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4-Amino-2-cyanopyrimidines: Novel scaffold for nonpeptidic cathepsin S inhibitors

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ABSTRACT

We describe here a novel 4-amino-2-cyanopyrimidine scaffold for nonpeptidomimetic cathepsin S selective inhibitors. Some of the synthesized compounds have sub-nanomolar potency and high selectivity toward cathepsin S along with promising pharmacokinetic and physicochemical properties. The key structural features of the inhibitors consist of a combination of a spiro[2.5]oct-6-ylmethylamine P2 group at the 4-position, a small or polar P3 group at the 5-position and/or a polar group at the 6-position of the pyrimidine.

Eleven members of the cysteine cathepsin family have been identified in the human genome (cathepsins B, C, H, F, K, L, O, S, V, W, and X).[1] Lysosomal cysteine proteases are important enzymes for processing proteins such as prohormones. Recent studies on their genes revealed that these cathepsins have specific individual functions which are important for the normal functioning of an organism. These functions are often associated with the restricted tissue localization of the cathepsins, as demonstrated for cathepsins S, V, and K. Cathepsin S (Cat S) is predominantly expressed in spleen, professional antigen presenting cells (APC), such as dendritic cells, B lymphocytes, and macrophages. The major role of Cat S in these cells 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 largely responsible for the last proteolytic cleavage step of the invariant chain that produces class II-associated leupeptin induced peptide (CLIP). Due to its role in the immunological system, Cat S is an attractive therapeutic target and selective Cat S inhibitors[2] may also modulate a number of other diseases such as rheumatoid arthritis, multiple sclerosis, myasthenia gravis, asthma, atherosclerosis, and neuropathic pain.[3]

We have recently reported a novel Cat K inhibitor **1** and its analogs having a 2-cyanopyrimidine scaffold.[4] However, 2-cyanopyrimidine derivatives were rapidly cleared from the circulation in

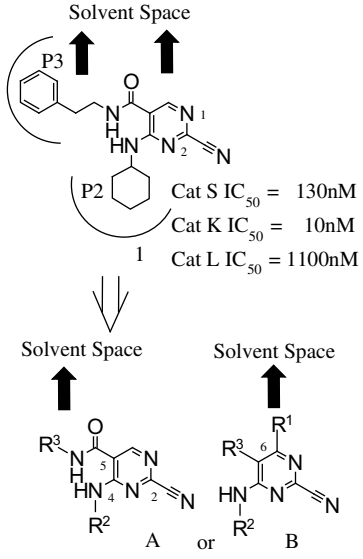

Figure 1. Design of new 2-cyanopyrimidine Cat S inhibitors.

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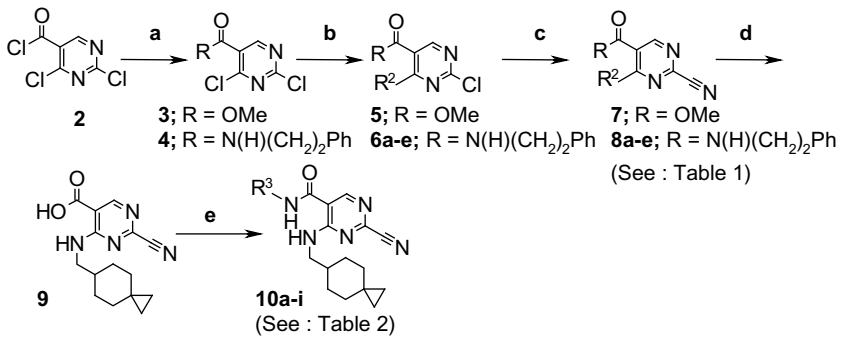
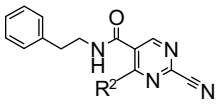


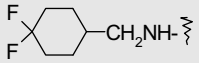
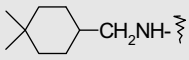
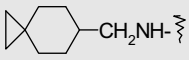
Scheme 1. Reagents and conditions: (a) MeOH or phenethylamine, NEt_3, CH_2Cl_2, 0 °C–rt, 69–86%; (b) R^2XH (X = NH or NMe), MeOH, rt–60 °C, 88–98%, or cyclohexylmethanol, NaH, THF, 0 °C–rt, 32%; (c) NaCN, DABCO, DMSO–H_2O, rt–80 °C, 62–96%; (d) LiOH, THF–H_2O, 0 °C, 1 h, 33–98%; (e) R^3NH_2, EDC–HCl, HOAt, DMF, 0 °C–rt, 10 h, 19–88%.

