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4-(3-Trifluoromethylphenyl)-pyrimidine-2-carbonitrile as cathepsin S inhibitors: N3, not N1 is critically important

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ABSTRACT

Using computer aided modelling studies, a new extended P2/S2 interaction was identified. This extended region can accommodate a variety of functional groups, such as aryls and basic amines. It was discovered that the N3 nitrogen of the pyrimidine-2-carbonitrile is critical for its cathepsin cysteine protease inhibition. N1 nitrogen also contributes to the inhibitory activity, but to a very limited degree. An 'in situ double activation' mechanism was proposed to explain these results.

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 optimisation.[2]

We[6,7] and others[8–17] reported non-peptide heteroaryl nitriles as cathepsins K and S inhibitors. One common feature of all these arylnitrile based cysteine cathepsin inhibitors is that the nitrile war-head is flanked between two electronically negative aromatic nitrogen atoms. This often over-activates the nitrile group which becomes too reactive and could potentially cause covalent binding to proteins in the microsomal protein binding assay.[18]

In our previous publication,[19] we reported that 4-EtO group on the P2 phenyl ring significantly improves cathepsin S inhibitory activity probably through hydrophobic interactions with S2 subsite residues Phe70, Phe211 and Val162 (Fig. 1). In view that the side chain of Phe211 at the bottom of the S2 pocket is quite mobile and 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, it was suggested that the P2 region of the molecule can be further expanded to take advantage of the Phe70 and Phe211 which could potentially have both hydrophobic or π–π interactions with either lipophilic groups, π-electron-containing groups or basic nitrogen containing groups. Thus basic nitrogen containing compounds are especially interesting as they could potentially improve compound properties, for

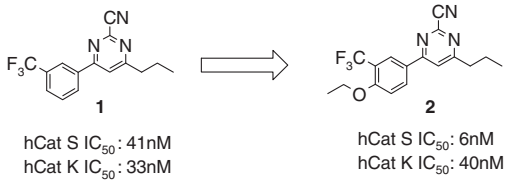

Figure 1.

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example, solubility, cellular activity and absorption. Optimisation of the activity will also allow the opportunity to replace the 'reactive' pyrimidine-2-carbonitrile core with less reactive alternatives. In this Letter, we will describe some brief SAR in the expanded S2 binding region and also our early efforts to obtain less reactive pyridine-carbonitrile based cysteine protease inhibitors.

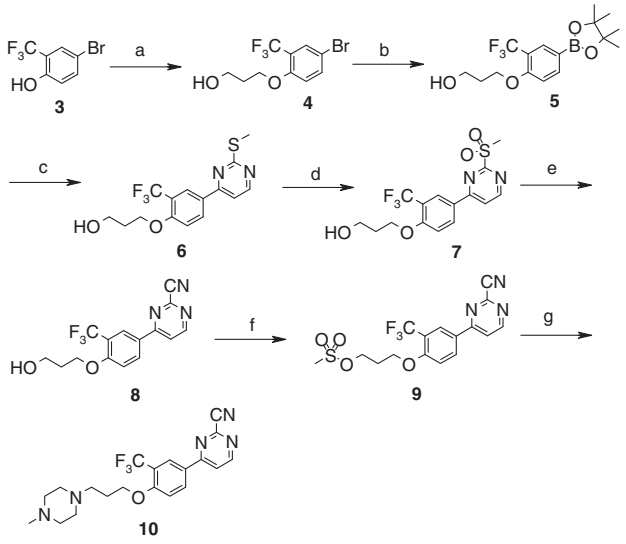
The synthesis of the P2 expanded pyrimidine compounds is shown in Scheme 1. Starting with commercially available 4-bromo-2-trifluoromethylphenol, O-hydroxypropylation gives compound **4** in very high yield. Standard palladium catalyzed conversion of aryl bromide provided boronic ester **5** in good yield. Suzuki coupling of **5** with 4-chloro-2-methylthiopyrimidine afforded compound **6**. Oxidation of sulphide using Oxone proceeded smoothly to the desired sulphone **7** which was then converted to the first desired pyrimidine-2-carbonitrile **8**. Conversion of the alcohol to amine mediated through methanesulphonate **9** went smoothly to give final product **10**. Results of these P2-expanded pyrimidine-2-carbonitrile compounds **8** and **10** are shown in Table 1.

Although the P1 propyl group was deleted from the molecule for the purpose of easy synthesis, both compounds **8** and **10** appeared to be more potent against human cathepsin S than the corresponding compound **2**.

With a view to reducing the undesired war-head reactivity of the above pyrimidine-2-carbonitrile core, a pyrimidine ring nitrogen deletion study was carried out in the hope that extra-binding from this expanded P2/S2 interactions could compensate for the loss of activity from reduced war-head reactivity as reported by Altmann et al.[13]

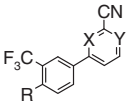
The pyridine analogues are synthesized according to Scheme 2. Suzuki coupling of readily available boronic ester **12** or **13** with 6- or 4-chloropyridine-2-carbonitrile gives the desired final products.

