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Abstract—We report a novel series of noncovalent inhibitors of cathepsin S. The synthesis of the peptidomimetic scaffold is described and structure–activity relationships of P3, P1, and P1′ subunits are discussed. Lead optimization to a non-peptidic scaffold has resulted in a new class of potent, highly selective, and orally bioavailable cathepsin S inhibitors.
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Cathepsin S (Cat S) is a lysosomal cysteine protease that is expressed in antigen presenting cells such as macrophages, dendritic cells, and B cells. Cat S plays a critical role in the targeted processing of the invariant chain (Ii), a chaperone protein for the major histocompatibility class II complex (MHC-II).[1–3] Proteolytic removal of Ii from the MHC-II binding groove is a prerequisite for productive antigen loading onto the MHC-II complex,[4] and consequently, inhibition of Cat S attenuates antigen presentation to CD4+ T-cells. The resulting immunosuppression is specific for CD4+ T-cells, making cathepsin S an attractive therapeutic target for the modulation and regulation of immune hyperresponsive diseases such as myasthenia gravis, multiple sclerosis, and rheumatoid arthritis.[5–7]

We have recently reported on a series of arylaminoethyl amides including compounds such as **1a** and **1b** (Fig. 1), which lack an electrophilic covalent warhead and act as reversible, competitive inhibitors of cathepsin S.[8–11] Compound **1a** ($K_i = 0.087$ μM) has only a narrow window of selectivity over closely related cathepsin L, while **1b** and many of its closely related analogs exhibit poor pharmacokinetic (PK) properties in rats.[11] Lead optimi-

1a R,R = H
Cat S K_i = 0.087 μM
Cat K K_i = 5.130 μM
Cat L K_i = 0.512 μM

1b R,R = CH$_3$
Cat S K_i = 0.011 μM
Cat K K_i = 16.9 μM
Cat L K_i = 0.810 μM

2
Cat S K_i = 0.662 μM
Cat K K_i = 8.79 μM
Cat L K_i = >30.0 μM

3
Cat S K_i = 0.260 μM
Cat K K_i = >30.0 μM
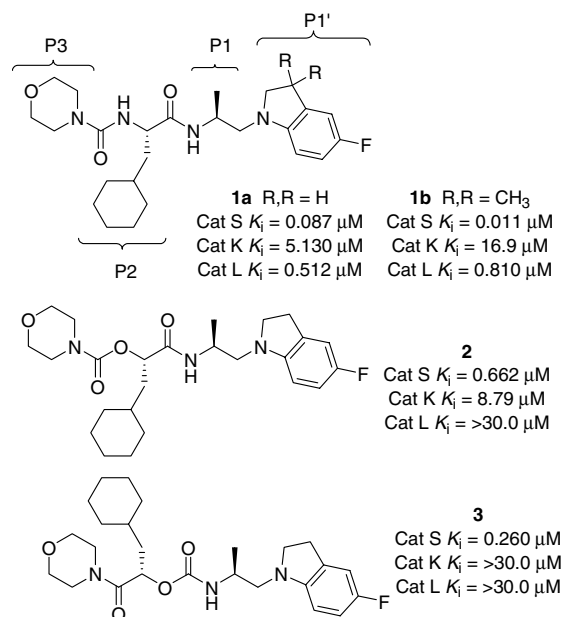Cat L K_i = >30.0 μM

Figure 1.

zation efforts have focused primarily on improving the PK properties by reducing the peptidic nature of the series while retaining the potency and selectivity of our earlier, more peptidic chemotypes.

Medicinal chemistry optimization efforts have been aided by structural data that we and others have reported involving co-crystal structures of active-site inhibitors bound to cathepsin S.[10–14] In peptidic Cat S inhibitors such as arylaminoethyl amides[10,11] or dipeptide nitriles,[13] the P2 amide-NH forms a hydrogen bond with the Cat S backbone carbonyl of Gly69. In order to determine whether this interaction was essential for binding to the protein, we synthesized the carbamate analog of urea **1a**. Lactic acid analog **2**, in which the P2 NH has been replaced by oxygen,[15] was not as active toward Cat S ($K_i = 0.662 \ \mu M$), but the corresponding drop-off in Cat L inhibitory activity was much more pronounced (>60×). Initial efforts to exploit this divergence resulted in compound **3** (Cat S $K_i = 0.260 \ \mu M$), in which the connectivity of the main chain has been directly transposed from **2**. The Cat S inhibitory activity was retained, and more importantly the selectivity over cathepsins K and L was improved to well over 100-fold. Furthermore, the kinetics of inhibition was shown to be fully reversible as the enzymatic activity was fully restored following dilution and dialysis of the enzyme–inhibitor complex.[16]