rat pharmacokinetic experiments (PK), and showed low aqueous solubility. The low solubility is largely due to the lipophilic nature of compounds that were designed to attractively interact with the lipophilic active site of cathepsins. There are, however, some regions in cathepsins that accept polar functional groups. The effective use of such regions is expected to afford water soluble Cat S specific inhibitors. After switching the selectivity preference to Cat S, we thus focused on improving the physicochemical properties, especially the thermodynamic solubility and the PK profiles, starting from the 2-cyanopyrimidine compound **1**.[4] Our strategy was to add a polar functional group at the 5- or the 6-position on the pyrimidine ring (Fig. 1). Herein, we describe the details of the novel scaffold, some of which turned out to be potent and orally bioavailable Cat S inhibitors with suitable PK and physicochemical properties.

The synthesis of 5-substituted pyrimidines, type A analogs in Figure 1, is shown in Scheme 1. Condensation of commercially available 2,4-dichloropyrimidine-5-carbonyl chloride **2** with methanol, as a protecting group, or phenethylamine as a P3 part gave a methyl ester **3** and an amide **4**. Treatment of P2 amines or sodium cyclohexylmethoxide with pyrimidine **3** or **4** followed by addition of sodium cyanide provided methyl ester **7** and compounds **8a–f** (Table 1). Hydrolysis of **7** under basic conditions provided a

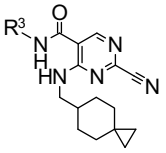
Table 1
Optimization of the P2 moiety

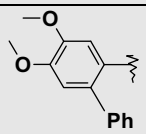
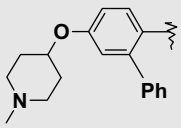
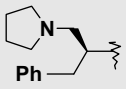
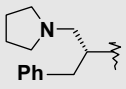
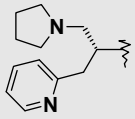
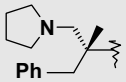
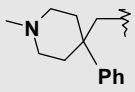
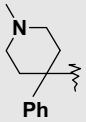
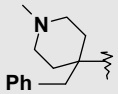


Compound	R^2	IC_{50}[a] (nM)		
		Cat S	Cat K	Cat L
1	Cyclohexyl-NH–	130	10	1100
8a	Cyclohexyl-CH_2NH–	22	15	>2000
8b	Cyclohexyl-$CH_2N(Me)$--	32	36	2000
8c	Cyclohexyl-CH_2O–	14	9	780
8d		22	130	>3000
8e		93	>3000	>3000
8f		15	>3000	>3000

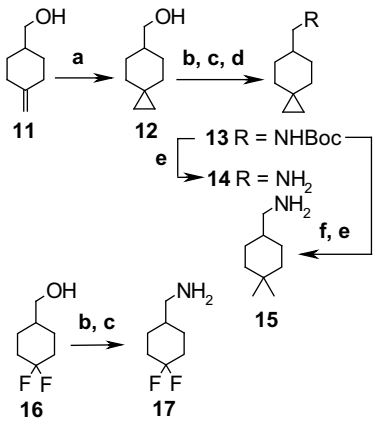
[a] Inhibition profiles were determined by a fluorometric assay with recombinant human Cat K, L, and S, employing Z-Phe-Arg-AMC (Cat K and L) and L-Leu-Leu-Arg-AMC (Cat S) as synthetic substrates.[4]

Table 2
Optimization of the P3 moiety



Compound	R^3	IC_{50}[a] (nM)		
		Cat S	Cat K	Cat L
10a		24	>1000	>3000
10b		3	–	840
10c		23	200	1500
10d		<1	44	150
10e		4	–	480
10f		21	–	840
10g		6	170	700
10h		7	–	510
10i		2	140	180

[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 L-Leu-Leu-Arg-AMC (Cat S) as synthetic substrates.[4]



