Design, Synthesis and Biological Evaluation of a Library of Thiocarbazates and Their Activity as Cysteine Protease Inhibitors

Zhuqing Liu[1,2], Michael C. Myers[1,2], Parag P. Shah[2,3], Mary Pat Beavers[2,3], Phillip A. Benedetti[1,2], Scott L. Diamond[2,3], Amos B. Smith, III[1,2] and Donna M. Huryn[*,1,2]

[1]*Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104-6323, USA*

[2]*Penn Center for Molecular Discovery, University of Pennsylvania, 1024 Vagelos Research Laboratories, Philadelphia, PA 19104-6383, USA*

[3]*Institute for Medicine and Engineering, University of Pennsylvania, 1024 Vagelos Research Laboratories, Philadelphia, PA 19104-6383, USA*

Abstract: Recently, we identified a novel class of potent cathepsin L inhibitors, characterized by a thiocarbazate warhead. Given the potential of these compounds to inhibit other cysteine proteases, we designed and synthesized a library of thiocarbazates containing diversity elements at three positions. Biological characterization of this library for activity against a panel of proteases indicated a significant preference for members of the papain family of cysteine proteases over serine, metallo-, and certain classes of cysteine proteases, such as caspases. Several potent inhibitors of cathepsin L and S were identified. The SAR data were employed in docking studies in an effort to understand the structural elements required for cathepsin S inhibition. This study provides the basis for the design of highly potent and selective inhibitors of the papain family of cysteine proteases.

Keywords: Thiocarbazates, cathepsin B, cathepsin S, cathepsin L, cysteine protease inhibitor, library.

INTRODUCTION

Cysteine proteases, ubiquitous in Nature, are frequent targets of drug discovery efforts due to roles they play in a number of physiological and pathophysiological processes. In mammals, three classes of cysteine proteases have been characterized: papain-like (such as the cysteinyl cathepsins), calpains, and caspases [1, 2]. Papain-like cysteine proteases play a role in protein turnover, and their overexpression or disregulation has been implicated in certain inflammatory diseases, in cancer, and in osteoporosis, and arthritis, among other diseases. Inappropriate activity of calpains has also been associated with a number of disease conditions including neurodegeneration, muscular dystrophy and diabetes. Caspases play a role in the inflammatory process and in apoptosis; their inhibition has been suggested as an approach to degenerative diseases such as arthritis and stroke. A number of infectious agents (bacteria, viruses, and protozoa) also utilize either host or their own cysteine proteases for infectivity, virulence and/or replication processes, and targeting these proteases has been a popular strategy for anti-infective drug discovery efforts [3].

Inhibitors of cysteine proteases typically rely on the presence of a "warhead" to provide a site for nucleophilic attack by the active site cysteine thiolate [4]. Examples of warheads include peptidyl halomethyl ketones [5], peptidyl diazomethanes [6], peptide aldehydes [7], acyloxymethyl ketones [8], epoxysuccinyl derivatives [9], epoxyketones [10], α-aminoalkyl epoxides [11], α-keto-aldehydes [12], azepanones [13], aziridines and azodicarboxamides [14, 15], vinyl sulfones [16], and aza peptides [17].

Recently, we disclosed a novel series of cathepsin L inhibitors characterized by a unique thiocarbazate warhead [18]. Through further optimization, potent and selective inhibitors of cathepsin L were developed; Compound **1** was characterized as a competitive inhibitor that exhibited slow binding and reversible kinetics [19-21]. Since this warhead had not been described, prior to our publication, we explored its potential to inhibit other proteases. Our study entailed the design and synthesis of a library of thiocarbazates and their biological characterization in a series of protease assays to define the potential of this chemotype to exhibit broad protease inhibition. Based on the results of these studies, the library was tested against specific cathepsins; docking studies to rationalize our findings were also carried out. The results from these efforts are reported herein.

