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Design, synthesis, and evaluation of inhibitors of cathepsin L: Exploiting a unique thiocarbazate chemotype

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Abstract—Recently, we identified a thiocarbazate that exhibits potent inhibitory activity against human cathepsin L. Since this structure represents a novel chemotype with potential for activity against the entire cysteine protease family, we designed, synthesized, and assayed a series of analogs to probe the mechanism of action, as well as the structural requirements for cathepsin L activity. Molecular docking studies using coordinates of a papain–inhibitor complex as a model for cathepsin L provided useful insights.

Human cathepsin L is an endosomal cysteine protease that has been implicated in a variety of physiological and pathophysiological processes.[1–3] Cathepsin L is widely distributed, and plays key roles in bone remodeling and the immune response, as well as in disease states such as cancer,[4] rheumatoid arthritis[5] and osteo-arthritis.[6,7] Furthermore, a number of infectious agents (e.g., Ebola, SARS, and Leishmania) have been reported to require cathepsin L or cathepsin L-like activity for viral processing and infectivity.[8–12] As such, the identification of inhibitors of cathepsin L would provide valuable tools to probe the role of this enzyme in biological systems, as well as to provide potential starting points for drug discovery efforts.

The Penn Center for Molecular Discovery (PCMD), a member of the Molecular Libraries Screening Center Network (MLSCN), has conducted a series of High-throughput Screening (HTS) campaigns of the Molecular Libraries Small Molecular Repository (MLSMR) to identify inhibitors of cysteine (cathepsins B,[13] L, and S) and serine (cathepsin G, Factor XIa, and XIIa) proteases.[14] This effort recently led to the identification[15] and characterization[16] of (−)-**1**, a novel and potent inhibitor of human cathepsin L (Fig. 1).[17]

Most cysteine protease inhibitors require the presence of an electrophilic warhead that provides a site of reaction (either reversible or irreversible) for the active site thiolate. Selectivity and potency are often dictated by the reactivity of the warhead in conjunction with additional binding interactions of the molecule across the enzyme active site. Classic warheads include epoxides, nitriles, activated carbonyls, vinyl sulfones, oxocarbazates, and aza-peptides. [2,18–20] Indeed, incorporation of such warheads has led to cathepsin K and cathepsin S inhibitors currently in clinical trials.[3] Potent inhibi-

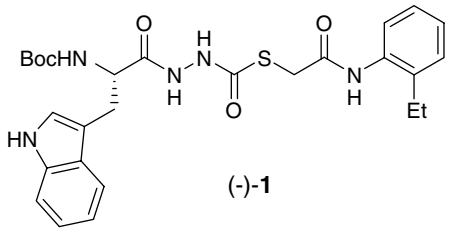

Figure 1. Thiocarbazate cathepsin L inhibitor (−)-**1**.

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tors of cathepsin L that incorporate azepanones and cyanamides have also been described recently.[21,22] To the best of our knowledge, thiocarbazates and their corresponding biological activity have not been described prior to our original report.[15] Since the thiocarbazate core embodies the potential for broad utility as a cysteine protease inhibitor scaffold, we sought to understand further the requirements for activity within this substructure.

In an effort to evaluate the potential binding mode of (−)-**1** with cathepsin L, we performed docking studies using the publicly available X-ray coordinates for papain complexed to a succinyl epoxide inhibitor (1cvz.pdb).[23] The papain model was a relevant model for cathepsin L due to the high degree of sequence homology between the binding sites of these two cysteine proteases. In these studies we observed the simultaneous occupation of the S2, S3, and S1′ subsites by hydrophobic and aromatic functionalities of thiocarbazate (−)-**1** as shown in Fig. 2; the indole side chain occupies the S2 subsite; the –NHBoc group occupies the S3 subsite, and the 2-ethylphenyl aniline occupies the S1′ subsite. A key hydrogen bond is observed between the Gly66 backbone NH and the amino acid derived carbonyl of the diacyl hydrazine. In other inhibitor systems, the absence of a hydrogen bonding interaction between Gly66 and inhibitor has been reported to lead to a loss of inhibition in numerous cathepsins, including cathepsin L.[22] Details of the molecular docking studies are reported elsewhere;[24] however, they suggest that (a) the thiocarbazate carbonyl is in sufficient proximity to the active site Cys25 to permit nucleophilic thiolate addition and (b) significant binding interactions (both hydrogen bonding and van der Waals) are present between the inhibitor and protease subsites. These observations support our hypothesis that specific binding interactions as well as appropriate reactivity of (−)-**1** are essential for the observed inhibitory properties.

