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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1486–1490
Bioorganic & Medicinal Chemistry Letters

Arylaminoethyl amides as noncovalent inhibitors of cathepsin S. Part 2: Optimization of P1 and *N*-aryl

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Received 2 November 2005; revised 9 December 2005; accepted 12 December 2005
Available online 18 January 2006

Abstract—A systematic study of anilines led to the discovery of a metabolically robust fluoroindoline replacement for the alkoxy aniline toxicophore in **1**. Investigations of the P1 pocket resulted in the discovery of a wide tolerance of functionality leading to the discovery of **11** as a potent and selective inhibitor of cathepsin S.

Cathepsin S is a papain-like cysteine protease that is predominantly expressed in antigen presenting cells. Cathepsin S plays an important role in the immune response by processing the invariant chain (li) of the major histocompatibility class II complex (MHC II). This proteolytic event is necessary for the loading of antigen onto the MHC II molecules, which subsequently leads to antigen presentation to $CD4^+$ T-cells.[1] Cathepsin S-deficient mice show major impairment in the degradation of the invariant chain in antigen presenting cells along with resistance to collagen-induced arthritis[2] and autoimmune myasthenia gravis.[3] Since these mice appeared normal in all other respects, small molecule inhibitors of cathepsin S are thought to be good candidates for the treatment of diseases that require modulation of the immune response.[4]

Compound **1** (Fig. 1) was reported by Altmann and coworkers as a noncovalent competitive inhibitor of cathepsin K but was not reported to be selective.[5] A high-throughput screening effort identified **1** as an inhibitor of cathepsin S.[6] Our previous report described the optimization of the P2 and P3 substituents to improve the potency and selectivity over related cathepsins.[7] The subject of this article is our continuing efforts in hit-to-lead optimization of the P1/P1′ moieties of this series for potency, selectivity, and removal of metabolic liabilities.[8]

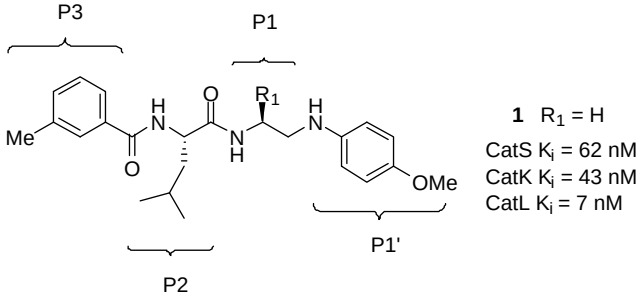

Figure 1. Screening hit for the inhibition of cathepsin S.

The methoxyaniline moiety in compound **1** is a known toxicophore. The phenolic metabolite, as a result of O-demethylation, is further oxidized to 4-benzoquinone imine in vivo, which elicits toxicity by GSH depletion and covalent adducts with cellular macromolecules. Therefore, suitable replacements were explored.[9]

Scheme 1 shows the chemistry used to access the initial group of compounds. Leucine was acylated with *m*-toluoyl chloride under Schotten–Bauman conditions and then reacted with ethanolamine using EDC as the coupling agent to afford **2**. This material was then oxidized to the aldehyde with the Dess–Martin periodinane and reductively aminated with various anilines and indolines

Keywords: Cathepsin S; Noncovalent protease inhibitor; SAR.
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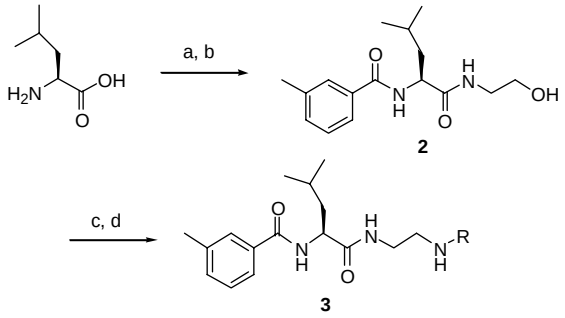
doi:10.1016/j.bmcl.2005.12.056



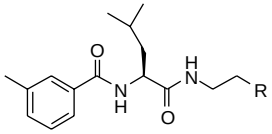
Scheme 1. Reagents: (a) 1 M NaOH, *m*-toluoyl chloride; (b) ethanolamine, EDC, HOBt, CH_2Cl_2; (c) Dess–Martin periodinane, DCM; (d) R-NH_2, $NaBH_3CN$, DMF, AcOH.

