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Dioxo-triazines as a novel series of cathepsin K inhibitors

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ABSTRACT

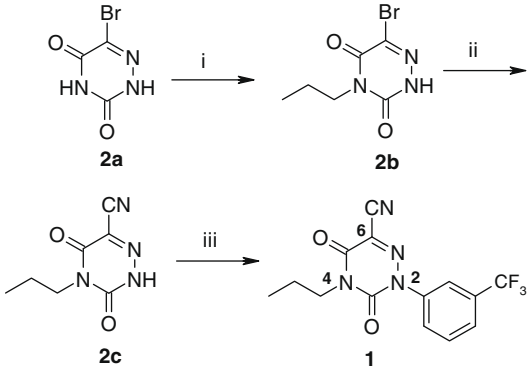
A novel dioxo-triazine series of cathepsin K inhibitors was identified from HTS. A rapid exploratory programme led to the discovery of potent and selective cathepsin K inhibitors, typified by compound **24** which displayed IC_{50} values of 17 nM against catK and >10,000 nM in catL, catB and catS assays.

Osteoporosis is a systemic, degenerative skeletal condition characterised by low bone mass and micro architectural deterioration of bone, leading to reduced bone strength.[1] It most commonly affects postmenopausal women, but also occurs in premenopausal women, and in men, often being induced by a variety of secondary causes. The resultant low bone mineral density (BMD) makes osteoporotic patients prone to fractures, which can lead to serious complications and fatality. The abundant and selective expression of cathepsin K in osteoclasts, cells involved in bone resorption, indicates its potentially critical role in bone remodelling.[2] Indeed, a number of cathepsin K mutations have been found to be associated with pycnodysostosis, a recessive bone sclerosing disorder characterised by increased bone density (osteopetrosis), dwarfism and skeletal abnormalities. Cathepsin K knockout mice display similar skeletal abnormalities, including osteopetrosis, further corroborating the key role of cathepsin K in bone remodelling.[3] Cathepsin K is a lysosomal cysteine protease from the papain family which contains a highly conserved catalytic triad Cys25, His159 and Asn175 within its active site (papain numbering).

Eleven members of this family are known to be expressed in the human genome, of which cathepsins L, S and V are most closely related to cathepsin K.[4] Development of selective cathepsin K inhibitors for treatment of osteoporosis attracted considerable attention in recent years.[5] Such compounds displayed ex vivo efficacy in osteoclast-based assays of bone resorption and in vivo efficacy in rodent[6] and primate models of postmenopausal osteoporosis[7] and most recently in patients within clinical settings.[8]

High throughput screening of the corporate compound file against cathepsin K led to the identification of 6-cyano-2-(3′-trifluoromethyl-phenyl)-3,5-dioxo-1,2,4-triazine **1**. Despite **1** being a singleton hit with poor catK potency (IC_{50} = 2000 nM) and low aqueous solubility (<1 mg/L), this was an interesting discovery since at the time only peptidic catK inhibitors were known in the literature.[9] Hence, an exploratory programme was instigated to evaluate progressability of this chemotype.

The synthesis of the dioxo-triazine **1** is described in Scheme 1.



Scheme 1. Reagents and conditions: (i) $KHCO_3$, *n*-PrI, DMSO, rt, 1 h; (ii) tetramethyl urea, CuCN, 145 °C, 3 h; (iii) $Cu(OAc)_2$, 3-$CF_3PhB(OH)_2$, CH_2Cl_2, rt, 18 h.

