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5-Aminopyrimidin-2-ylnitriles as Cathepsin K inhibitors

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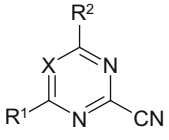
ABSTRACT

A series of pyrimidine nitrile inhibitors of Cathepsin K with reduced glutathione reactivity has been identified and Molecular Core Matching (MoCoM) has been used to quantify the effect of an amino substituent at C5.

The lysosomal cysteine protease Cathepsin K[1,2] is highly expressed in osteoclasts and has been implicated as a key driver of collagen breakdown. Cathepsin K is seen as an attractive target for intervention in the treatment of both osteoporosis and osteoarthritis.[2,3]



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High throughput screening against Cathepsin S led to the identification of the triazine scaffold (**1**; X = N) initially as a singleton hit.[4] Subsequent SAR evaluation demonstrated that the template could be replaced with pyrimidine (**1**; X = CH).[4] Further evaluation highlighted the template as a start point for broader inhibition of the Cathepsin enzyme family. Concurrently, the template has been the focus of research by other groups.[5–8] The carbon atom of the nitrile binds covalently, although reversibly, to the cysteine residue in the active site. Whilst this interaction makes an important contribution to binding, it cannot be the sole basis for inhibition because excessively reactive electrophiles present significant safety issues.[9] Selectivity with respect to other Cathepsins is also important and cannot be achieved unless molecular recognition elements other than the nitrile are exploited.[10]

Relationships between nitrile reactivity and structure were explored using Molecular Core Matching (MoCoM). This cheminformatic method complements approaches based on energies calculated using density functional theory.[11] Reactivity to glutathione, quantified as half life ($t_{1/2}$) was used as an indicator of inherent electrophilicity.[12,13] The rationale for applying MoCoM to these

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systems is that variable parts of molecules are insulated electronically from the reactive centers and not expected to contribute to differences in reactivity. MoCoM of the heteroaromatic nitriles was performed using the OELeatherface[14,15] molecular editor to trim[16] substitution of the nitrogen atoms at C4 and C6 to methyl or phenyl. The resulting molecular cores were matched as canonical[17,18] (unique) SMILES[19] strings.

The results presented in Table 1 show the beneficial effect of a 5-amino substituent on reactivity. Comparing mean values of $\log(t_{1/2})$ for the Core1/Core2 and Core3/Core4 pairs suggests that substitution at C5 with an amino group leads to an increase in $t_{1/2}$ of 0.6–0.7 log units. The range of 0.8 log units in $t_{1/2}$ measured for the compounds specified by Core5 reflects the sensitivity of reactivity to the substitution of the phenyl ring. The range and standard deviation in a property provide useful checks on the validity of the assumption that different structures with the same core are equivalent.

MoCoM is related to the RECAP[20] and Scaffold Tree[21] methods. The power of the approach lies in being able to partition molecules arbitrarily into core and peripheral regions in a user specified manner. The method represents a general approach to associating structurally related molecules. Data analysis is not restricted to comparisons of mean values of properties. For example, MoCoM can be used to define series in structurally diverse data sets for exploration of correlations between solubility and lipophilicity.

The effect of 5-amino substituent on nitrile reactivity was also explored (Fig. 1) using Matched Molecular Pair Analysis[15,22,23] (MMPA). On average, substitution at C5 with amino results in a fourfold (0.62 log units) increase in $t_{1/2}$. This technique addresses the question more directly than MoCoM because structures are identical except for the specific difference that is probed. However, the small number of exactly matched pairs does mean that less data is used in the analysis.

The C5 amino substituent was adopted in lead generation because it appeared to provide the best opportunity to achieve an adequate window between Cathepsin K inhibition and reaction with other cysteine thiols.

Table 1
Analysis of nitrile reactivity using molecular core matching (MoCoM) for neutral pyrimidine and triazine nitriles (keyed to **1**). The glutathione reactivity assay is described in Ref. 12

