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Discovery and SAR of novel pyrazole-based thioethers as cathepsin S inhibitors. Part 2: Modification of P3, P4, and P5 regions

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ABSTRACT

A novel class of tetrahydropyrido-pyrazole thioether amines that display potency against human Cathepsin S have been previously reported. Here, further SAR investigations of the P3, P4, and P5 regions are described. In particular, 4-fluoropiperidine is identified as a competent P3 binding element when utilized in conjunction with a (*S*)-2-hydroxypropyl linker-containing P5 moiety and oxamide or sulfonamide P4 substitution.

Cathepsin S (CatS) is a cysteine protease that mediates cleavage of major histocompatibility class II (MHC II)-associated invariant chain (Ii), crucial in the initiation of MHC II-related immune response to an antigen.[1] The invariant chain blocks the MHC II binding groove and prevents loading of the antigen into the groove for subsequent presentation on the cell surface to $CD4^+$ T-cells. Inhibition of the CatS enzyme slows the degradation of the invariant chain, thereby reducing antigen presentation. For this reason, CatS inhibitors have been proposed for treatment of various autoimmune disorders, as well as other diseases. Inhibitors of CatS are often covalent-binding active site-modifiers, though recently noncovalent inhibitors have been disclosed.[2,3]

In the preceding paper,[4] the synthesis and biological evaluation of a novel class of noncovalent thioether amine- and amide-containing tetrahydropyrido-pyrazoles led to the identification of a series of molecules (e.g., **1**, Fig. 1) with good in vitro potency against human CatS, as measured in an enzymatic assay (hCatS IC_{50}) and in an invariant chain degradation cellular assay in human JY cells (Ii IC_{50}); these compounds are hypothesized to access the S3 binding pocket of the enzyme, based on computational studies and preliminary X-ray crystal structure analysis. In an effort to improve potency and further elucidate the binding mode, additional investigations into the SAR of this template were undertaken, seeking to optimize the P3, P4, and P5 regions of this thioether amine series.

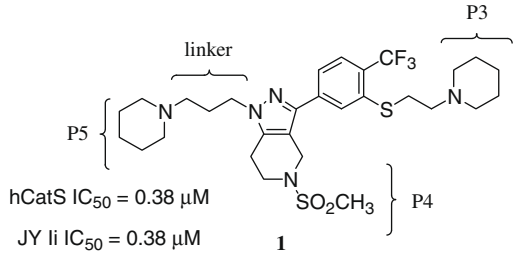
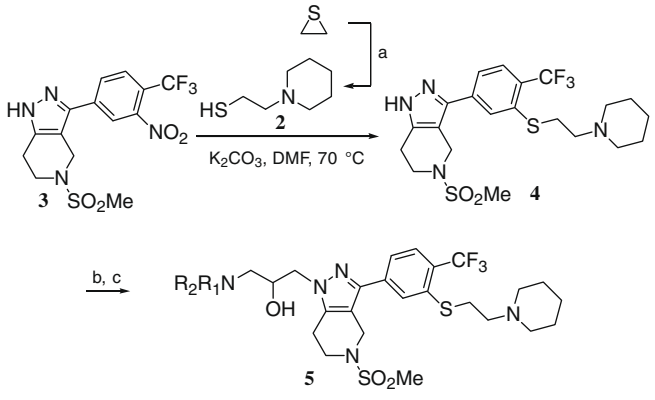


Figure 1.



Scheme 1. Reagents and conditions: (a) piperidine, toluene, 110 °C; (b) epichlorohydrin, Cs_2CO_3, DMF, rt; (c) NR_1R_2, EtOH, 80 °C.

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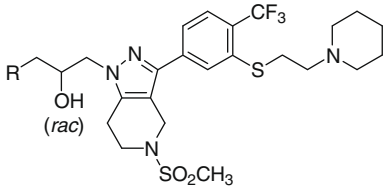
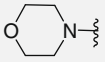
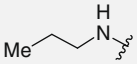
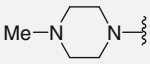
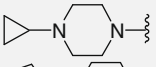
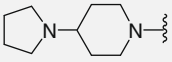
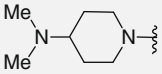
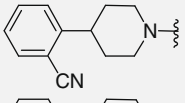
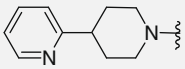
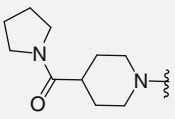
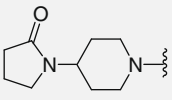
Variation of the P5 amine architecture was explored in conjunction with a 2-hydroxypropyl linker found in other pyrazole-based CatS inhibitor series reported previously.[3] The desired analogs were prepared according to the revised synthetic route shown in Scheme 1, which allowed for both introduction of the hydroxyl substituent as well as rapid variation of the P5 substituent. Treatment of thiirane with a secondary amine afforded the amino thiol **2**. This thiol was added in nucleophilic fashion to the unsubstituted tetrahydropyrido-pyrazole core **3**, prepared in an analogous fashion to similar cores previously described,[3] to afford pyrazole **4**. Alkylation with epichlorohydrin affords an epoxide intermediate, which is readily derivatized to the desired analogs **5** by simple treatment with various amines. The desired analogs can be prepared in enantiopure form by replacing epichlorohydrin with (*R*)- or (*S*)-glycidyl nosylate.

