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Synthesis and SAR of succinamide peptidomimetic inhibitors of cathepsin S

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Abstract—Peptidic, non-covalent inhibitors of lysosomal cysteine protease cathepsin S (**1** and **2**) were investigated due to low oral bioavailability, leading to an improved series of peptidomimetic inhibitors. Utilizing phenyl succinamides as the P2 residue increased the oral exposure of this lead series of compounds, while retaining selective inhibition of the cathepsin S isoform. Concurrent investigation of the P1 and P2 subsites resulted in the discovery of several potent and selective inhibitors of cathepsin S with good pharmacokinetic properties due to the elimination of saturated aliphatic P2 residues.

Cathepsin S (Cat S) is a papain-like cysteine protease that is expressed in the lysosome of antigen presenting cells such as macrophages, dendritic cells, and B cells. A key enzymatic function of cathepsin S is the targeted degradation of the invariant chain that is associated with the MHC class II complex.[1–3] This proteolytic degradation step is required prior to productive loading of an antigen onto the MHC II complex.[4] Cat S deficient mice exhibit a resistance to the development of autoimmune diseases such as experimental autoimmune myasthenia gravis[5] and collagen-induced arthritis[6] in comparison to wild type mice, suggesting Cat S may be an attractive therapeutic target for immunosuppression.[7]

As part of a broader program to discover novel cathepsin S inhibitors,[8–10] we recently reported the discovery of potent non-covalent inhibitors of cathepsin S arylaminoethyl amides such as **1**.[11] This compound is a potent Cat S inhibitor, but in general compounds bearing a P3 morpholine urea displayed limited Cat L selectivity (Fig. 1) and poor to moderate pharmacokinetic properties (Table 1). This was due in part to the cyclic aliphatic P2 residue and the inhibitors' peptidic nature. Interestingly, reducing the peptidic nature of **1** by removal of the P2 NH hydrogen bond donor led to a selective (albeit significantly less potent) Cat S inhibitor. We reported recently that the putative hydrogen bond formed between the P2 urea NH in compound **1** and the Cat S Gly69 carbonyl is not an essential interaction, as demonstrated by related analogs of compound **2**.[12] We felt that we could improve the interactions in the

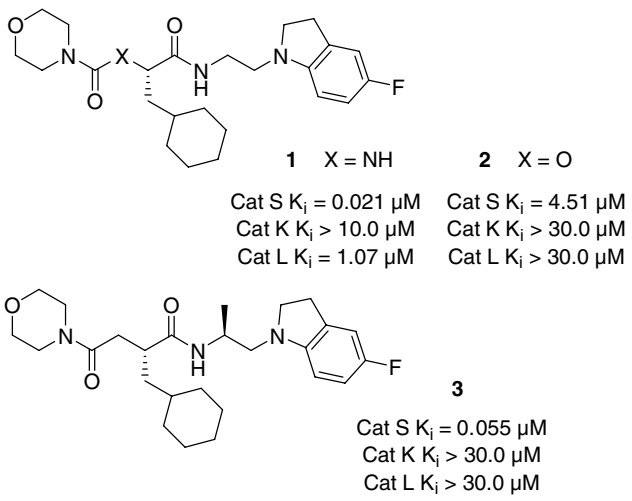

Figure 1.

Keywords: Cathepsin S; Lysosomal proteases; Succinamides.
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Table 1. Pharmacokinetics of succinamide derivatives[a]

Compound	AUC (h*nM)	C_{max} (nM)	T_{max} (h)	$T_{1/2}$ (h)
1	312	140	0.33	1.12
3	48	24	0.42	1.12
9	2778	983	1.5	0.87
11	752	220	1.83	1.23
12	831	241	1.7	1.06
14	2774	815	2.0	1.22
17	806	318	1.0	0.68
31	1914	647	0.7	0.92
32	1871	629	1.67	0.97
33	2821	720	2.0	1.82
34	2036	789	1.0	1.06
36	4629	946	2.0	2.26

[a] Pharmacokinetic data in fasted male Wistar rats (10 mpk, single dose), where the values are means of three individual experiments.

