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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2543–2546
Bioorganic & Medicinal Chemistry Letters

Orally bioavailable small molecule ketoamide-based inhibitors of cathepsin K

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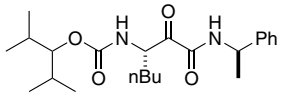
Received 30 January 2004; revised 23 February 2004; accepted 26 February 2004

Abstract—An orally available series of ketoamide-based inhibitors of cathepsin K has been identified. Starting from a potent inhibitor with poor oral bioavailability, modifications to P^1 and $P^{1'}$ elements led to enhancements in solubility and permeability. These improvements resulted in orally available cathepsin K inhibitors.

Bone resorption and bone formation are tightly coupled processes that maintain skeletal homeostasis.[1] However, when the pace of bone resorption outstrips that of bone formation, osteoporosis results, leading to decreased bone mass and increased susceptibility to fracture. Osteoclasts resorb bone by secreting acid and proteolytic enzymes that remove the mineral and matrix components of bone. Cathepsin K, a C1A family cysteine protease highly expressed in osteoclasts, is the major proteolytic enzyme responsible for degradation of bone matrix.[2] As part of a complex with glycosaminoglycans, it efficiently degrades type I collagen, the major component of bone matrix.[3] Small molecule inhibitors of cathepsin K have proven efficacious in attenuating bone resorption in animal models of osteoporosis.[4]

As part of a larger program to develop novel cathepsin K inhibitors, researchers from these laboratories recently reported the discovery of the ketoamide cathepsin K inhibitor **1** (IC_{50}=2.5 nM).[5] Although ketoamide **1** is a potent cathepsin K inhibitor that was able to attenuate bone resorption in a rat calvarial resorption assay,[6,7] it exhibited poor oral bioavailability in male Han Wistar rats ($F = 3.1\%$). This inhibitor showed reasonable permeability in the Madin–Darby Canine Kidney cell monolayer transport assay (MDCK, $P_{APP} = 57$ nm/sec),[8] but low solubility in fasted state-simulated intestinal fluid (FS-SIF, solubility=0.012 mg/mL).[9] Based on the premise that the oral bioavailability was absorption limited due to the low aqueous solubility of **1**, it was decided to vary the P^1 and/or $P^{1'}$ substituents to try to improve solubility within the series, and test whether these solubility enhancements would result in improved oral bioavailability.



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Two general routes were utilized to synthesize the α-ketoamides. The first process applied the acyl cyanophosphorane oxidative cleavage and amine coupling procedure of Wasserman and Petersen to generate the

Keywords: Cathepsin K; Ketoamide; Cysteine protease inhibitors.
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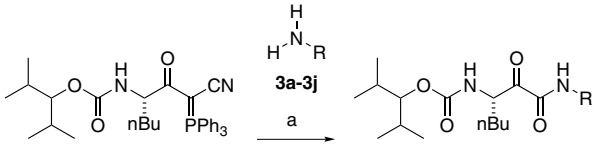
doi:10.1016/j.bmcl.2004.02.085

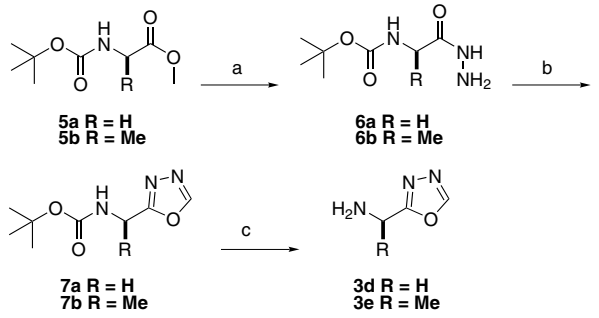


Scheme 1. (a) O_3, CH_2Cl_2, −78 °C; N_2; **3a–j**, −78 °C to rt; $AgNO_3$, THF, H_2O, 16–67%.

