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Design and optimization of a series of novel 2-cyano-pyrimidines as cathepsin K inhibitors

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ABSTRACT

Morphing structural features of HTS-derived chemotypes led to the discovery of novel 2-cyano-pyrimidine inhibitors of cathepsin K with good pharmacokinetic profiles, for example, compound **20** showed high catK potency (IC_{50} = 4 nM), >580-fold selectivity over catL and catB, and oral bioavailability in the rat of 52%.

Cathepsin K is a lysosomal cysteine protease of the papain family. Eleven members of this family are expressed in the human genome, of which cathepsins L, S and V are most closely related to cathepsin K.[1] The selective and abundant expression of cathepsin K in osteoclasts, cells involved in bone resorption, indicates its potentially critical role in bone remodeling. A large body of evidence accumulated over the last decade points towards cathespin K as an important therapeutic target for the treatment of osteoporosis.[2] As such, considerable interest has been generated in the development of selective cathepsin K inhibitors.[3] These compounds have been shown to display ex-vivo efficacy in osteoclast-based assays of bone resorption and in vivo efficacy in rodent[4] and primate models of bone resorption.[5]

Most recently, clinical data have been disclosed for selective cathepsin K inhibitors showing a reduction of biochemical markers of bone resorption and increases in bone mineral density (BMD).[6]

A rapid SAR exploration around a hit obtained from high throughput screening of the corporate compound collection against cathepsin K, led to identification of triazine **1**[7] (Fig. 1).

This compound exhibited high potency against cathepsin K (IC_{50} = 1 nM) and selectivity over cathepsins S (158 nM), L (1711 nM) and B (520 nM). However, although **1** showed good stability in human microsomes ($t_{½}$ >120 min) and hepatocytes ($t_{½}$ = 118 min), it was rapidly cleared in rodent microsomes (rat $t_{½}$ = 23 min; mouse $t_{½}$ = 4 min) and hepatocytes (rat $t_{½}$ = 6 min).

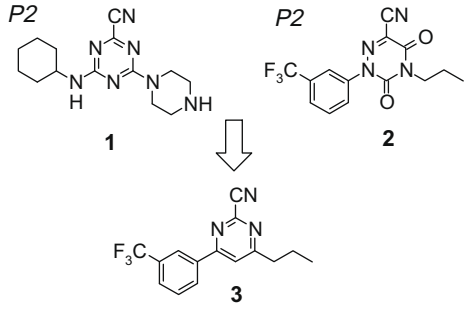

Figure 1. Morphing structures of two HTS-derived inhibitors, triazine **1** and dioxytriazine **2**, led to the design of pyrimidine **3**, a novel cathepsin K inhibitor with improved metabolic stability.

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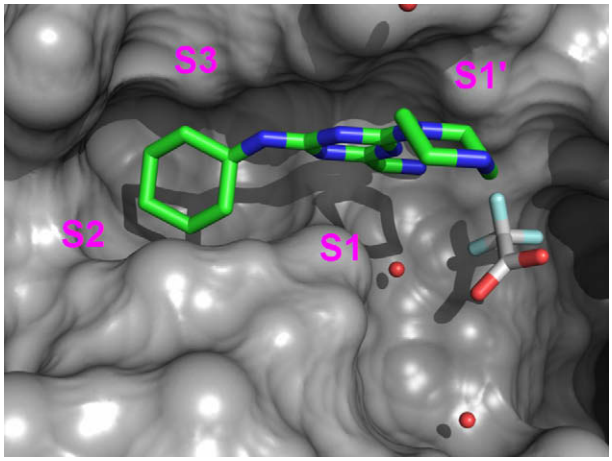


Figure 2. Co-crystal structure of triazine **1** with cathepsin K (1.8 Å resolution; PDB 3KW9). A reversible covalent thioimidate bond is formed between the nitrile and Cys25, which is similar to the previously reported binding mode of an aliphatic nitrile group (PDB structure 2F7D). A molecule of trifluoroacetic acid and a water molecule make additional contacts to the ligand in the prime side.