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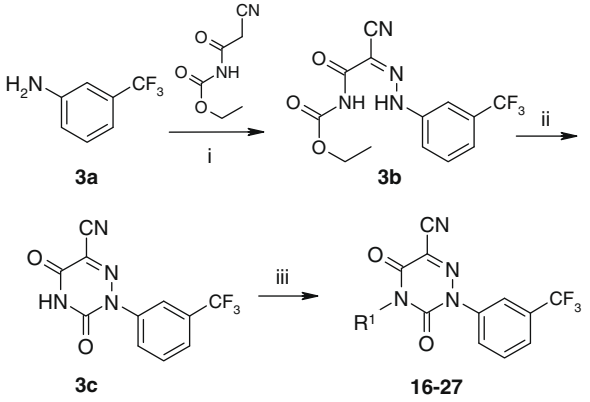
Selective N4-propylation of the 6-bromo-triazine-3,5-dione **2a** to obtain corresponding 4-propyl-triazinedione **2b** was achieved using *n*-propyl iodide and potassium bicarbonate at room temperature in DMSO. Bromo displacement with copper (I) cyanide in tetramethyl urea provided 6-cyano-4-propyl-triazinedione **2c**. Compound **1** was obtained by copper (II) acetate promoted N-arylation of **2c** with the appropriate arylboronic acid. The same synthetic route was employed to prepare aryl derivatives of **1** (**4**–**11**), whereas the aliphatic analogues **12**–**15** were obtained by alkylation of intermediate **2c** with the corresponding alkyl bromide ($KHCO_3$, DMSO, 60 °C, 48 h).

The SAR around the N4 substituent of dioxo-triazine **1** was explored using the synthetic route outlined in Scheme 2.

Intermediate **3c** was synthesized in two high-yielding steps following a literature method.[10] Alkylation using sodium hydride as a base produced derivatives **16–27** (**31** was prepared by the same route starting from 3-isopropylaniline).

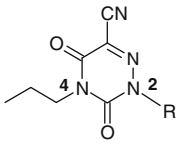
SAR exploration around the aryl moiety of **1** showed that the substituent in position 3 is critical for cathepsin K potency. For example, both CF_3 positional and deletion analogues (**4** and **5**, respectively) were inactive, whereas methyl derivative **6** showed catK potency similar to **1** (Table 1). Interestingly, the slightly larger iso-propyl **7** and *tert*-butyl **8** analogues displayed considerably improved potency (IC_{50} = 72 nM and 288 nM). Further increase in the size of the substituent in this position was not tolerated, for example, 3-biphenyl analogue **9** was inactive in catK assay. Similarly, replacement of the CF_3 group with more polar groups such as the methoxy in **10**, and hydroxymethyl in **11**, proved detrimental for catK potency. Substitution in position 2 of the aromatic ring generally resulted in loss of potency (data not shown). Considering the relatively tight SAR around the phenyl ring, it was interesting to find that replacement with a saturated ring system is tolerated (**12**, IC_{50} = 870 nM). Enlargement of the aliphatic ring proved beneficial, for example, cyclohexyl analogue **13** showed catK an IC_{50} 197 nM against catK. Further homologation of the ring yielded one of the most potent inhibitors in the series, cycloheptyl analogue **14** (IC_{50} = 38 nM). By comparison, insertion of a methylene linker between the two rings yielding **15**, was not optimal (IC_{50} = 1100 nM).

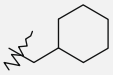
At this point we turned our attention to the propyl group of **1**. The chain length variations in **16**–**18** demonstrated that the propyl group is optimal for this region (Table 2). Interestingly, a larger cyclopentyl group was tolerated (**19**, IC_{50} = 2000 nM), whereas the corresponding phenyl analogue **20** showed a sixfold loss in potency compared to **1**. Although the benzyl analogue **21** showed a slight improvement



Scheme 2. Reagents and conditions: (i) $NaNO_2$, HCl, 2 h; (ii) xylene, 140 °C, 1 h; (iii) NaH, R^1Br, KI, DMSO, 70 °C, 1 h.