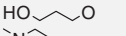
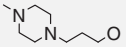
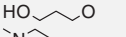
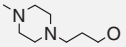
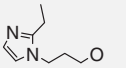
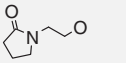
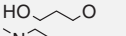
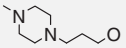
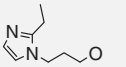
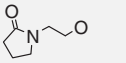
Other pyridine compounds were synthesized according to Scheme 3. Suzuki coupling of boronic ester **5** with 6- or 4-chloropyridine-2-carbonitriles gives the corresponding pyridine-carbonitrile compounds **16** and **21**, respectively, in high yields. The alcohol intermediates were then converted to the amine final products **18**, **19**, **22** and **23** mediated through the methanesulphonate intermediate **17**.



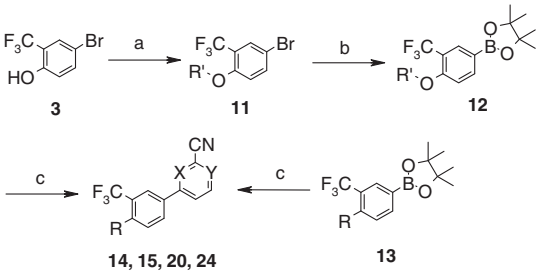
Scheme 1. Reagents and conditions: (a) 3-iodopropanol, K_2CO_3, MeCN, 85 °C, 3h; (b) bis(pinacolato)diboron, $PdCl_2$(DPPF), KOAc, dioxane, 100 °C, 3 h; (c) 4-chloro-2-methylthiopyrimidine, $Pd_2(DBA)_3$, K_3PO_4, (c-Hex)$_3$P, Dioxane–H_2O, 100 °C, 3 h; (d) Oxone, MeCN–H_2O, rt, 12 h; (e) KCN, DMSO, rt, 1 h; (f) MsCl, DIPEA, DCM, rt, 2 h; (g) 1-methylpiperazine, NMP, 90 °C, 20 min.

Table 1
Cathepsin S inhibitory activity of the pyrimidine- and pyridine-carbonitrile compounds

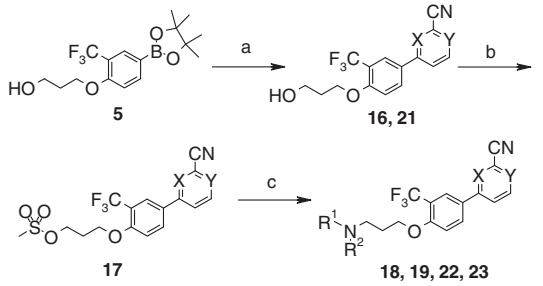


Compds	R	X	Y	IC_{50}[a] (nM)	
				Cat S	Cat K
2				6	40
8		N	N	4.5	50
10		N	N	1.3	13
14	H	N	CH	2884	>10,000
15	EtO	N	CH	1660	>10,000
16		N	CH	155	8900
18		N	CH	58	1660
19		N	CH	31	3090
20		N	CH	29	>10,000
21		CH	N	Inactive	Inactive
22		CH	N	Inactive	Inactive
23		CH	N	Inactive	Inactive
24		CH	N	Inactive	Inactive

[a] For assay conditions see Ref. 6; all pyridine compounds showed no activity against human cathepsins B, L, and V.



Scheme 2. Reagents and conditions: (a) R′OH, DIAD, Ph_3P, 12 h; (b) bis(pinacolato)diboron, $PdCl_2$(DPPF), KOAc, dioxane, 100 °C, 3 h; (c) 6- or 4-chloropyridine-2-carbonitrile, $Pd_2(DBA)_3$, K_3PO_4, (c-Hex)$_3$P, Dioxane–H_2O, 100 °C, 3 h.



Scheme 3. Reagents and conditions: (a) 6- or 4-chloropyridine-2-carbonitrile, $Pd_2(DBA)_3$, K_3PO_4, (c-Hex)$_3$P, Dioxane–H_2O, 100 °C, 3 h; (b) MsCl, DIPEA, DCM, rt, 2 h; (c) amine, NMP, 100–120 °C, 10–40 min.

The results of all pyridine compounds together with the results from pyrimidine derived compounds are shown in Table 1.