P1 and P2 subsites and could recapitulate the Cat S potency without the P2 putative H bond.

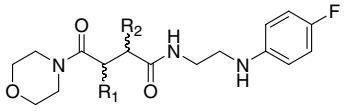
Given this important result of compound **2**, a parallel effort employing a succinamide P2 moiety was initiated. The succinic acid scaffold has previously been reported in various protease inhibitors such as TACE and HDAC inhibitors to reduce their peptidic characters and improve oral bioavailability.[13] Our initial efforts to utilize the succinamide scaffold resulted in **3**, a potent Cat S inhibitor (K_i = 55 nM) with excellent selectivity over related cathepsin K and L. In addition to determination of biochemical activities,Table 1 illustrates our parallel effort to evaluate the oral pharmacokinetic properties of the inhibitors. We employed a 'rapid-rat' protocol where only the oral dosing arm was performed.[14] As demonstrated in Table 1, the oral exposure of lead compound **3** was lower than that of **1**, but the observed selectivity over Cat L prompted us to concurrently investigate the SAR and the oral bioavailability of this series.

We initially investigated the regiochemistry and stereochemistry of the succinamide P2 unit (Table 2). The P2 substituent can access the S2 pocket from either carbon of the succinamide backbone (Table 2, compounds **4** and **6**). The absolute configuration is required to be *R*. It is noteworthy that all of these compounds were completely selective for Cat S over Cat L and Cat K. While these initial results were encouraging, the moderate potency of **4** and **6** and the poor oral exposure of **3** required us to investigate P1 and P2 substitutions that would have a good selectivity profile and improved bioavailability.

A systematic parallel investigation of P1 and P2 residues is depicted in Table 3. Our previous experience on P1 optimization demonstrated a broad-SAR landscape in this pocket (Table 3) using a fixed P2 and P3.[9] Cyclohexylmethyl succinamides were synthesized from the commercially available succinic acid **i** and P1 diamine **ii**[9–11] as shown in Scheme 1. Addition of a small P1 alanine sidechain improves potency by 6-fold (Table 3, compound **9**). Replacement of the indoline moiety in **3** with *p*-trifluoromethoxy aniline **9** dramatically improved the oral exposure (Table 1) and is consistent with results obtained with related analogs of **1**.[11] Other P1 alkyl groups increase Cat S inhibitory activity, but also increase potency significantly on the K and L cathepsin isoforms and lower oral exposure, such as **11** and **12** (Table 1). Of particular interest are aromatic P1 sidechains such as phenethyl analog **13** and phenyl derivative **14** which has a good selectivity profile. The phenylglycine derived P1 **14** also retained similar oral exposure to **9** (Table 1). Addition of a polar functionality as in the methionine sulfones **15** and **16** also improves potency while improving aqueous solubility, albeit with reduced selectivity. Interestingly, the unnatural *R* configuration in the P1 subsite of **16** is also active.

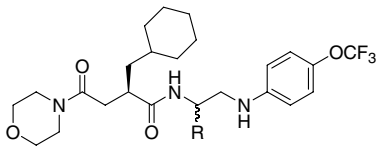
Table 4 outlines the significant P1/P2 cross-talk in determining the potency and selectivity within this succinate series of cathepsin S inhibitors. One of the key goals of this effort was to eliminate the metabolic liabilities of the cyclohexylmethyl P2 group while still utilizing our previously established SAR with α-amino acids.[8] Detailed metabolite ID studies of a number of analogs from this series suggested the main metabolic products are due to hydroxylation, particularly on the P2 moiety. The synthesis of non-commercially available succinic acids is depicted in Scheme 2 and starts from their corresponding carboxylic acids using Evans oxazolidinone methodology.[15] The first set of analogs reduced the cyclohexyl group by one methylene unit to lessen the likelihood of metabolic oxidation. Interestingly, replacement of the cyclohexyl group in **9** with a cyclopentyl analog **17** did not change Cat S potency, but greatly reduced oral exposure contrary to our original hypothesis (Table 1). There was no significant difference in

