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# Arylaminoethyl Amides as Inhibitors of the Cysteine Protease Cathepsin K—Investigating $P_1'$ Substituents

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**Abstract**—Modeling, synthesis and in vitro activities of a series of arylaminoethyl amide based inhibitors of the cysteine protease cathepsin K are described.


The identification of cysteine proteases which are selectively expressed in specific tissues has raised considerable interest in this class of enzymes as potential therapeutic targets.[1–4] As a result, the search for novel, potent and selective cysteine protease inhibitors has evolved into a highly competitive area of research.[5] In this context we have recently reported the discovery of arylaminoethyl amides as novel, non-covalent inhibitors[6] of cathepsin K. Cathepsin K,[7] a lysosomal cysteine protease abundant in osteoclasts, exhibits potent collagenolytic activity against type I collagen and is also involved in the degradation of other important bone proteins.[8] The crucial role cathepsin K plays in bone resorption is demonstrated by data obtained from cathepsin K null mice[9] which display an osteopetrotic phenotype in the absence of any other overt pathological signs. Mutation of the gene expressing cathepsin K in humans results in pycnodysostosis,[10] a rare bone disease characterized by increased bone density, osteosclerosis and bone fragility. These observations indicate that cathepsin K inhibition might be useful for the treatment of diseases characterized by excessive bone loss, such as osteoporosis.

As part of our ongoing efforts in the area of arylaminoethyl amide-based cathepsin K inhibitors we have now further probed the SAR around our lead structure benzyloxycarbonyl-L-leucine 4-methoxy-phenylamino-ethylamide **1a** (Fig. 1) through incorporation of extended $P_1'$ substituents and the replacement of the $P_3$ benzyloxycarbonyl group by different acyl moieties.

Molecular modeling of lead **1a** into the active site of h cathepsin K (Fig. 2) indicates aromatic–aromatic

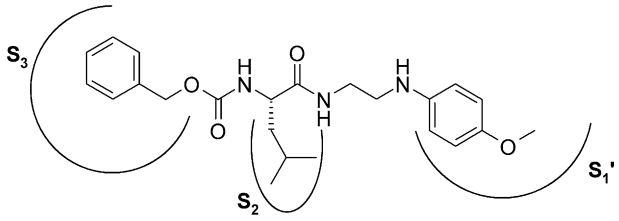


**Figure 1.** Schematic representation of the assumed binding mode of inhibitor **1a** within the active site of h cathepsin K. The Cbz moiety is assumed to bind in the $S_3$ pocket, the isobutyl group of leucine within the hydrophobic $S_2$ binding pocket and the 4-methoxy-phenyl ring extends into the $S_1'$ site.

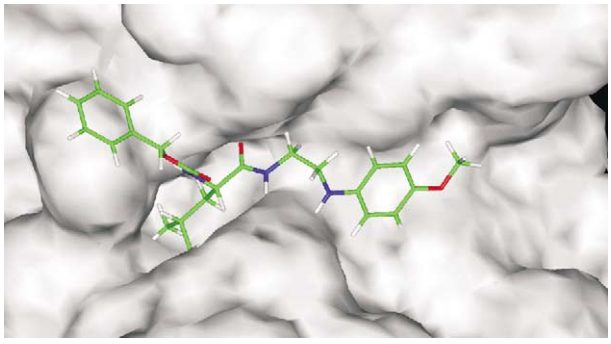

**Figure 2.** Compound **1a** docked into the h cathepsin K active site.[11]

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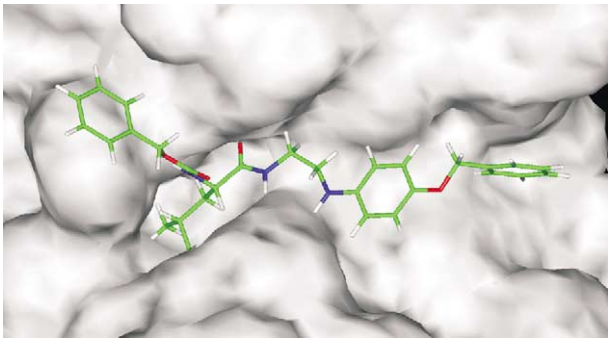


