Bioorganic & Medicinal Chemistry Letters 22 (2012) 5563–5568

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Isosteric replacements for benzothiazoles and optimisation to potent Cathepsin K inhibitors free from hERG channel inhibition

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ARTICLE INFO

Article history:
Received 28 May 2012
Revised 29 June 2012
Accepted 4 July 2012
Available online 15 July 2012

Keywords:
Cathepsin K
Covalent inhibitor
Molecular complexity
Structure based design
hERG
Pharmacokinetics
Matched molecular pair analysis
MMPA
Synthesis

ABSTRACT

The discovery of nitrile compound **4**, a potent inhibitor of Cathepsin K (Cat K) with good bioavailability in dog is described. The compound was used to demonstrate target engagement and inhibition of Cat K in an in vivo dog PD model. The margin to hERG ion channel inhibition was deemed too low for a clinical candidate and an optimisation program to find isosteres or substitutions on benzothiazole group led to the discovery of **20**, **24** and **27**; all three free from hERG inhibition.

The lysosomal cysteine protease Cathepsin K (Cat K) is highly expressed in osteoclasts and plays a key role in bone resorption by the degradation of type I cartilage.[1] Cat K knockout mice exhibit osteopetrosis (abnormally dense bone) and abnormal joint morphology[2] indicating the potential role of this enzyme in key bone pathologies such as osteoporosis (OP), osteoarthritis (OA), and metastatic bone disease (MBD).[3–5] Osteoarthritis is a group of degenerative disorders characterised by joint pain, and loss of function in the absence of chronic autoimmune or autoinflammatory mechanisms.[6] They have an increasing prevalence with age and are thus a growing socioeconomic burden.[2] Articular cartilage breakdown is a prominent feature of joint degeneration and, as a result, has received significant attention from the pharmaceutical industry. Several companies have trialed compounds in the OP and OA disease areas. Novartis has completed Phase II studies in OP and OA with Balicatib (**1**).[7] The Phase II OP trial reported positive outcomes on bone mineral density measures. Merck has completed a Phase II trial in OP with Odanacatib (**2**), is recruiting for Phase III in MBD and has abandoned plans for a Phase II study in OA.[8–10] Both compounds are electrophilic nitrile-containing compounds that bind covalently and reversibly to the Cat K enzyme preventing type I collagen degradation.

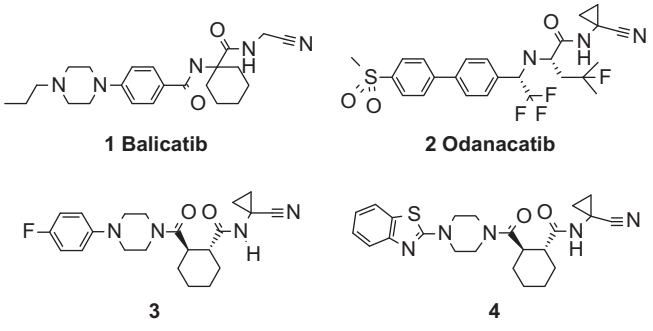

Figure 1. Nitrile Cathepsin K inhibitors.

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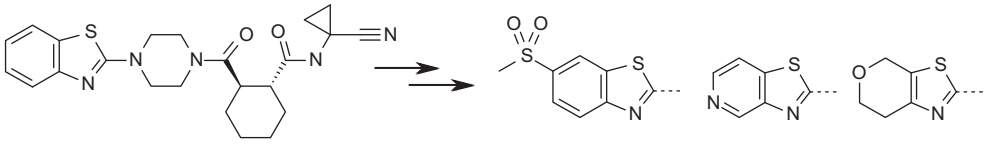
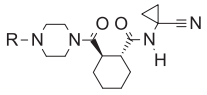
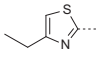
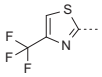
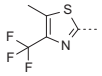
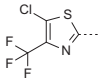
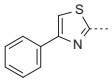
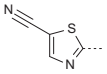
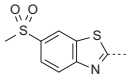
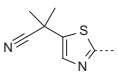
http://dx.doi.org/10.1016/j.bmcl.2012.07.012

Table 1
SAR for thiazole variations



Compound	**4**	**20**	**24**	**27**
Cat K pIC_{50}	8.0	7.6	7.8	7.6
hERG pIC_{50}	5.1	<4.0	<4.0	<4.0



