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## Active Site Mapping of Human Cathepsin F with Dipeptide Nitrile Inhibitors

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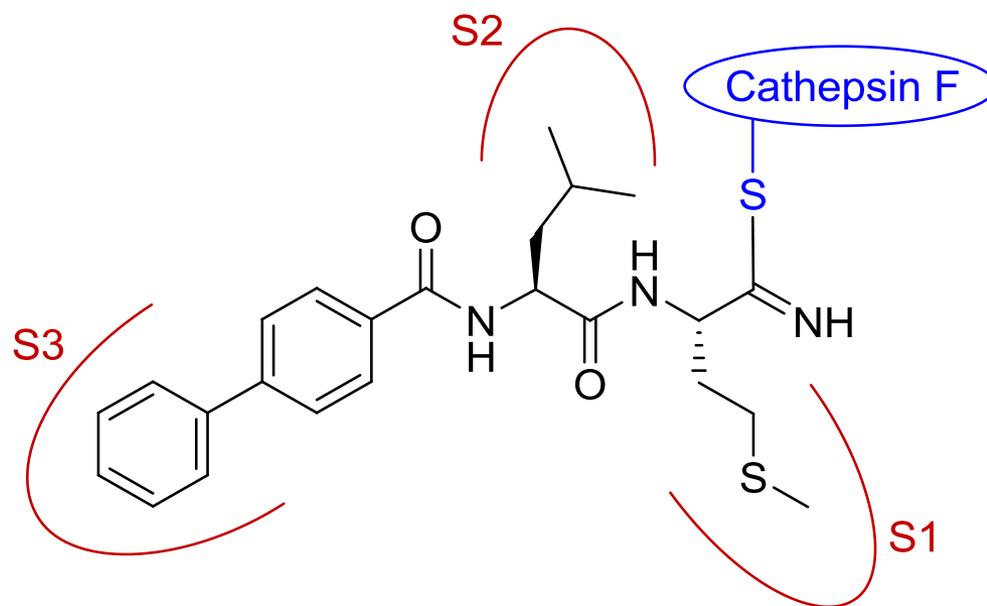
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# Active Site Mapping of Human Cathepsin F with Dipeptide Nitrile Inhibitors

## Graphical Abstract



**Abstract:** Cysteine cathepsins are lysosomal cysteine proteases which play roles in many physiological processes. Cathepsin F is predominantly expressed in macrophages. Major histocompatibility complex class II molecules (MHC-II) are expressed by antigen-presenting cell types including macrophages, B cells, and dendritic cells. The cleavage of the invariant chain is the key event in the pathway of MHC-II complexes. Cathepsin S was described as the major processing enzyme of the invariant chain, but it was shown that cathepsin F can adopt its role in cathepsin S deficient mice. Low molecular weight inhibitors for cathepsin F have not been investigated so far. We have chosen the dipeptide nitrile chemotype to develop covalent-reversible inhibitors for this target.

An active site mapping with a library of 52 nitrile-based cathepsin inhibitors was performed at human cathepsin F to draw structure-activity relationships. With the kinetic data in hand, new compounds with optimized residues in P1, P2 and P3 position were synthesized and evaluated. With all dipeptide nitriles including the newly synthesized derivatives, a 3D activity landscape was generated to visualize similarity-activity relationships of this series of cathepsin F inhibitors.

**Keywords:** cysteine proteases; human cathepsins; nitrile inhibitors ; 3D activity landscapes



# Introduction

Cysteine cathepsins are lysosomal proteases which play crucial roles in various physiological processes, such as bone remodeling, osteoporosis, neurological disorders, autoimmune response and cancer, and represent the largest and best characterized group of cathepsins.<sup>[1,2]</sup> One of its representatives, cathepsin F, is predominantly expressed in macrophages, but not in dendritic cells or B cells.<sup>[3]</sup>

The cleavage of the invariant chain is the key event in the trafficking pathway of major histocompatibility complex class II. Cathepsin S constitutes the major processing enzyme of the invariant chain, but cathepsin F acts in macrophages as its functional synergist which is as potent as cathepsin S in invariant chain cleavage.<sup>[3-6]</sup> The implication of cathepsin F in various physiological conditions should provide the impetus for the development of low-molecular weight inhibitors or activity-based probes as possible therapeutic agents or tool compounds, respectively, to further elucidate the physiological role(s) of this protease.



# Introduction

Given that cathepsins F and S are the most important processing enzymes in antigen-presenting macrophages, their proteolytic synergism might be addressed in the design of inhibitors as potential therapeutics for macrophage-related disorders. Much effort has been made to develop inhibitors for human cathepsin S, which hold potential for the treatment of autoimmune diseases,<sup>[7]</sup> but little is known about inhibition of cathepsin F by low-molecular weight compounds.

In this study, a library of dipeptide nitriles was applied for active site mapping of the non-primed binding region of human cathepsin F. Stepwise structural modifications aimed at developing optimized inhibitors and elucidating structure-activity relationships by means of enzymatic assays and a 3D activity landscape.