Core	X	R^1	R^2	N[a]	Mean $\log(t_{1/2})$[b]	SE[c]
Core1	CNH_2	NHMe	NMe_2	27	3.38	0.03
Core2	CH	NHMe	NMe_2	3	2.67	0.16
Core3	CNH_2	NMe_2	NMe_2	9	3.26	0.03
Core4	CH	NMe_2	NMe_2	4	2.56	0.05
Core5[d]	CNH_2	NMe_2	NHPh	9	3.00	0.10
Core6	CH	NMe_2	NHPh	1	2.12	
Core7	CNH_2	NMe_2	OPh	2	2.98	0.14
Core8	CNHMe	NMe_2	NHPh	1	2.91	
Core9	COMe	NMe_2	NHPh	3	2.31	0.05
Core10	CBr	NHMe	NMe_2	1	2.19	
Core11	CNO_2	NHMe	NMe_2	1	< 2.08	
Core12	N	NMe_2	NHPh	1	2.07	

[a] Number of compounds with core.
[b] Mean log(half life in minutes) for compounds with core.
[c] Standard error in mean.
[d] The $\log(t_{1/2})$ values for this core range from 2.60 to 3.41.

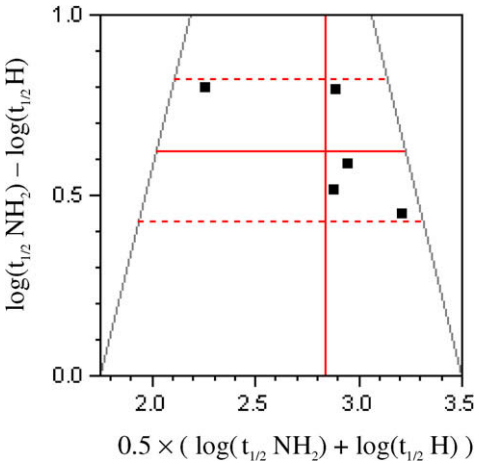
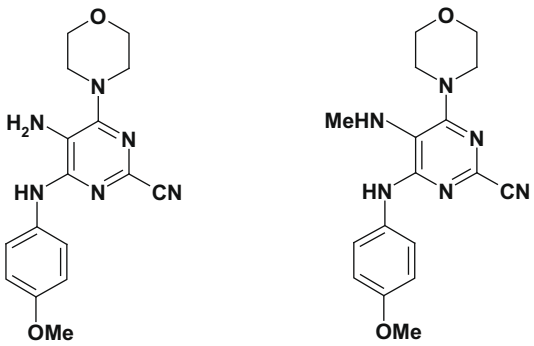


Figure 1. Matched molecular pair analysis of effect of 5-amino substituent on reactivity of pyrimidin-2-yl nitriles. Solid red lines indicate mean values for the two quantities being plotted and broken red lines mark the 95% confidence interval for the mean difference (0.62) in reactivity.



6 **7**

The binding of **6** to Cathepsin K has been modelled (Fig. 2).[24] The aromatic ring sits in the lipophilic S_2 pocket and the morpholine substituent is substantially exposed to solvent. Synthesis of analogues of **6** is summarized in Scheme 1.

Nitration of **2** and subsequent conversion to the corresponding dichloro analogue **3** is straightforward, and can be carried out on multi-gram scale. Introduction of the first amine substituent can be achieved rapidly and selectively at room temperature, with **4** being isolated in high yield. The change in electronic properties then reduces the overall reactivity of the molecule, and subsequently more forcing conditions are necessary to incorporate the second amino substituent, to generate **5**. Cyanide addition has to

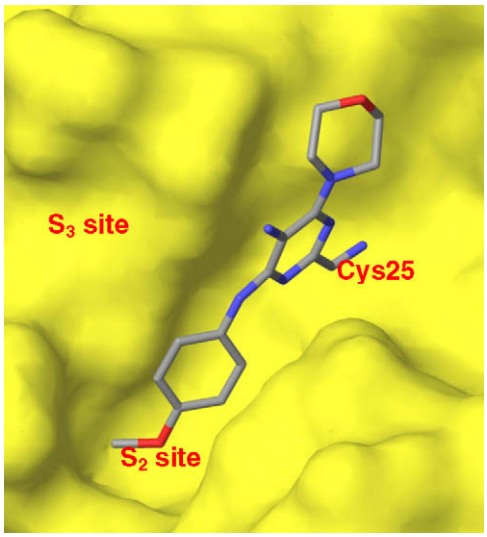


Figure 2. Potential binding mode of adduct of **6** with Cathepsin K showing molecular surface of protein. The covalent bond is formed between the nitrile carbon atom and the sulfur atom of the catalytic cysteine (hidden by surface).

be undertaken prior to reduction of the nitro group, in order to utilize its beneficial electron withdrawing effect.