Compd	R - group	Cat K pIC_{50}[a]	Cat L pIC_{50}[a]	Cat S pIC_{50}[a]	Cat B pIC_{50}[a]	log $D_{7.4}$	Mol W./Da	hERG inhib. pIC_{50}	Aqueous solubility pH 7.4 (μM)	HLM[c] μL/min/mg	RHC[d] μL/min/10^6cells
3		7.8	<4.0	5.9	6.0	2.1	398.5	4.5	>4300	9.2	2.5
4		8.0	<5.1	6.2	5.8	2.6	437.6	5.1	1000	<2.0	64
10		7.6	<4.0	5.8	5.6	1.3	387.5	<4.0	3100	6.9	36
11		7.7[b]	<4.0[b]	5.6[b]	5.6[b]	-	415.6	<4.0	-	10	140
12		7.7[b]	<4.0[b]	5.5[b]	5.5[b]	2.1	415.6	<4.0	3300	9	69
13		7.7[b]	<4.0[b]	5.4[b]	5.3[b]	-	427.6	<4.0	-	21	71
14		8.0[b]	<4.0[b]	5.8[b]	5.5[b]	3.3	443.6	5.2	280	174	130
15		7.2[b]	<4.0[b]	5.8[b]	5.2[b]	2.4	455.5	4.6	-	13	26
16		7.6[b]	<4.0[b]	5.7[b]	5.3[b]	2.9	469.5	4.8	-	27	51
17		7.5	<4.0	5.8	5.3	3.4	489.9	4.6	230	32	-
18		7.8	<4.0	5.8	5.4	3.5	463.6	4.6	96	20	34
19		6.7[b]	-	-	5.4	1.2	412.5	-	-	-	-
20		7.6	5.0	<4.0	5.1	1.5	515.7	<4.0	-	<2.0	4.9
21		7.4	<4.0	5.5	5.3	1.9	454.6	<4.0	2600	<2.0	9.3

[a]Binding affinity Cat K, L, S, B versus FRET substrate, mean of greater than $n = 4$ tests, unless where stated.
[b]Mean of $n = 2$ tests.
[c]In vitro human liver microsomal turnover mean of at least $n = 2$ tests (μL/min/mg).
[d]In vitro rat liver hepatic clearance (RHC) turnover mean of at least $n = 2$ tests (μL/min/10^6 cells).

Electrophilic nitrile-containing cysteine protease inhibitors, such as **1** and **2** (Fig. 1), have been observed to interact with Cat K by reversible formation of a covalent bond between the nitrile and an active site cysteine (Cys-25). Screening of nitrile containing compounds from the corporate collection identified (*R*,*R*)-1,2-cyclohexyl-diamide-acetonitriles as potent and efficient inhibitors of the enzyme. We developed this lead series with an initial focussed library of rigid tertiary amides to bring groups into contact with the S3 groove,[11–13] and identified piperazines, such as **3** and **4**, as potential starting points for optimisation. Both **3** and **4** had good Cat K inhibition, physical properties and stability to in vitro human liver microsomes (Table 1).[13]

Studies in rat pharmacokinetics experiments showed 4-fluorophenylpiperazine **3** was the inferior of the two compounds when no appreciable compounds levels were detected after oral dosing compared to benzothiazole-piperazine **4** with bio-availability of 20%. It was unclear why **3** had poor PK as the compound had good rodent in vitro stability and aqueous solubility. The in vitro rat hepatic stability for **4** was significantly higher and led us to suspect an absorption issue with **3** (Table 1). An excellent pharmacokinetic profile was found when **4** was dosed orally and intravenously to dog: bio-availability of 78%, CL 13.0 mL/min/kg from doses of 1.0 mg/kg. The blood samples from this PK study were analysed further by measuring the concentration of C-telopeptide (CTX-I), the degradation product of the action of Cat K on type I collagen.[14–16] As blood concentrations of **4** rose after oral dosing to dog, a rapid decrease in CTX-I levels was observed as Cat K enzyme activity was abated as expected (Fig. 2). A large effect was observed in both dogs, demonstrating target engagement and inhibition of enzyme activity. As blood levels of **4** diminished, so enzyme activity returned, as shown by rising CTX-I levels over the second 12-hour period of the experiment. Compound **4** was clearly a good quality tool compound suitable for further pre-clinical disease model work, which will be published in due course.