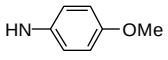
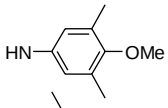
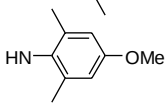
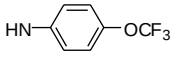
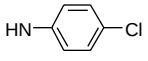
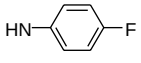
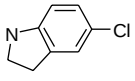
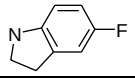
to afford the desired adducts **3a**–**h**. The aniline was used in a 3-fold excess to ensure that product **3** did not undergo further reductive amination.

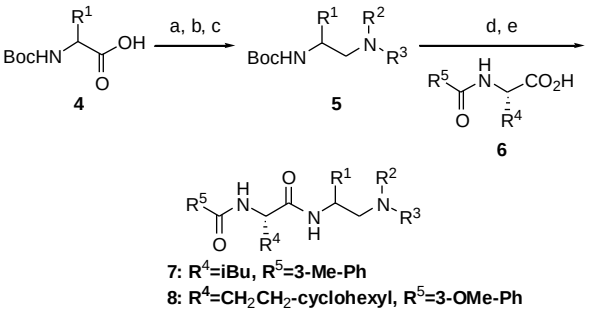
Compounds **3a** and **3b** indicated that there was additional space around the aniline for steric hindrance (Table 1). Specifically, **3b** was interesting because it demonstrated that the interaction between the aniline and the enzyme was not significantly perturbed by the *ortho*-substituted methyl groups. *para*-Substituted compounds **3c**, **3d**, and **3e** showed that the methoxy unit was not required for inhibitory activity, but the halogens appeared to be less preferred. HPLC analysis of a sample of **3c**, which was treated with rat liver microsomes, showed a new peak whose weight corresponded to the trifluoromethoxy group being replaced with a hydroxyl group. It has been reported that *para*-substituted fluoroanilines can be similarly metabolized.[10] It is likely that this metabolism proceeds via a homolytic process starting at the aniline hydrogen. We were therefore pleased to see that compounds **3f**–**h** retained activity upon replacement of the aniline with an indoline. The 5-fluoroindoline analog **3h** remained the most attractive, as it had an activity profile similar to that of initial hit **1**. In metabolite ID studies, there was no product observed that lost a fluorine from the indoline in the analogues of **3h**.[11]

Exploration of the P1 position was initiated by installation of a side chain on the ethylenediamine linker. As shown in Scheme 2, Boc protected amino acids were reduced to the alcohol, oxidized to the aldehyde, and then reductively aminated with the appropriate aniline or indoline to afford **5**. The Boc group was removed and the resulting amine then coupled to the appropriate

Table 1. Inhibition of the cathepsin S, K, and L—variation of the aniline on the hit structure



Compound	R	Cathepsin S, K_i (nM)	Cathepsin K, K_i (nM)	Cathepsin L ,K_i (nM)
1		68	91	26
3a		107	125	20
3b		132	131	14
3c		104	125	120
3d		458	408	92
3e		563	478	62
3f		230	310	88
3g		196	139	17
3h		98	80	11



Scheme 2. Reagents and condition: (a) i—*i*-butyl chloroformate, NMM, THF, −10 °C; ii—$NaBH_4$, H_2O; (b) Dess–Martin periodinane, DCM; (c) R^2R^3-NH, $NaBH_3CN$, DMF, AcOH; (d) TFA/DCM (1:1); (e) HATU, DIEA, DMF.

N-acylated amino acid **6** (prepared as in Scheme 1) using standard peptide coupling conditions.

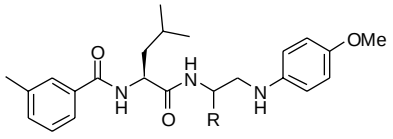
First, we determined that the *S*-diastereomer **7a** is more active than the *R*-diastereomer **7b** as shown in Table 2. It should be noted that there was also a 5-fold increase in activity versus **1** when an alanine side chain was introduced at P1.

We then went on to do a more thorough investigation of the nature of tolerated P1 substituent as shown in Table 3. We noted that for intermediates **5** where R^1 is larger than methyl racemization of the amino acid stereocenter occurred during the coupling step. We chose to analyze the mixture on the assumption that the other diastereomer would have significantly less activity (vide supra).