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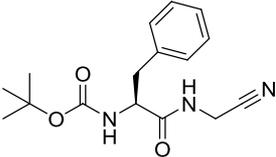
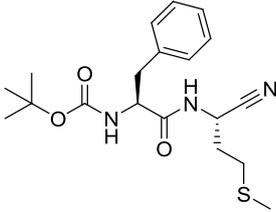
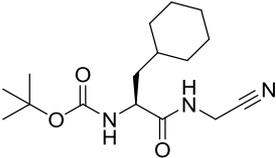
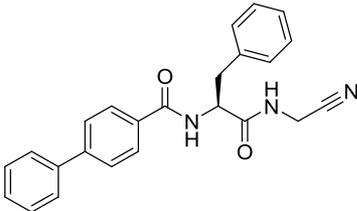
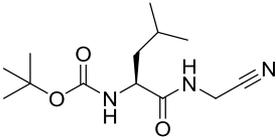
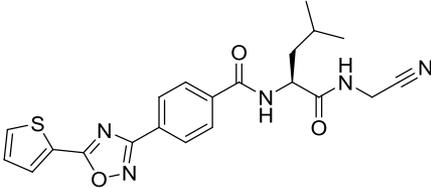
## Results and discussion

As a starting point of this project, a library of 52 dipeptide nitriles<sup>[8-11]</sup> with different aminonitrile moieties in position P1, different amino acids in position P2 and various capping groups at the N-terminus was investigated at human cathepsin F to draw structure-activity relationships (selected compounds **1-6** are shown in Table 1). With the kinetic data in hand, new compounds **7-12** with optimized fragment combinations in R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> position were designed and synthesized. These compounds were evaluated at human cathepsins F, B, L, K and S by performing assays with fluorogenic or chromogenic peptide substrates (Table 2). Dipeptide nitrile **12** ( $K_i = 7.3$  nM) was the most potent cathepsin F inhibitor obtained in the course of this study. Inhibitor **12** is selective for cathepsin F over cathepsins B and L, similarly active against cathepsin S, and even more potent towards human cathepsin K, indicating similar binding properties for peptidic ligands in the S3-S1 pockets of the three proteases.

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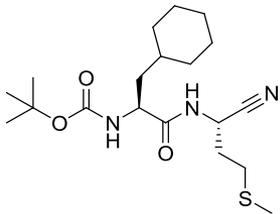
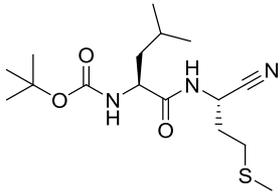
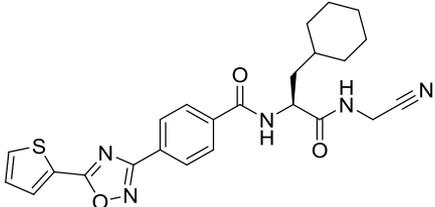
**Table 1.** Inhibition of human cathepsin F by dipeptide nitriles 1-6

compd	$K_i$ ( $\mu\text{M}$ )	compd	$K_i$ ( $\mu\text{M}$ )
1 	8.48 $\pm 0.35$	4 	4.36 $\pm 0.19$
2 	4.40 $\pm 0.15$	5 	0.687 $\pm 0.046$
3 	1.50 $\pm 0.04$	6 	0.0124 $\pm 0.0006$

$\text{IC}_{50}$  values were obtained from duplicate measurements in the presence of five different inhibitor concentrations. Progress curves were followed over 20 min and analyzed by linear regression.  $\text{IC}_{50}$  values were determined by nonlinear regression using equation  $v_s = v_0/(1+[I]/\text{IC}_{50})$ , where  $v_s$  is the steady-state rate,  $v_0$  is the rate in the absence of the inhibitor, and  $[I]$  is the inhibitor concentration. Standard error of the mean (SEM) values refer to this nonlinear regression.  $K_i$  values  $\pm$  SEM were calculated from  $\text{IC}_{50}$  values by applying the equation  $K_i = \text{IC}_{50}/(1+[S]/K_m)$ , where  $[S]$  is the substrate concentration.