The α-hydroxyacids **4** were prepared from their respective lactic acid or amino acid precursors. The synthesis of carbamate **3** and related analogs with the P3 morpholine amide is shown in Scheme 1. Morpholine amides **5**, prepared by standard peptide coupling conditions using PyBOP, were activated as the nitrophenyl carbonates **6**, and then condensed with the desired *N*-aryl diamines **7a–e** (Fig. 2).[17]

The SAR of a representative set of examples is illustrated in Table 1. It is clear that an alkyl group in the P1 position of this arylaminoethyl carbamate series is required for sub-micromolar activity as demonstrated by compound **8** (Cat S $K_i = 2.95 \ \mu M$). This is in contrast to the arylaminoethyl amide series described in previous reports which were typically very potent even without a P1 substituent.[8–11] Presumably a different mode of crosstalk between P1 and the other subsites predominates in the case of this non-peptidic carbamate scaffold in such a manner that the conformation assumed by the inhibitor allows for a more favorable interaction (particularly P1–S1 and P1′–S1′) with the enzyme than exists with the peptidic scaffold for compounds such as **1a**
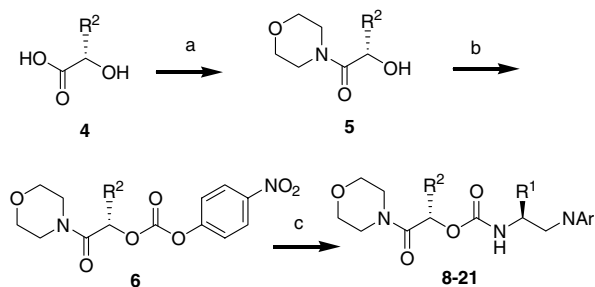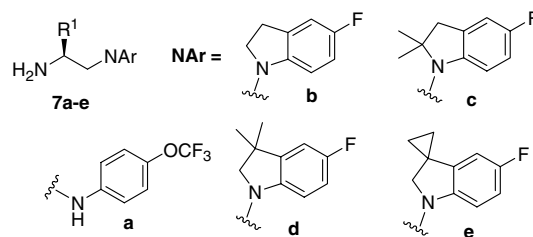
Figure 2. *N*-Aryl diamines (Ref. 16).

and **1b**. This becomes more apparent with an increasing size of the P1 alkyl substituent, as the hydroxymethyl **9** is nearly equipotent with **3**, while the benzyloxymethyl **10** analog (Cat S $K_i = 0.008 \ \mu M$) gains over 30-fold in inhibitory activity over compound **3**. Moreover, compound **10** is highly selective (>1000-fold) over both cathepsins K and L—a major contrast to the arylaminoethyl amides described previously which typically exhibit a decrease in selectivity over Cat L with increasing Cat S potency that is derived from larger P1 alkyl substituents.[9–11]

Replacement of the 5-fluoroindoline moiety at the prime side with a 4-trifluoromethoxyaniline led to a slight improvement (∼2.5×) in potency (**11** vs. **3**), while larger alkyl groups in P1 cooperatively contributed to an additional boost in the potency of cathepsin S inhibitors (**12**, **13**, and **14**). The cyclohexylmethyl group has previously been shown to be a preferred P2 group for cathepsin S inhibitors of varied chemotypes, in particular arylaminoethyl amides[8–11] and dipeptide nitriles,[13] however, other hydrophobic groups are often well tolerated.[5] In contrast, neither the benzyl (**15**) nor the *tert*-butylmethyl (**16**) P2 moieties are well tolerated by Cat S in the context of this current carbamate series. The well-defined hydrophobic S2 pocket of Cat S is known from reported X-ray structural data to be a comparatively deep pocket (versus Cat K and L) with a narrow entrance.[10–14] It is possible, in this case, that the trajectory of suboptimal P2 groups emanating from a non-peptidic backbone leads to hydrophobic clash with the narrow entrance of the S2 pocket.

In light of this SAR, it appeared that the cyclohexylmethyl was the optimal choice for the P2 substituent both in terms of potency and selectivity regardless of the P1 and P1′ moieties. Continuing with the optimization of the P1′ moiety led to the 2,2-dimethyl-5-fluoro-indoline **17**, which showed a moderate (3×) improvement in potency over the unsubstituted 5-fluoroindoline **3**. A more significant improvement was seen with the 3,3-*gem*-dimethyl indoline analog **18**, which is considerably more potent than the unsubstituted indoline **3** (>10×), and especially noteworthy considering it did not require a P1 substituent larger than a methyl group for this activity. This is further exemplified with compound **19**, which is equipotent with **18** despite bearing a larger cyclopropyl group in P1. Moreover, the selectivities of indolines **17**, **18**, and **19** over cathepsins K and L are greater than three orders of magnitude. Compound **20** further illustrates the influence that the

Scheme 1. Reagents and conditions: (a) morpholine, PyBOP, CH_2Cl_2, 0 °C to rt, 74–89%; (b) 4-nitrophenylchloroformate, pyridine, CH_2Cl_2, rt, 62–91%; (c) **7a–d**, DIEA, DMF, rt, 48–89%.

