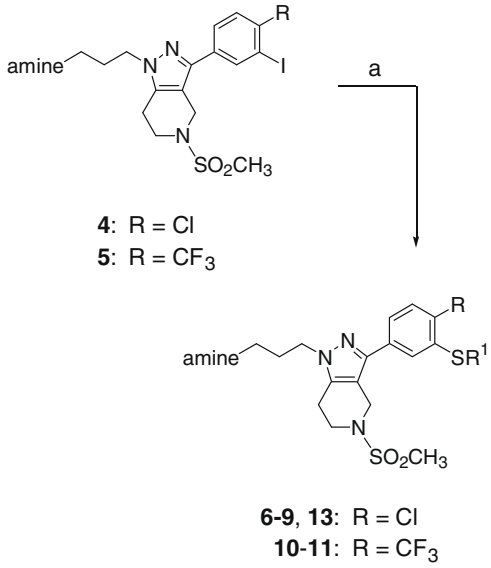
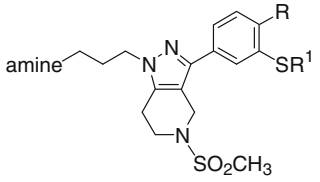


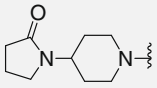
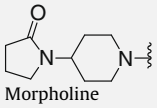
Figure 1. Previous CatS inhibitors with tetrahydropyrido-pyrazole core.



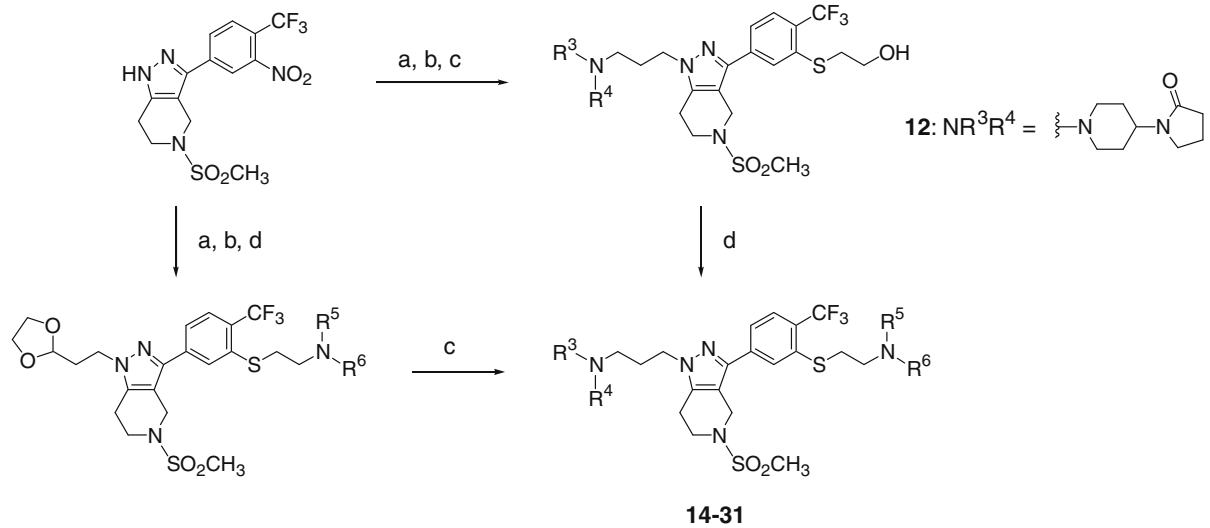
Scheme 1. Reagents and conditions: (a) HSR^1, $Pd_2(dba)_3$, dppf, Et_3N, DMF or NMP, 70–80 °C (36–95%). For $HSCH_2CH_2OH$: CuI, neocuproine, NaO*t*-Bu, toluene, 110 °C (43%). R^1 is defined in Table 1.

