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Discovery of selective and nonpeptidic cathepsin S inhibitors

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ABSTRACT

Nonpeptidic, selective, and potent cathepsin S inhibitors were derived from an in-house pyrrolopyrimidine cathepsin K inhibitor by modification of the P2 and P3 moieties. The pyrrolopyrimidine-based inhibitors show nanomolar inhibition of cathepsin S with over 100-fold selectivity against other cysteine proteases, including cathepsin K and L. Some of the inhibitors showed cellular activities in mouse splenocytes as well as oral bioavailabilities in rats.

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Cathepsin S (Cat S) is a lysosomal cysteine protease belonging to the papain superfamily, which is expressed in spleen, antigen presenting cells, such as dendritic cells, B cells, and macrophages.[1] The major role of Cat S is the processing of the major histocompatibility complex (MHC) class II associated invariant chain, which is essential for the normal functioning of the immune system. Cat S is thus an attractive therapeutic target for the treatment of autoimmune disorders. It is also reported that Cat S is implicated in various diseases such as cancer, Alzheimer's disease, and neuropathic pain.[2] Other cysteine proteases, Cat K and L, play a significant role in numerous important physiological and pathological processes, such as bone resorption, cancer progression, and atherosclerosis.[3] Herein, we describe the discovery of novel selective Cat S inhibitors, which should be safer therapeutic agents than nonselective inhibitors by avoiding off-target side effects.[4,5]

The X-ray crystal structures of human Cat S, K, and L in complex with inhibitors have been disclosed.[6–8] The high sequence homology among Cat S, K, and L reflects the similarity in 3D structures of the proteins. Although the design of specific Cat S inhibitors represents a considerable challenge, there are some differences in the subsite functionalities among the cathepsins. The S2 subsite in Cat S has Gly137 and Gly165, while the corresponding regions in the S2 subsites comprise of Ala134/Ala163 in Cat K and Ala135/Gly164 in Cat L (Fig. 1). This means that the S2 subsite of Cat S accepts slightly bulkier groups than that of Cat K and L. The S3 subsite in the Cat S enzyme has Phe70 and Lys64, which exhibit distinctive electrostatic features compared to Tyr67/Asp61 in Cat K and Leu69/Glu63 in Cat L, respectively. The positively charged ammonium group of Lys64 in the S3 subsite of Cat S could make a favorable electrostatic interaction with the negatively charged functional group on the P3 moiety of ligand, such as a carboxylate group, although this functional group should have electrostatic repulsion with the negatively charged side chains of Asp61 and Glu63 in the S3 subsites of Cat K and L, respectively. Those structural features in both subsites gave us a clue to the discovery of Cat S selective compounds against Cat K and L.

Recently, we reported a novel and potent nonpeptidic pyrrolopyrimidine Cat K inhibitor **1**[9] (Fig. 2). Our strategy for the discovery of selective Cat S inhibitors was to switch selectivity preference to Cat S starting with compound **1**, which exhibits a modest potency against Cat S (IC_{50} = 460 nM) and good synthetic feasibility for the modification of both P2 and P3 parts. Initial efforts to improve potency and selectivity were focused on finding an optimal size for the P2 functionalities of compound **1** by replacing the neopentyl group.

Our parallel synthetic approach to explore SAR on the P2 part is shown in Scheme 1. The key intermediate **4** was prepared from commercially available 4-amino-5-bromo-2-chloropyrimidine **2**. Treatment of **2** with NaCN and a catalytic amount of DABCO in

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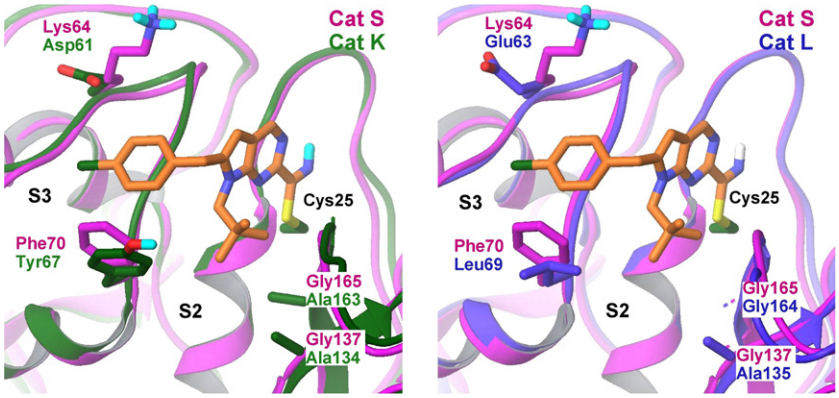


Figure 1. Compound **1** docked with human cathepsin S (Magenta), K (Green)—left, and L (Violet)—right showing 3D arrangement of subsite side chains.

