Journal of Medicinal Chemistry

Volume 50, Number 4 February 22, 2007

Letters

2-Cyano-pyrimidines: A New Chemotype for Inhibitors of the Cysteine Protease Cathepsin K

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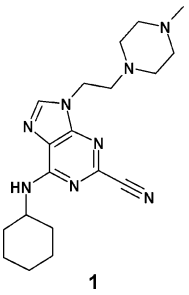
Received November 22, 2006

Abstract: Starting from the purine lead structure **1**, a new series of cathepsin K inhibitors based on a pyrimidine scaffold have been explored. Investigations of P3 and P2 substituents based on molecular modeling suggestions resulted in potent cathepsin K inhibitors with an improved selectivity profile over other cathepsins.

Physiological remodeling of bone tissue is an important and complex process that is crucial for the maintenance of a healthy skeleton. Bone remodeling depends on the tightly coupled activity of two highly specialized cell types, osteoclasts and osteoblasts. Osteoclasts are responsible for bone matrix resorption, whereas osteoblasts build new bone. Elevated osteoclast activity leads to pathological conditions such as osteoporosis. Modulation or inhibition of osteoclastic bone resorption is one of the current therapeutic approaches to osteoporosis treatment. Predominant expression of cathepsin K, a lysosomal cysteine protease, in osteoclasts[1] and evidence for the critical role of this enzyme in the degradation of essential proteins of the bone matrix[2,3] has turned this protease into an attractive target for the development of novel anti-resorptive drugs.[4]

We have disclosed recently the discovery of purine nitriles[5] as a novel class of cathepsin K inhibitors. Although these purine nitriles are potent inhibitors of cathepsin K, as exemplified by **1** (Figure 1), they exhibit only moderate specificity toward the highly homologous cathepsin L and S antitargets.

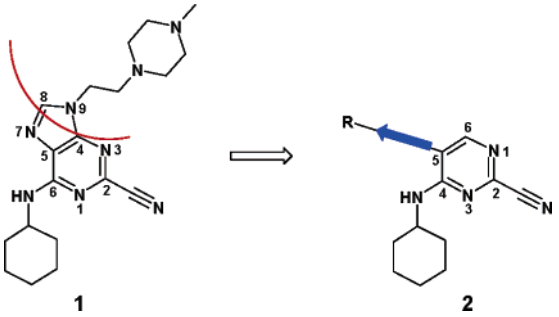
Several X-ray cocrystal stuctures of these purine-based inhibitors within the active site of cathepsin K[5] revealed that these derivatives do not extend into the S3 subsite of cathepsin K but utilize solely lipophilic interactions within the S1/S2 subsites. Based on prior experience with other series of cathepsin K inhibitors, we hypothesized that an optimally designed P3 moiety might provide an essential contribution to high specificity. In addition, due to the rather hydrophilic nature of the S3 subsite of the enzyme, polar substituents would be tolerated as part of this P3 group, and we hoped to be able to exploit this feature to fine-tune the physicochemical properties of our inhibitors. Therefore, one of our strategies toward the development of improved templates included the assessment of heterocyclic scaffolds that would allow the attachment of a P3 substituent. Our X-ray cocrystal structures offered opportunities for computer-assisted modeling studies. The strategy that we pursued, as schematically outlined in Figure 2, is based on the removal of those parts of our original purine-based inhibitor **1** that do not specifically interact with the substrate binding site. Molecular modeling of pyrimidine template **2** into the active site of cathepsin K (Figure 3) indicates that the 5-position could be used to introduce potential P3 substituents, which should



	IC_{50} [nM]
Cat K	6
Cat L	89
Cat S	150

1

Figure 1. Purine lead structure **1**.



1 **2**

Figure 2.

