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N-Arylaminonitriles as Bioavailable Peptidomimetic Inhibitors of Cathepsin B

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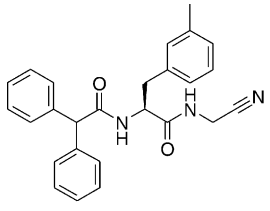
Abstract—To improve the pharmacokinetics of a previously reported series of dipeptidyl nitrile cathepsin B inhibitors, the P_2–P_3 amide group was replaced with an arylamine. Further optimization of this template resulted in highly potent and selective inhibitors with excellent oral availability.

Cathepsin B (cat B), a lysosomal cysteine protease, has been implicated in the pathology of a number of important human diseases. Cat B is upregulated in the synovium of patients with rheumatoid arthritis[1] and selective cat B inhibitors have been found to block proteoglycan release from articular cartilage.[2] Cat B has also been implicated in the progression and invasion of tumors, by aiding in extracellular matrix breakdown.[3] More recently, cat B−/− mice have been found to be resistant to TNF-α mediated hepatocyte apoptosis, revealing a potential contributory role in various types of liver injury.[4] It also appears that cat B facilitates $CD8^+$ T-cell mediated cytotoxicity, by protecting these cells from their own lethality.[5] This may indicate a central role for cat B in host defense. The wide-ranging functions of this enzyme therefore make it an attractive drug discovery target.

Our group has previously reported on a series of dipeptidyl nitrile inhibitors of cat B.[6] These compounds were designed with a carboxylate-containing substituent at the P_1 position, which was capable of interacting with His110 within the S_2' site of cat B. Thus, the moderately potent (50 nM) but nonselective compound **1** was optimized to compound **2**, a 6 nM cat B inhibitor with 100-fold selectivity versus cathepsins S and L. Unfortunately, compound **2** and its analogues were found to have very poor pharmacokinetic properties and could not be used for in vivo testing. It was hypothesized that the highly peptidic nature of compounds such as **2** was contributing to this problem, perhaps through amide hydrolysis or low intestinal permeability. This report describes our efforts to use a structure-guided approach to partially depeptidize these compounds, resulting in highly potent and selective cathepsin B inhibitors with much more favorable pharmacokinetic properties.



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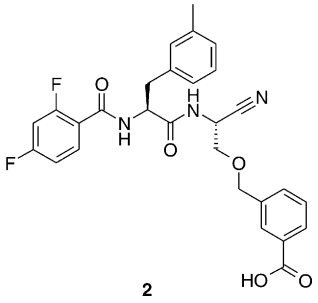

2

The X-ray structure of a compound **1**–cat B complex[6] revealed hydrogen-bonding interactions between the backbone of the enzyme and both NH's of the inhibitor, as well as the P_2 (phenylalanyl) carbonyl group. However, the P_3 (diphenylacetyl) carbonyl does not appear to play any role in cat B binding (Fig. 1). With this in

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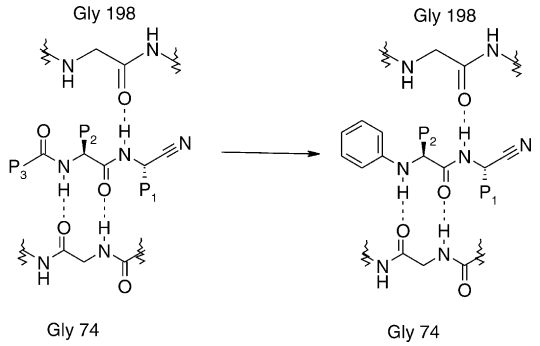
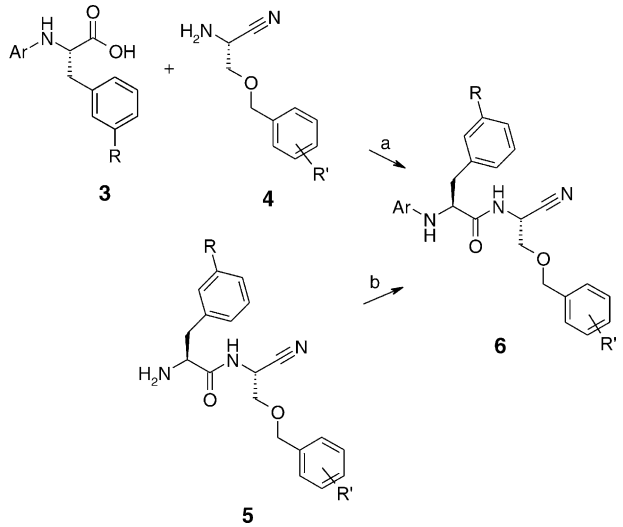