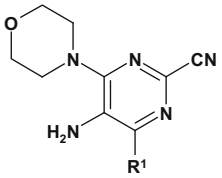
Alkylation of the 5-amino group was considered as an approach to exploiting the S_3 pocket. The *N*-methyl analogue **7** shows equivalent Cathepsin K activity to **6**. However, the glutathione reactivity of **7** ($t_{1/2}$ = 800 min) is greater that that of **6** ($t_{1/2}$ = 2500 min). It appears that the additional bulk of the *N*-methyl substituent compromises the ability of the substituents at C4, C5, and C6 to release electrons to the heteroaromatic ring. Based on this result, the focus of the work shifted to optimization of the P_1 and P_2 substituents.

The S_2 pocket of Cathepsin K is a well-defined hydrophobic cavity and the relevant SAR is summarized in Table 2. The glutathione stability of compounds with alkylamino R^1 substituents approached that of Nilvadipine which was used as a reactivity benchmark.[12] Compound **8**, with an anilino R^1 substituent is both less potent and less stable than its cyclohexylamino analogue **11**. To some extent increasing the size of the alkylamino R^1 substituent leads to an increase in potency as reflected by the 40-fold difference in IC_{50} values measured for **9** and **10**. Although compounds such as **15** show excellent potency, they are too lipophilic to be of interest, as the poor solubility and high metabolic turnover of these motifs present significant challenges to the optimization process. The S_2 pocket appears intolerant of ligand polarity as the tetrahydrofuran substituent **16** shows ~25-fold loss of potency compared to **10**. Further expansion of the SAR demonstrated that cyclic amines were also tolerated in this region (**17**,**18**). Small, branched lipophilic substituents such as isobutylamino appeared to give a more balanced profile with greater scope to achieve the desired overall parameters for progression.

The opportunity to modify the physicochemical and DMPK parameters of the series is most easily achieved through modification of R^2. As can be seen in Table 3, a wide variety of substitution is tolerated. Glutathione reactivity and selectivity, with respect to other Cathepsins, can be controlled by variation of this substituent. The presence of a basic centre in R^2 leads to an increase in Cathepsin K potency and a decrease in GSH $t_{1/2}$. Conversely, introduction of an acid results in an increase in stability but loss of potency. These differences may reflect electrostatic interactions between ionized centres in the substituent and the anionic carboxylate groups of glutathione.

A more detailed evaluation of key compounds was undertaken (Table 4). Basic functionality in R^2 (**19**) leads to increases in Cathepsin K, L, and B potency, solubility, and free fraction but a decrease in GSH $t_{1/2}$. Better selectivity with respect to other Cathepsins was observed for **19** than for **12** or **13** despite the lower

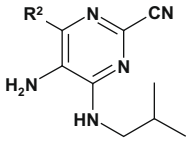
Table 2
Effect of P_2 substituent on potency and stability



Compd	R^1	IC_{50}[a] (nM)	GSH $t_{1/2}$[b] (min)
8	Anilino	110	1200
9	Isopropylamino	560	3200
10	Cyclopentylamino	13	2000
11	Cyclohexylamino	23	2000
12	Isobutylamino	18	2700
13	Neopentylamino	17	3600
14	*sec*-Butylamino	300	3300
15	Norbornan-2-ylamino	2	2900
16	Tetrahydrofuran-3-ylamino	340	1900
17	Pyrrolidin-1-yl	60	2000
18	1-Piperidyl	22	1700

