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2-Phenyl-9*H*-purine-6-carbonitrile derivatives as selective cathepsin S inhibitors

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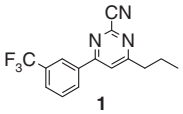
ABSTRACT

Starting from previously disclosed equally potent cathepsin K and S inhibitor 4-propyl-6-(3-trifluoromethylphenyl)pyrimidine-2-carbonitrile **1**, a novel 2-phenyl-9*H*-purine-6-carbonitrile scaffold was identified to provide potent and selective cathepsin S inhibitors.

Eleven members of the cysteine cathepsin family proteases have been identified in the human genome (cathepsins B, C, H, F, K, L, O, S, V, W, and X).[1] Two of these, cathepsins K and S have been the subject of extensive effort in the pharmaceutical industry.[1–3] Cathepsin S is highly expressed in antigen presenting cells and plays a major role in the degradation of the invariant peptide chain associated with the major histocompatibility complex and affects antigen presentation. Selective cathepsin S inhibitors should then be useful therapeutics for autoimmune disorders, for example, rheumatoid arthritis (RA) and multiple sclerosis (MS). More recently cathepsin S has also been indicated for neuropathic pain.[4] Several cathepsin K inhibitors have progressed into human clinical trials for osteoporosis,[5] but as yet there are no reports of any cathepsin S inhibitors in advanced stages of human clinical trials. Several different cathepsin S inhibitor chemotypes have been reported in the literature, most of which contain some kind of peptide feature within the molecule and pose some challenges in terms of pharmacokinetic property optimization.[2]

We[6,7] and others[8–17] have recently reported non-peptide heteroaryl nitriles as cathepsin K and S inhibitors. Here we describe our latest effort in identifying selective cathepsin S inhibitors through P2 residue optimization and scaffold hopping starting from recently disclosed non-selective cathepsin S and K inhibitor 4-propyl-6-(3-trifluoromethylphenyl)pyrimidine-2-carbonitrile (Fig. 1).[7]

There are several major differences between the highly homologous cathepsin S and K binding sites. The cathepsin K S3 pocket contains an aspartic acid residue (Asp61) against a lysine for cathepsin S (Lys64). This major difference has been applied successfully for identifying selective cathepsin K inhibitors.[8] With the cathepsin S enzyme, the S2 pocket could be regarded as the



1
hCat S IC_{50}: 41nM
hCat K IC_{50}: 33nM

Figure 1.

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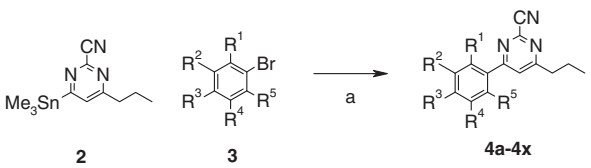
most important binding site for inhibitor design. There are five significant differences between cathepsin S and K in this site[18] namely Phe70, Gly137, Val162, Gly165, and Phe211 for cathepsin S and Tyr67, Ala133, Leu157, Ala160, and Leu209 for cathepsin K. Two smaller glycine residues at the bottom of the pocket provide cathepsin S with a slightly deeper (wider) S2 pocket than the corresponding S2 pocket for cathepsin K. The Phe211 sits at the end of the S2 pocket. The side chain of Phe211 is quite mobile, but is preferentially found in an 'open' conformation which increases the space available at the end of the S2 pocket by opening a narrow passage between Phe70 and Phe211. The equivalent residues for cathepsin K, Tyr67 and Leu209, are quite close and do not afford any additional space between them. Val162 sits on side of the cathepsin S S2 pocket, and makes the S2 pocket for cathepsin S slightly narrower in that region. As reported previously, the trifluoromethylphenyl group of the compound **1** analogs binds to the cathepsin K S2 pocket. Considering the high overall and binding site similarity between cathepsin K and S, it is reasonable to believe that this group also binds to the S2 pocket in cathepsin S. For this reason, we envisaged that further optimization of the trifluoromethylphenyl region could improve activity against cathepsin S and also selectivity over cathepsin K.

The analogs of **1** were synthesized by applying our previously reported chemistry[7] as shown in Scheme 1. The results of this study are shown in Table 1.