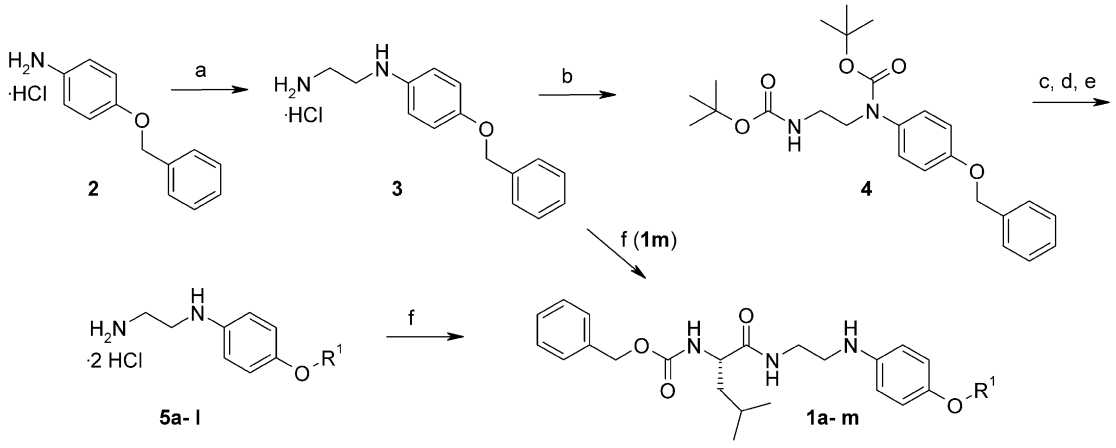
**Figure 3.** Compound **1m** docked into the h cathepsin K active site.[11]

interaction between Tyr 67 and the Cbz phenyl ring in $S_3$ and tight binding of the isobutyl side chain of leucine into the lipophilic $S_2$ pocket. Furthermore, from this model we hypothesized that modifying **1a** to extend deeper into the $S_1'$ site could improve potency and/or selectivity of this class of compounds (Fig. 3).

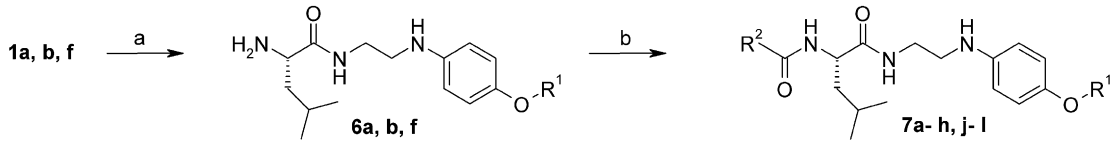
In this communication we report on the effects of these modifications on the potency and selectivity for cathepsin K inhibition.

The synthesis of these analogues proceeded through the common intermediate **4**, which was obtained through decarboxylative ring opening of 2-oxazolidinone[13] with 4-benzyloxyaniline **2** (Scheme 1). Bis-Boc protection of the resulting 2-aminoethyl-4-benzyloxy aniline **3** followed by hydrogenolytic removal of the benzyl moiety provided the free phenol, which was *O*-alkylated with different bromides in the presence of $Cs_2CO_3$ or by treatment with the appropriate alcohol, $Ph_3P$ and diethyl azodicarboxylate. Simultaneous cleavage of both Boc groups with 4N HCl in $Et_2O$ furnished diamines **5a–l**, whose reaction with Cbz-L-leucine-*N*-hydroxysuccinimide ester resulted in exclusive acylation of the primary amino group to yield the desired secondary amides **1a–l**. Compound **1m** was obtained through direct acylation of **3** with Cbz-L-leucine-*N*-hydroxysuccinimide ester, while phenoxy derivative **1n** was synthesized via Borch reduction of 4-phenoxyaniline with *t*-butyl-N-(2-oxoethyl)carbamate followed by cleavage of the Boc-group and again reaction of the resulting amine with Cbz-L-leucine-*N*-hydroxysuccinimide ester. In order to vary the acyl moiety attached to the leucine amino group, the Cbz group was removed from analogues **1a**, **b** and **f** by catalytic hydrogenation and the resulting intermediates **6a**, **b**, **f** were converted into inhibitors **7a–h**, **j–l** by BOP-mediated coupling with benzoic acids or 1H-indole-2-carboxylic acid (Scheme 2). Analogues **7i** and **7m** were prepared by reaction of amine **3** with Boc-L-leucine (EDC/HOBt) followed by treatment of the coupling products with neat formic acid and subsequent BOP-mediated coupling of the resulting amines with 4-ethyl benzoic acid and 1H-indole-2-carboxylic acid, respectively.