Table 1. Inhibition of cathepsin S, K, and L[a]

Core structure: morpholine-C(=O)-CH(R²)-O-C(=O)-NH-CH(R¹)-CH₂-NAr

Compound	R²	R¹	NAr	Cat S K_i (µM)	Cat K K_i (µM)	Cat L K_i (µM)
3	cyclohexylmethyl	–CH₃	5-fluoroindoline	0.226	>30	>30
8	cyclohexylmethyl	–H	5-fluoroindoline	2.95	>30	>30
9	cyclohexylmethyl	–CH₂OH	5-fluoroindoline	0.143	>30	>100
10	cyclohexylmethyl	–CH₂OCH₂Ph	5-fluoroindoline	0.008	8.71	17.7
11	cyclohexylmethyl	–CH₃	NH-C₆H₄-OCF₃	0.100	>30	>100
12	cyclohexylmethyl	–CH₂CH₂CH₃	NH-C₆H₄-OCF₃	0.016	1.39	5.47
13	cyclohexylmethyl	–CH(CH₃)₂	NH-C₆H₄-OCF₃	0.008	1.18	6.03
14	cyclohexylmethyl	–CH₂CH₂Ph	NH-C₆H₄-OCF₃	0.018	3.97	9.79
15	benzyl	–CH₃	NH-C₆H₄-OCF₃	12.6	>100	>100
16	neopentyl (tert-butyl-CH₂)	–CH₃	NH-C₆H₄-OCF₃	1.05	0.355	>100
17	cyclohexylmethyl	–CH₃	2,2-dimethyl-5-fluoroindoline	0.080	>100	>100
18	cyclohexylmethyl	–CH₃	3,3-dimethyl-5-fluoroindoline	0.019	>100	>100
19	cyclohexylmethyl	cyclopropyl	3,3-dimethyl-5-fluoroindoline	0.019	>30	>30
20	cyclohexylmethyl	–CH₃	3,3-spiro-cyclopropyl-5-fluoroindoline	0.122	>30	>30

prime side indoline has on binding, as the subtle variation to the 3,3-*spiro*-cyclopropyl indoline resulted in a 6-fold drop-off in potency.

Exploration of the SAR in P3 was done with direct analogs of compound **18**, as the 5-fluoro-3,3-dimethylindoline P1′ group was considered to confer the optimal properties for both potency and selectivity. The synthesis of these P3 analogs is shown in Scheme 2. Protection of α-hydroxyacid **4a** as the benzyl ester **21** was followed by conversion to the *para*-nitrophenylcarbonate **22**. The activated carbonate **22** was condensed with diamine **7d**,
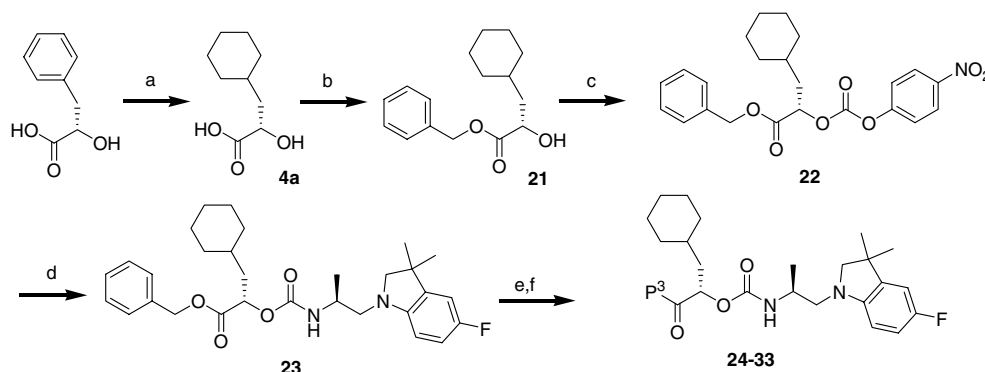
Scheme 2. Reagents and conditions: (a) H_2, 1000 psi, 5% Rh/C, 10% aq EtOH, 50 °C, 18 h, 90%; (b) $PhCH_2Br$, Et_3N, DMF, rt, 87%; (c) 4-nitrophenylchloroformate, pyridine, THF, 60 °C, 83%; (d) **7d**, DIEA, THF, 57%; (e) H_2 (40 psi), 5% Pd/C, EtOH, 94%; (f) P3 amine, HATU, DIEA, DMF, 63–87%.