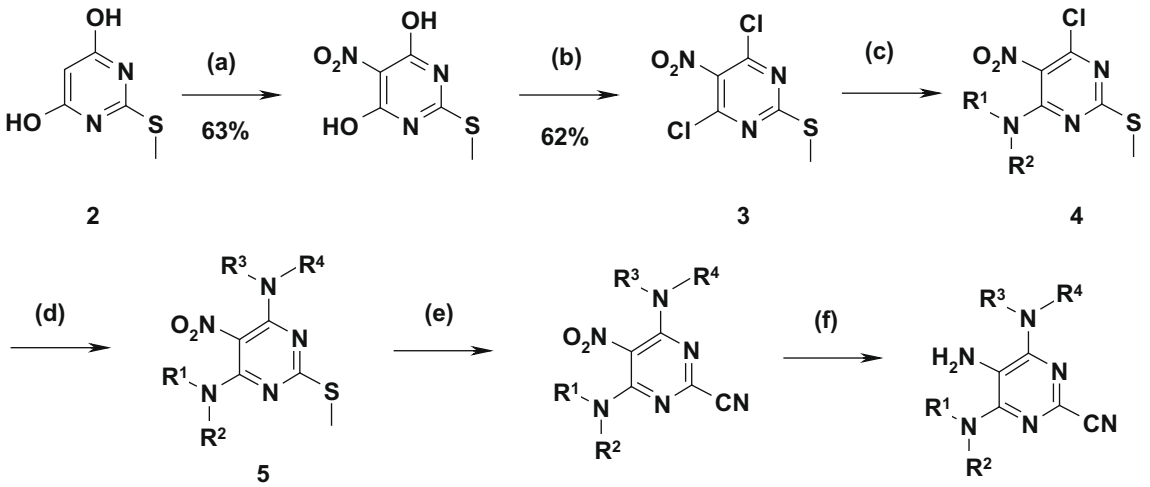
[a] Values are means of at least three experiments.
[b] The glutathione reactivity assay is described in Ref. 12.

Table 3
Effect of P_1 substituent on potency and stability



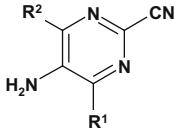
Compd	R^2	IC_{50}[a] (nM)	GSH $t_{1/2}$[b] (min)
12	Morpholino	18	2700
19	Piperazin-1-yl	6	440
20	2-Methoxyethylamino	24	6000
21	4-Acetylpiperazin-1-yl	9	1800
22	(1,1-Dioxothiolan-3-yl)amino	14	2600
23	3-Hydroxyazetidin-1-yl	32	3000
24	1*H*-Tetrazol-5-ylmethylamino	62	>9999

[a] Values are means of at least three experiments
[b] The glutathione reactivity assay is described in Ref. 12.



Scheme 1. Reagents and conditions: (a) Fuming nitric acid; (b) $POCl_3$ *N*,*N*-diethylaniline; (c) R^1R^2NH, THF room temp.; (d) R^3R^4NH, THF, reflux; (e) (i) MCPBA, CH_2Cl_2; (ii) NaCN, DMSO; (f) H_2, 10% Pd/C, EtOAc.

Table 4
Profiles for Cathepsin K inhibitors.



Parameter	12	19	13
R^1	Isobutylamino	Isobutylamino	Neopentylamino
R^2	Morpholino	Piperazin-1-yl	Morpholino
CatK IC_{50} nM	18	6	17
CatS IC_{50} nM	560	890	910
CatL IC_{50} nM	2000	1400	>10,000
CatB IC_{50} nM	>10,000	6100	>10,000
GSH $t_{1/2}$ min [a]	2700	440	3600
log *D*	2.8	0.7	3
Solubility μM	67	1000	25
PAMPA 10^6 cm/s	27	2	NT[e]
Human % free	16	47	5.3
hERG IC_{50} μM	>100	52	70
Ames	−ve	−ve	NT[e]
CYP IC_{50} μM[b]	>10 (5:5)	>10 (5:5)	>10 (5:5)
Clint Rat Heps[c]	<2	4.7	19
Clint Human Mics[d]	11	<2	18
Rat Cl ml/min/kg	13	14	10
Rat $t_{1/2}$ h	5.1	5	3
Rat V_{dss} l/kg	3.1	3	2.7
Rat bioavailability	70%	36%	42%

[a] The glutathione reactivity assay is described in Ref. 12.
[b] Inhibition of cytochrome P450 isoforms: 1A2, 2C9, 2C19, 2D6, and 3A4.
[c] Intrinsic clearance, rat hepatocytes (μl/min/10^6 cells).
[d] Intrinsic clearance, human microsomes (μl/min/mg).
[e] Not tested.

GSH $t_{1/2}$ measured for **19**. This observation provides evidence that the R^1 and R^2 substituents make significant contributions to free energy of binding to Cathepsin K.

Good physicochemical and in vivo pharmacokinetic profiles were observed for these compounds. Acceptable half-lives were achieved with modest volumes and the compounds were readily bioavailable. Despite the presence of the aniline-like amino group, neither **12** nor **19** showed activity in the Ames test either in the presence or absence of metabolizing enzymes. The good pharmacokinetic properties of compounds **12**, **13,** and **19** combined with the eightfold dynamic range in GSH $t_{1/2}$ suggest value as tools with which to investigate the link between glutathione reactivity and covalent adduct formation.

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