The effects of 4-alkoxy-substituents on the aminophenyl ring on inhibitory potency and the selectivity profile are summarized in Table 1. Compounds **1a–f**, in which the ether substituent $R^1$ was varied, exhibit similar $IC_{50}$ values in the range of 36–81 nM. The presence of a phenoxy substituent in compound **1n** leads to a 6-fold reduced potency as compared to the corresponding cyclohexyloxy derivative **1d**. In contrast, the activity of

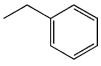
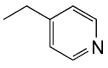
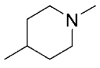
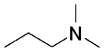
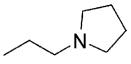
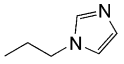



**Scheme 1.** (a) 2-oxazolidinone, 2-(2-methoxyethoxy)ethanol, 160–175 °C, 16 h, 20%; (b) $Boc_2O$, *N*-ethyl-diisopropylamine, DMF, room temp, 18 h, 84%; (c) $H_2$, Pd/C (10%), MeOH, room temp, 3 h, 98%; (d) $R^1$-Br, $Cs_2CO_3$, DMF, 16–20 h, 70–98% (**5a–c**, **e**, **j–l**) or $R^1$-OH, $Ph_3P$, DEAD, THF, 5–6 h, 52–68% (**5d**,**f–i**); (e) 4*N* HCl in $Et_2O$, room temp, 2 h, 92–98%; (f) Cbz-Leu-OSu, N-ethyl-diisopropylamine, DMF, room temp, 16 h, 59–91%. For identities of substitutents $R^1$ cf. Table 1.




**Scheme 2.** (a) $H_2$, Pd/C (10%), MeOH, room temp, 1 h, 93–98%; (b) $R^2$-COOH, benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP), *N*-ethyl-diisopropylamine, DMF, room temp, 63–95%.

**Table 1.** Inhibition of rh cathepsins K, L and S catalytic activity—Variation of the alkoxy substituent

| Compd | $R^1$ | h Cath K $IC_{50}$ (μM)[a] | h Cath L $IC_{50}$ (μM)[b] | h Cath S $IC_{50}$ (μM)[c] |
|---|---|---|---|---|
| **1a** |  | 0.070 | 4.0 | 4.0 |
| **1b** |  | 0.036 | >10 (41%) | 1.2 |
| **1c** |  | 0.081 | 6.0 | 4.4 |
| **1d** |  | 0.043 | 10 | 1.2 |
| **1e** |  | 0.069 | >10 (40%) | 2.8 |
| **1f** |  | 0.049 | >10 (43%) | 1.1 |
| **1m** |  | 0.054 | >10 (31%) | 9.5 |
| **1n** |  | 0.280 | >10 (40%) | 5.8 |
| **1g** |  | 0.152 | 10 | 3.4 |
| **1h** |  | 0.190 | 10 | 4.6 |
| **1i** |  | >1.0 (43%) | 10 | 4.6 |
| **1j** |  | >1.0 (27%) | >10 (29%) | >10 (34%) |
| **1k** |  | >1.0 (40%) | >10 (43%) | 9.5 |
| **1l** |  | 0.855 | >10 (45%) | 5.7 |

[a]Inhibition of recombinant human (rh) cathepsin K activity in a fluoresence assay using 48 μM Cbz-Phe-Arg-AMC as substrate in 100 mM $NaH_2PO_4$, 1 mM EDTA, 20 μM Tween 80, 2 mM DTT, pH 7.
[b]Inhibition of rh cathepsin L activity using 3 μM Cbz-Phe-Arg-AMC as substrate in 100 mM NaOAc, 1 mM EDTA, 0.005% Brig 35, 1 mM DTT, pH 5.5.
[c]Inhibition of rh cathepsin S activity using 11 μM Cbz-Leu-Leu-Arg-AMC as substrate in 100 mM NaOAc, 1 mM EDTA, 0.01% Triton X-100, 1mM DTT, pH 5.5. Data represent mean of 2 experiments performed in duplicate, individual data points in each experiment were within a 3-fold range with each other.

benzyloxy-containing analogue **1m** ($IC_{50}$ for cathepsin K of 54 nM) is similar to that of cyclopentylmethyloxy and cyclohexyloxy derivatives **1f** and **1d**. These data show clearly that extension into the $S_1'$ site with lipophilic substituents does not improve the potency of our inhibitors. However, compounds with larger alkoxy groups display high selectivity for cathepsin K over cathepsin L[14] while selectivity against cathepsin S[14] is not affected. We identified **1m** as exhibiting the best selectivity profile of all Cbz-derivatives studied (>400 fold for cathepsin K over cathepsins L and 176–fold for cathepsin K over S).