Scheme 2. Reagents and conditions: (a) Et_2Zn, CH_2I_2, toluene, rt–60 °C, 20 h, 34%; (b) TsCl, NMe_3–HCl, NEt_3, CH_2Cl_2, 0 °C–rt, 1 h, quant.; (c) i—NaN_3, DMF, rt–60 °C, 3 h; ii—PPh_3, THF–H_2O, rt, 10 h, 77–99% (2 steps); (d) Boc_2O, THF, 0 °C–rt, 0.5 h, quant; (e) HCl–AcOEt, rt, 0.5 h, quant; (f) H_2, PtO_2, AcOH, AcOEt, rt, 23 h, 96%.

carboxylic acid **9** as a versatile intermediate for the variation of P3. Compound **9** was coupled to a variety of P3 amines[5] with EDC–HCl and HOAt to afford the corresponding compounds **10a–i** (Table 2).[6]

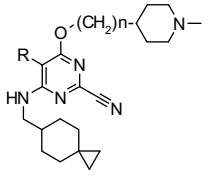
The preparation of 4,4-disubstituted cyclohexylmethyl-amines **14**, **15**, and **17** is shown in Scheme 2. Simmons-Smith cyclopropanation of commercially available 4-methylenecyclohexylmethanol **11** provided spiro[2.5]oct-6-ylmethanol **12**, which was converted to an amine **14**. Hydrogenation of Boc-protected cyclopropane derivative **13** with PtO_2 in AcOH–EtOAc gave 4,4-dimethylcyclohexylmethanol **15** after removal of the Boc group under acidic conditions. Application of the same condition allowed conversion of commercially available 4,4-difluorocyclohexylmethanol **16** to 4,4-difluorocyclohexylmethylamine **17**.

The synthesis of 6-substituted 2-cyano-4-amino-pyrimidines, type B analogs, is described in Scheme 3. Addition of LDA at −78 °C to commercially available 4,6-dichloro-2-methylsulfanyl-pyrimidine **18** in THF followed by treatment of carbon dioxide as an electrophile afforded a 5-substituted pyrimidine **19**. Condensation of benzylamine, methylamine, or phenethylamine with the acid chloride derived from carboxylic acid **19** gave amide **20**. Introduction of the optimum P2 amine **14**–**18** and **20** under basic conditions provided 4-amino pyrimidines **22** and **21**, respectively. Conversion of the methyl sulfides to nitriles **23** and **24** was performed by oxidation with *m*CPBA followed by treatment with potassium cyanide. Addition of an alkoxy group at the 6-position on the pyrimidine followed by removal of the Boc group and reductive amination with HCHO and $NaBH_3(CN)$ provided the desired compounds **25a–e** (Table 3) and **26**. Halogenation of compound **26** at the pyrimidine 5-position was achieved by HBr_3–pyridine to provide bromide **27**.

In order to obtain compounds with high potency and selectivity before exploring the 5- and the 6-substitution on the 2-cyanopyrimidine, we initially attempted to optimize the P2 moiety as the S2 subsite of cathepsins is a crucial binding site. The crystal structures of human Cat S (PDB code 1MS6), K (PDB code 2R6N), and L (PDB code 3BC3) suggest that the S2 subsite of Cat S is larger due to the absence of a methyl group on the enzyme compared with that of Cat K and L.[7–9] The S2 subsite in the Cat S enzyme has Gly137 and Gly165, while the corresponding regions in the S2 subsites in Cat K and L are comprised of Ala134/Ala163 and Ala135/Gly164, respectively. This structural feature of the Cat S S2 subsite was considered a clue to the discovery of potent and selective Cat S inhibitors.

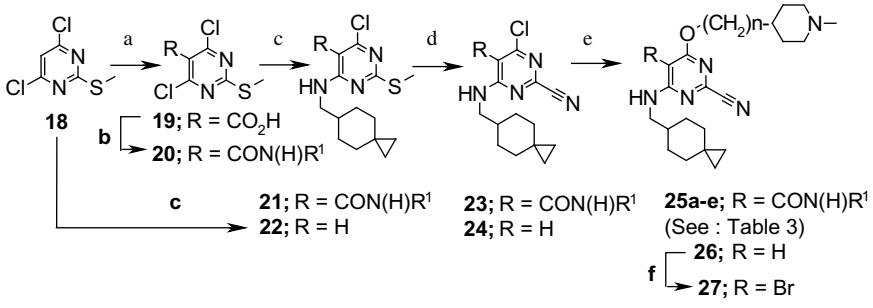
The result of the SAR study for optimization of the P2 moiety is shown in Table 1. Lengthening the P2 substituent on the pyrimidine by an additional methylene group gained potency against Cat S also improving the selectivity to Cat K and L. The cyclohexyl group was already an appropriate size for a Cat K inhibitor (Table 1; **1** vs **8a**). *N*-Alkylation with a methyl group at the 4-position on pyrimidine **8b** did not give any advantage compared with the secondary amine **8a**. Replacement of the nitrogen atom by oxygen resulted in a loss of selectivity against Cat L (Table 1; **8a** vs **8c**). Substitution of the cyclohexyl ring at the 4-position by difluorides **8d** improved selectivity but not the potency. Alternatively