Similarly, good stability of **1** in human plasma ($t_{½}$ >150 min) was contrasted by its poor rodent plasma stability (e.g., rat $t_{½}$ <17 min). Consequently, the compound demonstrated a poor pharmacokinetic (PK) profile in rat (iv 2 mpk; po 10 mpk), with CL = 159 ml/min/kg, AUC_{inf} = 64 ng h/ml, $t_{½}$ = 2.6 h and oral bioavailability = 6%.

Metabolite identification studies indicated that the uniformly poor rodent plasma stability of compounds in this series was a result of hydrolysis of the nitrile group into the corresponding acid and primary amide. This then might account for the clearance being higher than hepatic blood flow in the rat. Consequently, such poor rodent PK was considered prohibitive for further development of this chemotype, despite the acceptable human in vitro profile. At this point, in order to improve stability of the nitrile warhead in **1** we considered reducing its electrophilicity by replacing the triazine with a pyrimidine core.[8]

The most prominent features of a high resolution X-ray structure of **1** bound in the active site of cathepsin K are the thioimidate moiety formed by covalent interaction between the nitrile warhead and Cys25 in the cathepsin K catalytic site, and the cyclohexyl group deeply buried into the hydrophobic S2 pocket. The piperazine moiety is bound to the S1′ pocket, with the basic amino group exposed to solvent (Fig. 2).

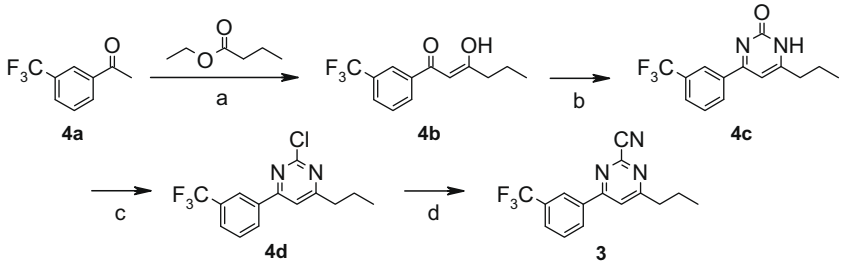
We have previously observed a very similar binding mode in a dioxo-triazine series of cathepsin K inhibitors.[9] This suggested that morphing the structural features of triazine **1** and dioxo-triazine **2** into a pyrimidine chemotype could be a feasible medicinal chemistry strategy towards novel cathepsin K inhibitors with improved metabolic stability. This was indeed confirmed by a hybrid 2-cyano-pyrimidine **3** with moderate cathepsin K potency (IC_{50} = 33 nM), Fig. 1.[10] The synthesis of pyrimidine **3** is shown in Scheme 1.

In order to explore SAR around the P2 aryl moiety of **3**, analogues were prepared according to either of the routes depicted in Scheme 2. The results of this study are shown in Table 1.