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10.1021/jm0613525 CCC: $37.00
Published on Web 01/27/2007

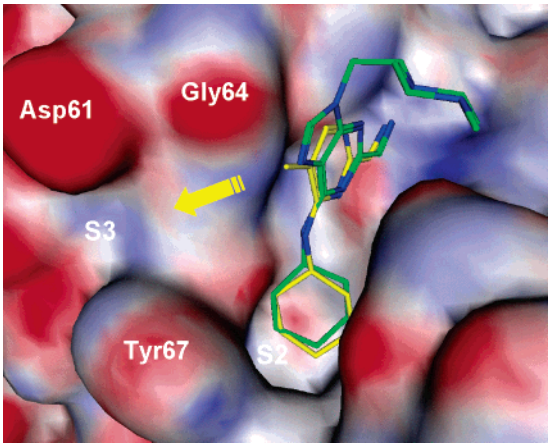
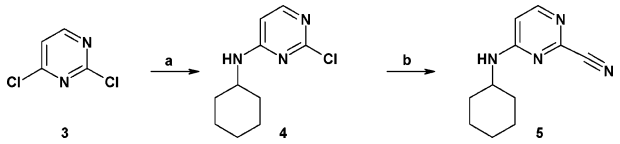


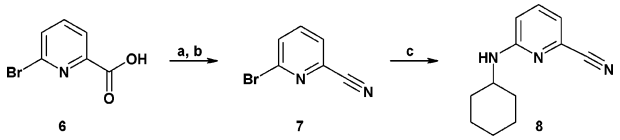
Figure 3. Pyrimidine core structure **2** (yellow) docked into the cathepsin K active site of the cocrystal structure of **1** (green) (PDB 1U9V).

Scheme 1[a]



[a] Reagents and conditions: (a) cyclohexylamine, Et_3N, EtOH, 70 °C, 15 h, 67%; (b) NaCN, DMA, 160 °C, 18 h, 35%.

Scheme 2[a]



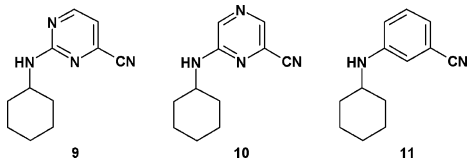
[a] Reagents and conditions: (a) (1) 1,1-carbonyldiimidazole, THF, rt, 10 min; (2) NH_3 (aq 25%), rt, 3 h, 81%; (b) trifluoroaceticacidanhydride, Et_3N, THF, −5 to 0 °C, 2 h, 97%; (c) cyclohexylamine, LiHMDS (1M in toluene), (*R*)-(−)-1-[(*S*)-2-(dicyclohexylphosphino)-ferrocene]ethyl-di-*t*-butylphosphine, $Pd(OAc)_2$, DME, 85 °C, 18 h, 13%.

extend into S3.[6] At the synthetic level, we wanted a simple entry into the desired heterocyclic scaffold, which would enable the introduction of a wide range of substituents at a late stage of the synthesis.

Before looking at such 5-substituted 2-cyano-pyrimidines, we explored whether the presence of nitrogen atoms in the aryl moiety is required for biological activity. Two different strategies were followed for the synthesis of these structures (**5**, **8**, **9**, **10**, and **11**), which are exemplified in Schemes 1 and 2 for compounds **5** and **8**, respectively. Thus, commercially available 2,4-dichloro-pyrimidine (**3**) was reacted with cyclohexylamine to afford the 4-substituted pyrimidine **4**, which was then converted into 2-cyano-4-cyclohexylamino-pyrimidine **5** by displacement of the 2-chloro substituent with cyanide.

To obtain target nitrile **8** (Scheme 2), 6-bromo-pyridine-2-carboxylic acid (**6**) was reacted with carbonyldiimidazole. The resulting imidazolide was quenched *in situ* with aqueous NH_3 to provide the amide, which was then converted to the nitrile **7** by $(CF_3CO)_2O$-mediated dehydration.[7] The 6-cyclohexylamino-substituted pyridine nitrile **8** was produced from aryl bromide **7** by a Buchwald reaction using a ferrocene ligand.[8]

From the examples prepared in this series, it is evident that a pyrimidine core is required for cathepsin K inhibition, because all other derivatives investigated proved to be significantly less active. 2-Cyano-pyridine **8** exhibited an IC_{50} (concentration that inhibits to 50% of control activity) value of 1 μM versus cathepsin K, while the 2-cyano-pyrazine **10** and benzonitrile **11** had potencies of >1 μM. The most active derivative within



this series was 4-cyano-pyrimidine **9** (IC_{50} for cathepsin K of 120 nM). Within this system, the introduction of a P3 group at the required position is not possible because it is occupied by one of the nitrogen atoms. For this reason, we focused our attention on 5-substituted derivatives of 2-cyano-pyrimidine **5** (IC_{50} for cathepsin K of 170 nM), which is directly derived from the purine family of cathepsin K inhibitors.

