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Identification of a novel class of succinyl-nitrile-based Cathepsin S inhibitors

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Abstract—The synthesis and in vitro activities of a series of succinyl-nitrile-based inhibitors of Cathepsin S are described. Several members of this class show nanomolar inhibition of the target enzyme as well as cellular potency. The inhibitors displaying the greatest potency contain *N*-alkyl substituted piperidine and pyrrolidine rings spiro-fused to the α-carbon of the P1 residue.

Antigen processing and presentation is a process during which foreign proteins are degraded into smaller peptides by antigen presenting cells (APCs) and then delivered to the cell surface in association with major histocompatibility complex (MHC) class II molecules.[1] The recognition of the MHC class II/peptide complex by $CD4^+$ T cells triggers an immune response.[2] The MHC class II molecules are associated in the endoplasmic reticulum with invariant chain (Ii) which, acting as a chaperone peptide,[3] mediates the delivery of the MHC class II–Ii complexes into the endocytic compartment where the antigenic peptides are generated. The invariant chain must be proteolyzed prior to MHC binding of the antigenic peptides. The proteolysis of the invariant chain involves the participation of endosomal/lysosomal proteases, such as aspartate and cysteine proteases.[4]

Cathepsin S (Cat S) is a 24 kDa monomeric cysteine protease, which is a member of the papain super-family.[5] Cat S is involved in the last step of the proteolysis of Ii, where it degrades the remaining invariant chain p10 oligomer (Iip10) to a small peptide called CLIP. CLIP is subsequently released from the MHC class II binding groove by association with a third protein called HLA-DM.[6,7] Due to its involvement in the antigen processing and presentation process, Cat S became an attractive target for medicinal chemists as inhibition may modulate autoimmune diseases such as rheumatoid arthritis (RA) and multiple sclerosis (MS).[8]

The catalytic site of Cat S contains a thiolate anion formed by the Cys25 and His164 pair. Nucleophilic addition of the thiolate anion at the carbonyl carbon of the peptide bond followed by hydrolysis produces the proteolyzed Ii. Most cysteine protease inhibitors reported in the literature are peptide based and act through the chemical interaction of an electrophilic 'warhead' with the thiolate anion of the active site.[9,10]

We previously reported the design and synthesis of such inhibitors of Cat S (Fig. 1) and their inhibitory activities as well as their cellular potencies. From this study we ascertained that the nitrile 'warhead' is critical for potency against Cat S.[11] It was thought that such dipeptide inhibitors may suffer pharmako-kinetic limitations commonly seen with peptidic compounds. With an attempt to reduce peptide character of our first series our search

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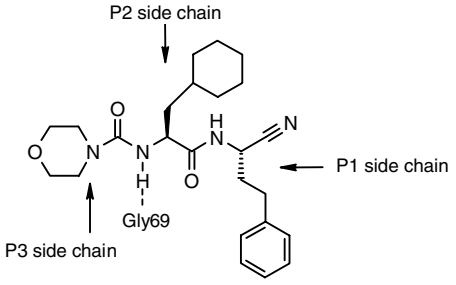


Figure 1. Dipeptide based inhibitor of Cathepsin S.

for new and novel Cat S inhibitors led us to a series of succinate-based compounds (Fig. 2) which are reported herein.

From our earlier work on peptidic inhibitors,[11,12] we were aware of the importance of the hydrogen bond established between the P2 amide hydrogen and the carbonyl of Gly69 of the protein. However, we envisioned overcoming any loss in binding potency caused by the removal of this key hydrogen bond interaction through structural modifications of the P1 and P2 side chains.

During the course of this study several structural models and X-ray structures of Cat S complexed with inhibitors were elucidated.[11] The structural information was particularly helpful in guiding the design and optimization of the side chains. We also took advantage of structure–activity relationships generated from the peptide series.[11,13]

To examine the optimal substitution in the P2 side chain, several compounds were made using alkylated succinic acid derivatives which were either commercially available or synthesized using the general process described in Scheme 1.

The requisite acid chlorides **6** were synthesized from carboxylic acids **1** using a standard malonate ester homologation sequence. Reduction of the carboxylic acid **1** with lithium aluminum hydride gave the primary alcohol **2**. Compound **2** was converted to bromide **3** using hydrobromic acid. Reaction of **3** with the anion of diethyl malonate gave malonic ester **4** which was hydrolyzed and decarboxylated to the corresponding carboxylic acid **5** using potassium hydroxide. Reaction of **5** with oxalyl chloride gave the acid chloride **6**.