Generally, analogs with the 2-hydroxypropyl linker-containing a variety of amines as P5 elements display good potency in the enzymatic and cellular assays, comparable to the piperidine P5 parent **6** (Table 1). Tolerated P5 substituents include pyrrolidine and morpholine (**7** and **8**) as well as acyclic amine substituents (**9**). Notably, the presence of a second basic amine in the six-membered ring is deleterious (**10**), though decreasing the basicity of that second basic amine can restore potency (**11**), as can exocyclic placement of the second basic amine (**12** and **13**). Significant improvement in enzymatic potency is realized only for analogs with aromatic substituents at the 4-position of the piperidine (**14** and **15**), though these compounds are significant inhibitors of the hERG K^+ channel as measured by an astemizole binding assay (data not shown). Given the reasonable potency and suitable secondary properties of the simple, relatively low molecular weight compound **6** (in contrast to potent but also larger analogs such as **14**–**17**), further optimization focused on this structure.

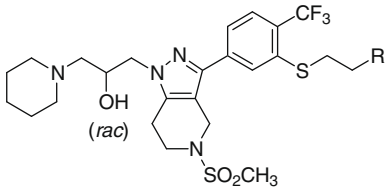
Table 1
Aminothioethers with 2-hydroxypropyl linker: variation of left-hand side amine (P5)

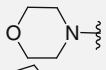
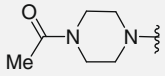
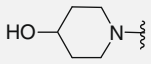
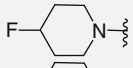
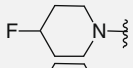
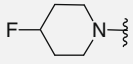


Compound	R	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a,b] (μM)
6		0.204	0.335
7		0.335	0.401
8		0.325	0.889
9		0.595	nd
10		1.15	nd
11		0.290	0.220
12		0.218	0.081
13		0.710	nd
14		0.010	0.160
15		0.010	0.062
16		0.090	0.096
17		0.040	0.152

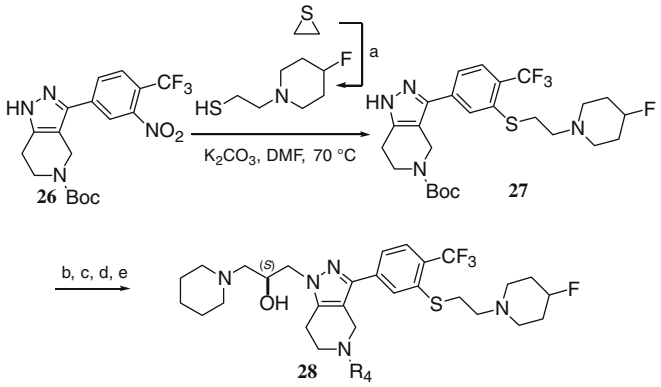
[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[3a,c] All IC_{50}s were within a twofold range.
[b] 'nd' denotes data not determined.

Table 2
Aminothioethers with 2-hydroxypropyl linker: variation of right-hand side amine (P3)



Compound	R	hCatS IC_{50}[a] (μM)	JY Ii IC_{50}[a,b] (μM)
18	NH_2	1.13	nd
19		0.420	2.517
20		0.712	0.458
21		1.035	nd
22		0.565	nd
23		0.149	0.493
24[c]		0.395	0.810
25[d]		0.058	0.120

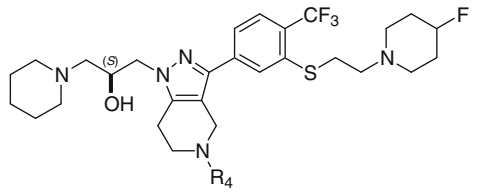
[a] CatS IC_{50} and JY Ii degradation IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[3a,c] All IC_{50}s were within a twofold range.
[b] 'nd' denotes data not determined.
[c] (*R*)-Enantiomer.
[d] (*S*)-Enantiomer.