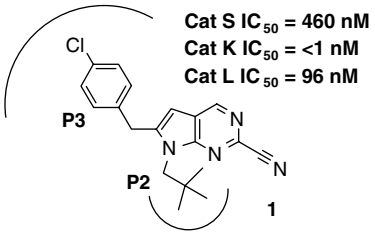
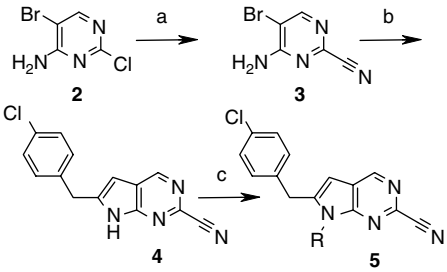
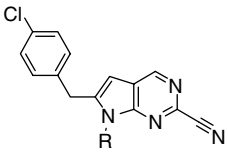


Figure 2. Nonpeptidic cathepsin K inhibitor.



Scheme 1. Reagents and conditions: (a) NaCN, DABCO, DMSO–H_2O, 60 °C, 4 h, 83%; (b) prop-2-ynyl-*p*-chlorobenzene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C, 7 h, 40%; (c) R-X (X = Cl, Br, or I), K_2CO_3, DMF, rt, 5 h, 65–80%, or R-OH, DEAD, PPh_3, THF, rt, 10 h, 45–60%.

Table 1
Inhibition of human Cat K, L, and S by compounds **1** and **5a–l**—initial optimization of P2



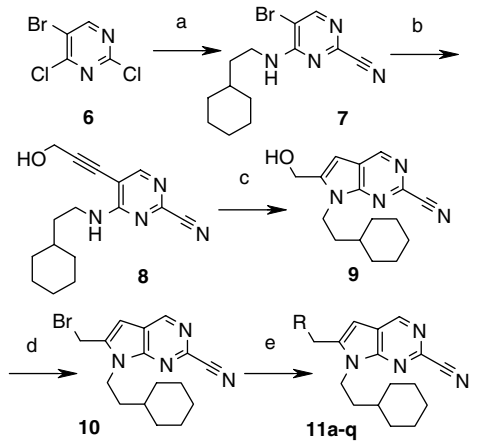
Compound	R	IC_{50}[a] (nM)		
		Cat S	Cat K	Cat L
1	$-CH_2{}^tBu$	460	<1	96
5a	$-(CH_2)_2{}^tBu$	2	<1	18
5b	$-(CH_2)_3{}^tBu$	320	100	>1000
5c	$-CH_2$-Cyclohexyl	110	6	370
5d	$-(CH_2)_2$-Cyclohexyl	21	220	320
5e	$-(CH_2)_3$-Cyclohexyl	150	>1000	>1000
5f	$-(CH_2)_2$-Cyclopentyl	7	17	260
5g	$-(CH_2)_2$-Cycloheptyl	33	>1000	840
5h	$-(CH_2)_2$-Cyclooctyl	99	>1000	>1000
5i	$-(CH_2)_2$-Phenyl	64	110	23
5j	$-(CH_2)_2$(*p*-Cl)Phenyl	19	>1000	86
5k	$-(CH_2)_2$(*m*-Cl)Phenyl	190	>1000	40
5l	$-CH_2CH_2-N$	>1000	>1000	>1000

[a] Inhibition profiles were determined by a fluorometric assay with recombinant human Cat K, L, and S, employing Z-Phe-Arg-AMC (Cat K and L) and Z-Leu-Leu-Arg-AMC (Cat S) as synthetic substrates.[9]

DMSO–H_2O, followed by a Castro-Stephens type reaction using catalytic amounts of $Pd(PPh_3)_2Cl_2$ and CuI in DMF, afforded pyrrolopyrimidine **4**. The Mitsunobu coupling reaction of alcohols or treatment of alkyl halides with the key intermediate **4** provided compounds **5a–l** bearing a variety of P2 parts, in order to allow the identification of the optimum P2 part for the S2 subsite in Cat S.