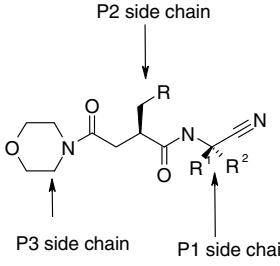
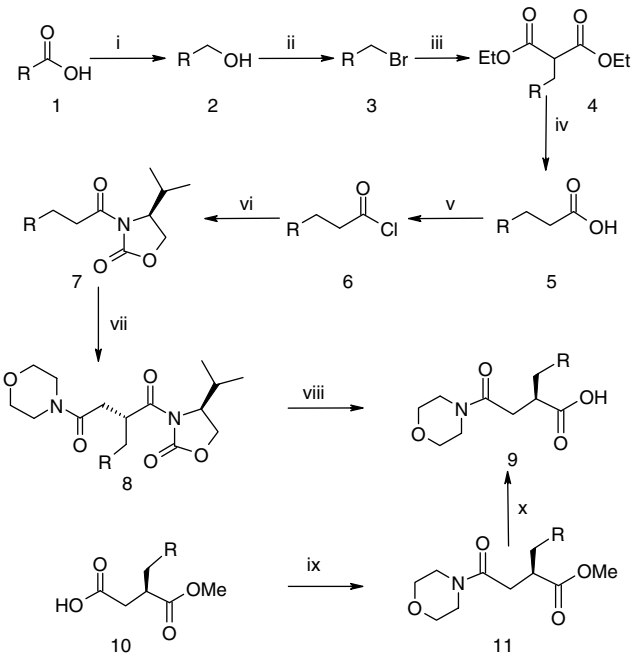


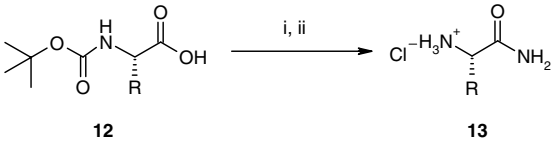
Figure 2. Succinate-based inhibitor of Cathepsin S.



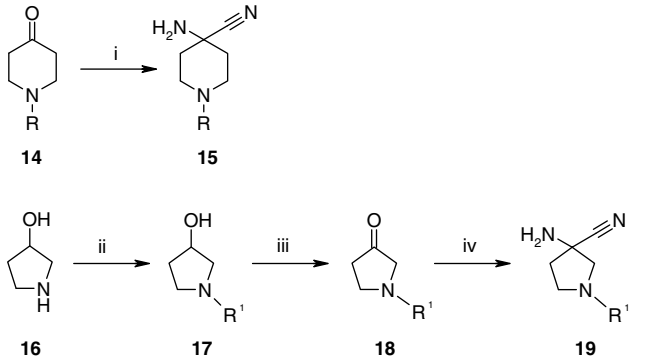
Scheme 1. Reagents and conditions: (i) $LiAlH_4$, Et_2O (98%); (ii) HBr, H_2SO_4 (82%); (iii) NaOEt, diethyl malonate (47%); (iv) KOH, MeOH, 180 °C (89%); (v) oxalyl chloride, CH_2Cl_2 (43%); (vi) (*S*)-(−)-4-isopropyl-2-oxazolidinone, *n*-BuLi, THF (77%); (vii) NaHMDS (1 M in THF), 2-bromo-1-morpholin-4-yl-ethanone, THF (17–47%); (viii) LiOH, H_2O_2, THF, H_2O (80%); (ix) morpholine, EDC, HOBt, DMF, 0 °C–rt (99%); (x) LiOH, THF, MeOH/H_2O (76%). The yields reported here were for R = 2-indanyl.