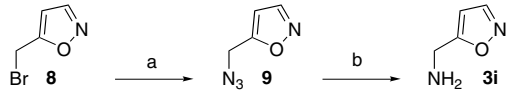
ketoamide functionality.[10] As displayed in Scheme 1, ozonolysis of the phosphorus–carbon double bond in the known phosphorane **2**[11] resulted in the in situ generation of an acyl nitrile. Displacement of cyanide by a $P^{1'}$ amine **3a–j** generated the desired ketoamides **4a–j**.

The $P^{1'}$ amines, 2-(aminomethyl)pyridine **3a**, 3-(aminomethyl)pyridine **3b**, and 4-(aminomethyl)pyridine **3c**, were commercially available, whereas the amines, 2-(aminomethyl)thiazole **3f**,[12] 5-(aminomethyl)thiazole **3g**,[13] 4-(aminomethyl)thiazole **3h**,[14] and (3*S*)-3-amino-2-piperidinone **3j**,[15] were known in the literature. The amines, 2-(aminomethyl)oxadiazole **3d** and (1*R*)-2-(1-aminoethyl)oxadiazole **3e**, were synthesized as depicted in Scheme 2. The commercially available esters **5a** and **5b** were heated at reflux with hydrazine to provide the hydrazides **6a** and **6b**.[16] Subsequent heating with triethylorthoformate at reflux afforded the oxadiazoles **7a** and **7b**,[17] which were deprotected with hydrogen chloride to give the $P^{1'}$ amines **3d** and **3e**.

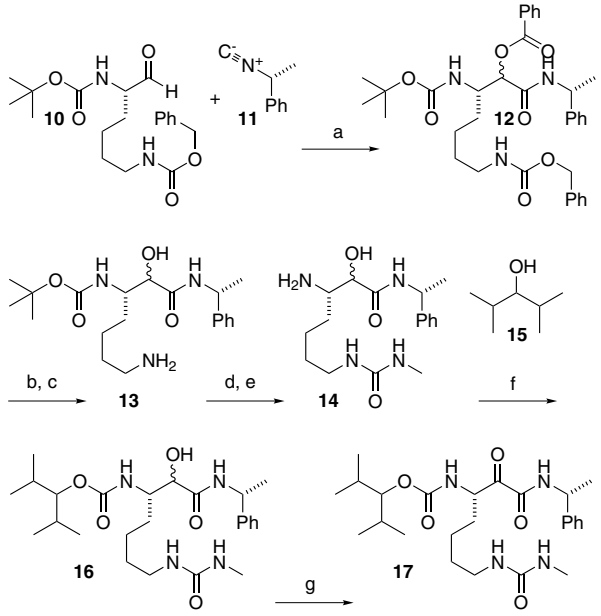
5-(Aminomethyl)isoxazole **3i** was synthesized as shown in Scheme 3. Treatment of the known bromide **8**[18] with sodium azide gave the azide **9**. Subsequent reduction of the azide **9** with triphenyl phosphine afforded the desired amine **3i**.[19]



Scheme 2. (a) H_2NNH_2, EtOH, reflux, 63–83%; (b) $HC(OEt)_3$, reflux, 24–57%; (c) HCl, EtOAc, 60–70%.



Scheme 3. (a) NaN_3, MeOH/H_2O (9:1), 44%; (b) PPh_3, THF, H_2O, 72%.

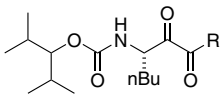


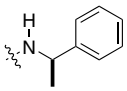
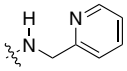
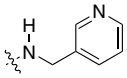
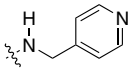
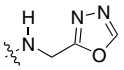
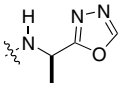
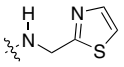
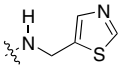
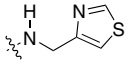
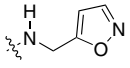
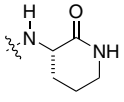
Scheme 4. (a) PhCOOH, CH_2Cl_2, 49%; (b) LiOH·H_2O, dioxane, H_2O, 99%; (c) H_2/Pd–C, EtOH, H_2O, 89%; (d) MeN=C=O, THF, 86%; (e) HCl, EtOAc, 99%; (f) **15**, 1.93 M $COCl_2$ in PhMe, THF, 0 °C to rt; **14**, *i*Pr_2NEt, MeOH, 79%; (g) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C; **16**; NEt_3, −60 °C to rt, 69%.