Identical to that recently reported for cathepsin K,[7] the *meta*-substitution of the P2 phenyl group also proved to be critical for cathepsin S inhibition. Both the unsubstituted **4a** and *ortho*-trifluoromethyl substituted **4b** compounds show no activity against human cathepsin S. The *para*-substitution by CF_3 group provided compound **4c** 30 times less active than the corresponding *meta*-substituted analog **1**. The trifluoromethyl substitution at the *meta*-position of the P2 phenyl group also appeared to be optimal for cathepsin S inhibition as all other compounds **4d–4q** are less active. A second substitution at position 5 (R4) of the phenyl ring is well tolerated, such as compound **4r**, which has a similar activity and selectivity profile to compound **1**. A chlorine substitution at position 6 **4s** (R5) of the P2 phenyl ring appeared to be detrimental for both cathepsin S and K inhibition, probably due to an unfavorable torsion angle between pyrimidine and benzene rings. A second substitution on position 4 (R3) enhances cathepsin S inhibition activity (compounds **4t–4y**) except compound 4**z** with 4-ethoxy substitution (**4x**) as the most active human cathepsin S inhibitor in the series. Although only a small improvement in most cases, the trend of these results is clear and it was expected from our computer aided docking studies. These groups on position 4 were expected to interact with Phe70, Val162, and Phe211 as shown in Figure 2.

Although all pyrimidine analogs tested showed no or very little activity against human cathepsin B and L, the selectivity over human cathepsin K is less than 10-fold in almost all cases. One of the reasons for this poor selectivity might be due to the high reactivity of the thio-trapping nitrile war-head of the pyrimidine-2-carbonitriles on top of the high level of binding site homology between human cathepsin K and S. Visualizing all the X-ray structures of aryl nitrile inhibitors complexed to the human cathepsin K protein, one feature is that the cys25 attacks the nitrile war-head from the P2 side of the molecule to form a planar thio-imidate with the NH on the prime side of the molecule (Fig. 3a). We envisaged that a purine template



Scheme 1. Reagents and condition: (a) $Pd(Ph_3P)_2Cl_2$, aryl bromide, DMF, 150 °C.

Table 1
Inhibitory activity of arylpyrimidine compounds against human cathepsin S and K

Compound	R1	R2	R3	R4	R5	IC_{50}[a] (nM)	
						Cat S	Cat K
1	H	CF_3	H	H	H	41	33
4a	H	H	H	H	H	>10,000	1122
4b	CF_3	H	H	H	H	>10,000	4070
4c	H	H	CF_3	H	H	1259	977
4d	H	Cl	H	H	H	148	60
4e	H	Br	H	H	H	174	44
4f	H	MeCO	H	H	H	447	316
4g	H	$MeSO_2$	H	H	H	93	148
4h	H	Me	H	H	H	950	182
4i	H	Et	H	H	H	83	71
4j	H	*n*-Pr	H	H	H	410	166
4k	H	*i*-Pr	H	H	H	195	56
4l	H	*i*-Bu	H	H	H	224	398
4m	H	*t*-Bu	H	H	H	191	11
4n	H	CF_3O	H	H	H	115	661
4o	H	*c*-Pr	H	H	H	200	195
4p	H	*c*-Bu	H	H	H	63	145
4q	H	*c*-Pen	H	H	H	155	457
4r	H	CF_3	H	CF_3	H	51	44
4s	H	CF_3	H	H	Cl	1047	1000
4t	H	CF_3	F	H	H	13	32
4u	H	CF_3	Cl	H	H	28	34
4v	H	CF_3	Me	H	H	10	17
4w	H	CF_3	MeO	H	H	15	151
4x	H	CF_3	EtO	H	H	6	40
4y	H	CF_3	CF_3O	H	H	32	65
4z	H	CF_3	*i*-PrO	H	H	58	91

[a] Inhibition of recombinant human cathepsin S, K, L, and B in a fluorescence assay, employing synthetic substrates. Data represents means of two experiments in duplicate. All compounds shown do not have inhibitory activity against human cathepsin B and L (IC_{50} >10,000 nM).

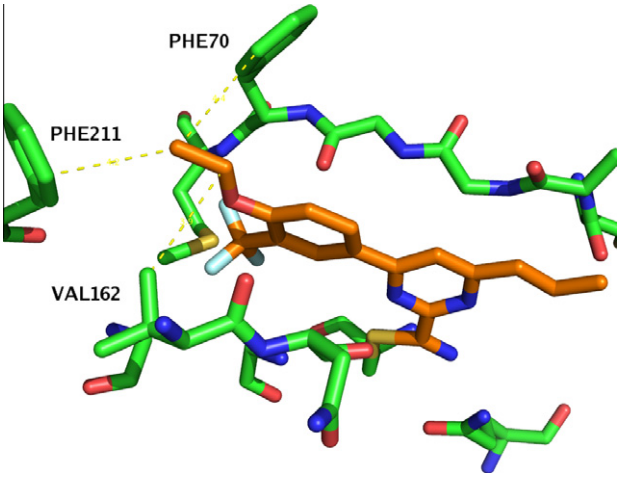


Figure 2. Compound **4x** (EtO at position 4 of the P2 phenyl ring) docked into cathepsin S active site showing hydrophobic interactions between Et and Phe70 (top), Phe211 (left), and Val162 (bottom).