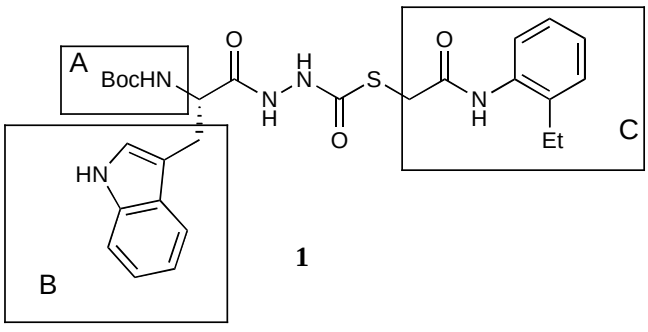


Fig. (1). Design of thiocarbazate library.

*Address correspondence to this author at the Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104-6323, USA; Tel: 215-746-3567; E-mail: huryn@sas.upenn.edu

1386-2073/10 $55.00+.00 **© 2010 Bentham Science Publishers Ltd.**

DESIGN OF THE THIOCARBAZATE LIBRARY

Our strategy involved the design of a library containing a thiocarbazate scaffold incorporating a variety of functional groups at three different positions A, B and C (Fig. **1**). From the outset, we sought to optimize diversity of the final products in terms of size, shape and functionality. At the same time, we focused on maintaining optimal physical properties to ensure solubility, permeability and other "drug-like" properties. Finally, we maintained a strict requirement for an expedient synthesis that would produce a minimum of 10 mg of final product in purities of at least 95% as determined by LC-MS (liquid chromatography/mass spectrometry) analysis. Modifications at the A position involved changes in size, as well as replacement of the *t*-butyloxycarbonyl group. Previous work in our laboratories identified an issue of instability with the presence of the free amine at this position; therefore all modifications took that possibility into consideration. Position B underwent the most extensive modifications, where changes in size, polarity, acidity, and functionality were incorporated. Thiocarbazates derived from natural amino acids, such as methionine, valine, alanine, glutamic acid, leucine, proline, phenylalanine, tyrosine, threnonine, serine, glutamic acid, lysine, arginine and histine, along with unnatural amino acids were prepared. Modifications at C involved incorporation of ring constraints, removal of the amide bond and exploration of size requirements; a variety of acetamides derived from aniline, primary amines, and methyl esters were included. Examples of substituents at position C include differentially substituted anilines, quinolines and isoquinolines, non-aromatic amines, morpholines, indolines, and pyridinones.

To ensure that the library contained compounds with a range of acceptable physical properties such as logP, molecular weight, polar surface area, etc., we relied on the cheminformatics tool, Leadscope [22]. Table **1** below details this analysis. The aggregated library exhibited excellent properties according to Lipinski-like [23] parameters: average LogP = 3.5, average H-bond acceptors = 4.8, average H-bond donors = 3.9. The average molecular weight (526) is typical of small molecule protease inhibitors, but slightly above the Lipinski target of <500. Consequently, the number of Lipinski violations on average was 1, which is reflective of the molecular weight characteristics. Polar surface area and rotable bonds were on average 134 Å and 16, respectively. While the PSA was within the range suggested by Veber [24] for orally available drugs, the rotatable bond count was somewhat higher than suggested as optimal.

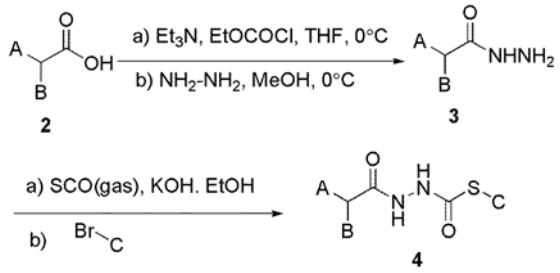
SYNTHESIS OF THE THIOCARBAZATE LIBRARY

Based on chemistry developed in our laboratory [18, 19] we designed a versatile synthetic strategy to prepare the thiocarbazate library as illustrated in Scheme **1**. A variety of acids were treated with ethylchloroformate to form the corresponding mixed anhydride, which were not isolated. Treatment with hydrazine monohydrate then furnished the hydrazides which were isolated after aqueous workup. Reaction with carbonyl sulfide gas in ethanol [25] formed the thiosemicarbazide intermediates that were directly