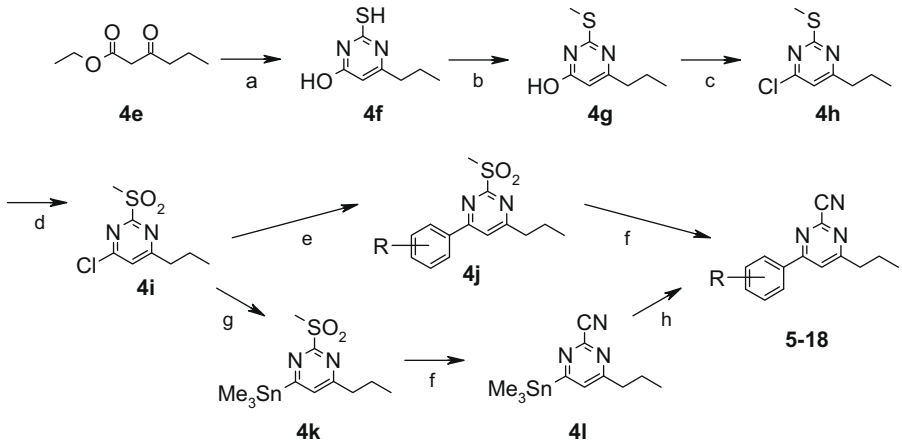
As in the dioxo-triazine series,[9] substitution in the 3-position of the P2 aryl group proved to be critical. For example, the CF_3 deletion analogue **5**, as well as both the 2- and 4-positional isomers **6** and **7**, showed several orders of magnitude lower potency in the cathepsin K assay (IC_{50} values 985 nM, 4074 nM, and 1000 nM, respectively). Small 4-substituents, in addition to the obligatory 3-CF_3 group, were generally tolerated (e.g., 4-Cl analogue **8**, IC_{50} = 34 nM), whereas 2-substitution was found to be detrimental (e.g., 2-Cl analogue **9**, IC_{50} = 1000 nM). The size and lipophilicity of the 3-substitutent was found to be more important than its ability to increase or reduce electron density in the aromatic ring. For instance, the *tert*-butyl analogue **10** showed a threefold higher potency (IC_{50} = 11 nM), whereas corresponding analogues with a slightly larger e.g. cyclopentyl (**11**) or methylsulfonyl (**12**) or smaller, for example, Me (**13**) or CN (**14**) substituent in this position, showed 6–10-fold lower potency when compared to the parent compound **3**. Interestingly, whilst the 3,4-dimethyl analogue **15** displayed only around threefold lower cathepsin K potency (96 nM), its selectivity over cathepsin S was significantly higher than that shown by the compound **3** (catS IC_{50} >10,000 nM vs 60 nM). Similarly, replacement of the aryl moiety with a cycloheptyl group produced inhibitor **16** with moderate cathepsin K potency (IC_{50} = 84 nM), but a significantly improved selectivity over cathepsin S (IC_{50} = 1300 nM). Analogue **17**, with a slightly larger cyclooctyl P2 group, showed further improved cathepsin K potency (IC_{50} = 11 nM) and over 200-fold selectivity over cathepsin S (IC_{50} = 2239 nM), whereas cyclohexyl derivative **18** displayed almost an order of magnitude lower potency for both enzymes (IC_{50} catK = 680 nM and catS >10,000 nM). Although cathepsin S inhibition was not considered detrimental, possibly even beneficial for this programme,[11] a high selectivity over cathepsins L and B was required. Gratifyingly, the majority of cathepsin K inhibitors in this series were shown to be inactive in both cathepsin L and B assays, for example, cat L and B IC_{50} values were >10,000 nM for all compounds in Table 1 (data not shown).

As in the dioxo-triazine series,[9] incorporation of an amine group such as the piperidinyl of compound **19**, resulted in not only improved solubility (from <1 mg/L in **3** to 379 mg/L in **19**), but enhanced cathepsin K potency, too (IC_{50} = 4 nM). Increased potency against the other cathepsins was also observed although, importantly, the high selectivity over catL and catB was maintained (Table 2). Interestingly, the potency enhancing effect of amine group incorporation was not observed for pyrimidine analogues with a cycloalkyl P2 group. For example, piperidinyl **22** showed similar catK potency and selectivity profile to the parent cycloheptyl analogue **16**.

The binding mode similarity of pyrimidine **19** (Fig. 3) and an analogous inhibitor from the dioxo-triazine series[9] co-crystallized with cathepsin K explain the observed SAR similarities between these two chemical series.

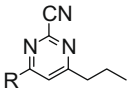


Scheme 1. Reagents and conditions. (a) $NaNH_2$, Et_2O, 60 °C; (b) urea, HCl/EtOH, 80 °C; (c) $POCl_3$, 100 °C; (d) CuCN, NMP, 200 °C.