Table 1
Effect of thioether substitution on cathepsin S activity[a]

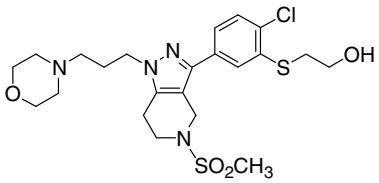


Compd	Amine	R	R^1	CatS IC_{50} (μM)
6	Morpholine	Cl	CH_2Ph	1.65
7	Morpholine	Cl	CH_2CH_2Ph	1.47
8	Morpholine	Cl	$CH_2CH_2CH_3$	3.60
9	Morpholine	Cl	CH_2CH_2OPh	0.60
10	Morpholine	CF_3	CH_2CH_2OPh	0.74
11		CF_3	CH_2CH_2OPh	0.22
12		CF_3	CH_2CH_2OH	0.20
13	Morpholine	Cl	CH_2CH_2OH	0.96

[a] CatS IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[2b]



Scheme 2. Reagents and conditions: (a) $HSCH_2CH_2OH$, K_2CO_3, DMF, 90 °C (75–89%); (b) 2-(2-bromoethyl)-1,3-dioxolane, Cs_2CO_3, DMF (95%); (c) (i) 1 N HCl (aq), acetone, 55 °C; (ii) HNR^3R^4, acetic acid, $NaBH(OAc)_3$, CH_2Cl_2 (6–51%, two steps); (d) (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (ii) HNR^5R^6, EtOH/DCE, 60 °C (33–65%, two steps). NR^3R^4 and NR^5R^6 are defined in Tables 2 and 3.



13

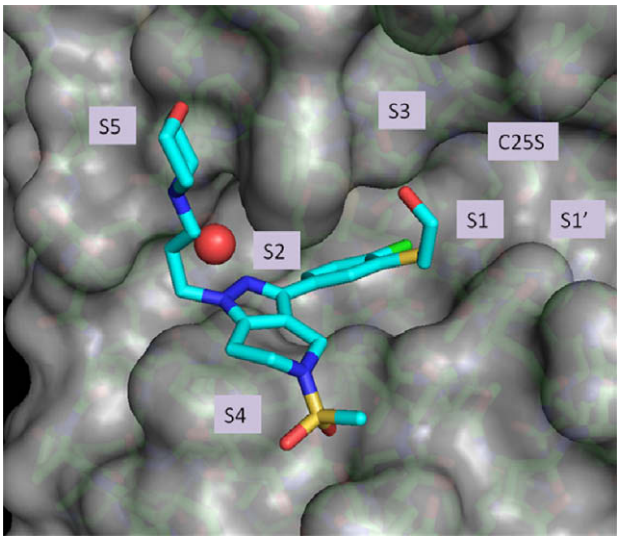


Figure 2. Crystal structure of **13** bound to a Cys25Ser mutant of cathepsin S and interacting with one molecule of water at the S2 pocket.

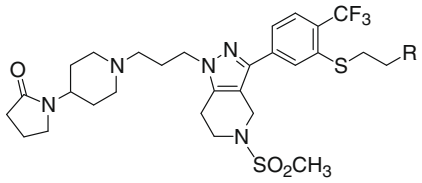
S1, as was observed for arylalkynes such as **3**. The close proximity of the S3 pocket as well as the tolerance for the hydroxyl group suggested that replacement with an amine functionality might be acceptable. To this end, analogs were prepared using alcohol **12** as an intermediate. Formation of the mesylate and reaction with various amines led to compounds **14–26**. Alternatively, by reversing the final two steps of the sequence, the right-hand side amine was held constant and the headgroup substitution was investigated, furnishing amines **27–31**.

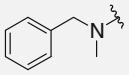
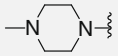
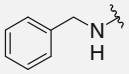
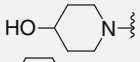
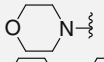
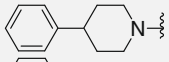
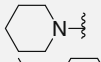
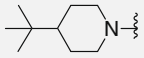
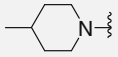
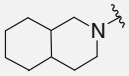
The enzymatic data in Table 2 for the right-hand side analogs suggests a preference for cyclic (**19–26**) over acyclic (**14–15**, **17–18**) amine substitution. Neither the display of an additional basic amine (**16**) nor truncation of the amine substitution (**15**, **17**) is favored. Double-digit nanomolar activity was attained through the incorporation of decahydroisoquinoline (**26**).