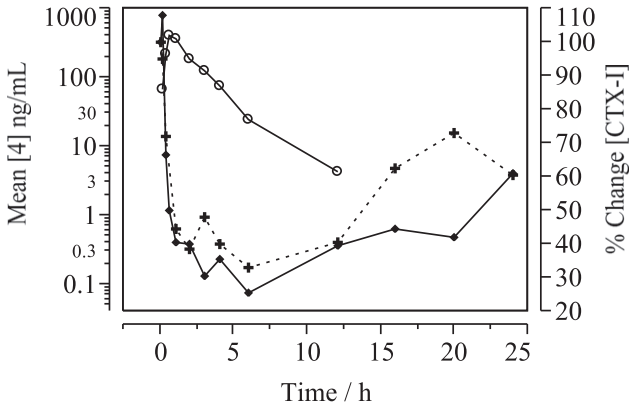
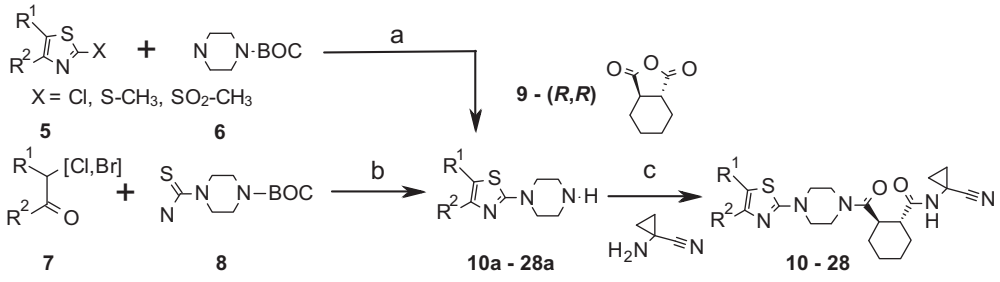


Figure 2. Dog PK/PD experiment for **4**—the blood samples taken at a given time point after dosing were split and used to measure [**4**] and [CTX-1]. ○—PK Mean [**4**] % ng/mL at 1.0 mg/kg uid to both dogs (406 and 476); ◇—[CTX-1] as % baseline 1.0 mg/kg uid Dog 406; +—[CTX-1] as % baseline 1.0 mg/kg for Dog 476. Dog enzyme Cat K inhibition for **4** was measured at 0.018 ± 0.010 μM and dog PPB 83% bound thus 1× free [**4**] was 49.2 ± 27.4 ng/mL.

Compound **4** was prioritised for further optimisation due to the in vivo profile above and good selectivity with respect to other cathepsins; at worst, 80-fold against Cat S. The absence of a basic centre in the molecule was also seen as important because basicity is associated with compound accumulation in lysosomes (lysosomotropism), which is considered a potential cause of toxicity.[17,18] However, the compound was not suitable for further clinical development as inhibition of the hERG ion channel, measured at pIC_{50} 5.1 (9.0 μM), needed improvement to provide a better safety margin.[19–21] With this in mind a set of thiazole piperazines (**10**–**19**) was designed as an initial training set to cover a range of lipophilicities and reduce the molecular complexity of the compounds so we could incorporate structure based design, using the results we obtained.

Thiazole piperazines were synthesised in one of two basic methods, either displacement of leaving group from the 2—position of the substituted thaizole or employing the classic Hantzsch reaction (Scheme 1). Nucleophilic aromatic substitution of a 2-chloro, 2-methylsuphide, 2-methylsulphone on thiazole **5** with carbamate protected piperazine **6** was high yielding but required heating at high temperatures. Although diversity was introduced early on, condensation of an α-haloketone (**7**) with carbamate protected piperazine thio-urea **8** was a short and convenient route. Following either route, simple deprotection with acid yielded the thiazole-piperazines (**10a**–**28a**) ready for coupling. Final compounds were synthesised by ring opening of chiral anhydride **9**—(*R*,*R*) at ambient temperature with the piperazines (**10a**–**28a**) to yield an intermediate acid. Final compounds (**10**–**28**) were then obtained by amide formation with 1,1-amino-cyclopropylnitrile and a suitable coupling reagent *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and di-*iso*-propylethylamine (DIPEA) in yields between 7% and 78%.[22] This method proved to be an excellent 'one-pot' convergent method and amenable to scale-up on multigram amounts; for example, 4.8 g of **4** was synthesised in an overall yield of 62%.