Insertion of a methylene group into the P2 part of compound **1** dramatically enhanced the inhibitory activity against Cat S because the S2 subsite in Cat S possesses a deeper pocket than that of Cat K and L (Table 1, **1** vs **5a**, **5c** vs **5d**). Extension of one more methylene group into the P2 subsite of **5a** significantly decreased Cat S affinity (**5a** vs **5b**, **5d** vs **5e**). We expected the structural plasticity of the S2 subsite in Cat S, because the Phe211 near the bottom of the S2 pocket is known to move its position to adjust to either a small or a bulky P2 side chain of ligands.[6] Replacement of the ^{t}Bu group on **5a** with a bulkier cycloalkane group improved selectivity toward Cat S (**5a** vs **5d**, **5f–h**). Introduction of phenyl derivatives with an ethyl spacer led to loss of selectivity against Cat L (**5d** vs **5i–k**), although compound **5j** having a *p*-chlorophenethyl group on the P2 moiety showed promising potency against Cat S and selectivity over Cat K. Substitution of a polar functional group on the P2 part was not tolerated due to the hydrophobic properties of the S2 susbsites in Cat S, K, and L (**5d** vs **5l**). This SAR study for the P2 optimization led to the conclusion that the cyclohexylethyl group was suitable for the P2 moiety on pyrrolopyrimidine because of an excellent balance between Cat S potency (**5d**; IC_{50} = 21 nM) and selectivity against Cat K and L (over 10-fold selectivity), while the cycloheptylethyl **5g** was also promising P2 moiety. We next set out to explore the optimization of the P3 part on the pyrrolopyrimidine scaffold to improve selectivity against Cat K and L.

A key intermediate, bromide **10**, was prepared following the previously described synthetic route for the parallel synthesis approach[7] to optimize the P3 moiety (Scheme 2). Suzuki-coupling reactions between the bromide **10** and commercially available aryl boronic acids with a catalytic amount of $Pd(dppf)Cl_2$ in THF gave corresponding 6-arylmethyl pyrrolopyrimidines **11a**, **b**. Treatment of commercially available anilines, thiol, or phenol derivatives with bromide **10** under basic conditions provided compounds **11d–q** having a variety of P3 moieties.[10]



Scheme 2. Reagents and conditions: (a) i—cyclohexylethylamine, Et_3N, MeOH, 0 °C to rt, 10 h, 76%, ii—NaCN, DABCO, DMSO–H_2O, 60 °C, 10 h, 83%; (b) propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C, 2 h, 96%; (c) DBU, DMF, 100 °C, 2 h, 56%; (d) CBr_4, Ph_3P, CH_2Cl_2, 0 °C, 0.5 h, rt, 3 h, 78%; (e) $ArB(OH)_2$, $Pd(dppf)Cl_2$-CH_2Cl_2, Cs_2CO_3, THF, 60 °C, 1 h, 46–77% for **11a**, **b** or ArXH, (X = NH, NMe, S, O), K_2CO_3, DMF, 0 °C to rt, 15–88% for **11c–q**.

Substitution of the P3 aromatic ring resulted in a loss of inhibitory activity to Cat S (Table 1, **5d** vs Table 2; **11a**, **b**). Saturation of the P3 phenyl ring decreased the Cat S potency (**5d** vs **11c**), due in part to the loss of π–π interaction between the phenyl ring on the pyrrolopyrimidine and Phe70 in the S3 subsite of Cat S. Insertion of a polar hetero atom, N, O, or S, between the P3 part and the pyrrolopyrimidine scaffold for changing the distance of the P3 aromatic ring from the pyrrolopyrimidine scaffold enhanced the Cat S affinity along with improved selectivity over Cat K and L (**5d** vs **11d**, **f**, **h**), presumably due to the structural feature unique to the Cat S enzyme owing to the Phe70 and Lys64 in the S3 subsite. The electronic requirement in the P3 moieties was investigated next. Replacement of the phenyl ring with a pyridyl ring caused a loss of selectivity against Cat K and L (**11f** vs **11g**). In order to give additional attractive interaction between the positively charged Lys64 in the S3 subsite of Cat S and negatively charged functional group, a carboxylic acid was introduced to the P3 phenyl group, which showed excellent potency (**11j**; IC_{50} = 6 nM) and high selectivity (>450-fold) against Cat K and L.

