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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4296–4299

Design and synthesis of tetracyclic nonpeptidic biaryl nitrile inhibitors of cathepsin K

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Received 6 March 2006; revised 17 May 2006; accepted 18 May 2006
Available online 5 June 2006

Abstract—The synthesis and biological profile of a novel series of potent and selective inhibitors of cysteine protease cathepsin K (Cat K) are described. Pharmacokinetic evaluation of **12** indicated that some members of this series could be suitable candidates to develop new orally active therapeutic agents for the treatment of osteoporosis.

Osteoporosis, defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, occurs as an imbalance between bone degradation and bone reconstruction. Cathepsin K (Cat K) is a cysteine protease of the papain superfamily that is highly and selectively expressed in osteoclasts. Osteoclasts are specialized multinuclear cells that promote bone degradation. Cat K is the proteolytic enzyme released by the osteoclasts that hydrolyzes type I collagen, the major component of bone matrix.[1] Thus, recently the use of selective Cat K inhibitors has been considered as a promising approach to treat diseases characterized by excessive bone loss such as osteoporosis.

Recently, we showed that compound **1** (Fig. 1) displayed excellent in vivo efficacy in the rhesus monkey model for inhibition of urinary collagen breakdown products associated with bone resorption.[2] This compound displayed a good PK profile in rhesus monkey and an acceptable potency in enzyme (K_i: 8 nM)[3] as well as in cellular assay (bone res. IC_{50}: 95 nM).

In this report, we describe the synthesis and biological activity of a novel series of nonpeptidic biaryl inhibitors with enhanced potency against Cat K. Compounds were tested against human cathepsins K, L, B, and S. They were also tested in an in vitro bone resorption assay that evaluates degradation of bovine bone by isolated rabbit osteoclasts.[4]

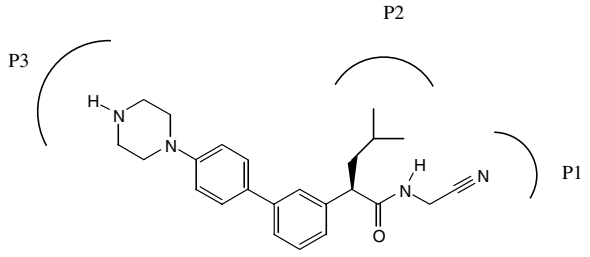

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Figure 1.

From our previous work in the nonpeptidic biaryl series,[2] as well as from the tricyclic P3 benzamide containing aminoacetonitriles[5] and 3,4-disubstituted azetidinone series,[6] we have shown that the S3 subsite of cathepsin K can accommodate fairly large moieties without adversely affecting potency and selectivity. This fact led us to conduct further SAR studies on the terminal P3 portion of the molecule.

The terminal portions of compounds **11**–**13** and **16** were obtained by following the general synthetic procedure depicted in Scheme 1. Assembly of the tri-ring system