Table 1. Physical Properties of Thiocarbazate Library

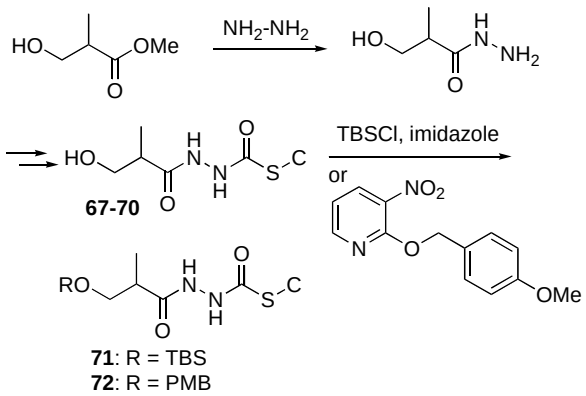
	Average	Range	Median	Mode
AlogP	3.5	1.0-6.0	3.5	4.1
H-Bond Acceptors	4.8	3-7	5.0	5.0
H-Bond Donors	3.9	2-7	4.0	4.0
Lipinski Violations	1.0	0-3	1.0	1.0
Molecular Weight	526.1	367.2-706.6	536.6	539.6
Polar Surface Area (Å)	134.3	95.5-210.8	132.6	125.6
Rotatable Bonds	16	9-23	16	16

treated with an alkylating agent to form the thiocarbazate products. The thiocarbazates were isolated and purified by HPLC to at least 95% purity. All compounds were characterized by high resolution mass spectrometry (HRMS), and a subset further characterized by ^{1}H NMR, ^{13}C NMR and IR. The general procedure for the preparation of thiocarbazates was amenable to all of the thiocarbazates produced in the library (Table **2**).



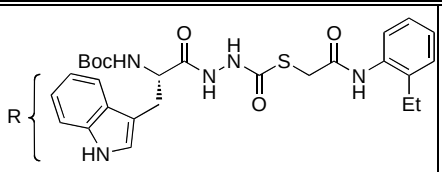
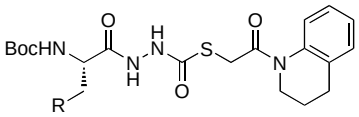
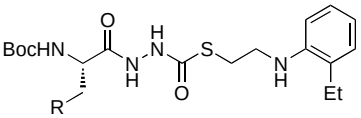
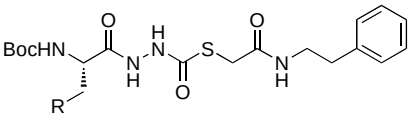
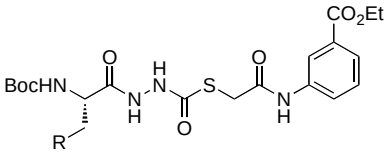
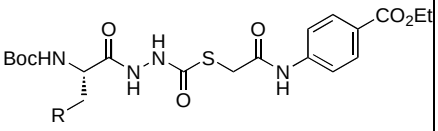
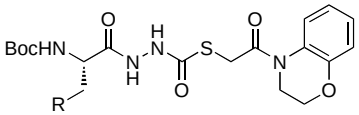
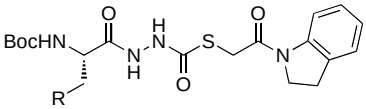
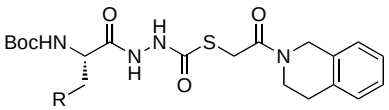
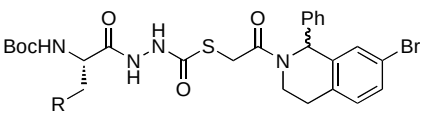
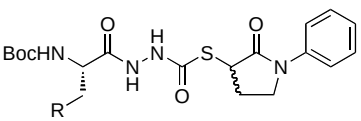
Scheme 1. General synthesis of thiocarbazates.

