Arylaminoethyl Amides as Novel Non-Covalent Cathepsin K Inhibitors

Eva Altmann,*,† Johanne Renaud,† Jonathan Green,† David Farley,† Brian Cutting,‡ and Wolfgang Jahnke‡

Arthrithis & Bone Metabolism Therapeutic Area and Central Technologies, Novartis Pharma Research, CH-4002 Basel, Switzerland

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Abstract: A series of N_α-benzyloxycarbonyl- and N_α-acyl-L-leucine(2-phenylaminoethyl)amide derivatives were prepared and evaluated for their inhibitory activity against rabbit and human cysteine proteases cathepsins K, L, and S. These data indicate that N_α-acyl-α-amino acid-(arylaminoethyl)amides represent a new class of selective non-covalent inhibitors of cathepsin K. Compounds **4b**, **4e**, and **4g** exhibit high potency toward rabbit and human cathepsin K (IC_{50} < 0.006 μM) and are characterized by an excellent selectivity profile vs human cathepsins L and S.

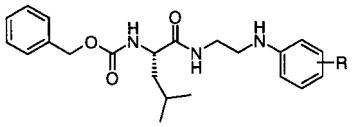
Cathepsin K is a cysteine protease of the papain superfamily that is highly and selectively expressed in osteoclasts.[1,2] Osteoclasts are highly specialized multinuclear cells that solubilize bone through acid and protease secretion. The fact that cathepsin K is the predominant cathepsin in osteoclasts[1,2] suggests that this enzyme may play an essential role in bone resorption. Cathepsin K exhibits potent collagenolytic activity against type I collagen and is involved in the degradation of other components of the extracellular bone matrix.[3,4] Cathepsin K deficient mice develop osteopetrosis,[5,6] confirming the importance of this enzyme for bone remodeling. Likewise, pycnodysostosis, a rare human disease characterized by bone abnormalities due to impaired osteoclastic bone resorption, has been linked to defects in the gene encoding cathepsin K.[7−9] Thus, selective inhibitors of cathepsin K may offer an efficacious treatment for diseases characterized by excessive bone loss such as osteoporosis.

Investigations of arylaminoethyl amides as potential cathepsin K inhibitors started with N_α-benzyloxycarbonyl-L-leucine(2-phenylaminoethyl)amide (**1a**) as a lead structure (Table 1). Compound **1a** inhibits rabbit cathepsin K with an IC_{50} of 470 nM. We were intrigued by the fact that this compound does not contain any electrophilic group that could covalently interact with the cysteine residue at the active site, in contrast to other cysteine protease inhibitors described so far.[10] We thus embarked in the preparation of various aminoethyl amide derivatives to get further insight into this series of compounds.

The synthesis of cathepsin K inhibitors related to lead structure **1a** is summarized in Schemes 1 and 2.

An efficient entry into the preparation of the 2-aminoethylanilines **3a**−**g** is the decarboxylative ring opening of oxazolidin-2-one with an aromatic amine (Scheme 1).[11] Subsequent reaction of **3** with Z-Leu-OSu furnished N_α-*Z*-protected amides **1a**−**g**.

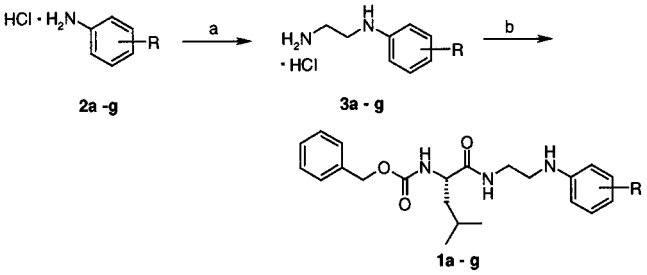
Table 1. Inhibition of Rabbit Cathepsin K



compd	R	IC_{50}[a] (μM)
1a	H	0.47
1b	3-Me	0.46
1c	4-Me	0.19
1d	3-Cl	>1
1e	4-Cl	0.35
1f	3-OMe	>1
1g	4-OMe	0.06