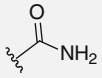
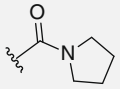
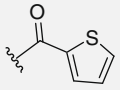
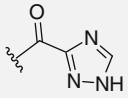
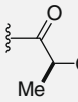
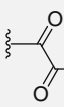


Scheme 2. Reagents and conditions: (a) 4-fluoropiperidine, toluene, 110 °C; (b) (*S*)-glycidyl nosylate, Cs_2CO_3, DMF, rt; (c) piperidine, EtOH, 80 °C; (d) 4 N HCl/dioxane, rt; (e) R_4-Cl, CH_2Cl_2; or R_4-OH, HATU, HOAt, *i*-Pr_2EtN, DMF; or R-NCO, CH_2Cl_2.

A variety of analogs (Table 2) exploring changes to the P3 substituent in conjunction with the 2-hydroxypropyl linker and the piperidine P5 moiety were prepared using the methods of Scheme 1 or methods similar to those reported previously.[4] Replacement of piperidine with a simple primary amine (**18**) decreases enzymatic inhibition, as does inclusion of rings such as morpholine, pyrrolidine, and substituted piperazine (**19–21**). Substitution of the piperidine at the 4-position with a hydroxyl was not beneficial (**22**). Though fluorine substitution on the piperidine, as in racemate **23**, does not appreciably affect potency, the stereochemical orientation of the hydroxyl moiety has a marked effect. Comparison of compounds **24** and **25** reveals that the (*S*) orientation is preferred to the (*R*) orientation. Indeed, the (*S*)-2-hydroxypropyl linker analog **25** offers the best combination of increased potency and low molecular weight, and, as such, was selected for further optimization through variation of the P4 binding element.

Table 3
Aminothioethers with (*S*)-2-hydroxypropyl linker: variation of P4 region



Compound	R_4	hCatS IC_{50}[a] (μM)
25	SO_2Me	0.058 (0.120)[c]
29[b]	H	9.6
30[b]	Me	9.0
31	SO_2iPr	0.35
32	SO_2Pr	0.30
33		0.235
34		1.19
35		0.355
36		0.205 (0.32)[c]
37		0.385
38		0.690
39		0.235
40		0.245
41		0.470
42		0.310
43[b]		0.810
44		0.07 (0.19)[c]

[a] CatS IC_{50} values are the mean of $n \geqslant 2$ runs and determined as described previously.[3a] All IC_{50}s were within a twofold range.

[b] Data reported are for the racemate.

[c] JY Ii degradation IC_{50} (μM) data are in parentheses. Values are the mean of $n \geqslant 2$ runs and determined as described previously.[3c] All IC_{50}s were within a twofold range.

Following a sequence similar to that shown in Scheme 1, replacement of the sulfonamide moiety to modify the P4 region of these molecules was accomplished as shown in Scheme 2. The sequence started from the Boc substituted pyrazole intermediate **26**, prepared analogously to the sulfonamide material. Following introduction of the P3 and P5 amino fragments, removal of the Boc group was accomplished using HCl, and this secondary amine intermediate was treated with a variety of acid chlorides, sulfonyl chlorides, carboxylic acids, or alkyl halides under the appropriate conditions to afford the desired analogs.

As shown in Table 3, removal of the P4 substituent altogether leads to a molecule without appreciable potency (**29**), as does replacement of the methyl sulfonamide with a simple methyl substituent (**30**). Other sulfonamides such as **31** and **32** are slightly less potent than the methyl sulfonamide parent **25**, as are ureas and aromatic amides (**33–36**). Simple acetamide and propionamide substituents (**37** and **38**), as well as substituted acetamides (**39–42**), are somewhat deleterious to potency. Basic amine substituents on the acetamide are not beneficial (**43**), though, importantly, preserving the overall length of the amino-acetamide substituent while also reducing the basicity in the context of an oxamide substituent as in analog **44** reproduces the enzymatic and cellular potency of sulfonamide analog **25**. Though none of these P4 variations successfully improved potency relative to the sulfonamide P4 element, the ability to retain potency with a non-sulfonamide entity offers opportunities for further exploration of other P4 substituents.

These studies with pyrazole-based CatS inhibitors have provided an expanded understanding of SAR within several relevant enzyme binding regions. The fluoro-piperidine P3 substituent, an enantiomerically-pure (*S*)-2-hydroxypropyl linker, and a variety of non-sulfonamide P4 substituents have been identified as beneficial, offering favorable enzymatic and cellular potency. Further investigations with these and other, related structures will be reported in due course.

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