While many of the amino acid and acid starting materials were commercially available, several required preparation. Hydrazides derived from commercially available *R* or *S*-2-methyl-3-hydroxypropionate were generated directly from the ester as shown in Scheme **2**, then converted to the corresponding thiocarbazates (e.g., **67**-**70**). Subsequent etherification of **69** afforded the *tert*-butyldimethylsilyl (TBS) [26], and *para*-methoxyl benzyl (PMB) [27] ether analogs **71** and **72**. Beta amino acids, such as those incorporated into thiocarbazates **74**-**86**, were prepared using a modified Arndt-Eistert protocol to furnish the desired β-amino acid in yields of 70-95% [28].

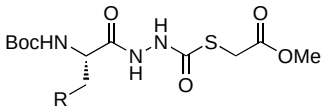
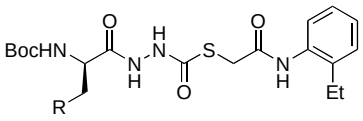
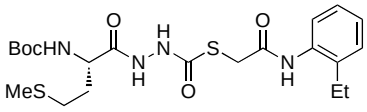
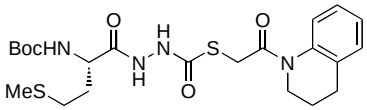
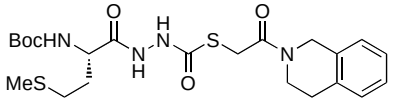
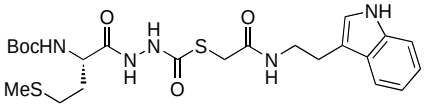
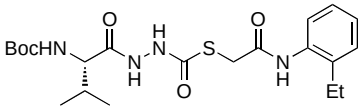
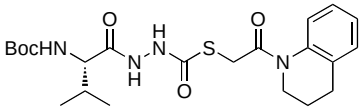
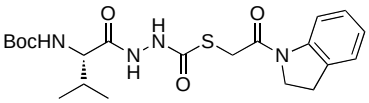
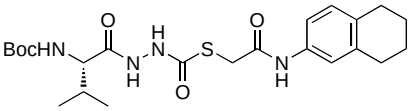
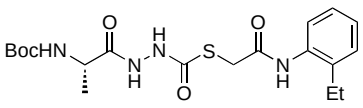
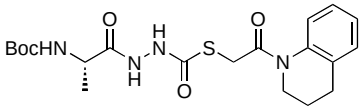


Scheme 2. Preparation of thiocarbazates **67**-**72**.

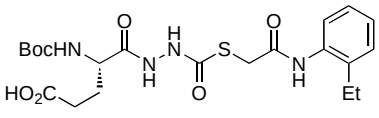
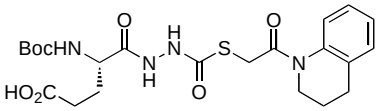
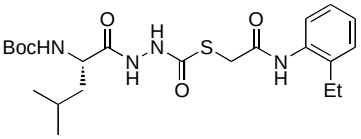
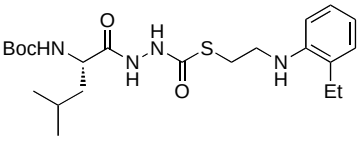
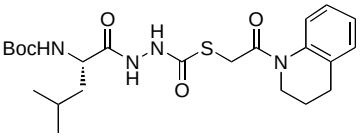
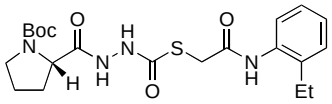
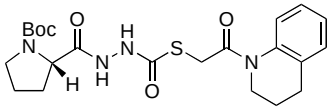
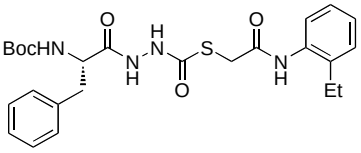
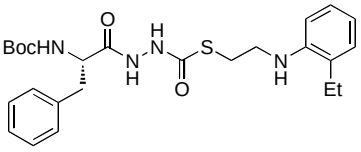
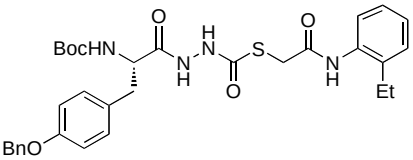
Table 2. Thiocarbazates Synthesized and Assayed Against Cathepsins B, L, and S