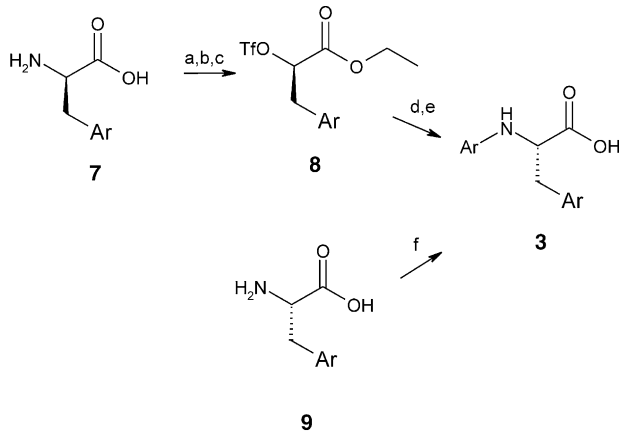
Figure 1. Schematic representation of H-bonding pattern of nitrile inhibitors to cat B, and proposed modification of P_3 amide.

mind, we designed compounds that would maintain the relatively acidic P_2 NH, but would lack the P_3 carbonyl group. To this end, replacement of the amide with an *N*-arylamine appeared to be attractive for several reasons: (1) the aniline NH was expected to have similar H-bond donating properties to the amide NH, (2) the aryl group still provided a hydrophobic substituent for binding in the S_3 pocket, and (3) the modification was not expected to affect the P_1 or P_2 substituent orientations, so that reoptimization of these portions of the molecule would probably not be necessary.

The desired compounds **6** were assembled in one of two ways (Scheme 1). When Ar = Ph, *N*-arylation was carried out on the pre-assembled amino nitrile **5** using triphenylbismuth diacetate (path b), as described by Barton.[7] For substituted aromatic groups, the *N*-aryl amino acid **3** was prepared and then coupled with an amino nitrile **4** (path a). Preparations of amino nitriles **4** and **5** were carried out as previously described.[6] To generate compounds with a carboxylic acid on the P_1 substituent, esters **4** or **5** were prepared (R′ = CO_2allyl) and deprotected in the final step.[6] The corresponding P_1-substituted tetrazoles were introduced with a cyanoethyl protecting group and were revealed using DBU in CH_2Cl_2.



Scheme 1. Reagents and conditions: (a) EDCI, HOBt, NMM, CH_2Cl_2; (b) $Ph_3Bi(OAc)_2$, $Cu(OAc)_2$, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) $NaNO_2$, NaOAc, HOAc; (b)–TsOH, EtOH, reflux; (c) Tf_2O, pyridine; (d) $ArNH_2$, THF; (e) LiOH, THF, water; (f) ArBr or ArI, $(P(o\text{-tol})_3)_2PdCl_2$, CuI, TEBA, K_2CO_3, TEA, DMF, water.

In the early stages of this effort, the *N*-aryl amino acids **3** were prepared through displacement of the corresponding triflated *R*-hydroxyester **8**, which were themselves prepared from *R*-amino acids **7** using standard methodology (Scheme 2). Compounds **14**–**16** were prepared in this manner (Table 1). Later, however, the more convenient Pd(0)/Cu(I)-catalyzed amino acid arylation conditions reported by Ma were employed using the appropriate aryl bromide or iodide as the coupling partner.[8] Compounds **17**–**20** and **23** were prepared in this manner.