Scheme 2. Reagents and conditions. (a) thiourea, NaOMe, MeOH, 0–100 °C; (b) KOH, MeOH, MeI; (c) $POCl_3$, 100 °C; (d) *m*CPBA, DCM; (e) $Pd[(Ph_3)P]_4$, K_2CO_3, aryl boronic acid, DME, 100 °C; (f) NaCN, DMSO; (g) $(SnMe_3)_2$, $Pd[(Ph_3)P]_4$, LiCl, 2,6-di-*tert*-butyl-4-methylphenol, dioxane, 100 °C; (h) $Pd(Ph_3P)_2Cl_2$, aryl bromide, DMF, 150 °C.

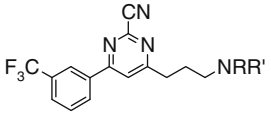
Table 1
Optimization of the P2 group of **3**

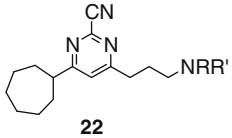


Compds	R	IC_{50}[a] (nM)	
		K	S
3	3-CF_3-phenyl-	33	60
5	phenyl-	985	>10,000
6	2-CF_3-phenyl-	4074	>10,000
7	4-CF_3-phenyl-	1000	1259
8	4-Cl-3-CF_3-phenyl-	34	28
9	2-Cl-3-CF_3-phenyl-	1000	1047
10	3-*tert*-Butyl-phenyl-	11	191
11	3-Cyclopentyl-phenyl-	457	155
12	3-Methylsulfonyl-phenyl-	148	93
13	3-Methyl-phenyl-	181	1300
14	3-Cyano-phenyl-	293	191
15	3,4-Dimethyl-phenyl-	96	>10,000
16	Cycloheptyl-	84	1300
17	Cyclooctyl-	11	2239
18	Cyclohexyl-	680	>10,000

[a] Inhibition of recombinant human cathepsin K and S in a fluorescence assay, employing Z-Phe-Arg-MCA as synthetic substrates. Data represent means of two experiments performed in duplicate.

Table 2
Optimization of the prime side of **3**





Compds	NRR′	IC_{50}[a] (nM)				
		K	S	L	B	ROC[b]
19	Piperidinyl-	4	23	>10,000	4200	22
20		4	47	6760	2340	34
21	NH-*tert*-Butyl-	10	20	9772	6457	56
22	Piperidinyl-	100	>10,000	>10,000	>10,000	ND

[a] Inhibition of recombinant human cathepsin K, S, L and B in a fluorescence assay, employing Z-Phe-Arg-MCA as synthetic substrates. Data represent means of two experiments performed in duplicate.
[b] Rabbit osteoclasts assay.[13]

Pyrimidine **19** was found covalently bound to Cys25 in the cathepsin K active site, with the aryl-CF_3 group deeply buried into the S2 pocket where it makes extensive hydrophobic contacts with the protein, as in the previously reported dioxo-triazine structure.[9] The propyl-piperidine moiety of **19** is angled towards the solvent exposed prime side where the basic nitrogen interacts via a sulfate molecule, an artifact of the crystallization, with Trp184 and Gln19. A similar interaction in situ via a bridging water molecule together with additional hydrophobic contacts may account for the positive effect of amine group incorporation into this region of the molecule.

The X-ray structure of **22** (Fig. 4) revealed the cycloheptyl ring bound in the S2 pocket, but not as deeply as the 3-CF_3-phenyl in **19**, which may explain the potency difference between the two compounds although as a more voluminous group, it forms tighter contacts to both sides of the pocket. Interestingly, the S2 pocket is slightly narrower in catS (there is a ~0.7 Å shift between cat K and cat S residues Leu160, Asn161, and His162), and deeper (Ala134 replaced by Gly in catS) which may explain the greater catS selectivity displayed by **22** (IC_{50} >10,000 nM).