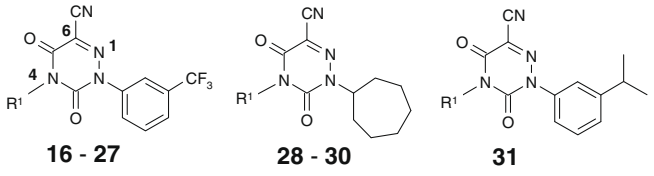
Table 1
Effect of N2 substitution on Cat K potency

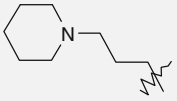
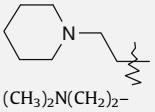
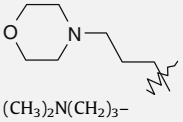
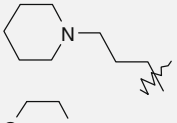
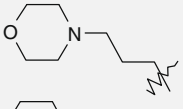
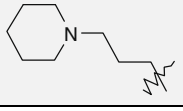


Compound	R	Cathepsin K inhibition IC_{50}[a] (nM)
1	3-CF_3-phenyl-	2000
4	4-CF_3-phenyl-	>10,000
5	Phenyl-	>10,000
6	3-Methyl-phenyl-	3500
7	3-*iso*-Propyl-phenyl-	72
8	3-*tert*-Butyl-phenyl-	288
9	3-Biphenyl-	>10,000
10	3-Methoxy-phenyl-	>10,000
11	3-Hydroxymethyl-phenyl-	>10,000
12	Cyclopentyl-	870
13	Cyclohexyl-	197
14	Cycloheptyl-	38
15		1100

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

Table 2
Effect of N4 substitution on Cat K potency



Compound	R^1	Cathepsin K inhibition IC_{50}[a] (nM)
16	$CH_3(CH_2)_3$–	7400
17	CH_3CH_2–	2500
18	CH_3–	4780
19	Cyclopentyl–	2000
20	Phenyl–	12,000
21	$PhCH_2$–	1000
22	$Ph(CH_2)_2$–	4460
23	$(CH_3)_2N(CH_2)_3$–	100
24		17
25		182
26	$(CH_3)_2N(CH_2)_2$–	810
27		112
28	$(CH_3)_2N(CH_2)_3$–	110
29		100
30		500
31		38

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

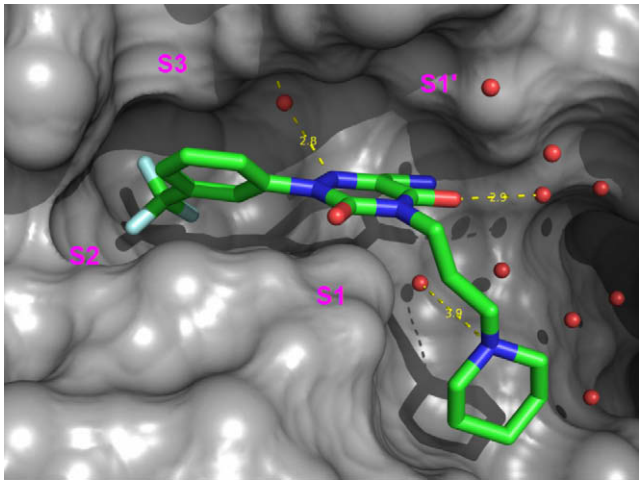


Figure 1. Co-crystal structure of dioxo-triazine **24** with cathepsin K (2 Å resolution; PDB entry 3KWB). The 3-CF_3-phenyl group is bound to the hydrophobic S2 pocket, whereas the propyl-pyperidine interacts with the enzyme prime side largely exposed to solvent. The core N1 atom forms a hydrogen bond with the conserved water in the S1 pocket (key interactions with water molecules are indicated with dashed yellow lines).

(IC_{50} = 1000 nM), further extension of the linker proved not to be beneficial for cathepsin K potency (**22**, IC_{50} = 4460 nM). Most gratifyingly, incorporation of dimethylamine group led to a 20-fold increase in potency (**23**, IC_{50} = 100 nM). Another fivefold improvement was achieved by replacing the dimethylamine with a piperidine group in **24** (IC_{50} = 17 nM). The *n*-propyl spacer was found to be optimal, as demonstrated by around 8–10-fold loss in potency of ethyl-linked analogues **25** and **26**. More polar amines such as morpholine **27** displayed uniformly lower potency in comparison to **24**.