In general, the cellular activity of these analogs, as measured by the invariant chain degradation in a human JY cell assay, is submicromolar and reasonably corresponds to the enzymatic data. Three examples (**16**, **19**, and **23**) exhibit enhanced cellular versus enzymatic inhibition. This anomaly has been reported previously for our tetrahydropyrido-pyrazoles and may be due to lysosomotropism.[2e,5]

Variation of the headgroup can also influence the enzymatic activity, as previously observed between compounds **10** and **11** (Table 1). Compound **23** is included in Table 3 for ease of comparison with these additional analogs. Notably, smaller headgroups such as morpholine (**27**), pyrrolidine (**29**), and piperidine (**30**) are less preferred over the parent compound **23**. The presence of an additional basic amine (**28**) is not favored in this region of the molecule, though not detrimental to activity as it was for compound **16**. Replacement of 1-piperidin-4-yl-pyrrolidin-2-one with the headgroup from **2** yields **31** and confers equipotent enzymatic inhibition (**2**: CatS IC_{50} = 0.023 μM).

Table 2
Effect of aminoethylthioether substitution on cathepsin S activity[a]



Compd	R	CatS IC_{50}, μM	JY Ii IC_{50} (μM)
14		2.01	ND
15		1.28	ND
16		1.06	0.13
17		0.80	ND
18		0.70	0.46
19		0.41	0.08
20		0.34	0.35
21		0.27	0.68
22		0.20	0.95
23		0.17	0.04
24		0.16	0.33
25		0.11	0.81
26		0.055	0.46

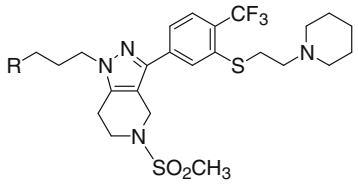
[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[2b] ND = not determined.

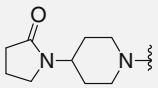
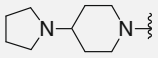
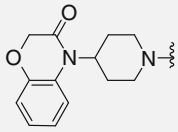
The observed cellular activity for compounds **28–30** closely matches the enzymatic data. However, both **27** and **31** experience a drop in their cellular activity with respect to their enzymatic inhibition, though **31** remains relatively potent compared to the other analogs with an IC_{50} = 210 nM.

Concomitantly, amide analogs were also explored using an alternative thioether intermediate (Scheme 3). The synthesis of these amides allowed for the incorporation of a 2-hydroxypropyl linker, which was featured in previous work in the tetrahydropyrido-pyrazoles and is exemplified by compound **2**. Though enzymatic and cellular activity is usually unchanged, the inclusion of this hydroxyl group can provide different physicochemical properties for the molecule. Alkylation with epichlorohydrin proceeded smoothly with the Boc-protected aminoethylthioether. Following nucleophilic opening of the epoxide and removal of the protecting group, standard coupling conditions enabled ready access to racemic mixtures of the amide analogs **32–36**. An additional deprotection step provided compound **37**. Notably, similar alkylating protocols were not regioselective with the hydroxyethylthioether intermediate from Scheme 2; alkylation would occur equally at either of the nitrogen atoms of the pyrazole ring.

There are few clear trends from the enzymatic data for these moderately active amides (Table 4). Substitution on the benzamide does little to affect enzymatic inhibition. In fact, neither the inclu-

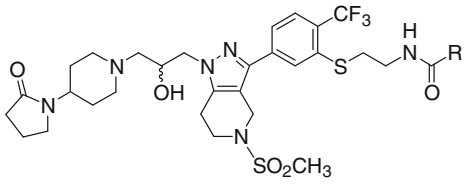
Table 3
Effect of varying the headgroup on cathepsin S activity[a]

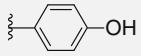
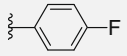
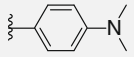
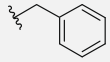
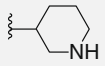


Compd	R	Cat S IC_{50} (μM)	JY Ii IC_{50} (μM)
23		0.17	0.04
27		0.81	3.57
28		0.56	0.32
29		0.52	0.47
30		0.38	0.38
31		0.03	0.21

[a] See Table 2 for details.