In the case of homocyclohexylalanine P2, it turned out that the parent compound, **8a**, did not have potent activity against cathepsin S, however, addition of a hydrophobic side chain in P1 afforded potent cathepsin S inhibitors. In addition, the selectivity over cathepsin L was highly dependent on the nature of the P1 side chain. The valine derived isopropyl analog **8b** gave only 10-fold selectivity over cathepsin L, while substitution with isobutyl **8c** gave a 64-fold selectivity over cathepsin L.

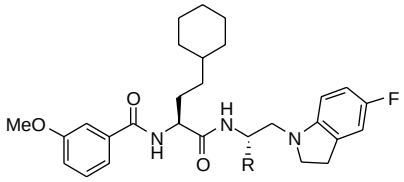
Compounds **8d**–**h** showed that aromatic substituents that were not directly attached to the side chain did not add any potency beyond their aliphatic counterparts. We then went on to try hydrophilic substituents

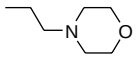
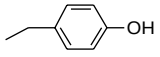
Table 2. Inhibition of the cathepsin S, K, and L—substitution on P1 side chain



Compound	R	Cathepsin S, K_i (nM)	Cathepsin K, K_i (nM)	Cathepsin L, K_i (nM)
1	H	68	91	26
7a	(*S*)-Me	15	55	1.6
7b	(*R*)-Me	212	337	75

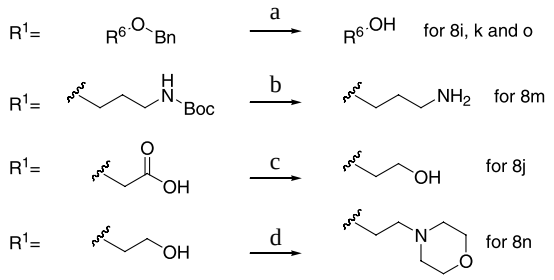
Table 3. Inhibition of the cathepsin S, K, and L—substitution on P1 side chain



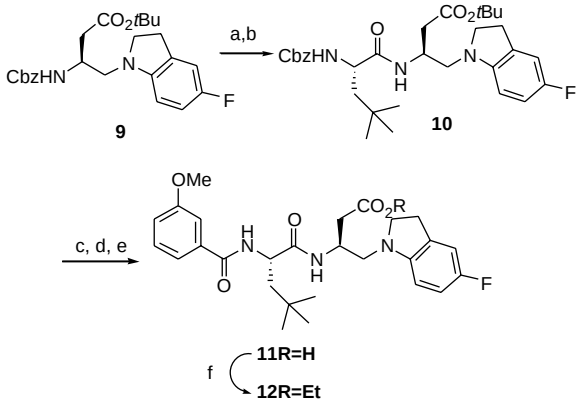
Compound	R	Cathepsin S, K_i (nM)	Cathepsin K, K_i (nM)	Cathepsin L, K_i (nM)
8a	H	2130	>100,000	>33,750
8b	$CH(CH_3)_2$	14	>10,000	150
8c	$CH_2CH(CH_3)_2$	28	>100,000	1800
8d	Ph	68	>100,000	>11,000
8e	CH_2Ph	133	>100,000	9200
8f	CH_2OBn	23	>30,000	380
8g	CH_2SBn	149	>100,000	5040
8h		15	4320	2
8i	CH_2OH	50	>100,000	15,530
8j	CH_2CH_2OH	208	>100,000	1800
8k		8	2750	20
8l	$CH_2CH_2SO_2Me$[a]	14	47	19
8m	$(CH_2)_3NH_2$	76	10,760	42
8n		7	2150	5
8o		96	>100,000	3410
8p	CH_2CO_2Bn	405	>37,250	>100,000
8q	CH_2CO_2H	88	>10,000	>37,000

[a] P2 residue is *t*-butylalanine.