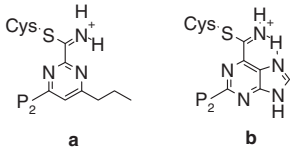
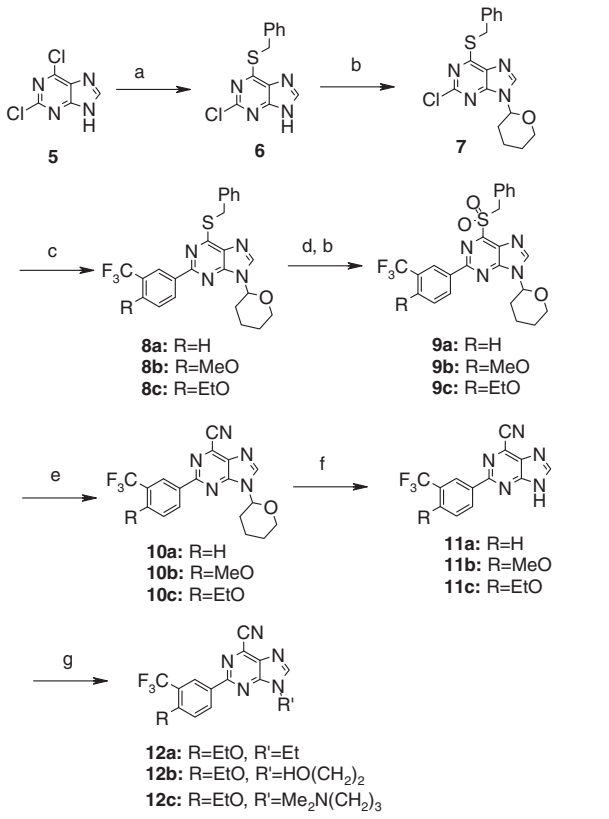


Figure 3. Covalent interactions between thio-trapping war-head nitrile group with active site cysteine thiol group.

could be an ideal replacement for the pyrimidine core as N7 (from imidazole ring) could potentially form an intramolecular hydrogen bond with thio-imidate NH (Fig. 3b) to further stabilize the transition state. This purine scaffold could potentially offer a more stable thio-trapping nitrile war-head.

The purine compounds were synthesized according to Scheme 2. Displacement of 6-chlorine of 2,6-dichloro-*9H*-purine with a benzylthio group followed by THP protection of the purine NH provided compound **7**. Suzuki coupling of compound **7** with a suitable boronic acid or ester gives compound **8**. Oxidation of sulfide by oxone provided sulfone with the concomitant deprotection of the THP group which was then re-installed to afford compound **9**. Replacement of the sulfone with cyano-group gives compound **10**. Removal of THP group delivers the desired compound **11a–11c**. Compound **11c** was further modified to afford compounds **12a–12c**. Biological results of compound **11a–11c** and **12a–12c** are shown in Table 2.

The purine-6-carbonitrile core appeared to be a good scaffold for cathepsin S inhibition. In comparison with pyrimidine compound **1**, compound **11a** roughly maintained the cathepsin S inhibitory activity while it is 15 times less active against human cathepsin K. The SAR in the P2 aryl region appeared to be in agreement with that observed in the pyrimidine series with EtO > -MeO > H for cathepsin S inhibition. These 4-ethoxy analogs **11c**, **12a**, and **12b** all have >50-fold selectivity over human cathepsin K and no detectable activity against human cathepsin B and L at 10 μM. With a view to improving solubility and cellular activity, a basic nitrogen side chain was attached to N9 nitrogen to give compound **12c**. This compound maintained cathepsin S inhibitory activity, however it lost some selectivity over cathepsin K. Although this basic nitrogen side chain seems to have no direct interactions with any specific cathepsin K binding site residues



Scheme 2. Reagents and conditions. (a) BnSH, TEA, EtOH, 60 °C, 1.5 h; (b) DHP, TsOH, EtOAc, 50 °C, 2 h; (c) $ArB(OH)_2$, $Pd(Ph_3P)_4$, K_2CO_3, NMP–H_2O, 130 °C, 7 min; (d) oxone, MeCN–H_2O, 16 h; (e) KCN, DMSO, 90 °C, 7 min; (f) MeOH–DCM, TsOH, 2 h; (g) R′OH, Ph_3P, DEAD, 1 h.