Table 2. Inhibition of cathepsin S, K, and L—initial optimization of P2

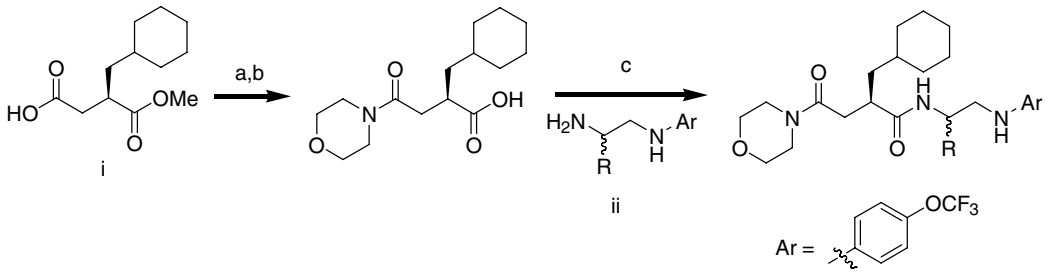


Compound	R_1	R_2	K_i (μM)		
			Cat S	Cat K	Cat L
4	H	(*R*)-CH_2-*c*-Hex	1.368	>30	>30
5	H	(*S*)-CH_2-*c*-Hex	>100	>100	>100
6	(*R*)-CH_2-*c*-Hex	H	1.721	>100	>100
7	(*S*)-CH_2-*c*-Hex	H	>100	>100	>100

Table 3. Inhibition of cathepsin S, K, and L—optimization of P1

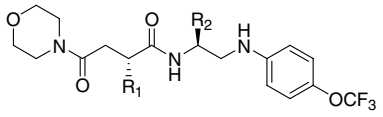


Compound	R	K_i (μM)		
		Cat S	Cat K	Cat L
8	H	0.194	>100	>30
9	(*S*)-Me	0.028	24.29	18.9
10	(*S*)-Et	0.016	0.907	0.267
11	(*S*)-*n*-Pr	0.013	0.557	0.285
12	(*S*)-*i*-Pr	0.011	0.995	0.193
13	(*S*)-CH_2CH_2Ph	0.018	1.48	0.636
14	(*S*)-Ph	0.062	>30	3.52
15	(*S*)-$CH_2CH_2SO_2Me$	0.005	0.26	0.098
16	(*R*)-$CH_2CH_2SO_2Me$	0.069	5.41	1.52



Scheme 1. Reagents and conditions: (a) HATU (1.2 equiv), Morpholine (2.0 equiv), CH_2Cl_2, 23 °C; (b) LiOH (1.2 equiv), MeOH/H_2O (2:1) 23 °C; 77% over 2 steps; (c) **ii** (1.2 equiv), DIPEA (3.0 equiv), CH_2Cl_2, 23 °C; 50–70%.

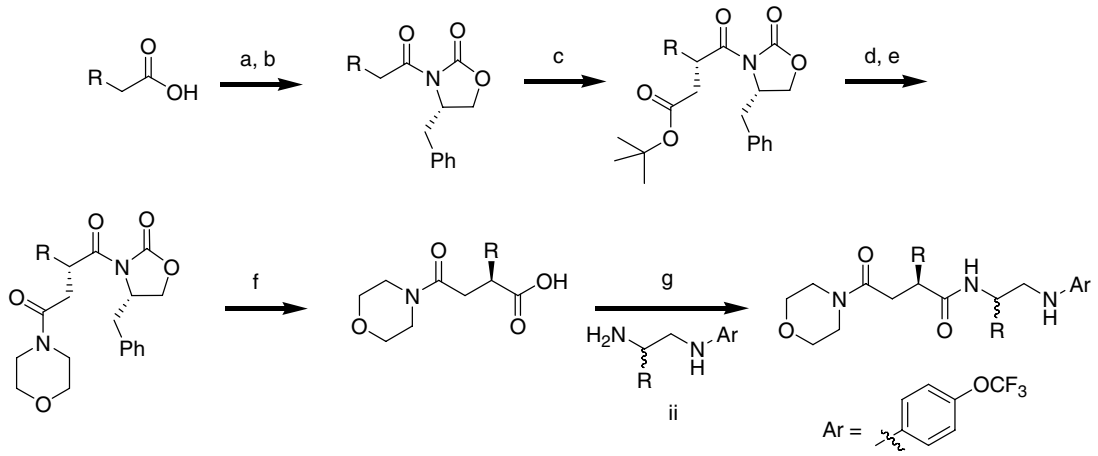
Table 4. Inhibition of cathepsin S, K, and L—optimization of P2/P1 cross-talk