Table 2. Inhibition of cathepsin S by P3 analogs[a]

Compound	P3	Cat S K_i (μM)
18	morpholine	0.019
24	dimethylmorpholine	0.297
25	MeO–CH2CH2–N(Me)	0.172
26	Me_2N–	0.227
27	azetidine	0.217
28	pyrrolidine	0.056
29	piperidine	0.039
30	thiomorpholine	0.023
31	thiomorpholine sulfone	0.422
32	H_3C–CO–piperazine	0.986
33	H_3C–SO_2–piperazine	1.08

debenzylated under hydrogenolysis conditions, and then reacted with the desired P3 amine using standard peptide coupling conditions to afford compounds **24–33**.

The SAR of a selected set of P3 analogs is shown in Table 2. All of the compounds listed in Table 2 were inactive against both cathepsins K and L in the concentration range tested ($K_i > 100$ μM), so only the cathepsin S inhibition constants are reported. Modification of the morpholine ring by addition of methyl groups (**24**) or replacement with a linear amide (**25**) was deleterious, leading to a 10-fold or more drop-off in activity. Likewise, the simpler dimethylamide (**26**) and azetidine amide (**27**) were roughly equipotent with **25**. However, increasing the ring size to the pyrrolidine (**28**), piperidine (**29**), and thiomorpholine (**30**) returned most of the inhibitory activity as compared to compound **18**. A six-membered ring is clearly preferred in the hydrophobic S3 pocket, with the enzyme being particularly sensitive to subtle changes in polarity of this P3 group. Although we have seen empirically from recent SAR involving a more peptidic scaffold that the S3 pocket can accommodate much larger hydrophobic P3 groups,[8] it is interesting to note that a further increase in the polarity and steric bulk at the 4-position was detrimental to the Cat S inhibitory activity, as was exemplified with sulfone **31** and substituted piperazines **32** and **33**.

The pharmacokinetic profiles of carbamates **17** and **18** were determined in male Wistar rats, and the data are presented in Table 3 alongside the arylaminoethyl amide analog **1b** for direct comparison of the two scaffold types. Despite its similarly high clearance ($C_1 = 52$ mL/min/kg), carbamate **18** exhibited an increased terminal half-life ($t_{1/2} = 100$ min), higher steady state volume of distribution ($V_{ss} = 4.10$ L/kg), and good oral bioavailability ($F = 42\%$), which was an order of magnitude improvement over its amide analog **1b** ($F = 4\%$). The high clearance for each of these compounds was suggestive of extensive first-pass metabolism. A detailed metabolite ID study following 30 min incubation in rat liver microsomes confirmed the primary metabolic prod-

Table 3. Pharmacokinetic data for selected analogs[a]

Compound	Single iv dose (3 mg/kg)			Single po dose (10 mg/kg)			
	$t_{1/2}$ (min)	C_l (mL/min/kg)	V_{ss} (L/kg)	AUC (min µg/mL)	C_{max} (nM)	$T_{1/2}$ (min)	F (%)
1b	43	52	1.76	8	118	51	4
17	82	52	3.10	44	513	62	23
18	100	52	4.10	81	774	69	42

[a] Pharmacokinetic data in male Wistar rats, where values are means of three individual experiments.

ucts arose from hydroxylation of the cyclohexyl P2 moiety and oxidation/dehydration on the indoline ring.

In summary, this report details the optimization of arylaminoethyl amides **1a** and **1b** by transforming the peptidic main chain into a carbamate scaffold, resulting in potent and highly selective cathepsin S inhibitors such as **10** and **18**. Moreover, this transformation resulted in a significant improvement in oral bioavailability, as compound **18** demonstrated a 10-fold enhancement over earlier lead **1b**. Subsequent reports will detail the logical progression of the medicinal chemistry efforts to further improve upon the pharmacokinetic and physicochemical properties of this series of cathepsin S inhibitors.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.07.032.

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15. Synthesis of compound **2**: (a) **4a**, **7b**, HATU, DIEA, DCM, 57%; (b) 4-morpholinecarbonyl chloride, NaH, DMF, rt, 42%.
16. Experimental details of the enzymatic assays and the dilution-dialysis experiment with compound **18** are included in the Supplementary material.
17. Diamines **7a**–**e** were prepared as previously described in Refs. 9–11, and the *spiro*-cyclopropyl indoline in diamine **7e** was synthesized as indicated below:
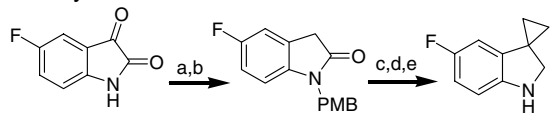
 (a) NaH, PMBCl, DMF, 0 °C, 82%; (b) H₂NNH₂, EtOH, reflux, 90%; (c) NaH, 1,2-dibromoethane, DMF, 0 °C, 61%; (d) TFA, 60 °C, 75%; (e) LAH, 0 °C, 60%.