Table 2
Inhibitory activity of purine compounds **11a–12c** against human cathepsin S, K and cellular activity in human JY cells (Lip10)

Compound	R	R′	IC_{50}[a] (nM)		Lip10[b]
			Cat S	Cat K	IC_{50} (nM)
11a	H	H	60	470	na
11b	MeO	H	23	630	na
11c	EtO	H	3.9	1072	>10,000
12a	EtO	Et	7.2	2291	na
12b	EtO	$HO(CH_2)_2$	4.5	282	509
12c	EtO	$Me_2N(CH_2)_3$	6.9	117	59

[a] Inhibition of recombinant human cathepsin S, K, L, and B in a fluorescence assay, employing synthetic substrates. Data represents means of two experiments in duplicate. All compounds shown do not have inhibitory activity against human cathepsin B and L (IC_{50} >10,000 nM).

[b] Measured by Western blot using human B lymphoblastoid cells, 500,000/ml, mouse anti-CD74 Pin. 1 monoclonal antibody, 50% of the maximum activity of LHVS in the same assay as IC_{50}; na, not available.

from our docking studies, the remote ionic interactions with two carboxylic residues (Asp61 and Glu59) might be the reason for the lost selectivity. In contrast, there are no negatively charged residues in human cathepsin S binding sites.

Three of the purine compounds were further assessed in the cell based Lip10 accumulation assay. Although neutral compounds **11c** and **12b** have low cellular activities, the basic compound **12c** has an IC_{50} of 59 nM. This high cellular activity is likely due to the previously reported lysosomotropic effect.[19,20]

Although compound **12c** possesses good cellular activity, one of its close analogs[21] showed a half life of 17 min in our NMR based nitrile stability studies. This poor stability prevented this class of compounds from progressing any further.

In summary, starting from a non-selective human cathepsins K and S pyrimidine-2-carbonitrile based inhibitor **1**, by using bio-X-ray structural data and computer aided molecule design, purine-6-carbonitrile compounds were synthesized to provide a novel class selective human cathepsin S inhibitor.