The Passerini reaction was exploited as a second technique to generate the required ketoamide functionality.[20] As shown in Scheme 4, the known aldehyde **10**[21] was reacted with the isonitrile **11**[5] and benzoic acid to give the α-acyloxyamides **12**. Hydrolysis of the esters **12**, followed by hydrogenation of the benzyl carbamates afforded the ϕ-amino-α-hydroxyamides **13**. Coupling of the amines **13** with methyl isocyanate, followed by acid catalyzed cleavage of the *tert*-butyl carbamates provided the β-amino-α-hydroxyamides **14**.[22] These amines **14** were then coupled to the chloroformate, generated from alcohol **15** and phosgene, to give carbamates **16**. Oxidation of alcohols **16** afforded the ketoamide **17**.

As shown in Table 1, replacement of the phenyl $P^{1'}$ moiety of inhibitor **1** (IC_{50}= 2.5 nM) by heterocycles resulted in a loss in inhibitory activity. The pyridine $P^{1'}$ analogs **4a–c**, bearing a positive charge at lysosomal pH, were approximately an order of magnitude less potent than the starting ketoamide **1**. Whereas the more neutral oxadiazole **4d**, the thiazoles **4f–h**, and the isoxazole **4i** also apparently suffered slight reductions in activity, the lactam **4j** (IC_{50}=1.0 nM) maintained similar inhibitory potency to ketoamide **1**. Since cathepsins contain a conserved tryptophan (^{184}Trp in cathepsin K) in their active site,[23] lactam **4j** could potentially form a favorable hydrogen bond to the indole NH of ^{184}Trp, rationalizing its enhanced potency relative to the other heterocyclic analogs tested. In the absence of an interaction with ^{184}Trp, the $S^{1'}$ subsite seems to prefer the hydrophobic phenyl group to more hydrophilic heterocycles.

Table 1. Inhibition of human cathepsin K by $P^{1'}$ analogs



#	R	IC_{50} nM[a]
1		2.5
4a		44
4b		23
4c		30
4d		19
4e		59
4f		18
4g		14
4h		13
4i		14
4j		1.0

[a] Inhibition of recombinant human cathepsin K activity in a fluorescence assay using 10 μM Cbz-Phe-Arg-AMC as substrate in 100 mM NaOAc, 10 mM DTT, 120 mM NaCl, pH = 5.5. The IC_{50} values are the mean of two or three inhibition assays, individual data points in each experiment were within a 3-fold range of each other.

Researchers from these laboratories had previously disclosed a series of aldehyde cathepsin K inhibitors.[24] Structure–activity relationships had revealed that the P^1 norleucine could be replaced by ornithine or lysine derivatives without attenuating activity. The lysine derived ketoamide **17** (IC_{50}=4.3 nM) shows that this substitution is also permissible in the ketoamide-based inhibitors. This is not surprising, since an X-ray crystal structure of one of the aldehyde inhibitors revealed that the S^1 subsite of cathepsin K is a shallow groove.[24] Thus, long P^1 residues should be completely exposed to solvent at their termini.

Although these ketoamides suffered attenuation in cathepsin K inhibitory activity, they were still suitable to test the hypothesis that increased aqueous solubility would result in improved oral bioavailability. As shown in Table 2, these inhibitors did indeed exhibit ~2–30-fold improvements in solubility in fasted state-simulated intestinal fluid at pH = 6.8. The calculated octanol/water partition coefficients (*c* log *P*) for the nonionized $P^{1'}$ analogs are also included in Table 2 for comparison with the measured solubilities. The decrease in *c* log *P* from 7.3 to 3.4–5.5 is further evidence of the increased hydrophilicity of these heterocyclic $P^{1'}$ analogs. The apparent MDCK permeability coefficients also improved. However, these small enhancements resulted in little increase in oral bioavailabilities for the pyridine $P^{1'}$ analogs **4a** ($F = 10\%$) and **4c** ($F = 5.2\%$). The P^1 lysine derivative **17** showed a small apparent improvement in oral bioavailability ($F = 10\%$). Notably, the oxazole analog **4d** ($F = 16\%$) and the lactam derivative **4j** ($F = 20\%$) showed significant increases in bioavailability. Furthermore, both the thiazole compound **4g** ($F = 55\%$) and the isoxazole **4i** ($F = 32\%$) exhibited an order of magnitude improvement in bioavailabilities.