In order to provide adequate throughput for assessment of oral exposure, compounds were subjected to a single timepoint plasma drug level measurement as an initial screen. To accomplish this, compounds were dosed orally to rats.[9] Plasma concentrations were then measured 4 h post-dosing, using HPLC/MS.

Data for the compounds are presented Table 1. We were gratified to find that replacement of the P_3 amide with a simple phenyl group (compound **10**) resulted in a nanomolar cat B inhibitor. Although this compound was somewhat less potent than **2**, its 4-h

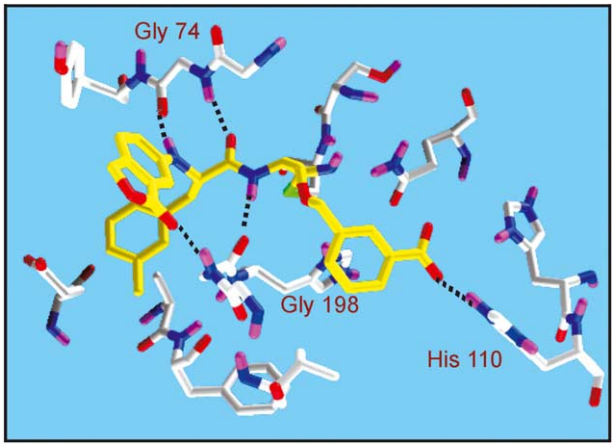


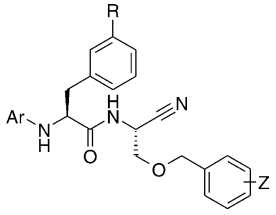
Figure 2. Molecular model of compound **16** bound to cat B, showing key H-bonds between enzyme and inhibitor.

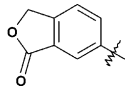
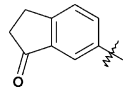
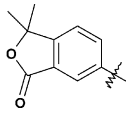
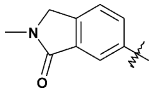
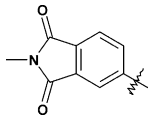
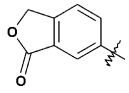
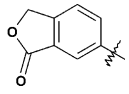
plasma concentration was much higher than that of **2** (1.1 μM for **10** vs 0.09 μM for **2**).

As expected, the P_2 and P_1 substituent SAR for the *N*-aryl amines was identical that of the P_3-acyl series:[6] removal (**11**) or repositioning (**12**) of the P_1 carboxylate resulted in reduced potency, as did removal of the 3-methyl group on the P_2 phenyl ring (**13**).

To improve the in vitro potency of this series, we examined a model of compound **10** bound to cat B, which was derived from the compound **1**–cat B X-ray structure.[6] It appeared from the model that an H-bond acceptor attached to the *meta*-position of the arylamine would be correctly positioned to interact with the backbone NH of Gly198 (Fig. 2). A *meta*-sulfone substituent at this position resulted in a 4-fold increase in potency

Table 1. Structures and data for compounds discussed in paper



Compd	Ar	R	*Z*	Cat B IC_{50} (nM)	Cat L IC_{50} (nM)	Cat S IC_{50} (nM)	Plasma concn.[a] (μM)
10	Ph	Me	3-COOH	49	1800	2000	1.1
11	Ph	Me	H	194	220	1200	—
12	Ph	Me	4-COOH	282	2800	3900	—
13	Ph	H	3-COOH	832	6100	2600	—
14	3-($MeSO_2$)–Ph	Me	3-COOH	13.3	—	—	0.037
15	3-(COOMe)–Ph	Me	3-COOH	8.6	210	710	—
16		Me	3-COOH	6.5	—	—	0.44
17		Me	3-COOH	5.3	—	—	0.07
18		Me	3-COOH	5.3	560	780	0.11
19		Me	3-COOH	9.3	> 1000	> 1000	0.038
20		Me	3-COOH	4.9	> 1000	> 1000	0.015
21	Ph	Me	3-OH	> 1000	—	—	—
22	Ph	Me	3-Tetrazole	16.5	—	—	0.23
23		Me	3-Tetrazole	5.0	2400	1000	0.037
24		Me	3-COOH, 4-F	4.1	1100	1000	0.056
25	Ph	Me	3-COOH, 4-F	12.2	1400	1600	5.27
26	Ph	Me	3-COOH, 4-Cl	8.7	1400	1700	1.88

— No data available for this compound.
[a]Plasma concentration is measured 4 h after a 30 mg/kg po dose.