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
1		[M+Na]+ 562.2126 562.2100 4.62	4.45	0.056	0.96
5		[M+Na]+ 574.2102 574.2100 0.35	4.46	0.096	0.93
6		[M+H]+ 526.2488 526.2483 0.95	>25.0	>25.0	>25.0
7		[M+Na]+ 562.2094 562.2100 0.96	6.59	0.029	0.32
8		[M+Na]+ 606.1995 606.1998 0.5	>25.0	0.062	0.68
9		[M+Na]+ 606.1995 606.1998 0.5	>25.0	0.057	0.58
10		[M+Na]+ 576.1874 576.1893 0.33	3.23	0.22	.95
11		[M+Na]+ 560.1943 560.1944 0.2	2.60	0.11	1.65
12		[M+Na]+ 574.2109 574.2100 1.50	3.25	0.019	0.53
13		[M+Na]+ 728.1520 728.1518 0.27	>25.0	1.03	2.90
14		[M+Na]+ 560.1924 560.1944 3.50	14.04	1.05	2.07

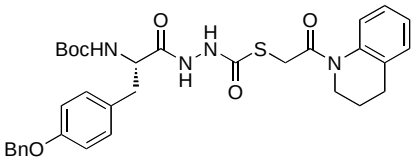
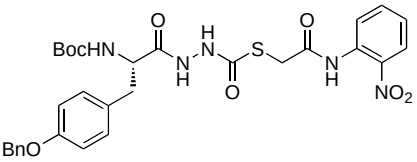
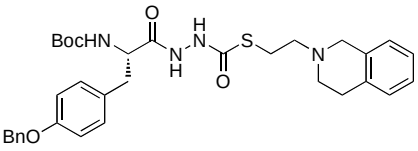
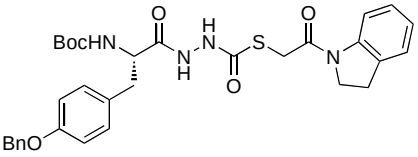
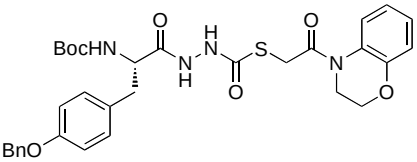
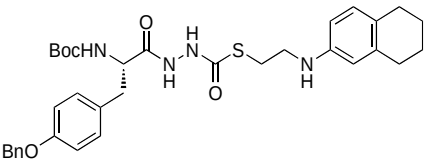
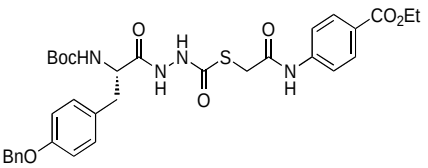
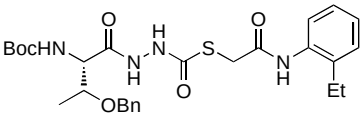
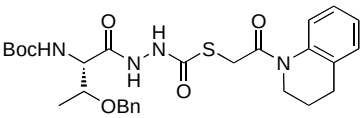
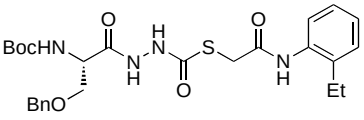
(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
15		[M+Na]+ 473.1458 473.1471 2.75	6.33	0.098	0.57
16		[M+Na]+ 562.2126 562.2100 4.62	>25.0	>25.0	>25.0
17		[M+Na]+ 507.1713 507.1712 0.19	2.03	1.29	0.69
18		[M+Na]+ 519.1699 519.1712 0.59	2.21	1.34	0.63
19		[M+Na]+ 519.1709 519.1712 0.96	2.31	0.52	0.41
20		[M+Na]+ 546.1807 546.1821 2.56	2.42	0.33	0.31
21		[M+Na]+ 475.2003 475.1991 2.5	0.87	0.66	3.74
22		[M+Na]+ 487.1994 487.1991 0.62	0.63	0.68	4.24
23		[M+Na]+ 473.1838 473.1835 0.63	0.44	0.45	7.24
24		[M+Na]+ 501.2155 501.2148 0.14	>25.0	0.11	0.62
25		[M+Na]+ 447.1685 447.1678 1.46	>25.0	>25.0	>25.0
26		[M+Na]+ 459.1674 459.1678 1.31	>25.0	>25.0	>25.0