The decrease in hydrophobicity of the analogs resulted in an apparent decrease in the steady state volume of distribution (V_{SS} = 90–1600 mL/kg) compared to the starting ketoamide **1** (V_{SS} = 1900 mL/kg). Also, the total clearance for most of the inhibitors increased (analogs **4d**, **4g**, **4i**, **4j**, **17** C_l = 24–33 mL/min/kg), indicating increased rates of elimination. These combined changes resulted in attenuation of the terminal half-lifes for most of these analogs (analogs **4a**, **4d**, **4g**, **4i** $t_{1/2}$ = 21–150 min). Furthermore, it is likely that the increased rate of elimination for these analogs, reflected in the increase in total clearance as compared to ketoamide **1**, functioned to lower the oral bioavailability via enhanced first-pass metabolism. This, at least partly, negated any gains made by improving the adsorption of compounds within the series.

In summary, this report highlights improvements in oral bioavailability of a potent series of cathepsin K inhibitors. Starting from a ketoamide cathepsin K inhibitor **1**, the P^1, and $P^{1'}$ substituents were replaced with more hydrophilic moieties. The resulting general decline in potency was compensated by overall enhancements in aqueous solubility and membrane permeability compared to the ketoamide lead **1**. In addition, these changes resulted in improvements in oral bioavailability with analogs **4g** and **4i** being 55% and 32% orally bioavailable in male Han Wistar rats, respectively. Subsequent reports will detail efforts to further improve the drug properties of these cathepsin K ketoamide-based inhibitors.

Table 2. Pharmacokinetics of combination analogs

#	MDCK[a] P_{APP}[b] (nm/sec)	*c* Log *P*	Sol. FS-SIF[c] (mg/mL)	$t_{1/2}$[d] (min)	C_l[e] (mL/min/kg)	V_{SS}[f] (mL/kg)	*F*[g] (%)
1	57	7.3	0.012	290	8.7	1900	3.1
4a	510	—	0.028	77[h]	5.2	240	10
4c	—	—	0.062	240[i]	7.1	90	5.2
4d	510	3.4	0.15	21[j]	33	980	16
4g	480	5.3	0.051	150[j]	28	1300	55
4i	410	5.0	0.063	52[j]	29	1200	32
4j	310	4.4	0.35	220[k]	44	1600	20
17	—	5.5	0.032	260[j]	24	1500	10

[a] Madin–Darby Canine Kidney cell monolayer transport assay.
[b] P_{APP} is the apparent permeability coefficient for apical to basolateral flux in nanometers per second. The error in the assay averaged 10%.
[c] FS-SIF is the equilibrium solubility in fasted state-simulated intestinal fluid @ pH = 6.8. The values are the mean of two measurements.
[d] $t_{1/2}$ is the iv terminal half-life dosed as a solution in male Han Wistar rats. All in vivo pharmacokinetic values are the mean of two experiments.
[e] C_l is the total clearance.
[f] V_{SS} is the steady state volume of distribution.
[g] *F* is the oral bioavailability.
[h] Dosed as a solution in sulfobutylether-7-cyclodextrin/methane sulfonic acid, pH = 3.0.
[i] Dosed as a solution in sulfobutylether-7-cyclodextrin/citrate, pH = 3.3.
[j] Dosed as a solution in 40% hydroxypropyl-β-cyclodextrin, pH = 8.9.
[k] Dosed as a solution in 15% solutol/citrate, pH = 3.5.

Acknowledgement

The authors would like to thank Robert B. McFadyen for critical reading of the manuscript.

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