Removal of N1 nitrogen (Y) of the pyrimidine-2-carbonitrile compounds results in the loss of just over 30-fold activity (**16**/**8** and **18**/**10** pairs) against human cathepsin S. Extension of the ethyl of compound **15** to a hydroxypropyl **16** resulted in a 10-fold improvement of cathepsin S inhibitory potency. It is probable that the extra CH_2 has contributed to further hydrophobic interactions in that region.[20] The piperazine containing analogue **18** is threefold better than the hydroxypropyloxy analogue **16**. The most active compounds identified are the imidazo- and γ-lactam containing analogues **19** and **20**. These results show that this extended S2 pocket is very accommodative in terms of binding interactions. Compound **18** has an EC_{50} of 138 nM in the human JY cells based Lip10 assay; however all other lower pK_a or neutral analogues in the same series are all >10 μM in the same assay likely due to the lack of lysosomotropic effect. Removal of N3 nitrogen (X) of the pyrimidine-2-carbonitrile compounds resulted in a total loss of cathepsins S and K inhibitory activity as shown by analogues **21–24**. The difference in the nitrile war-head chemical reactivity between the two pyridine types should be very small. Using the method described by Oballa et al.[18] to estimate the reactivity of a cysteine thiol towards the nitrile from the left hand side (P2 side), we obtained values of approximately −8 kcal/mol for pyrimidine, −5 kcal/mol for 'N3-pyridine' (X = N, Y = CH), and −3.5 kcal/mol for 'N1-pyridine' (X = CH, Y = N). The 'N1-pyridine' is predicted to be less reactive; however, the complete lack of activity is surprising. NMR measurements of nitrile reactivity towards glutathione showed that compound **18** (N3-pyridine) is in fact slightly more stable ($t_{1/2}$ 150 h) than compound **22** (N1-pyridine) ($t_{1/2}$ 120 h).[21] However it should be pointed out that this NMR based reactivity study does not consider the spatial direction limitation of the thio-nitrile reaction in the enzymatic case.

Removal of N3 nitrogen should also have little impact on the torsion angle between the pyridine ring and the P2 phenyl ring as this biaryl normally adopts torsion angle 15–40°, consistent with our X-ray structures of nitrile-pyrimidine and nitrile-pyridine based inhibitors. The X-ray structure of compound **18** complexed with human cathepsin S protein was obtained (Fig. 2). The X-ray structure showed that this N3 nitrogen is not involved in any final binding interactions with the cathepsin S protein. However this structure and others of similar compounds bound to both cathepsins K and S[6,7,22] revealed that the active site cysteine thiol always attacks the nitrile war-head from the left hand side (P2 side) of the inhibitors. These structural data suggest that a possible 'in situ double activation' mechanism with an electrostatic interaction between the active site cysteine thiol proton and the pyridine ring nitrogen as shown in Figure 3. This is supported by our docking studies. This interaction should make the thiol more nucleophilic and the nitrile carbon more electrophilic. It is obvious that this double activation is not possible for the 'N1' pyridine compounds.

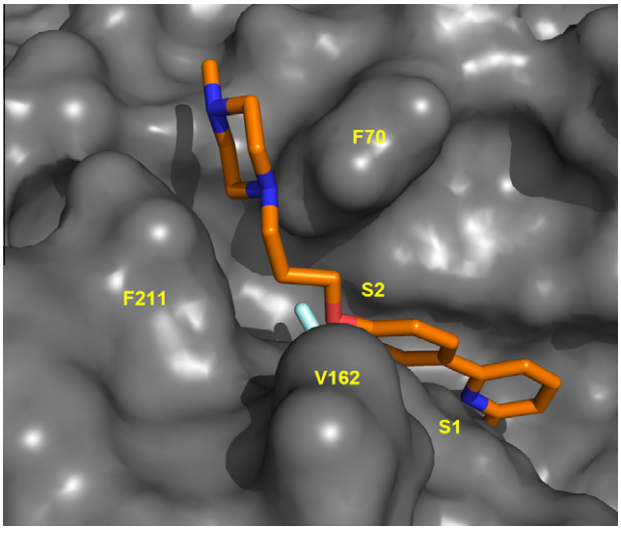


Figure 2. Crystal structure of **18** covalently bound to human cathepsin S enzyme, solved at 2.4 Å resolution (PDB code: 3N3G). There was no clear electron density for the piperazine ring, and it has been modelled in a suitable orientation, based on the structure of a similar ligand.

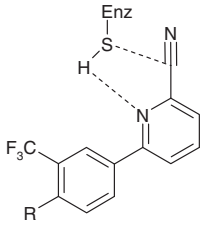


Figure 3. An 'in situ double activation' mechanism: electrostatic interaction between the thiol SH proton and the 'N3' pyridine nitrogen activates both thiol and nitrile simultaneously with a favourable five-membered ring transition state.

In summary, by using X-ray structural information and computer aided molecule design, a new expanded S2 pocket for cathepsin S enzyme containing Phe70, Phe211 and Val162 was identified. This pocket contributes nearly 100-folds towards cathepsin S inhibitory activity in the current aryl nitrile series. It is discovered that N3 nitrogen of the well-reported pyrimidine-2-carbonitrile is critical for its cathepsin family cysteine protease inhibitory activity. An 'in situ double activation' mechanism was proposed to explain the results. The N1 nitrogen contributes only 30- to 50-fold towards activity. It is our intention that this in situ activation could be used for other families of biological targets where covalent binding is often necessary, such as lipases or other proteases to reduce the potential risk of idiosyncratic toxicity related to non-specific irreversible covalent binding to off-target tissues and proteins.

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20. The *n*-PrO compound is not prepared in this series, however in a closely related imidazopyridine series, *n*-PrO is about 5- to 10-fold less active than corresponding EtO.
21. Half life ($t_{1/2}$) was measured by NMR with inhibitor (1.25 mM) and glutathione (7.1 mM) dissolved in 1:1 CD_3OD and D_2O at 20 °C at p*D* 7.5.
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