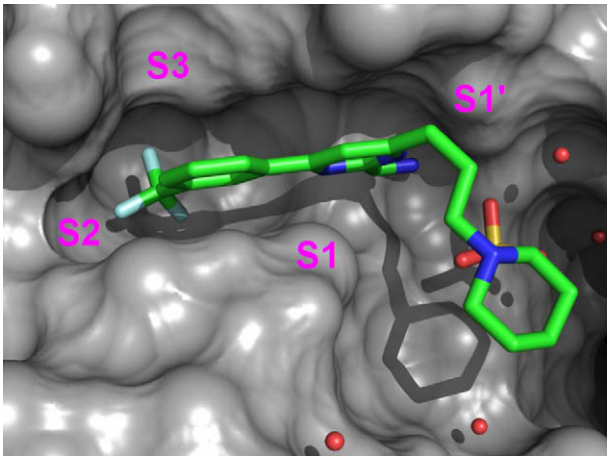


Figure 3. Co-crystal structure of pyrimidine **19** with cathepsin K (1.9 Å resolution; PDB 3KWZ). The sulfate molecule in the prime side is likely to be an artifact of the crystallization.

The crystal structure of **19** also explains our findings that SAR is highly flexible around the 'prime side' binding region of the molecule, which was shown to tolerate a wide range of amine substitu-

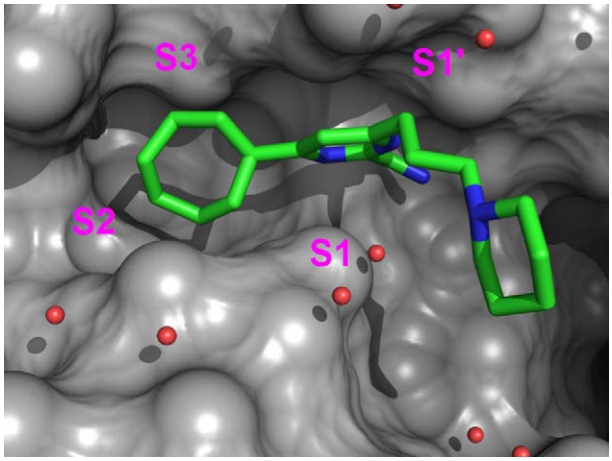


Figure 4. Co-crystal structure of cycloheptyl analogue **22** with cathepsin K (1.7 Å resolution; PDB 3KX1).

Table 3
Pharmacokinetic properties in male Sprague–Dawley rats (iv 2 mpk; po 10 mpk) and hERG potency

Compds	V_{ss} (L/kg)	CL (ml/min/kg)	$t_{½}$ (h)	F (%)	hERG[a] IC_{50} (μM)
19	3.3	13.3	4.1	23	0.16
20	3.5	13.5	3.5	52	0.32
21	12	30	5.7	61	1.1

[a] Inhibition of [^{3}H]-Dofetilide binding to hERG K^+ channel in HEK293 cells following a 10 μM dose of test compound. Data represent means of two experiments performed in duplicate.

ents of different size, basicity and structure (data not shown). However, we focussed mainly on small, secondary amine analogues, such as neopentyl (**20**) and *tert*-butyl (**21**) due to their generally superior PK profile (Table 3).

As initially predicted, both chemical and metabolic stability were greatly improved within the pyrimidine series as compared with the original triazine and dioxo-triazine chemotypes. The high potency of **19**–**21** in the cathepsin K biochemical assay was well translated into efficacy in the functional rabbit osteoclast bone resorption assay (Table 2). However, these compounds were not progressed further due to their prohibitively high hERG potency (Table 3). The significant hERG block is probably related to the relatively high lipophilicity and basicity of these compounds, for example, **19** *c* log P = 4 and pK_a = 9.19.[12]

In summary, biostructurally guided morphing of the triazine and dioxo-triazine cores led to the discovery of novel 2-cyano pyrimidine inhibitors of cathepsin K with good rat PK properties. Compounds in this series showed high catK potency and efficacy in the rabbit osteoclast bone resorption assay, as well as selectivity over catL and catB, but were not progressed due to their high hERG potency. Results of hERG optimization efforts and efficacy of compounds from this series on bone resorption markers in primates will be reported in future publications.

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