Keywords: Cathepsin K inhibitors; Nonpeptidic biaryl nitriles.
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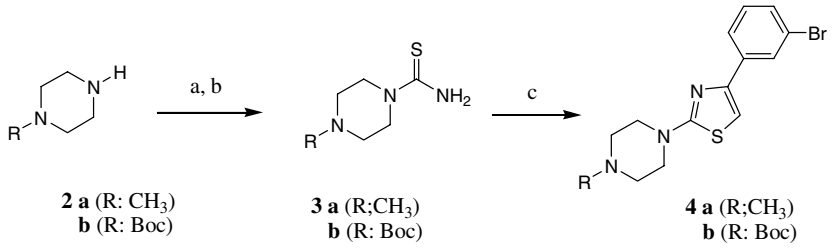
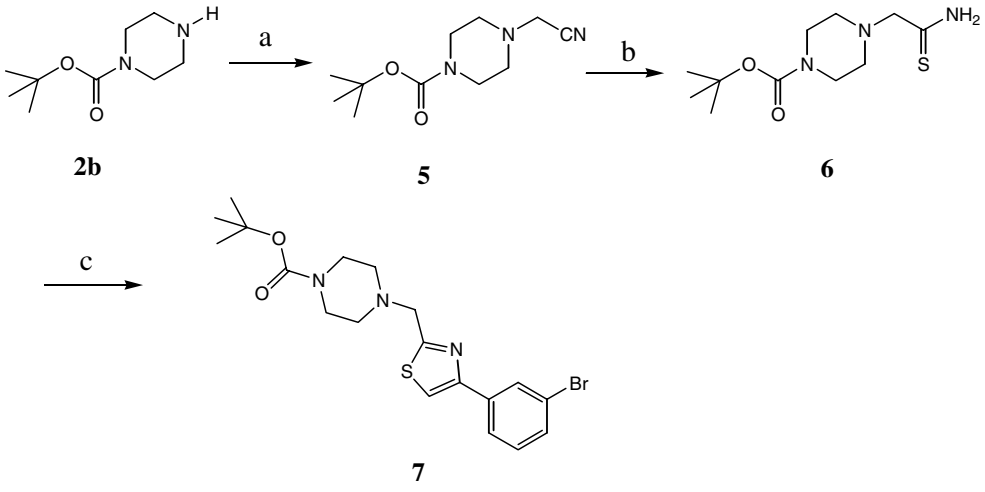
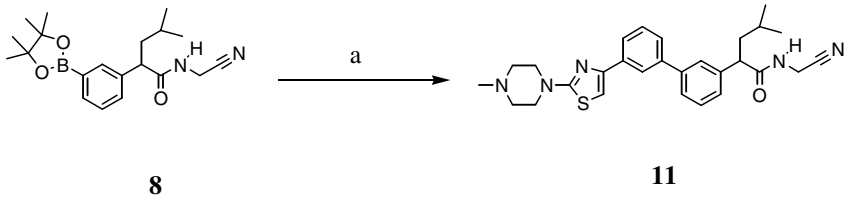
doi:10.1016/j.bmcl.2006.05.061



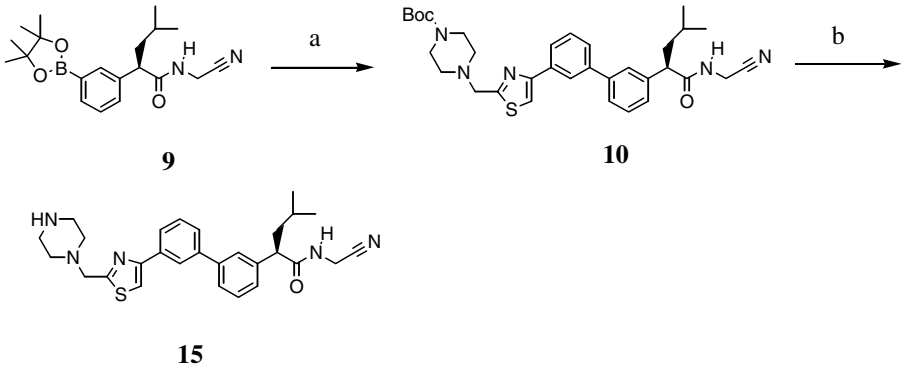
Scheme 1. Reagents and conditions: (a) $CSCl_2$, Et_3N, THF, 0 °C to rt; (b) NH_3/EtOH (2.0 M), rt, 16 h; (c) 2,3′-dibromoacetophenone, EtOH, 75 °C, 4 h.



Scheme 2. Reagents and conditions: (a) $BrCH_2CN$, K_2CO_3, CH_3CN, rt, 16 h; (b) H_2S, Et_3N–pyridine (3:1), rt, 16 h; (c) 2,3′-dibromoacetophenone, EtOH, 75 °C, 4 h.



Scheme 3. Reagents and conditions: (a) compound **4a**, $PdCl_2$(dppf), 2.0 M aq Na_2CO_3, DMF, 85 °C, 4 h.



Scheme 4. Reagents and conditions: (a) compound **7**, $PdCl_2$(dppf), 2.0 M aq Na_2CO_3, DMF, 85 °C, 4 h; (b) CH_3SO_3H, THF, rt, 16 h.