Such 5-substituted 2-cyano-4-cyclohexylamino-pyrimidine inhibitors were prepared from **12** as exemplified in Scheme 3 for compound **16a**.

Conversion to the 2,4-dichloro-pyrimidine-5-carbonylchloride (**13**) was achieved by heating carboxylic acid **12** in a mixture of phosphoroxychloride and phosphorus pentachloride. Addition of phenethylamine to the acid chloride **13** in the presence of diisopropylethylamine furnished amide **14**. Subsequent reaction with cyclohexylamine followed by the displacement of the 2-chloro substituent of **15** with cyanide provided nitrile **16a**.

Compound **16a** was one of the first compounds prepared in this series, and it displays highly potent cathepsin K inhibition combined with at least reasonable specificity toward the antitargets cathepsins L and S (Table 1).

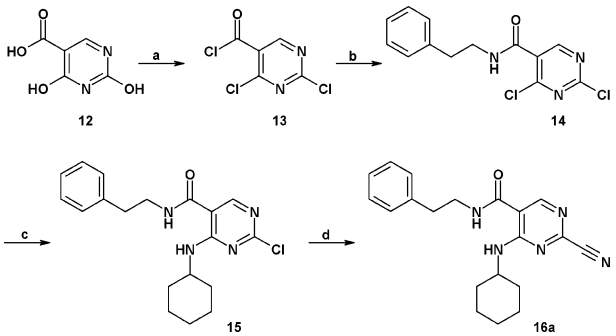
Attachment of a 4- or 3-methoxy group at the phenyl ring in **16a** (compounds **16b** and **16c**) did not result in any significant change in potency against cathepsin K, but both compounds suffered from a loss in antitarget selectivities (Table 1). In contrast, para substitution of **16a** with a pyrrolidine ring (**16d**) had a very unfavorable effect for potency against cathepsin K. Rather disappointingly, even the introduction of a 4-methylpiperazine substituent (as in **16e**), which was intended to interact with aspartic acid residue 61 (Asp61), did not lead to improved activity.

X-ray crystallographic analysis of compound **1** bound within the active site of cathepsin K[5] suggested that the potential hydrophobic interactions within the wide lipophilic S2 subsite of cathepsin K were not fully exploited with the cyclohexyl P2 substituent. Therefore in parallel to the modification of the P3 moiety of this 2-cyano-pyrimidine scaffold, we investigated a series of lipophilic P2 groups. Of the various types of substituents investigated (e.g., benzyl, phenethyl, isobutyl), a neopentyl proved to be the most favorable by far. Table 2 summarizes the activities and selectivities of a series of representatives compounds, which are based on a 2-cyano-4-neopentylamino-pyrimidine-5-carboxamide scaffold.

We were pleased to find that compounds **17a**−**17d** consistently showed improved potency vs cathepsin K and a significantly increased specificity profile compared with their 6-cyclohexyl-substituted analogues **16b**−**16e**. Encouraged by this observation, we continued to pursue this series and, in a next step, tried to refine the interactions between the P3 group and the S3 subsite of cathepsin K.

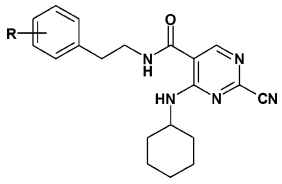
Starting from **17d**, we incorporated spacers between the phenyl ring and the appending basic group, in order to bring this group closer to Asp61 in the S3 pocket. While incorporation of a single atom spacer, such as in **17f** or **17g**, did not produce any change in potency, the latter proved to be significantly more

Scheme 3[a]



[a] Reagents and conditions: (a) $POCl_3/PCl_5$, 115 °C, 6 h, 75%; (b) 2-phenylethylamine, DIEA, CH_2Cl_2, rt, 15 h, 57%; (c) cyclohexylamine, Et_3N, DMF, 40 °C, 2 h, 70%; (d) KCN, DABCO, DMSO/H_2O, 80 °C, 3 h, 47%.

