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Arylaminoethyl carbamates as a novel series of potent and selective cathepsin S inhibitors

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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.

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 optimization 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.

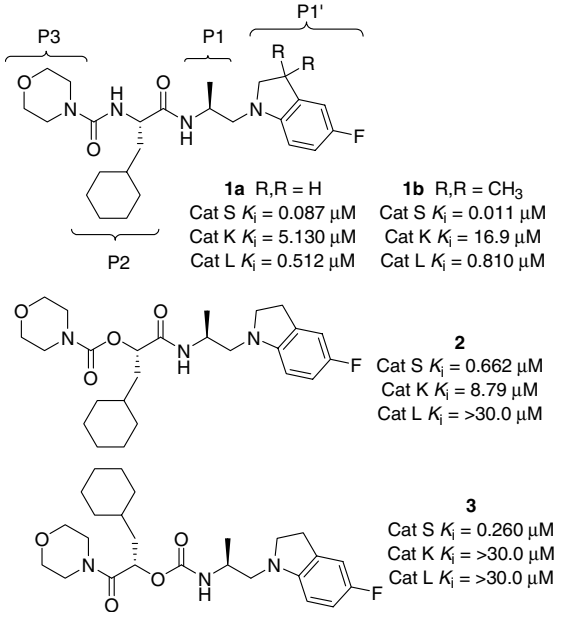

Figure 1.

Keywords: Cathepsin; Cysteine protease inhibitor; Non-covalent inhibitor; Peptidomimetic.
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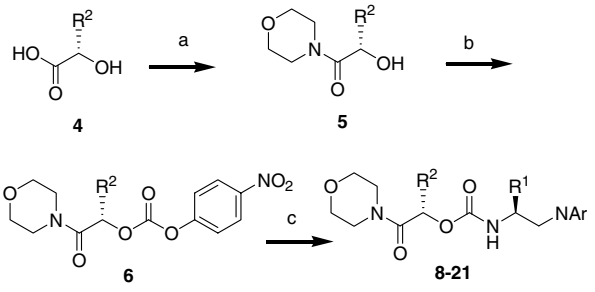
Medicinal chemistry optimization efforts have been aided by structural data that we and others have reported involving co-crystal structures of active-site inhibitors bound to cathepsin S.[10–14] In peptidic Cat S inhibitors such as arylaminoethyl amides[10,11] or dipeptide nitriles,[13] the P2 amide-NH 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 μM), 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 μM), 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 μM). 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 cross-talk 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**



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%.

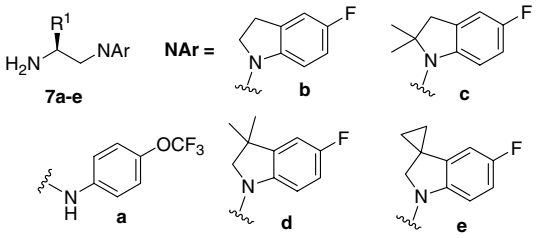


Figure 2. *N*-Aryl diamines (Ref. 16).

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 μM) 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-fluoroindoline **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