Table 1 details the results of the initial set of thiazole Cat K inhibitors compared to **4**. The simple thiazole **10** showed that the benzothiazole ring of **4** does not add significant Cat K potency (or indeed change selectivity), however activity was diminished along with inhibition at the hERG channel. The drop in lipophilicity, in line with prediction, resulted in thiazole **10** achieving a high point in ligand lipophilicity efficiency (LLE) for this series at 6.3. Compounds **11** through **17** steadily increase in molecular size and lipophilicity, which as a set made very little difference in Cat K inhibition. The more lipophilic examples were less stable to in vitro microsomal and hepatic incubation experiments (e.g.,



Scheme 1. Synthesis of cyclohexyl-1,2-diamides **10–27**. Reagents and conditions: (a) (i) EtOH, 50 °C, 1 h, concentrate, (ii) 5 equiv 1.0 M HCl in dioxane, rt, 1 h or TFA, CH_2Cl_2, overall 15–52% yield; (b) (i) DIPEA, DMF, toluene, xylene, 60–140 °C, 1–5 h, (ii) 5 equiv 1.0 M HCl in dioxane, rt, 1 h or TFA, CH_2Cl_2, Overall 25–68% yield; (c) (i) **9**—(*R*,*R*), CH_2Cl_2, rt, 1 h, concentrate, (ii) DMF or CH_2Cl_2, DIPEA (5 equiv), HATU (1.1 equiv), $NCC(CH_2CH_2)NH_3Cl$ (1.0 equiv), rt, 12 h. 12–78% yield.

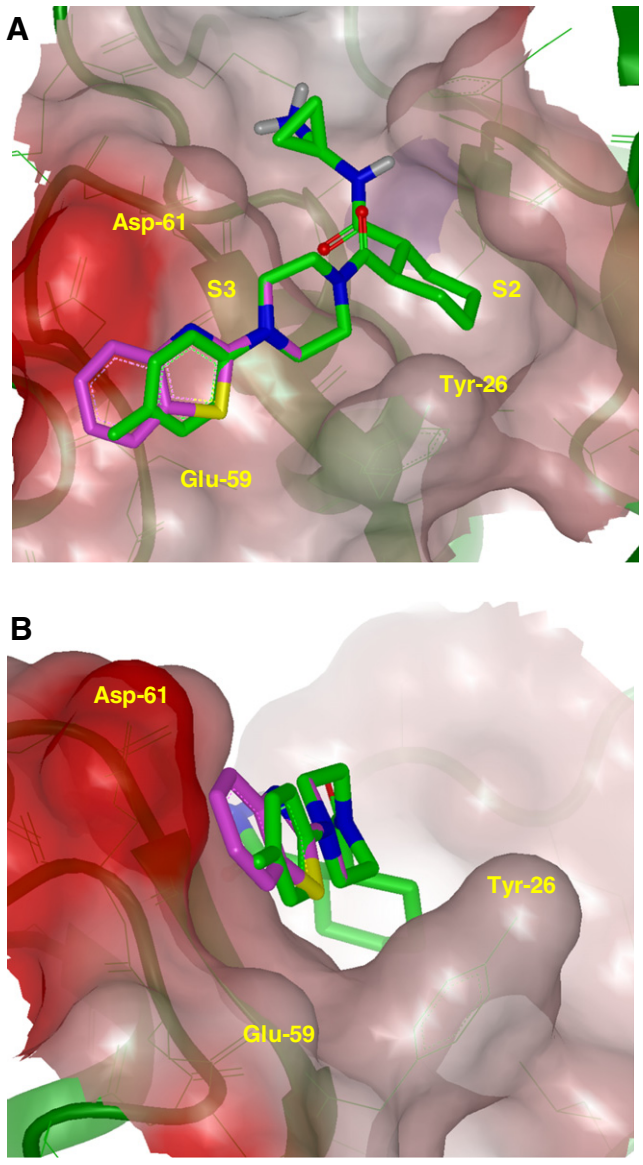


Figure 3. Compound **3** (green) bound to Cathepsin K (PDB 4DMX) and with **4** (pink) overlaid by atom substitution. Structures shown from two aspects, where **B** illustrate the open nature of the S3 groove.