Compound	R_1	R_2	K_i (μM)		
			Cat S	Cat K	Cat L
17	CH_2cyclopentyl	CH_3	0.031	3.706	>30
18	CH_2cyclopentyl	*i*-Pr	0.038	1.14	8.99
19	CH_2cyclopentyl	*n*-Pr	0.012	0.217	2.678
20	CH_2CH_2cyclohexyl	CH_3	0.095	3.706	>30
21	CH_2CH_2cyclopentyl	*i*-Pr	0.011	>26.4	0.777
22	CH_2CH_2cyclopentyl	CH_2CH_2Ph	0.020	>30	2.31
23	CH_2-*t*-Bu	CH_3	0.408	2.331	>100
24	CH_2CH_2-*t*-Bu	CH_3	0.070	>100	>100
25	CH_2Ph[a]	CH_3	>24	>30	8.86
26	CH_2CH_2Ph	Et	0.060	0.291	0.588
27	CH_2CH_2Ph	*i*-Pr	0.027	0.184	0.372
28	$CH_2CH_2CH_2Ph$	*i*-Pr	0.014	2.117	0.197
29	Ph	CH_2CH_2Ph	0.037	0.710	3.936
30	Ph	Ph	0.261	10.54	48.37
31	Ph	*i*-Pr	0.025	11.53	11.53

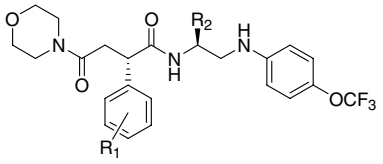
[a] 5-Fluoroindoline used instead of 4-trifluoromethoxyaniline portion.

potency in P1 SAR in the cyclopentylalanine P2 series (compounds **17**–**19**), and while an *n*-propyl P1 unit (**19**) improved potency, an isopropyl unit in P1 did not (**18**). This small P2 modification has a significant effect on the associated P1 SAR, which we observe repeatedly in this series: a homocyclohexyl group (**20**), which was



Scheme 2. Reagents and conditions: (a) $SOCl_2$ (1.2 equiv), CH_2Cl_2, cat. DMF, 23 °C, 100%; (b) (*S*)-benzyloxazolidinone (1.0 equiv), *n*-BuLi (1.0 equiv), cat. Ph_3CH, THF, −78 °C, 85–95%; (c) LiHMDS (1.1 equiv), THF, −78 °C, followed by *tert*-butylbromoacetate, −78 °C, 90–100% yield; (d) TFA/CH_2Cl_2 (75:25), 23 °C, 100% yield; (e) HATU (1.0 equiv), morpholine (5.0 equiv), CH_2Cl_2, 23 °C, 92–95%; (f) LiOH (2.0 equiv), H_2O_2 (4.0 equiv), H_2O, THF, 0 °C, 70–80%; (g) **ii** (1.0 equiv), HATU (1.0 equiv), DIPEA (3.0 equiv), 23 °C, 60–70%.