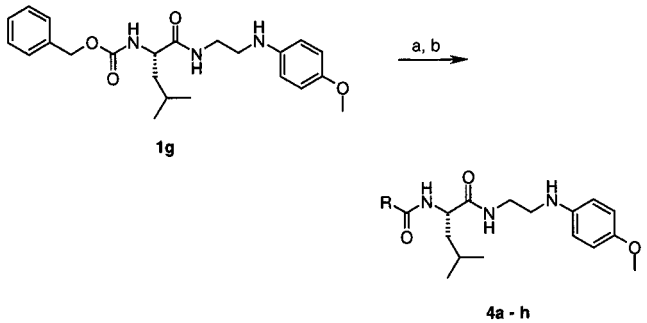
[a] Inhibition of recombinant rabbit cathepsin K in a fluorescence assay. Data are means of two experiments performed in duplicate.

Scheme 1[a]



[a] Reagents: (a) 2-oxazolidinone, 2-(2-methoxyethoxy)ethanol, 160−175 °C, 16−21 h, 34−60%; (b) *Z*-Leu-OSu, DMF, DIEA, room temp, 16 h, 41−83%. For specific structures, see Table 1.

Scheme 2[a]



[a] Reagents: (a) H_2, Pd/C (10%), MeOH, room temp, 2 h, 100%; (b) R−COOH, BOP (**4a**, **4b**, **4g**, **4h**), or HATU (**4c**−**f**), DIEA, DMF, room temp, 3−16 h, 34−75%. For specific structures, see Table 2.

In the first optimization cycle, we investigated the effects of substituents on the anilinophenyl ring on inhibitory activity. As can be seen from Table 1, the 3- and the 4-methyl derivatives **1b** and **1c** as well as the 4-chloro derivative **1e** have approximately the same potency as **1a**, while compounds **1d** and **1f**, incorporating a 3-chloro- or a 3-methoxy substituent on the phenyl ring, are only micromolar inhibitors of rabbit cathepsin K. A significant improvement in potency is realized in the 4-methoxy compound **1g**, which exhibits an IC_{50} of

* To whom correspondence should be addressed. Phone: +41 61 696 12 27. Fax: +41 61 696 60 71. E-mail: eva.altmann@pharma.novartis.com.
† Arthrithis & Bone Metabolism Therapeutic Area.
‡ Central Technologies.

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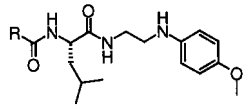
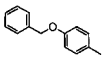
Table 2. Inhibition of Rabbit Cathepsin K



Cpd	R	IC_{50} (μM)[a]	K_i (μM)
4a		0.014	nd
4b		<0.003	0.015
4c[b]		<0.003	nd
4d		<0.003	nd
4e		<0.003	nd
4f		<0.003	nd
4g		<0.003	nd
4h		<0.003	0.011

[a] Inhibition of recombinant rabbit cathepsin K in a fluorescence assay. Data are means of two experiments performed in duplicate. [b] TFA salt.

Table 3. Selectivity Data on the Inhibition of Homologous Cathepsins

	IC_{50} (μM)		
compd	rh cath K[a]	rh capth L[b]	rh cath S[c]
4b	0.006	>10	9.5
4c	<0.003	<0.03	1.2[d]
4d	<0.003	0.05	5.7
4e	<0.003	1.5	7.2
4g	0.006	2.3	1.9
4h	0.009	>10	0.19

[a] Inhibition of recombinant rh cathepsin K. [b] Inhibition of rh cathepsin L. [c] Inhibition of rh cathepsin S. Data represent the mean of two independent experiments each performed in duplicate. [d] The reported IC_{50} for rh cath S of 1.2 μM represents the mean of four experiments carried out in duplicate (SEM = ∓0.52 μM).

64 nM and is thus 7-fold more potent as an inhibitor of rabbit cathepsin K than the lead structure **1a**.

On the basis of this latter result, we embarked on a second optimization round that involved replacement of the benzyloxycarbonyl group in **1g** by different acyl moieties. This strategy resulted in a number of highly potent cathepsin K inhibitors such as **4a**−**h**, all of which are characterized by the presence of a N-terminal para-substituted benzoic acid, a 5-substituted pyridine-2-carboxylic acid, or a phenyltriazole carboxylic acid residue **4g**,**h** (Table 2).