in order to lower the lipophilicity of the inhibitors. When hydroxyl containing side chains **8i**, **8j**, and **8k** were made, we found that these were not only tolerated but had a roughly similar SAR to the aliphatics in that chain substitution increases potency against cathepsin S but decreases selectivity. With this observation in hand, we pressed forward with hydrophilic substituents and found that a sulfone **8l** was accepted (although the P2 residue in this compound was *t*-Bu alanine, the difference in activity with homocyclohexyl P2 is one of absolute potency and not so much selectivity—vide supra **8q** vs **11**) and amines (**8m** and **8n**) were tolerated but lost selectivity against cathepsin L and K. We also wanted to test whether a negative charge is accepted and **8o** implied that this might be the case (although it is not certain whether the phenol is ionized under the assay conditions). Finally, we tested both the ester **8p** and the acid **8q** of the aspartate side chain and contrary to what one might expect from substrate specificity study,[12] these turned out not only to be tolerated but to confer a great deal of selectivity to the molecule. The positively charged guanidinium group at the S1 pocket emanating from Arg141 represented a distinct feature of cathepsin S that may account for the specificity of **8q**. The same region is occupied by Asn158 in cathepsin K and Asp162 in cathepsin L.[13]



Scheme 3. Reagents and condition: (a) H_2, Pd/C, MeOH; (b) TFA:DCM (1:1); (c) i—*i*-butyl chloroformate, NMM, THF, −10 °C; ii—$NaBH_4$, H_2O; (d) Dess–Martin periodinane, DCM; (e) Morpholine, $NaBH_3CN$, DMF, AcOH.



Scheme 4. Reagents: (a) H_2, 10% Pd/C, MeOH; (b) HATU, DIEA, DMF, Cbz-*t*-Bu Ala (77% over 2 steps); (c) H_2, 10% Pd/C, MeOH; (d) HATU, DIEA, DMF, anisic acid (57% over 2 steps); (e) TFA/DCM (1:1) (86%); (f) CSA, EtOH, triethylorthoformate (44%).

Scheme 3 shows the chemistry used to access the compounds that required transformation of the side chain after coupling in Scheme 2. In this scheme, R^6 refers to an alkyl, aryl or carbonyl group.

Scheme 4 shows the synthesis of the more optimized compound **11** and an ethyl ester **12**, synthesized as single diastereomers by using *N*-carbamate protected amino acids as building blocks.

The indoline conjugate **9** (prepared according to Scheme 2, except using a Cbz protecting group) was deprotected and coupled to Cbz-*t*-Bu Ala using standard conditions to afford **10**. It was crucial to use the *t*-Bu protecting group on the ester because once the nitrogen is deprotected from **10**, there is a strong tendency to cyclize. Indeed, when **10** is deprotected and coupled to anisic acid, the conjugate is obtained in only 57% yield. Deprotection of the ester affords **11** in 86% yield and the acid is esterified to **12** with CSA and TEOF in ethanol. Table 4 shows the activities of these compounds.

The inhibitory activity of **11** against cathepsin S proved to be high with an acceptable selectivity window over cathepsin K and complete selectivity over cathepsin L. Compound **11** shows that the presence of the acid in P1 increases the selectivity of this class of inhibitors for cathepsin S over cathepsins K and L. We also showed that **11** is a reversible inhibitor through a dialysis experiment.[14] Table 5 shows the in vivo PK parameters of **11** and **12**.

Table 4. Inhibition of the cathepsin S, K, and L

Compound	Cathepsin S, K_i (nM)	Cathepsin K, K_i (nM)	Cathepsin L, K_i (nM)	Selectivity K/S
11	6	460	>15,000	77
12	16	184	1754	11.5

Compounds **11** and **12**.

Table 5. PK parameters of **11** and **12** (Both dosed in Wistar rats at 10 mpk)

Compound	PO AUC (min μg/mL)	PO C_{max} (nM)	$T_{1/2}$ (h)	%*F*
11	6	45	0.56	2.0
12[a]	18.5	293	N/A	8.0

[a] Dosing 12 and monitoring for 11.

As is evident from Table 5, the use of the ethyl ester prodrug leads to a 4-fold improvement in oral bioavailability over the acid itself and generates a C_{max} that is 50-fold greater than the K_i.

In summary, we showed that there is a wide tolerance for functional groups in the S1 pocket of cathepsin S including carboxylic acid functionality which gave excellent selectivity over other related cathepsins. This effort represents the most thorough investigation of the P1 region reported to date. We have successfully replaced the methoxyaniline toxicophore with a fluoroindoline while maintaining potency and selectivity as exemplified by **11**. The synthesis of compound **12**, the prodrug of **11**, resulted in a modestly bioavailable, potent, and selective inhibitor of cathepsin S. Further strategies based on this work will be reported in due course.

Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.12.056.

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