Table 3
Optimization of the 5- and 6-position on the pyrimidine ring



Compound	*n*	R	IC_{50}[a] (nM)		
			Cat S	Cat K	Cat L
25a	0	–CONHCH2Ph	1	—	100
25b	1	–CONHCH2Ph	<1	—	64
25c	2	–CONHCH2Ph	<1	—	270
25d	2	–CONHMe	2	—	120
25e	2	–CONH(CH2)2Ph	<1	—	10
26	2	–H	22	240	870
27	2	–Br	2	100	280

[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 L-Leu-Leu-Arg-AMC (Cat S) as synthetic substrates.[4]



Scheme 3. Reagents and conditions: (a) LDA, THF, −78 °C, 0.5 h, then CO_2, 0.5 h, 98%; (b) $(COCl)_2$, cat. DMF, CH_2Cl_2, 0 °C–rt, 0.5 h, then $BnNH_2$ or $MeNH_2$ or phenethylamine, NEt_3, THF, 0 °C–rt, 4 h, 78–94%; (c) **14**, NEt_3, CH_2Cl_2, rt, 10 h, quant.; (d) i—*m*CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C–rt, 10 h; ii—KCN, $nBu_4N^+Br^-$, 18-crown-6, CH_2Cl_2–H_2O, rt, 3 h, 61–77% (2 steps); (e) i—$HO(CH_2)_n$(4-piperazine-NBoc), NaH, THF, 0–60 °C, 6 h, 54–88%; ii—HCl–AcOEt, rt, 0.5 h; iii—HCHO, $NaBH_3(CN)$, AcOH, THF, 0 °C–rt, 1.5 h, 47–69%; (f) pyridine–HBr_3, CH_2Cl_2, rt, 12 h, 89%.

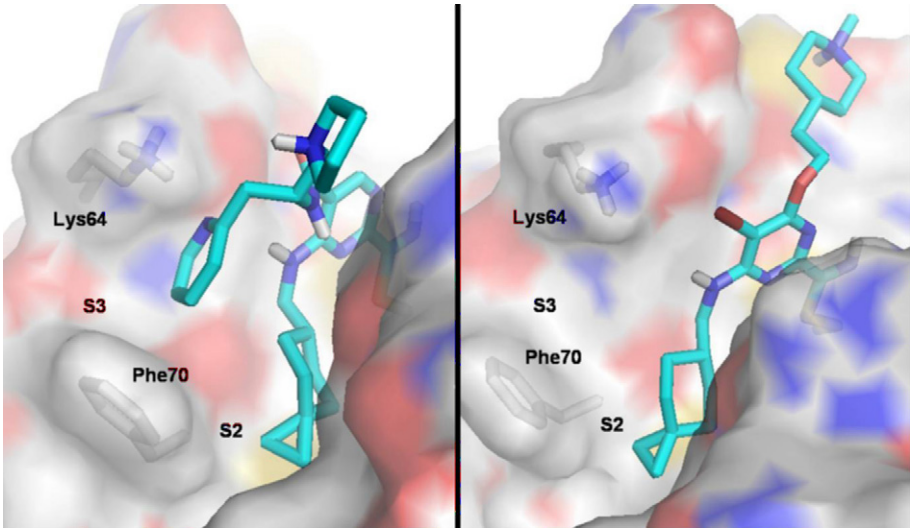


Figure 2. Designed compounds **10e** (type A; left) and **27** (type B; right) docked with the cathepsin S enzyme.