Table 4
Effect of amidoethylthioether substitution on cathepsin S activity[a]



Compds	R	CatS IC_{50} (μM)
32		0.38
33		0.39
34		0.36
35		0.53
36		0.13
37		0.42

[a] See Table 1 for details. These compounds were tested as racemic mixtures.

sion of an aniline (**35**) nor a more basic piperidine group (**37**) impacts activity. Only the insertion of a methylene group (benzamide **32** vs phenylacetamide **36**) results in a threefold increase in CatS inhibition. Ultimately, additional analogs in this amide series, including des-hydroxy linker-containing compounds, as well as further study of individual enantiomers were deprioritized due to general lack of cellular activity (IC_{50} >10 μM).

In conclusion, novel thioethers have been discovered as CatS inhibitors, where the thioether portion of the molecule appears to serve as a potential S3 binding element. The amidoethylthioethers demonstrate moderate enzymatic inhibition despite variations of the amide substitution. On the other hand, the aminoethylthioethers are easily influenced by amine choice at the headgroup or at the right-hand side of the molecule. Efforts to improve cellular activity within this thioether series will be reported in due course.

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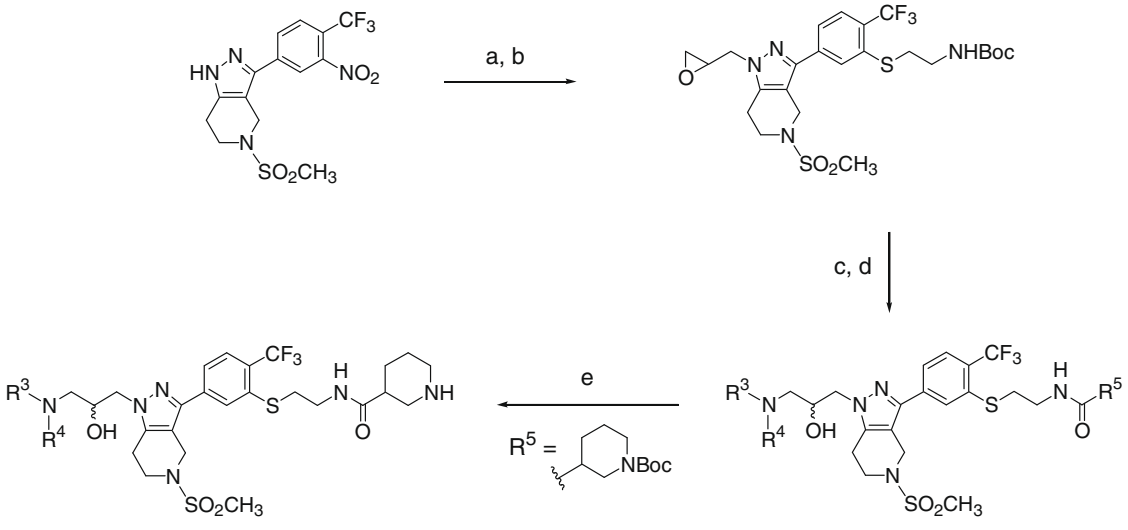

Scheme 3. Reagents and conditions: (a) $HSCH_2CH_2NHBoc$, K_2CO_3, DMF, 70 °C (86%); (b) epichlorohydrin, Cs_2CO_3, DMF (40%); (c) HNR^3R^4, EtOH, 90 °C (88%); (d) (i) Et_3SiH, TFA, CH_2Cl_2; (ii) R^5CO_2H, HATU, HOAt, $(i\text{-Pr})_2EtN$, DMF (13–58%, two steps); (e) Et_3SiH, TFA, CH_2Cl_2 (30%). NR^3R^4 and R^5 are defined in Table 4.

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4. The crystal structure of compound **13** could not be uploaded into the PDB because structure factors were not provided by Sunesis Pharmaceuticals, presumably because they were not required for submission into the PDB at the time this structure was solved (2004).
5. 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.