The docking studies were validated by the synthesis of analogs in which key residues occupying the S2, S3, and S1′ subsites were modified. Specifically, replacements of the indole side chain, the –NHBoc, and the 2-ethylphenyl anilide of (−)-**1** were targeted. Thiocarbazates for this study were prepared from the requisite hydrazides exploiting our previously developed chemistry.[15] In a one-pot reaction, hydrazides were treated with carbonyl sulfide gas followed by an appropriate electrophile (i.e., R^2–Br). Preparative reverse phase HPLC was employed to purify the final products,[25] which were assayed for their ability to inhibit cathepsin L.[26]

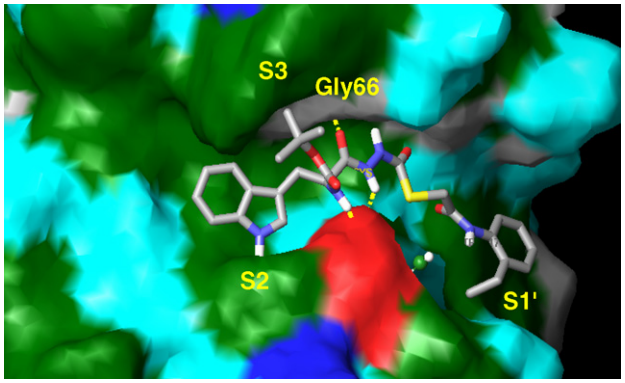

Figure 2. Thiocarbazate (−)-**1** (IC_{50} = 56 nM) in the binding subsite of papain. The indole forms hydrophobic contacts within the S2 subsite, the –NHBoc group forms hydrophobic contacts within the S3 subsite, and the 2-ethylphenyl anilide occupies the S1′ subsite.

As illustrated by the results listed in Table 1, occupation of the S2 subsite is essential for cathepsin L inhibition. Partial occupation, as in (−)-**2** where the indole side chain is replaced with the smaller phenyl group, results in less potent activity (IC_{50} = 115 vs 56 nM). Thiocarbazate **3**, in which the entire indole side chain has been eliminated, exhibits no inhibition. Also pronounced are the –NHBoc group's contributions to potency, as illustrated by thiocarbazate **4**'s significantly reduced activity (IC_{50} = 22 μM). In this case, we reason that the loss of a key hydrogen bond between the –NHBoc group and the Asp158 residue leads to diminished activity. These results support the importance of maintaining hydrophobic and hydrogen bonding interactions in the active site, consistent with the mode of docking proposed.

From the docking studies of thiocarbazate (−)-**1**,[16,24] we hypothesized that additional room for structural modifications and ring constraints was available in the anilide portion of this thiocarbazate (S1′ subsite). Based on this observation, a tetrahydroquinoline anilide (−)-**5** was substituted for the 2-ethylphenyl anilide moiety (Table 1).[27] An improvement in potency was observed (IC_{50} = 41 nM), further supporting our hypothesis. To explore this area further, two additional analogs were prepared: thiocarbazate **6**, in which constraints were imposed by incorporation of an *N*-phenyl pyrrolidinone group, and a methyl ester thiocarbazate (−)-**7**. [28,29] Both analogs **6** and (−)-**7** exhibited reduced activity against cathepsin L with IC_{50} values of 110 and 201 nM, respectively.

Our thiocarbazates are structurally related to oxocarbazates (e.g., **A**) and aza-peptides (e.g., **B**), known protease inhibitors (Fig. 3)[30–32] that are active by the virtue of their activated carbonyl groups. Depending on the nature of the leaving group present, these inhibitors often bind and react with the active site serine or cysteine, resulting in the formation of a stable acyl–enzyme complex, which then undergoes slow hydrolysis.[30,31] Alternatively, oxocarbazate and aza-peptide inhibitors with poor leaving groups are believed to form stable tetrahedral intermediates without acyl–enzyme adduct formation. [18,33,34] To further understand the cathepsin L inhibitory activity of thiocarbazates, (−)-**1** was incubated in the presence of stoichiometric amounts of cysteine or cathepsin L over prolonged time periods in the presence of assay buffer. The reactions were monitored by LC–MS for the disappearance of (−)-**1** as well as the appearance of reaction products such as cysteine adducts and products of hydrolysis. [35] In both experiments, thiocarbazate (−)-**1** was found to remain