Table 4
Thermodynamic solubility (pH 6.8, 25 °C) and pharmacokinetic parameters for **10b**, **d**, **g**, and **27** in male Sprague–Dawley rats (iv 1 mg/kg; po 3 mg/kg), where values are means of $n = 3$

Compound	Solubility (mg/L)	Cl (L/h/kg)	$_{iv}t_{1/2}$ (h)	F (%)	$_{po}$AUC[c] (nM h)
10b	2	0.8	>8.0	66	2551
10d[a]	27	1.0	5.7	42	916
10g[b]	89	10.4	3.3	100	225
27	11	5.1	4.6	100	489

[a] Single iv; 1 mg/kg and po; 3 mg/kg dose.
[b] iv; $n = 2$.
[c] Dose-normalized to 1 mg/kg.

substituting at the 4-position with the bulkier dimethyls **8e** decreased the potency. The spiro[2.5]oct-6-ylmethyl group, **8f** was an optimum size for the S2 subsite based on both potent and selectivity toward Cat S.

Having identified the optimum P2 moiety, we next explored the P3 part by a parallel synthesis approach with the key intermediate **9**. Our computer-assisted modeling studies suggested that attachment of a polar functional group at the 5-position (type A) or at the 6-position (type B) on the 2-cyanopyrimidine would be tolerated, because the substituent would orient itself toward the solvent space (Fig. 2).

The inhibition profiles for type A analogs are shown in Table 2. Addition of a polar functional group, such as a tertiary amine or an ether directed toward the solvent space was tolerated (**10a**, **b**). Removal of a phenyl group from the lipophilic compounds **10a**, **b** maintained the potency and selectivity toward Cat S (**10d**, **g–i**). Suitable orientation of the polar functional group increased the potency to Cat S inhibition with over 100-fold selectivity against Cat L. The absolute configuration of the P3 substitution was crucial for the inhibitory activity to Cat S (Table 2; **10c** vs **10d**). Compound **10d** is a sub-nanomolar Cat S inhibitor with suitable thermodynamic solubility (Table 4; 27 mg/L at pH 6.8, 25 °C). The IC_{50} values for compounds **10b** and **10g** were 3 and 6 nM, respectively. Furthermore, **10b**, **10d**, and **10g** exhibited cellular activity as measured by invariant chain (Ii) degradation at 1 μM in mouse A20 cells.[10]

As shown in Table 3, introduction of a substituent at the 6-position on pyrimidine (type B in Fig. 1) also provided excellent Cat S inhibition. The replacement of a polar functional group, such as *N*-methyl piperidine, from the 5- to the 6-position on the pyrimidine ring was well tolerated (Table 2; **10h** vs Table 3; **25a**). The ethoxy linker at the 6-position on the pyrimidine had an optimal based on the potency and selectivity toward Cat S (**25a–c**). Compound **25c** is a sub-nanomolar Cat S inhibitor with excellent selectivity against Cat L (>270-fold). The reduction of lipophilicity on the P3 moiety by removal of the phenyl ring was also tolerated (**25c** vs **d**), although elongation of the P3 spacer by one methylene group decreased the selectivity against Cat L despite the well preserved sub-nanomolar potency for Cat S (**25c** vs **e**). While nonsubstitution at the 5-position on the pyrimidine ring significantly decreased the potency (**25c** vs **26**), replacement of the amide bond to halogens in order to improve the physicochemical properties was tolerated in terms of both Cat S affinity and selectivity against other cathepsins (**25** vs **27**). In addition, compounds **26** and **27** displayed potent cellular activity at 1 μM in mouse A20 cells.

Selected compounds were evaluated by PK profiles in cassette dosing experiments[11] in male Sprague–Dawley rats. Representative PK results, along with thermodynamic solubility (at pH 6.8, 25 °C), are shown in Table 4. Compounds **10d** (type A) and **27** (type B) had excellent bioavailabilities (42% and 100%) and low clearances (1.0 and 5.1 L/h/kg, respectively) with acceptable thermodynamic aqueous solubility (27 and 11 mg/L, respectively).

In summary, two types of novel compounds having a 2-cyanopyrimidine scaffold were derived from the Cat K inhibitors, leading us to the development of sub-nanomolar Cat S inhibitors with excellent selectivity against Cat L. The compounds had potent cellular activity in mouse A20 cells as well as suitable physicochemical and PK profiles. The in vivo activity of these series in rodent will be reported in future publications.

Acknowledgments

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