Bioorganic & Medicinal Chemistry Letters 19 (2009) 1658–1661

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

5-Aminopyrimidin-2-ylnitriles as Cathepsin K inhibitors

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ARTICLE INFO

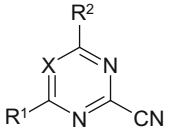
Article history:
Received 7 January 2009
Revised 29 January 2009
Accepted 30 January 2009
Available online 6 February 2009

Keywords:
Cathepsin K
Cheminformatics
Covalent inhibitor
Covalent adduct
Cysteine protease
Electrophilicity
Glutathione
Leatherface
Matched molecular pair analysis
MMPA
MoCoM
Molecular core matching
Molecule editor
SMARTS

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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doi:10.1016/j.bmcl.2009.01.110

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.

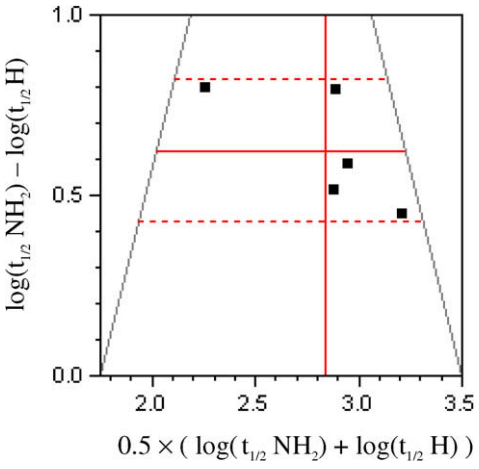
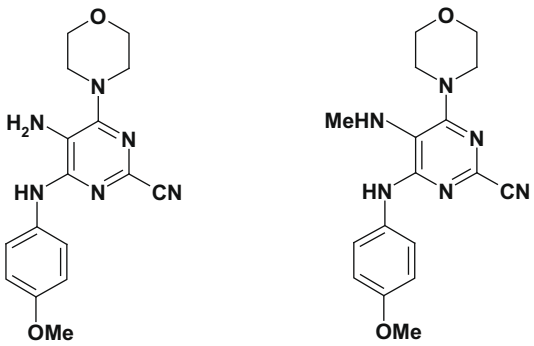


Figure 1. Matched molecular pair analysis of effect of 5-amino substituent on reactivity of pyrimidin-2-yl nitriles. Solid red lines indicate mean values for the two quantities being plotted and broken red lines mark the 95% confidence interval for the mean difference (0.62) in reactivity.



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

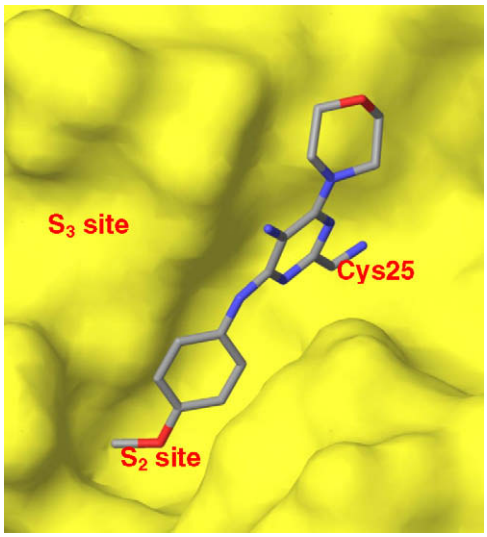


Figure 2. Potential binding mode of adduct of **6** with Cathepsin K showing molecular surface of protein. The covalent bond is formed between the nitrile carbon atom and the sulfur atom of the catalytic cysteine (hidden by surface).