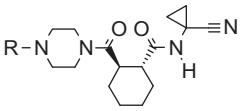
14), although some stability was regained with fluorine substitution (**15**–**17**). A near *iso*-lipophilic compound **15**, compared to **4**, did not have the same level of hERG inhibition (3.2-fold) suggesting structural elements and not just lipophilicity were controlling this. This observation was supported by examples **16**–**18**, where Log $D_{7.4}$ was higher than **4**, but hERG inhibition was lessened. These observations are consistent with molecular models for this ion channel, where either charged alkyl amino groups or aromatics bind in a ring of phenyl anilines and additional lipophilicity increases affinity.[23–26] These data indicated we should aim to keep lipophilicity down to Log $D_{7.4}$ of 2.0 or less and avoid benzothiazoles (The data indicated a weak correlation between hERG inhibition and Log $D_{7.4}$—see Supplementary data).

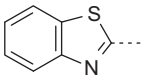
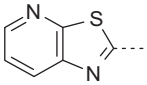
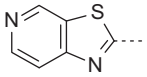
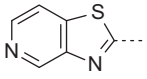
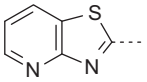
X-ray structure determination of a co-crystal of **3** with Cat K revealed the cyclohexane ring was occupying the relatively shallow S2 pocket of the enzyme[27,28] which placed the tertiary amide, and thus the piperazine, in the comparatively open S3 pocket with the carbonyl oxygen exposed to solvent (Fig. 3—**A**). The protein molecular surface in the S3 pocket was relatively flat and lacked concave polar regions that could be exploited. The piperazine group was between a glycine rich loop and Tyr-26 such that only the 'edge' was in contact with the protein surface (Fig. 3—**B**). This projected the 4-fluorophenyl in the same plane as the piperazine so that two aromatic C–H groups were pointing towards the protein surface. Therefore only half the piperazine/aromatic surface atoms made energetically favourable interactions with the protein. Virtual substitution of the 4-fluorophenyl of **3** with benzothiazole (**4**) and molecular minimisation logically placed this aromatic in the same plane as the piperazine, to maintain electronic interaction, such that sulphur contacts the protein surface in preference to nitrogen, which pointed towards solvent (Fig. 3—**B**). This result in the benzenoid moiety placed further down the groove between Asp-61 and Glu-59 towards a more polar area and open area. Applying knowledge-based design using matched molecular pair analysis we chose to make the methylsulfonyl substituent at C6, which has been shown to improve the physicochemical properties of compounds[29] and human liver microsomal stability[30–32], in the hope this group would be tolerated or even yield a favourable interaction. This produced **20**, with reduced hERG binding, increased in vitro stability, but a three-fold drop in Cat K inhibition, showing we were incorrect. The orientation of the thiazole (sulphur towards protein) was consistent with our experimental observations, in that alkyl substituents from the C4 position of thiazole (**12**–**16**) would orientate away from any favourable Van-de-Waals interactions with the protein, so explaining the fairly consistent level of Cat K inhibition. Therefore combining the knowledge gained we refined our tactics to focus on (i) substitution at the C5 position, maintaining Log $D_{7.4}$ equal or below 2.0, or (ii) finding a non aromatic isostere for benzothiazole that could pick up interactions with Asp-61 or Glu-59, to increase Cat K inhibition and eliminate hERG binding.

The first of these approaches, substitution at C5 position, proved disappointing with the best example represented by **21** (Table 1), where the C5—isobutyronitrile group gave a compound with Log $D_{7.4}$ = 1.9 and hERG inhibition pIC_{50} <4.0, but with inhibition of Cat K reduced to pIC_{50} 7.4 (fourfold less). Focusing more on the second approach, we found that Walczyński et al., as part of a program towards optimised H_3-antagonists, had made all four aza-benzothiazoles to reduce lipophilicity and find a polar interaction.[33] The routes outlined in their paper fitted our synthetic approach to Cat K inhibitors and all the four aza-benzothiazoles were made and profiled (Table 2). Using the virtual structure of benzothiazole **4** we reasoned upfront that 7-azabenzothioazole **22** would be less potent as the nitrogen lone pair was likely to point towards a non polar surface region. Despite this we still chose to make **22** for further medicinal chemistry understanding and as predicted it was less active but pleasingly free from hERG liability and remarkably stable in in vitro incubations, which is noteworthy. All other three isosteres were below pIC_{50} of 4.0 for hERG, and in a similar range for Cat K inhibition. The best example, 5-azabenzothiazole **24**, had similar Cat K inhibition to **4**, as well as good physical properties and in vitro stability making this an attractive isostere. The 5-aza nitrogen lone pair would, we predict, point away from protein surface and towards either solvent or Asp-61, which fits with the inhibition result. By the same rationale, we expected 4-aza compound **25** to show an increase in inhibition by better alignment with Asp-61. To our surprise a more significant drop was observed, which we cannot rationalise at this point. Finally for three isomers we measured cytochrome P450 inhibition potential using isolated enzymes (3A4, 2D6, 2C9, 2C19 and 1A2). **4** and **25** are in-active against all five isoforms, whereas **23** and **24** were inhibitors of three out the five so ruling these out as suitable compounds (see Supplementary data).