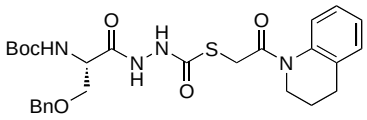
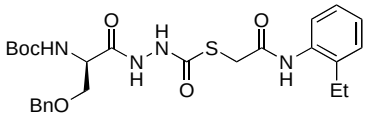
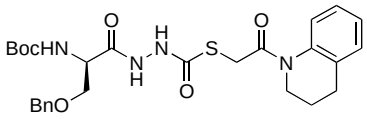
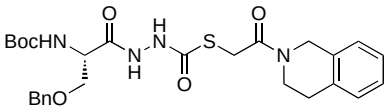
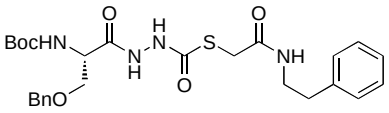
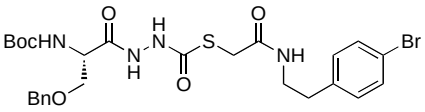
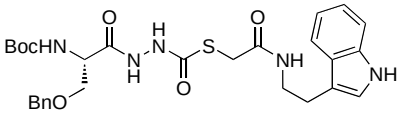
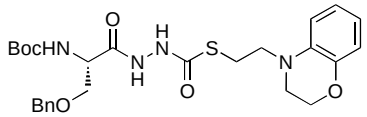
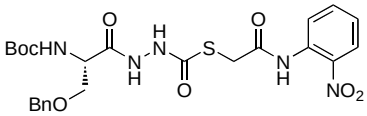
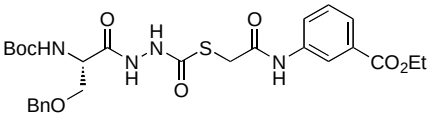
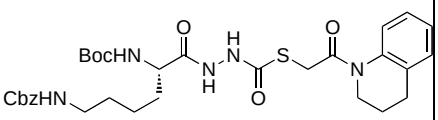
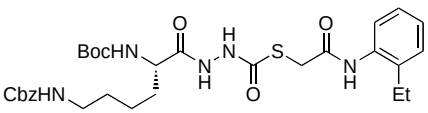
(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
27		[M+Na]+ 505.1733 505.1733 0	>25.0	>25.0	>25.0
28		[M+Na]+ 517.1725 517.1733 1.54	>25.0	>25.0	>25.0
29		[M+H]+ 467.2318 467.2328 0.21	2.63	1.09	1.65
30		[M+H]+ 453.2516 453.2536 0.44	>25.0	>25.0	>25.0
31		[M+H]+ 479.2327 479.2328 0.28	2.72	1.64	1.64
32		[M+Na]+ 473.1819 473.1835 3.34	>25.0	>25.0	>25.0
33		[M+H]+ 463.2014 463.2015 0.22	>25.0	>25.0	>25.0
34		[M+Na]+ 523.1977 523.1991 2.67	3.06	0.12	0.93
35		[M+H]+ 487.2383 487.2379 0.82	>25.0	>25.0	>25.0
36		[M+Na]+ 629.2419 629.2410 1.43	5.08	0.83	2.83