Incorporation of a heteroatom into the $P_1'$ subunit, such as the replacement of the benzyloxy- by a 4-pyridinylmethoxy substituent (**1m**→**1g**) and the substitution of a 4-tetrahydro-pyranyloxy moiety for the cyclohexyloxy group (**1d**→**1h**) led to slightly reduced

**Table 2.** Inhibition of rh cathepsins K, L and S catalytic activity—Variations in $P_3/P_1'$

| Compd | $R^2$ | $R^1$ | h Cath K[a] $IC_{50}$ (μM) | h Cath L[b] $IC_{50}$ (μM) | h Cath S[c] $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **7a** | Phenyl | $CH_3$ | 0.490 | 0.15 | 0.32 |
| **7b** | 3-Methylphenyl | $CH_3$ | 0.372 | <0.03 (67%) | 0.07 |
| **7c** | 4-Methylphenyl | $CH_3$ | 0.044 | <0.03 (52%) | 0.58 |
| **7d** | 3-Methoxyphenyl | $CH_3$ | 0.419 | 0.13 | 0.47 |
| **7e** | 4-Methoxyphenyl | $CH_3$ | 0.009 | 0.80 | 1.55 |
| **7f** | 4-Methoxyphenyl | $CH_2CH(CH_3)_2$ | <0.003 (71%) | 0.58 | 0.48 |
| **7g** | 4-Methoxyphenyl | $CH_2(C_5H_9)$ | <0.003 (56%) | 0.75 | 0.53 |
| **7h** | 4-Ethylphenyl | $CH_3$ | <0.003 (80%) | 0.03 | 0.66 |
| **7i** | 4-Ethylphenyl | $CH_2(C_6H_5)$ | <0.003 (72%) | 1.3 | 4.9 |
| **7j** | 1H-Indol-2-yl | $CH_3$ | <0.003 | 0.09 | 0.16 |
| **7k** | 1H-Indol-2-yl | $CH_2CH(CH_3)_2$ | <0.003 (71%) | >10 (22%) | >10 (46%) |
| **7l** | 1H-Indol-2-yl | $CH_2(C_5H_9)$ | 0.003 | >10 (27%) | 7.5 |
| **7m** | 1H-Indol-2-yl | $CH_2(C_6H_5)$ | 0.004 | 6.9 | 4.5 |

[a]Inhibition of recombinant human (rh) cathepsin K activity in a fluoresence assay using 48 μM Cbz-Phe-Arg-AMC as substrate in 100 mM $NaH_2PO_4$, 1 mM EDTA, 20 μM Tween 80, 2 mM DTT, pH 7.
[b]Inhibition of rh cathepsin L activity using 3 μM Cbz-Phe-Arg-AMC as substrate in 100 mM NaOAc, 1 mM EDTA, 0.005% Brig 35, 1 mM DTT, pH 5.5.
[c]Inhibition of rh cathepsin S activity using 11 μM Cbz-Leu-Leu-Arg-AMC as substrate in 100 mM NaOAc, 1 mM EDTA, 0.01% Triton X-100, 1 mM DTT, pH 5.5. Data represent mean of 2 experiments performed in duplicate, individual data points in each experiment were within a 3-fold range with each other.

inhibitory potency. As expected from modeling, the introduction of a 1-methyl- piperidinyloxy, a dimethylamino-, a pyrrolidinyl- or imidazolyl-ethoxy substituent in the $P_1'$-fragment resulted in significantly less potent inhibitors (**1i**–**1l**), most likely due to the polar group which cannot adjust within the hydrophobic $S_1'$ pocket.

As illustrated by the results summarized in Table 2, replacement of the Cbz group by appropriate acyl moieties afforded a series of highly potent cathepsin K inhibitors (**7e**–**m**). Replacement of the Cbz moiety by an unsubstituted benzamide (**1a**→**7a**) led to a loss in activity and selectivity. Introduction of a meta substituent at the benzamide moiety (**7b**, **7d**) reversed the selectivity profile in favour of cathepsins S and L. However, a 4-substituted benzamide or 1H-indolyl moiety as the $P_3$ substituent provides analogues with activities in the low nanomolar range. Within this series of inhibitors the size of the $P_1'$ substituent had an impact on the selectivity profile. Inhibitors **7e**, **h** and **j** all incorporating a 4-methoxy substituent on the aminophenyl ring are the least selective for cathepsin K over cathepsins L and S. Extended and lipophilic $P_1'$ substituents such as an isobutyloxy, a methylcyclopentyloxy- or a benzyloxy moiety in compounds **7f**, **g**, **i**, **k**–**m** were found to be significantly more selective inhibitors for cathepsin K. Out of this series compound **7k** emerged as the most attractive analoge investigated, which apart from high potency for cathepsin K inhibition exhibits the most favorable selectivity profile (>3300-fold for cathepsin K over cathepsins L and S).

In conclusion we have described the synthesis and in vitro activities of a series of *Nα*-benzyloxycarbonyl- and *Nα*-acyl-L-leucine(2-phenylaminoethyl)amide derivatives which incorporate extended $P_1'$ substituents. Expanded lipophilic $P_1'$ moities do not improve the potency of our inhibitors, however they generally lead to an increased specificity for cathepsin K over the two highly homologues cathepsins L and S. The appropriate combination of $P_3/P_1'$ subunits results in highly potent cathepsin K inhibitors with an excellent selectivity profile.

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