Table 3 shows the comparison data for partially saturated fused bicyclic rings which allow the introduction of an oxygen atom to help control lipophilicity or indeed to introduce lipophilicity capacity so that additional alkyl groups can be added to increase

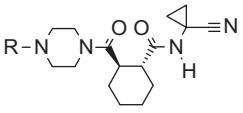
Table 2
SAR for aza-benzothiazoles

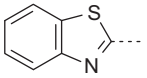
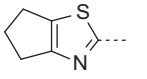
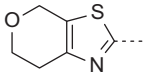
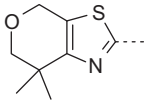


Compd	R—group	Cat K pIC_{50}[a]	Cat L pIC_{50}[a]	Cat S pIC_{50}[a]	Cat B pIC_{50}[a]	Log$D_{7.4}$	Mol Wt. (Da)	hERG inhib. pIC_{50}	Aqueous solubility pH 7.4 (μM)	HLM[c] (μL/min/mg)	RHC[d] (μL/min/10^6 cells)
4		8.0	<5.1	6.2	5.8	2.6	437.6	5.1	1000	<2.0	64
22		6.5[b]	<4.0[b]	<5.0[b]	4.6[b]	—	438.6	<4.0	—	<2.0	—
23		7.5	<4.0	<5.7	5.4	1.6	438.6	<4.0	>1600	11	<2.0
24		7.8	<4.0	<5.6	5.5	1.7	438.6	<4.0	>1500	4.2	5.2
25		7.3	<4.0	5.8	5.4	1.4	438.6	<4.0	>2000	<2.0	<2.0

[a] Binding affinity Cat K, L, S, B versus FRET substrate, mean of greater than $n = 4$ tests, unless where stated.
[b] Mean of $n = 2$ tests.
[c] In vitro rat liver microsomal turnover mean of at least $n = 2$ tests (μL/min/mg).
[d] In vitro rat liver hepatic clearance (RHC) turnover mean of at least $n = 2$ tests (μL/min/10^6 cells).

Table 3
SAR for partially saturated benzothiazoles



Compd	R—group	Cat K pIC_{50}[a]	Cat L pIC_{50}[a]	Cat S pIC_{50}[a]	Cat B pIC_{50}[a]	Log$D_{7.4}$	Mol Wt. (Da)	hERG inhib. pIC_{50}	Aqueous solubility pH 7.4 (μM)	HLM[c] (μL/min/mg)	RHC[d] (μL/min/10^6 cells)
4		8.0	<5.1	6.2	5.8	2.6	437.6	5.1	1000	<2.0	64
26		7.9[b]	<4.3[b]	5.5[b]	5.7[b]	>3.0	427.6	—	—	11	160
27		7.6	<4.0	5.6	5.5	1.4	443.6	<4.0	>3600	<2.4	<3.0
28		7.9	<4.0	<5.6	5.6	2.4	471.6	<4.0	1700	33	23

[a] Binding affinity Cat K, L, S, B versus FRET substrate, mean of greater than $n = 4$ tests, unless where stated.
[b] Mean of $n = 2$ tests.
[c] In vitro rat liver microsomal turnover mean of at least $n = 2$ tests (μL/min/mg).
[d] In vitro rat liver hepatic clearance (RHC) turnover mean of at least $n = 2$ tests (μL/min/10^6 cells).

inhibition by either protein contact or hydrophobicity. Firstly the lipophilic compound **26** did not increase potency, showing that hydrophobicity alone was not a suitable approach to increasing inhibition. This was not surprising given the openness and exposure of the S3 groove to solvent. However, the 6,7-dihydro-4*H*-pyrano[4,3-*d*][1,3]thiazol-2-yl group of **27** proved to be a good isostere, with LLE of 6.1 driven by Cat K inhibition of $pIC_{50} = 7.6$ for a Log$D_{7.4}$ of 1.4, but with the benefit of greatly improved in vitro stability. Encouraged by this we designed **28**, using the combined SAR of **27** with **14**, the most active C4-alkyl, yielding an additive effect on Cat K inhibition and the desired increase in hERG margin (9000-fold). Sadly human in vitro stability was increased at least 10-fold compared to **4** and **27**, although rat hepatic clearance was improved relative to **4**, otherwise this would have been the desired isostere.