Interestingly, combinations of the best R and R^1 groups were not additive. For example, in contrast to the profound effect observed in the 3-CF_3-phenyl series, incorporation of an amine group into cycloheptyl **13** or 3-isopropy-phenyl **6** analogues was either deleterious (**28–30**)[11] or neutral (**31**) with respect to catK potency.

Considering the structural novelty of this chemotype we were pleased to be able to obtain a high resolution X-ray structure of inhibitor **24** bound in the active site of cathepsin K (Fig. 1). The structure showed that a covalent thioamidate bond is formed between the nitrile and Cys25, similarly to previously reported aliphatic nitrile inhibitors (PDB structure 2F7D). C5 Carbonyl group of the dioxo-triazine core forms a hydrogen bond with an extensive water network in the prime side, whilst the other carbonyl moiety is exposed to solvent. A conserved water molecule in the S1 pocket forms a bridge between the NH of Gly 66 and a N1 atom of the dioxo-triazine core. The aryl ring binds into the unprime side of the active site, with the CF_3 group deeply buried into the hydrophobic S2 pocket, formed by the side chains of Tyr67, Met68, Ala134, Ala163 and Leu209 (papain numbering). The S2 pocket, the only true binding pocket within a relatively shallow active site of cathepsin K, is the primary determinant of enzyme specificity which favours small hydrophobic groups.[5] This could rationalise the observed preference for small hydrophobic aryl or cyclic aliphatic rings in N2 position of the dioxo-triazine core (Table 1). The propyl-piperidine moiety binds in the prime side, and makes a number of hydrophobic contacts in the S2′/S3′, as well as an interaction with a conserved water in the S1′ pocket. The piperidine amino group is at 3.9 Å distance to a conserved water in S1′ pocket, indicating possibility of a weak interaction.

Inhibitors in this series were generally found highly selective over closely related anti targets such as cathepsins B and L, as well as the less critical cathepsin S. For example, compounds **24**–**26** were shown inactive in catB, catL and catS assays at concentrations of up to 10 μM. However, poor chemical and plasma stability (human and rat $t_{1/2}$ <5 min) which proved to be intrinsic to this chemotype prevented further progression of compounds in this series. LCMS studies identified the nitrile hydrolysis to corresponding primary amide as the major route of degradation in plasma.

In summary, a novel non-peptidic dioxo-triazine series of cathepsin K inhibitors was identified from HTS. Potent and highly selective inhibitors were identified, that is, compound **24** displayed IC_{50} values of 17 nM in catK and >10,000 nM in catL, catB and catS assays. Intrinsically poor chemical and plasma stability precluded further progression of this series. However, the discovery of new S2 pocket binding groups as well as the SAR and biostructural information generated around this chemotype proved invaluable in the design of novel cathepsin K inhibitors with excellent pharamacokinetic profile, as we describe in the following article in this issue.

Acknowledgements

We thank Han Kok and Wim Koot for running fermentors to produce CatK protein.

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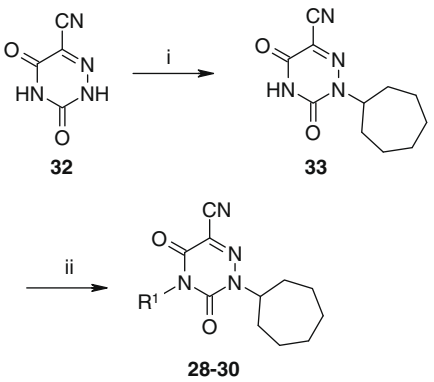
11. Synthesis of the cycloheptyl derivatives **28**–**30** is shown below:



Reagents and conditions: (i) $KHCO_3$, c-heptylBr, KI, DMSO, 70 °C, 2.5 h; (ii) NaH, R^1Br, KI, DMSO, 70 °C, 1 h.