The absolute stereochemistry of the P2 side chain was set using Evans' chiral oxazolidine methodology.[14,15] The chiral auxiliary was introduced by reacting acid chloride **6** with the lithium anion of (*S*)-(−)-4-isopropyl-2-oxazolidinone. The alkylation of **7** with 2-bromo-1-morpholin-4-yl-ethanone, which was prepared via the reaction of morpholine with bromoacetic anhydride, provided the desired compound **8** which was identified by ^1H NMR as a single diastereomer. Oxidative hydrolysis of the chiral auxiliary gave the key carboxylic acid intermediate **9**. Compound **9** could also be obtained through amide coupling of morpholine with commercially available succinic acid derivatives **10** followed by hydrolysis of the resulting methyl esters **11**.

Several P1 amino amides **13** were prepared by treatment of the commercially available *N*-Boc-amino acids **12** with EDC, HOBt, and NH_4OH followed by Boc removal with HCl (Scheme 2). Additional P1 residues, amino nitriles **15** and **19** identified from our earlier work



Scheme 2. Reagents and condition: (i) EDC, HOBt, NH_4OH, DMF, 0 °C–rt (90%); (ii) HCl (4 N in 1,4-dioxane) (>99%). The yields reported here were for R = CH_2CH_2Ph.



Scheme 3. Reagents and conditions: (i) NH_4Cl, KCN, H_2O, 60 °C (53%). The yield reported here was for R = Me; (ii) aldehydes or ketones, AcOH, $Na(OAc_3)BH$, THF (92%); (iii) oxalyl chloride, DMSO, CH_2Cl_2, Et_3N, −78 °C–rt (56%); (iv) NaCN, NH_4Cl, NH_3 (2.0 M in MeOH), MgSO4, 60 °C (51%). The yields reported here were for R^1 = 3,3-dimethyl-butyl.

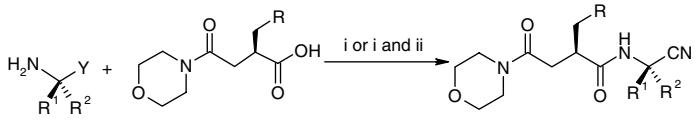
on dipeptide inhibitors,[13] were synthesized from commercially available cyclic piperid-4-ones **14** and pyrrolidin-3-ol **16**, respectively (Scheme 3). In the first case, Strecker reaction of commercially available ketone **14** with ammonium chloride and potassium cyanide in water gave compound **15**. In the second case, reductive amination of the pyrrolidin-3-ol **16** with aldehydes or ketones afforded **17** in high yields. Swern oxidation of **17** gave ketones **18**. Strecker reaction of **18** with ammonium chloride, ammonia, and potassium cyanide in methanol gave amino-nitriles **19** in good yields.

The targeted inhibitors **20** were then prepared via standard peptide coupling conditions of **9** and amino nitriles **15**, **19** or amino-amide salts **13** (Scheme 4). In the latter case the intermediate amide products were dehydrated using cyanuric chloride. Further experimental details of the preparation of these succinate inhibitors of Cat S are available in the patent literature.[16]

The synthesized succinates were tested in a Cat S binding assay as described in our previous published studies[11] and in a similar cellular assay to that developed by Riese et al.[17] in which the presence or absence of the p10 invariant chain fragment (the natural Cathepsin S substrate) was determined and the *minimal inhibitory concentrations* (MICs) at which the p10 band can be detected were reported.

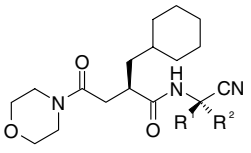
Our optimization work began at the P1 site and the results are summarized in Tables 1 and 2. The P2 side chain was kept as cyclohexylmethyl, which was previously determined to be a preferred P2 substituent.[11] Table 1 shows several P1 substituents that were selected and incorporated in the succinate series based on previous dipeptides SAR.[11,13] Compound **21b** with benzyloxy-methyl as P1 showed an improvement over **21a** in the binding activity and gave a hint of cellular potency. Compound **21e** with a piperidine based P1 group showed potent cellular activity despite poorer binding affinity; the boost in cellular activity could be explained by the presence of the basic nitrogen in **21e** as identified in our previous investigations.[13] Several N-substitution variants, such as **21f**, were prepared but they showed no improvement over **21e**.

Further investigations were performed using N-substituted pyrrolidines as P1 groups. These compounds are generally more potent in both the binding and cellular



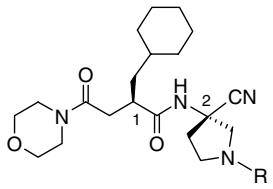
Scheme 4. Reagents and conditions: (i) EDC, HOBt, Hunig's base , DMF, 0 °C–rt (69%); (ii) cyanuric chloride, DMF, 0 °C (57%). The yields reported here were for compound **22h**.