Table 5. Inhibition of cathepsin S, K, and L—phenylsuccinamide P2 SAR



Compound	R_1	R_2	K_i (μM)		
			Cat S	Cat K	Cat L
32	4-F	*i*-Pr	0.024	11.53	11.53
33	4-Cl	*i*-Pr	0.021	>100	>100
34	4-OCH_3	*i*-Pr	0.105	>30	>65
35	4-CH_3	*i*-Pr	0.134	>30	>100
36	4-CF_3	*i*-Pr	0.043	>100	>100
37	3-CF_3	*i*-Pr	18.53	>100	>30
38	2-CF_3	*i*-Pr	>100	>100	>100
39	4-CF_3	CH_3	1.59	>100	>100
40	4-CF_3	Cyclopropyl	0.616	>100	>100
41	4-CF_3	H	15.6	>100	>100

quite potent in the previously reported amino acid P2 series, reduced potency in the corresponding succinamide P2 series.[8,9] Interestingly, the homocyclopentylalanine analog **21** improved potency for cathepsin S inhibition when compared to the cyclopentylalanine analog **18** but also increased Cat L activity. A *tert*-butylalanine unit, which had also been successfully used previously as a α-amino acid P2 residue, reduced Cat S potency in the succinamide series (**23** and **24**).

Since we observed a significant departure in the SAR of the succinamide P2 series from the α-amino acid series, we decided to broaden our hydrophobic P2 scan. Surprisingly, the introduction of a phenylalanine analog **25** is surprisingly inactive on Cat S despite the previously described potency in α-amino acids.[8] Adding methylene units increased Cat S potency, but also increased activity on cathepsin L and K (**26**–**28**). We also eliminated the methylene spacer in **25** to provide a phenylsuccinamide derivative **29** that was quite potent on Cat S. The cross-talk between P1 and P2 residues was quite pronounced, as a valine derived P1 residue provided a highly selective 25 nM Cat S inhibitor (**31**). We were pleased to see that the oral exposure of **31** was improved compared to cycloaliphatic analogs. The small P1 isopropyl group in **31** together with a conformationally constrained phenylsuccinamide P2 residue made this an attractive candidate for further optimization.

Table 5 outlines our SAR efforts to further optimize the phenylsuccinamide unit for improved Cat S inhibitory activity. The syntheses were performed from commercially available phenylacetic acids according to Scheme 2. It should be noted that the phenylsuccinic acids are susceptible to racemization under standard amide coupling conditions, requiring careful monitoring of the reaction, and the exclusive use of methylene chloride in the coupling reactions. The use of small halogens at the para position maintains Cat S potency (**32** and **33**), and these substituted phenylsuccinamides slightly increased oral exposure compared to **31** (Table 1). In

addition, electron-donating substituents such as *p*-methoxy analog **34** or a small aliphatic group analog **35** reduce Cat S potency, without any improvement in oral exposure (Table 1). We found that a strong electron-withdrawing group, such as a trifluoromethyl group **36**, retains Cat S potency while improving selectivity on Cat K and L. The CF_3 group must reside in the *para*-position as demonstrated by inactive isomers **37** and **38**. The isopropyl P1 group has a dramatic effect on Cat S potency as an alanine derived P1 **39** loses 100-fold potency and cyclization of the P1 isopropyl group to cyclopropyl derivative **40** also significantly diminishes Cat S activity. Complete removal of the P1 substituent (**41**) reduces Cat S inhibition by 1000-fold. In addition to favorable hydrophobic interactions in the S1 pocket, the P1 isopropyl group may also be playing a key role in the conformation of the P2 phenylsuccinamide group and its subsequent trajectory into the P2 pocket.[16] We were pleased to find that **36** displayed a 2-fold improvement in the AUC as compared to **31**. Analog **36** was later determined to have a modest oral bioavailability of 34% and represents a ~100-fold improvement in AUC when compared to lead compound **3**.

In conclusion, the use of a succinic acid backbone in non-covalent cathepsin S inhibitors provided a novel set of potent and selective compounds. The replacement of the cyclohexyl P2 moiety with a substituted phenyl group ring improved the selectivity profile and oral exposure, as demonstrated by analog **36**. The use of a 'rapid-rat' protocol allowed for testing a wider variety of analogs to address the low oral exposure of the early compounds and allowed for the parallel optimization of potency, selectivity, and oral exposure.

Acknowledgments

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