Table 1. Inhibition of Human Cathepsins K, L, and S



compd	R	IC$_{50}$ [nM][a] Cat K	Cat L	Cat S
16a	H	0.01	1.1	0.13
16b	4-MeO	0.022	0.17	0.7
16c	3-MeO	0.009	0.31	0.23
16d	4-	0.75	5.1	2.3
16e	4-	0.047	0.45	0.48

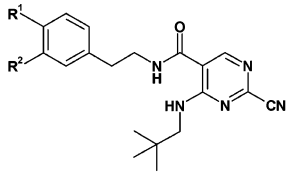
[a] Inhibition of rh cathepsins K, L, and S in a fluorescence-based assay, employing Z-Phe-Arg-AMC (cathepsins K and L) and Z-Leu-Leu-Arg-AMC (cathepsin S) as synthetic substrates. Data represent means of two experiments performed in duplicate. Individual data points in each experiment were within a 2-fold range with each other.

selective, in particular with regard to cathepsin S inhibition (K/L = 130, K/S = 770). The most interesting compound in this series was **17h**, which incorporates an imidazolyl ethyloxy substituent and which is a highly selective, single digit nanomolar inhibitor of cathepsin K (K/L =173, K/S =300).

In order to fully explore the concept of topographical separation of the positively charged amine moiety from the phenyl ring in the core structure, we also investigated a number of derivatives with the amine part attached to the meta position on the phenyl moiety. Rather surprisingly, this change in the substitution pattern did not result in any significant changes in the inhibitory profile (**17a** vs **17b**, **17d** vs **17e**, **17h** vs **17i**). In contrast, the combination of a para and a meta substituent, as in example **17j**, produced a 10-fold loss in potency and greatly compromised specificity.

A number of the above compounds were selected for PK analysis in a cassette dosing experiment in rats (iv 4 μM/kg and po 10 μM/kg). Unfortunately, all compounds investigated were rapidly cleared from the circulation (e.g., $t_{1/2}$ = 48 min, Cl = 27.7 L/(h·kg) for **17h**) resulting in very low to even nondetectable oral bioavailability. In line with these findings, capillary HPLC/MS/MS analysis of plasma, urine, and liver homogenate samples after iv dosing of compound **17d** revealed the presence of seven phase 1 metabolites (apart from very low levels of parent compound). The information gained from these *in vivo* studies will be useful for the design of metabolically more stable pyrimidine-based cathepsin K inhibitors.

Table 2. Inhibition of Human Cathepsins K, L, and S



compd	R^1	R^2	IC$_{50}$ [nM][a] Cat K	Cat L	Cat S
17a	CH_3O	H	< 0.003	0.34	1.6
17b	H	CH_3O	<0.003	1.2	0.9
17c		H	0.3	>10	>10
17d		H	0.025	3.9	3.1
17e	H		0.011	0.82	1.8
17f		H	0.011	0.33	2.5
17g		H	0.013	1.7	10
17h		H	0.003	0.52	0.9
17i	H		0.003	0.68	0.51
17j	CH_3O		0.031	0.58	2.6

[a] Inhibition of rh cathepsins K, L, and S in a fluorescence-based assay, employing Z-Phe-Arg-AMC (cathepsins K and L) and Z-Leu-Leu-Arg-AMC (cathepsin S) as synthetic substrates. Data represent means of two experiments performed in duplicate. Individual data points in each experiment were within a 2-fold range with each other.

In summary, we have discovered a potent new class of cathepsin K inhibitors, which are based on a pyrimidine scaffold. In general, these compounds exhibit enhanced specificity over the original purine-based lead structure. One of the key structural features responsible for the attractive specificity profile of these new inhibitors is the presence of a suitable P3 group extruding from the 5-position of the pyrimidine ring.

Acknowledgment. The authors thank Doris Huerzeler and Gérard Schreiber for excellent technical assistance.

Supporting Information Available: Description of the inhibition assays; experimental procedures for the synthesis of compounds **5**, **8**, and **16a**; and characterization (^{1}H NMR and HRMS) of compounds **5**−**8**, **9**−**11**, **16a**−**e**, and **17a**−**j**). This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0613525