Table 4 summarises rodent in vivo and in vitro pharmacokinetics for **20**, **24** and **27**, compared to the lead **4**. All compounds are comparable with respect to protein binding in rodent and human

Table 4
Serum protein binding and pharmacokinetic data

Compd	Species	Protein binding[a] (% free)	In vitro hepatocyte clint[b] (μL/min/10^6 cells)	Clp (mL/min/kg)	V_{dss} (L/kg)	iv half life (h)	Bioavailability (%)
4	Rat[c]	17	64 ± 1.3	47 ± 5.1	1.5 ± 0.3	0.8 ± 0.2	20 ± 9.0
	Dog[d]	16	5.6 ± 0.2	13 ± 4.9	1.5 ± 0.5	1.4 ± 0.2	78 ± 21
	Human	17	<2.0 (n = 4)				
20	Rat[c]	43	7.1 ± 6.5	23[e]	0.54	1.4	3.3[e]
	Human	42	<2.0 (n = 2)				
24	Rat[c]	64	5.2 (n = 1)	19[e]	0.8	0.63	17[e]
	Human	35	<3.0 (n = 2)				
27	Rat[c]	53	<3.0 (n = 4)	32 ± 8.1	1.3 ± 0.3	2.0 ± 0.3	33 ± 11
	Human	49	<2.0 (n = 5)				

[a] All results are a mean at least n = 2 experiments.
[b] (·)—number of repeat experiments were result was below limit of detection.
[c] Alderley Park Han Wistar male rats—dosed to fed animals, po 2.0 mg/kg as a suspension in 5% DMSO/95% HPMC/Tween, iv dosed as solution in 40% DMA/Water at 2.0 mg/kg.
[d] Beagle dog—dosed to fasted animals, po 1.0 mg/kg as a suspension in 5% DMSO/95% HPMC/Tween, iv dosed as solution in 10% DMSO/90% Sorensons at 1.0 mg/kg. All pharmacokinetic experiments reported were single dosed compounds in at least two experiments involving two animals in each, unless otherwise stated.
[e] From a single experiment with two animals.

plasma, and are highly unbound. Scaling in vitro rat hepatic clearance to whole liver predicted the measured in vivo clearance of **4** but under predicts for the rest of the compounds. We suspect that clearance was higher for these compounds due to renal elimination. Disappointingly, sulphone **20** has low bio-availability, which given the low clearance and polarity of the compound must be due to poor passive absorption (not indicated by CACO2 or MDCK assays). All four compounds had low human hepatic in vitro clearance suggesting HLM was predicting well for the series. The 5-azabenzothaizole **24** was orally bio-available and similar to **4** with lower clearance and overall represented the best isostere found from these studies. Finally **4** and **27** were tested in a low through-put in vitro primary human cell osteoclast 'pit' resorption assay and were found to have similar potency of pIC_{50} 7.2 and 6.9, respectively. This technically demanding assay measured the ability of compounds to stop the osteoclast cells dissolving cartilage and bone, thereby stopping the observed formation of 'pits' in the bone sample (see Supplementary data). Results were only considered qualitatively as no relationship was found to PD end points in the dog CTX-I model (data not shown).

In conclusion our studies of benzothiazole **4** found this potent inhibitor of Cat K had good dog PK and as such was used to study in vivo target engagement and pharmacodynamic effects successfully. We strove to improve on this start point by increasing the margin to hERG binding by the combined tactics of reducing lipophilicity and structure based design. Whilst clear improvements were made, we failed to maintain or increase Cat K inhibition, or indeed selectivity for other cathepsins, which remained fairly flat across the compounds in this study. However substituted benzothiazole **20**, and isosteres **21**, **24** and **27** were all within 4 fold of Cat K inhibition, below the assay limit for hERG binding and maintained similar rodent PK properties when compared to **4**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.012.

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