References and notes

1. Gupta, S.; Singh, R. K.; Dastidar, S.; Ray, A. *Expert Opin. Ther. Targets* **2008**, *12*, 291.
2. Leroy, V.; Thurairatnam, S. *Expert Opin. Ther. Patents* **2004**, *14*, 301.
3. Cai, J.; Jamieson, C.; Moir, J.; Rankovic, Z. *Expert Opin. Ther. Patents* **2005**, *15*, 33.
4. Bromme, D.; Lecaille, F. *Expert Opin. Invest. Drugs* **2009**, *18*, 585.
5. Clark, A. K.; Yip, P. K.; Grist, J.; Gentry, C.; Staniland, A. A.; Marchand, F.; Dehvari, M.; Wotherspoon, G.; Winter, J.; Ullah, J.; Bevan, S.; Malcangio, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 10655.
6. Rankovic, Z.; Cai, J.; Fradera, X.; Dempster, M.; Mistry, A.; Mitchell, A.; Long, C.; Hamilton, E.; King, A.; Boucharens, S.; Jamieson, C.; Gillespie, J.; Cumming, I.; Uitdehaag, J.; Zeeland, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1488.
7. Rankovic, Z.; Cai, J.; Kerr, J.; Fradera, X.; Robinson, J.; Mistry, A.; Hamilton, E.; McGarry, G.; Andrews, F.; Caulfield, W.; Cumming, I.; Dempster, M.; Waller, J.; Scullion, P.; Martin, I.; Mitchell, A.; Long, C.; Baugh, M.; Westwood, P.; Kinghorn, E.; Bruin, J.; Hamilton, W.; Uitdehaag, J.; Zeeland, M.; Potin, D.; Saniere, L.; Fouquet, A.; Chevallier, F.; Deronzier, H.; Dorleans, C.; Nicolai, E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1524.
8. Altmann, E.; Cowan-Jacob, S. W.; Missbach, M. *J. Med. Chem.* **2004**, *47*, 5833.
9. Irie, O.; Ehara, T.; Iwasaki, A.; Yokokawa, F.; Sakaki, J.; Hirao, H.; Kanazawa, T.; Teno, N.; Horiuchi, M.; Umemura, I.; Gunji, H.; Masuya, K.; Hitomi, Y.; Iwasaki, G.; Nonomura, K.; Tanabe, K.; Fukaya, H.; Kosaka, T.; Snell, C. R.; Hallet, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3959.
10. Irie, O.; Yokokawa, F.; Ehara, T.; Iwasaki, A.; Iwaki, Y.; Hitomi, Y.; Konishi, K.; Kishida, M.; Toyao, A.; Masuya, K.; Gunji, H.; Sakaki, J.; Iwasaki, G.; Hirao, H.; Kanazawa, T.; Tanabe, K.; Kosaka, T.; Hart, T. W.; Hallet, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4642.
11. Irie, O.; Kosaka, T.; Kishida, M.; Sakaki, J.; Masuya, K.; Konishi, K.; Yokokawa, F.; Ehara, T.; Iwasaki, A.; Iwaki, Y.; Hitomi, Y.; Toyao, A.; Gunji, H.; Teno, N.; Iwasaki, G.; Hirao, H.; Kanazawa, T.; Tanabe, K.; Hiestand, P.; Malcangio, M.; Fox, A. J.; Bevan, S. J.; Yaqoob, M.; Culshaw, A. J.; Hart, T. W.; Hallet, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5280.
12. Irie, O.; Kosaka, T.; Ehara, T.; Yokokawa, F.; Kanazawa, T.; Hirao, H.; Iwasaki, A.; Sakaki, J.; Teno, N.; Hitomi, Y.; Iwasaki, G.; Fukaya, H.; Nonomura, K.; Tanabe, K.; Koizumi, S.; Uchiyama, N.; Bevan, S. J.; Malcangio, M.; Gentry, C.; Fox, A. J.; Yaqoob, M.; Culshaw, A. J.; Hallet, A. *J. Med. Chem.* **2008**, *51*, 5502.

13. Altmann, E.; Aichholz, R.; Betschart, C.; Buhl, T.; Green, J.; Irie, O.; Teno, N.; Lattmann, R.; Tintelnot-Blomley, M.; Missbach, M. *J. Med. Chem.* **2007**, *50*, 591.
14. Teno, N.; Miyake, T.; Ehara, T.; Irie, O.; Sakaki, J.; Ohmori, O.; Gunji, H.; Matsuura, N.; Masuya, K.; Hitomi, Y.; Nonomura, K.; Horiuchi, M.; Gohda, K.; Iwasaki, A.; Umemura, I.; Tada, S.; Kometani, M.; Iwasaki, G.; Cowan-Jacob, S. W.; Missbach, M.; Lattmann, R.; Betschart, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6096.
15. Teno, N.; Masuya, K.; Ehara, T.; Kosaka, T.; Miyake, T.; Irie, O.; Hitomi, Y.; Matsuura, N.; Umemura, I.; Iwasaki, G.; Fukaya, H.; Toriyama, K.; Uchiyama, N.; Sugiyama, I.; Kometani, M. *J. Med. Chem.* **2008**, *51*, 5459.
16. Teno, N.; Irie, O.; Miyake, T.; Gohda, K.; Horiuchi, M.; Tada, S.; Nonomura, K.; Kometani, M.; Iwasaki, G.; Betschart, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2599.
17. Morley, A. D.; Kenny, P. W.; Burton, B.; Heald, R. A.; MacFaul, P. A.; Mullett, J.; Page, K.; Porres, S. S.; Riberio, L. R.; Smith, P.; Ward, S.; Wilkinson, T. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1658.
18. Gauthier, J. Y.; Black, W. C.; Courchesne, I.; Cromlish, W.; Desmarais, S.; Houle, R.; Lamontagne, S.; Li, C. S.; Masse, F.; Mckay, D. J.; Ouellet, M.; Robichaud, J.; Truchon, J.-F.; Truong, V.-L.; Wang, Q.; Percival, M. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4929.
19. 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.
20. MacIntyre, A.; Cutler, D. J. *Biopharm. Drug Dispos.* **1988**, *9*, 513.
21. Half life ($t_{1/2}$) was measured by NMR with the final concentration of inhibitor at 1.6 mM and glutathione at 6.7 mM in 1:1 CD_3OD and D_2O at 20 °C at pD 7.5. Structure of the compound measured is shown below which is synthesized according to Scheme 2.