For a selected number of compounds, activity against recombinant human (rh) cathepsin K and selectivity against rh cathepsins L and S were evaluated. The results of these studies are summarized in Table 3. While all compounds in Table 3 are also low nanomolar

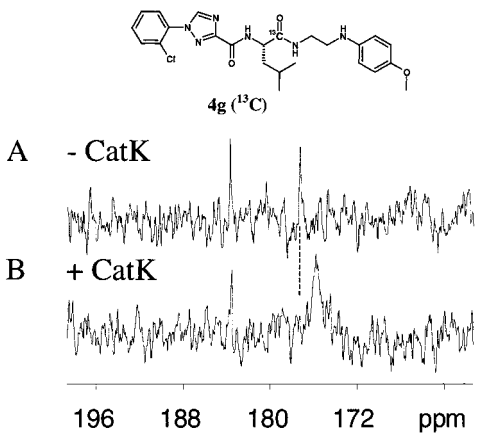


Figure 1. ^{13}C NMR spectra (150.9 MHz ^{13}C frequency) of compound **4g**(^{13}C) in the absence (top) and presence (bottom) of cathepsin K, indicating noncovalent binding of **4g**(^{13}C) to cathepsin K. The resonance at 184 ppm is an artifact of the instrument.

inhibitors of rh cathepsin K, profound differences in their selectivity profile vs rh cathepsins L and/or S are observed depending on the specific nature of the substituents present on the N_α-benzoyl- or -phenyltriazolyl moieties, respectively. In the N_α-benzoyl series, replacement of a linear alkyl chain (**4c**) by a branched alkyl group (**4d**) improves selectivity toward rh cathepsin L, while a phenoxy substituent (**4b**) dramatically improves selectivity toward rh cathepsin L and S, making **4b** a particularly selective, highly potent inhibitor of cathepsin K. Replacement of the N-terminal 4-isopropylbenzoyl moiety in **4d** with a corresponding pyridine carboxylic acid residue (**4e**) results in a 30-fold increase in selectivity toward rh cathepsin L, thus making **4e** completely selective for cathepsin K. In the N_α-phenyltriazolyl series, the ortho chlorophenyl derivative **4g** proved to be a potent and highly specific cathepsin K inhibitor, while the dichlorophenyl analogue **4h** is inactive (IC_{50} > 10 μM) against rh cathepsin L but exhibits low micromolar activity against rh cathepsin S.

The kinetics profiles of these novel cathepsin K inhibitors were investigated. The equilibrium constants for inhibition were determined using progress curves (changes in fluorescence versus time), following the hydrolysis of *Z*-Phe-Arg-AMC in the absence and presence of inhibitor. When standard assay conditions for rabbit cathepsin K were used, the K_i values for **4b** and **4h** were obtained and are given in Table 2. In addition, Lineweaver−Burk analysis of **4b** and **4h** demonstrated that these compounds are purely competitive inhibitors. Reversible binding was established by an experiment in which the inhibitors **4b** and **4h** were preincubated with cathepsin K at high concentration to ensure complete loss of enzyme activity. After dialysis and dilution into assay buffer containing substrate, the entire enzyme activity was restored.

The ^{13}C spectrum of **4g**(^{13}C) is shown in Figure 1A. Incubation of **4g**(^{13}C) with rh cathepsin K results in a broadening of the ^{13}C resonance of the labeled carbonyl carbon and a minor change in chemical shift from 177.2 to 175.6 ppm (Figure 1B). Both effects are consistent with a noncovalent binding mode of the inhibitor.

In conclusion, we have discovered a novel class of potent and selective cathepsin K inhibitors whose activity does not depend on the covalent interaction with the cysteine residue at the active site.

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Supporting Information Available: Description of the inhibition assays, details of kinetics and NMR experiments, and characterization (^{1}H NMR and HRMS) of compounds **1a**−**g**, **4a**−**h**, **and 4g**(^{13}C). This material is available free of charge via the Internet at http://pubs.acs.org.

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