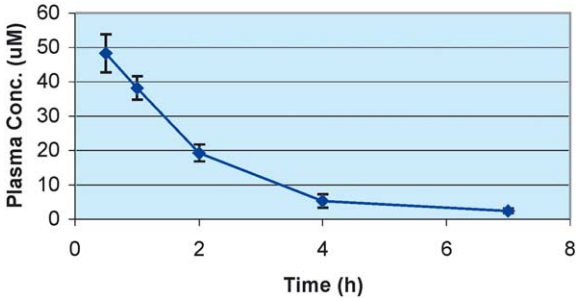


Figure 3. Plasma concd (μM) versus time after 30 mg/kg po dose in rats.

(**14**). An even greater enhancement was observed with a methyl ester (**15**). The best potencies were obtained from cyclized carbonyl compounds, in which the carbonyl is locked in the preferred conformation: lactones (**16**, **18**), lactam (**19**), phthalimide (**20**), and cyclic ketone (**17**). Unfortunately, none of these compounds displayed 4-h plasma levels as high as **10**, and were not pursued further. However, this endeavor established the feasibility of preparing arylamines with potencies comparable to the best P_3 amide inhibitors.

Our previous work established the importance of the P_1 carboxylate, which is thought to form an electrostatic interaction with His110 and His111 of the S_2' pocket of cat B (see Fig. 2).[10] With this in mind, we investigated the effect of carboxylate acidity on cat B potency. *ortho*-Substitution with a fluoro or chloro group (**25**, **26**) is expected to decrease the pK_a of the carboxylate by approximately 1.0 and 1.3 log units,[11] and increased the potency by 4- and 5.6-fold, respectively. In addition, these compounds maintain micromolar plasma concentrations at the 4-h timepoint. Replacement of the carboxylate with a tetrazole also resulted in a 4-fold increase in potency (**22**), but this compound displayed lower plasma levels than the carboxylates. The additive effect of the P_3 and P_1 modifications can be observed in the enhanced potency of compounds **23** and **24**.

As can be seen in Table 1, all compounds containing the P_1 carboxylate are selective for cat B over cat L and cat S. The lower selectivity (as well as potency) of compound **11** supports the hypothesis that the S′ carboxylate recognition site is unique for cat B. Incorporation of esters or lactones into the P_3 phenyl group increases potencies against all three enzymes, although the lactam and imides are highly selective (**19** and **20**, respectively). Halogen substitution on the P_1 benzoate improves potency only against cat B. Because of this, compounds **25** and **26** have >100-fold selectivity against the other cathepsins.

Table 2. Pharmacokinetic parameters for compound **25**

T_{max} (h)	0.50±0.07
$t_{1/2}$ (h)	0.24±0.02
CL (mL/min/kg)	64.8±0.61
Vss (L/kg)	1.22±0.07
F (%)	128±59

A full PK assessment in the rat was made for compound **25**. Figure 3 shows that micromolar plasma concentrations are maintained for at least 7 h after a 30 mg/kg po dose, with a very high C_{max}. Table 2 provides the PK parameters for this compound, indicating excellent oral availability (%F), although clearance and $t_{1/2}$ values are still not optimal.

In conclusion, we have demonstrated that structure-based peptidomimetic design has allowed for the identification of a series of potent, selective and highly orally bioavailable inhibitors of cathepsin B. These compounds are currently being profiled in animal models to further delineate the role of this enzyme in disease processes.

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