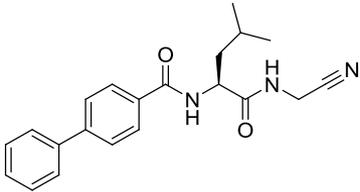
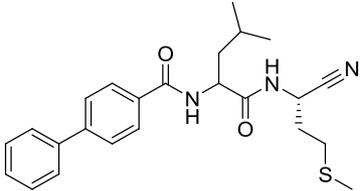
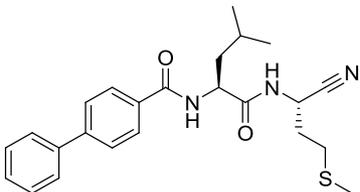


**Table 2.** Inhibition of Human Cathepsins by Dipeptide Nitriles **7-12**

compd	$K_i$ ( $\mu\text{M}$ )				
	cat F	cat B	cat L	cat K	cat S
7 	1.33 $\pm 0.03$	16.5 $\pm 0.8$	1.88 $\pm 0.13$	2.62 $\pm 0.35$	0.203 $\pm 0.008$
8 	0.510 $\pm 0.027$	42.6 $\pm 1.6$	0.854 $\pm 0.085$	0.0288 $\pm 0.0014$	0.0806 $\pm 0.0052$
9 	0.139 $\pm 0.016$	4.94 $\pm 1.31$	0.781 $\pm 0.116$	0.154 $\pm 0.011$	0.106 $\pm 0.009$



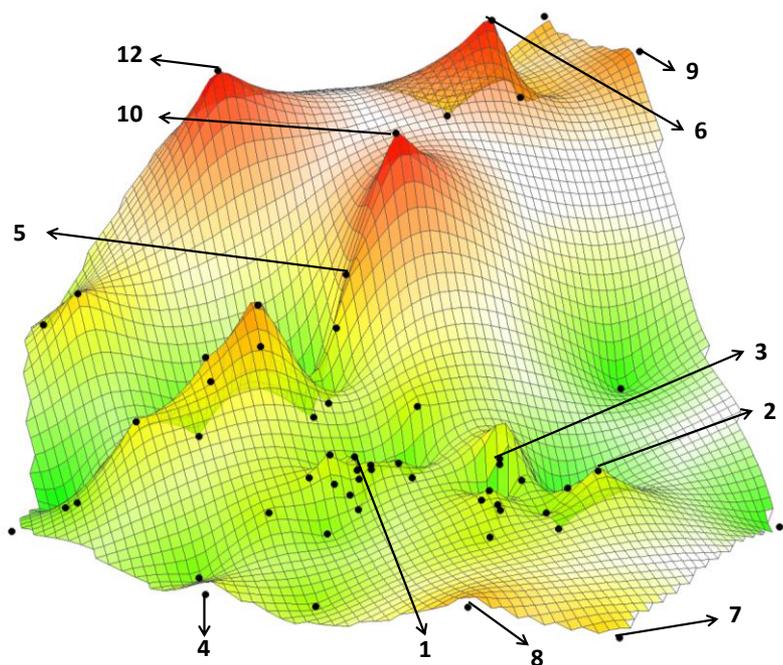
**Table 2.** continued

compd	$K_i$ ( $\mu\text{M}$ )				
	cat F	cat B	cat L	cat K	cat S
10 	0.00779 $\pm 0.00074$	1.79 $\pm 0.14$	1.36 $\pm 0.07$	0.000561 $\pm 0.000048$	0.0387 $\pm 0.0032$
11 	0.0118 $\pm 0.0010$	0.801 $\pm 0.043$	1.03 $\pm 0.05$	0.000479 $\pm 0.000035$	0.0347 $\pm 0.0016$
12 	0.00728 $\pm 0.00063$	0.333 $\pm 0.025$	0.563 $\pm 0.067$	0.000261 $\pm 0.000019$	0.0171 $\pm 0.0021$

For details see Table 1.



With all dipeptide nitriles, including the newly synthesized derivatives, a 3D activity landscape was generated (Figure 1). The activity cliff concept can reveal whether small chemical changes lead to significant potency differences. The inhibitors' affinities clearly reflect the characteristics of the non-primed binding region of human cathepsin F. Compounds in the upper red part of the landscape belong to the most potent inhibitors within this series including **6**, **9**, **10**, and **12**, bearing large aromatic P3 substituents.



**Figure 1.** A 3D activity landscape representation for the cathepsin F inhibitor series. Euclidean fingerprint distances were calculated for 57 inhibitors (compound 11 was not included). The surface is colored from green (lowest potency) to red (highest potency). Black spheres represent compound data points.



## Conclusions

Despite the involvement of human cathepsin F in important physiological and pathophysiological processes, this enzyme has thus far not been a focal point of drug development efforts. This prompted us to perform an active site mapping by employing a library of peptide nitriles, known to interact with cysteine cathepsins in a covalent and reversible manner. A stepwise structural optimization afforded two highly potent cathepsin F inhibitors, **10** and **12**, with  $K_i$  values of less than 10 nM. The structure-activity relationships obtained for 57 dipeptide nitriles have been illustrated by 3D activity landscape representations. The inhibitors' affinities clearly reflect the characteristics of the non-primed binding region of human cathepsin F. Our data and, in particular, the structures of inhibitors **10** and **12** might represent a starting point for the future design of therapeutics against autoimmune diseases. Moreover, these peptidomimetic inhibitors are expected to be valuable tools for investigations on the role of cathepsin F in macrophage-related disorders, atherogenesis or lysosomal storage defects.



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