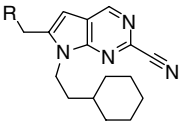
While compound **11j** had both excellent potency and selectivity for Cat S, it was inactive at 10 μM in a secondary cellular assay[12] measuring MHC II associated invariant chain degradation in mouse splenocytes. Another compound **11i** (IC_{50} = 16 nM) with a carboxylic acid also showed no cellular activity at 10 μM, although compound **5a** and LHVS[12] as a positive control, demonstrated high sensitivity of cellular activity at 1 μM. From these results, it was anticipated that a carboxyl group would decrease permeability into cells. We thus turned to improving the cellular potency.

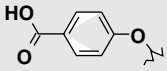
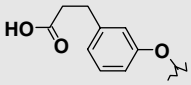
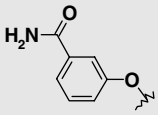
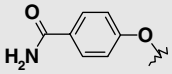
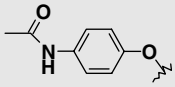
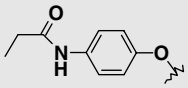
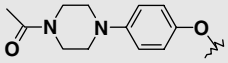
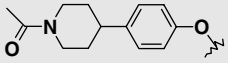
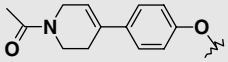
Replacement of the carboxyl group with amide was well tolerated by Cat S (**11i** vs **11l**). Extension of the P3 part on **11l** into the deep S3 subsite of Cat S resulted in high affinity (IC_{50}; 9–15 nM) to Cat S with excellent (~100-fold) selectivity against Cat K and L (**11m–q**). Interestingly, compounds **11o** and **11q** showed cellular activities at 1 μM in mouse splenocytes.

Selected compounds, **11l**, **o**, **p**, were evaluated for pharmacokinetic properties in cassette dosing experiments[13] with male Sprague–Dawley rats (Table 3). All compounds had acceptable oral bioavailabilities of greater than 21% and moderate to high plasma clearances of 2.5–5.3 L/h/kg and moderate terminal elimination half-lives of 0.7–1.4 h after intravenous administration.

In summary, potent and selective nonpeptidic Cat S inhibitors were discovered. The novel pyrrolopyrimidine scaffold **1** derived from the Cat K inhibitors was modified by stepwise optimization utilizing key structural and electrostatic features of the S2 and S3 subsites of the Cat S enzyme. Some of the selective Cat S inhibitors identified had potent cellular efficacies in mouse splenocytes and modest PK profiles in rats. The biological activities in rodents by

Table 2
Inhibition of human Cat S, K, and L by compounds **11a–q**—optimization of P3



Compound	R	IC_{50}[a] (nM)		
		Cat S	Cat K	Cat L
11a	4-MeO-Phenyl-	110	280	>1000
11b	1-Naphtyl-	440	—	—
11c[11]	Cyclohexyl-	140	—	>1000
11d	Phenyl-N(H)-	24	>1000	>1000
11e	Phenyl-N(Me)-	52	>1000	>1000
11f	Phenyl-O-	10	>1000	>1000
11g	2-Pyridyl-O-	11	250	790
11h	4-Pyridyl-S-	6	480	>1000
11i		16	>2000	>2000
11j		6	>5000	>5000
11k		7	390	>1000
11l		2	280	>1000
11m		8	>1000	>1000
11n		12	>1500	>4000
11o		9	>1500	>4000
11p		15	>1000	>1000
11q		13	>1000	>1000

[a] Inhibition profiles were determined by a fluorometric assay with recombinant human Cat K, L, and S, employing Z-Phe-Arg-AMC (Cat K and L) and Z-Leu-Leu-Arg-AMC (Cat S) as synthetic substrates.[9]

Table 3
Pharmacokinetic parameters of **11l** and **11o–q** in male Sprague–Dawley rats (**11l**; iv 1.6 mg/kg; po 4.0 mg/kg, **11o–q**; iv 1 mg/kg; po 3 mg/kg), where values are means of $n = 3$

Compound	Cl (L/h/kg)	$_{iv}t_{1/2}$ (h)	F (%)	$_{po}C_{max}$[a] (nM)	$_{po}AUC$[a] (nM h)
11l	5.0	0.7	21	25	108
11o	4.0	1.2	47	34	242
11p	5.3	1.3	25	17	103
11q	2.5	1.4	38	43	346

[a] Dose-normalized to 1 mg/kg.

their Cat S specific inhibition will be described in future publications.

Acknowledgments

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11. Preparation of **11c**; A Sonogashira reaction of **7** with prop-2-ynylcyclohexane as an alkyne under the same condition as (b) followed by a cyclization condition (c) in Scheme 2, yielded **11c**.
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