Table 1. SAR of the *P*1 side chain

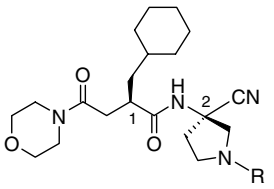


Compound	R^1	R^2	IC_{50} (nM)	K_d (nM)	[p10 MIC][17](nM)
21a	H	$-CH_2CH_2Ph$	171–187	25	5000
21b	H	$-CH_2OCH_2Ph$	37–63	4.39	1000
21c	H	$-CH_2OCH_2(p\text{-Cl})Ph$	62	4.86	5000
21d	$-CH_2CH_2-$		135–154	12	5000
21e	$-CH_2CH_2NMeCH_2CH_2-$		251–641	177	50
21f	$-CH_2CH_2N(CH_2Ph)CH_2CH_2-$		207–347	52	1000

Table 2. SAR of the *P*1 side chain



22 (1R, 2S)

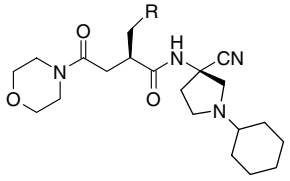


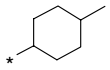
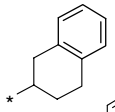
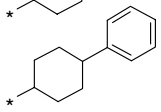
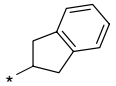
22 (1R, 2R)

Compound	Configuration[a]	R	IC_{50} (nM)	K_d (nM)	[p10 MIC][17] (nM)
22a	(1*R*,2*R*)	H	141	123	100
22b	(1*R*,2*R*)	Me	155–172	161	1000
22c	(1*R*,2*R*)	*i*-Pr	66–82	60.6	100
22d	(1*R*,2*R*)	$-CH_2CH(CH_3)_2$	39–89	55.3	1000
22e	(1*R*,2*R*)	$-CH_2CH(CH_2CH_2)$	22–39	20	100
22f	(1*R*,2*R*)	Cyclopentyl	75–78	n.d.	100
22g	(1*R*,2*R*)	Cy	23–49	17.8	50
22h	(1*R*,2*S*)	$-CH_2Cy$	41–79	13.8	100
22i	(1*R*,2*R*)	Cycloheptyl	23–35	11	100
22j	(1*R*,2*S*)	$-CH_2Ph$	29–31	3.64	100
22k	(1*R*,2*R*)	$-CH_2CH_2Ph$	22.32	15.2	100

[a] The diastereomers were separated by reverse-phase HPLC. The assignment of relative stereochemistry was performed by analogy based on the assignments of similar analogues in the dipeptide series.[13] X-ray crystal structure determination of inhibitor **22h** co-crystallized with Cathepsin S confirmed our assignment of the relative stereochemistry.

Table 3. SAR of the *P*2 side chain



Compound	R	IC_{50} (nM)	K_d (nM)	[p10 MIC][17] (nM)
22g		23–49	17.8	50
23a		47–62	77.3	100
23b		14–17	13.8	100
23c		1.9	5.4	100
23d		3136	nd[a]	nd
23e		63–74	59	100
23f		42	23.1	100

[a] nd, not determined.

assays. A variety of N-substituents investigated are well tolerated. This exploration revealed **22g** as the best inhibitor in this series in terms of binding and cellular potency Table 3.

Next, we sought to optimize compound **22g** by fine-tuning the cyclohexylmethyl P2 residue. Several substituted cyclohexyl and related P2 side chains were incorporated in an attempt to properly fill the lipophilic S2 pocket of the enzyme and enhance the binding and cellular potency of this series. This exercise demonstrated that a variety of sterically demanding groups are tolerated at the P2 position and identified inhibitor **23c** (K_d = 5 nM) as the most potent Cat S inhibitor in the series in terms of binding. However, we did not observe a corresponding increase in cellular potency.

In conclusion, this study shows that the novel succinate scaffold approach was successful in providing several potent and cellularly active Cat S inhibitors such as **22g** and **23c**. These compounds are being evaluated in other assays and the results will be reported in due course.

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