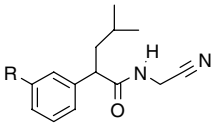
(**4a/b**–**7**) was accomplished by heating the corresponding thiourea or thiamide (**3a**,**b** or **6**) with 2,3′-dibromoacetophenone (Schemes 1 and 2) in ethanol for 4 h (Schemes 3 and 4). The syntheses of the key intermediates **8** and **9** were performed using the procedures described by Robichaud et al.[2] Suzuki cross-coupling

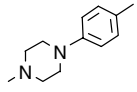
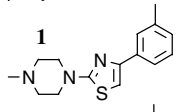
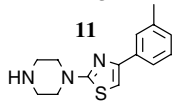
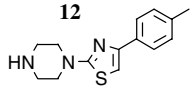
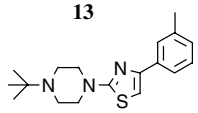
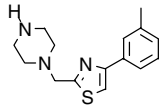
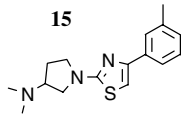
between the pinacoldiborane **8** and **9** with the corresponding aryl bromide intermediate (**4a/b**–**7**) afforded the tetracyclic final product, or a boc-protected intermediate (**10**) which was subsequently deprotected with methanesulfonic acid in THF at room temperature (Scheme 4).

Knowing that the introduction of a thiazole moiety boosted potency in the benzamide series,[5] we decided to insert this group between the distal phenyl group and the piperazine of our lead structure **1**. As shown in Table 1, the introduction of a thiazole ring clearly increased the selectivity against Cat B and S. When the thiazole–piperazine group was placed at the *meta*-position of the distal phenyl, the modification yielded a more potent Cat K inhibitor (**12**, K_i: 0.8 nM) despite the fact that it was an enantiomeric mixture. Docked structure of compound (*R*)-**12** into in-house Cat K protein showed that the proximal *meta*-substituted phenyl ring orients the P3 residue deep into the S3 pocket of the enzyme, acting as a proper surrogate for the amide bond present in other series[5,6] (Fig. 2). This binding mode was found to be comparable to the binding mode of compound **17** to Cat K established by X-ray structure (Fig. 3).[5]

The enantiomeric mixture **12** also displayed, overall, a better PK profile in rat than compound **1**, when dosed intravenously (Table 2). Unfortunately, the increase in Cat K potency did not translate well in cell since the bone resorption assay value (IC_{50}: 349 nM) showed a more than 400-fold shift as compared to 12-fold for the lead compound. Low solubility of compounds **12** and **16** in the cellular assay medium was thought to be in part responsible for the shift between enzyme and cellular assay potencies. Other factors, such as cellular permeability and nonspecific protein binding, may also play a role, although we did not specifically quantify these in the context of the assays. When this mixture of compounds was administered orally in rat at a dose of 10 mg/kg, it had a very good bioavailability (*F*: 85%) and a terminal $t_{1/2}$ of 3.9 h (Table 3). Capping

Table 1. Inhibition of human cathepsins K, L, S and B by tetracyclic analogues



R	Stereo chem.	Cat K K_i (nM)	Cat L K_i (nM)	Cat B K_i (nM)	Cat S K_i (nM)	Bone res. IC_{50} (nM)
	*R*	8.0	4641 (×580)	3843 (×481)	1695 (×212)	95
1 	*R*,*S*	1.3	390 (×300)	22,000 (×16,923)	1800 (×1384)	59
11 	*R*,*S*	0.8	190 (×237)	18,000 (×22,500)	5600 (×7000)	349
12 	*R*,*S*	3.5	2500 (×714)	12,000 (×3428)	9500 (×2714)	12
13 	*R*	1.3	360 (×276)	37,000 (×28,461)	5600 (×4307)	28
14 	*R*	4.8	680 (×141)	51,000 (×10,625)	7400 (×1541)	43
15 	*R*	3.0	540 (×180)	51,000 (×17,000)	3600 (×1200)	172
16						

Selectivities over Cat K are shown in brackets.[8]

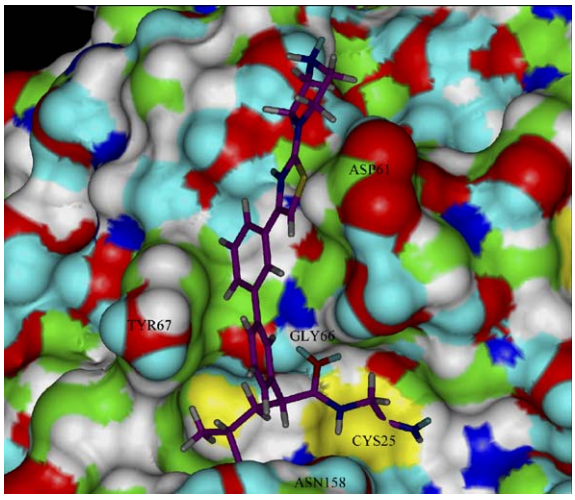
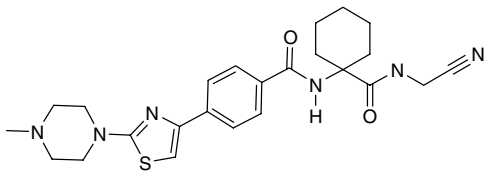


Figure 2. Modeled structure of compound (*R*)-**12** bound to Cat K.[7]



17

Figure 3.

Table 2. Rat PK parameters after iv administration at 0.5 mg/kg

Compound	V_{ss} (l/kg)	MRT (min)	CL (ml/kg/min)
1	14.8	155	98
11	1.7	54	32
12	4.3	176	25
13	12.0	106	114
14	6.6	109	60
15	9.1	140	65

Table 3. Rat PK data for compound **14**

Compound	Dose (mg/kg)	C_{max} (mM)	T_{max} (min)	AUC (uM min)	$t_{1/2}$ (h)	*F* (%)
12	10	1.1	200	560	3.9	85

the nitrogen with a methyl (**11**, K_i: 1.3 nM) yielded an enantiomeric mixture with similar potency against Cat K and higher potency in cell (bone res. IC_{50}: 59 nM), a finding that is in agreement with results found in other series.[5]

Interestingly, when the thiazole–piperazine pair was attached to the *para*-position of the distal phenyl, the resulting mixture of compounds (**13**, K_i: 3.5 nM against Cat K) turned out to be about 4-fold less potent than the *meta*-substituted analogue with a poorer overall PK profile, illustrated by its higher clearance (114 ml/kg/min, Table 2). To our surprise, the mixture **13** showed only a 3.4-fold shift between enzyme and cellular assay values.

In order to explore how far away the basic nitrogen[9] could be placed without affecting potency, we synthesized compound **15**. We have found that this one carbon homologation rendered the molecule slightly less potent against Cat K (4.8 nM), possibly due to a combination of the size and a certain loss of rigidity of the terminal portion at which the basic nitrogen is attached.

In summary, we have shown that the insertion of a thiazole moiety between the piperazine and the distal phenyl ring of the biaryl structure **1** enabled us to synthesize a series of more potent and selective Cat K inhibitors in enzyme and, many instances, more potent in bone resorption assay as well. Preliminary pharmacokinetic evaluation of the enantiomeric mixture **12** in rat demonstrated that members of this series could be suitable candidates to develop new orally active therapeutic agents for the treatment of osteoporosis.

Acknowledgments

The authors thank Dr. Paul Grothaus, Dr. Cameron Black and Mr. Leland Burrill for their critical reviews of this manuscript.

References and notes

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3. The reported Cat K IC_{50} of compound **1** in Ref. 2 is 3 nM.
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7. Docking experiment was done by using GOLD 3.0 software from Cambridge Crystallography Data Center. Water molecules were removed from the protein and hydrogen bond constrain was added between ligand and Asn^{158}.
8. Same enzyme assay conditions as described in Ref. 5.
9. The presence of a basic nitrogen at the distal position of the molecule was shown to be essential for potency and selectivity in other series. See Refs. 4–6.