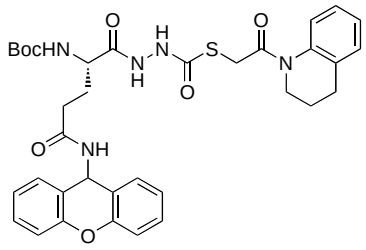
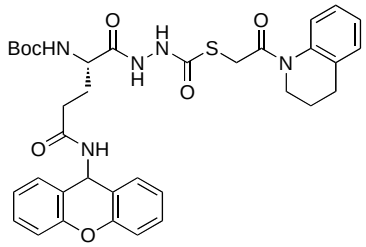
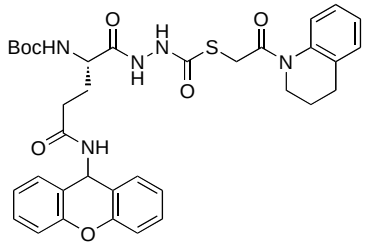
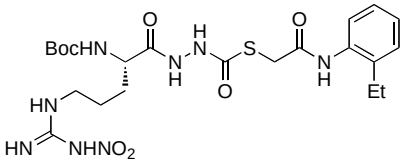
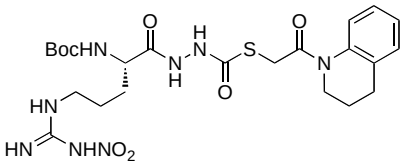
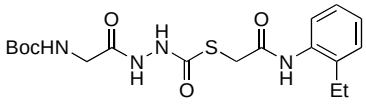
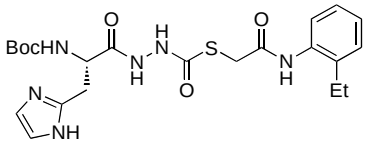
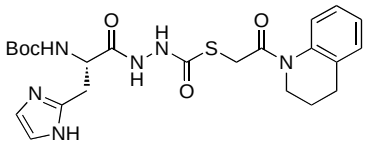
(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
37		[M+Na]+ 619.2406 619.2410 0.64	3.94	0.63	3.34
38		[M+Na]+ 646.1946 646.1948 0.31	2.77	0.59	2.08
39		[M+Na]+ 641.2401 641.2410 1.43	3.19	0.086	1.31
40		[M+Na]+ 627.2245 627.2253 1.27	3.12	0.70	8.00
41		[M+Na]+ 643.2224 643.2202 3.42	3.03	1.56	3.98
42		[M+Na]+ 655.2554 655.2566 1.83	>25.0	0.50	1.90
43		[M+Na]+ 673.2322 673.2308 2.10	>25.0	0.14	1.08
44		[M+Na]+ 567.2239 567.2253 2.47	3.67	13.7	3.32
45		[M+Na]+ 579.2236 579.2253 2.93	4.35	>25.0	5.15
46		[M+Na]+ 553.2101 553.2097 2.53	14.3	1.27	2.15

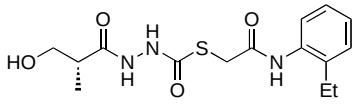
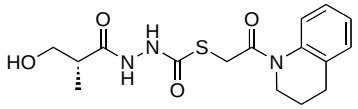
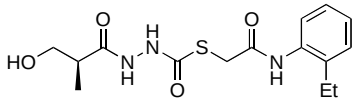
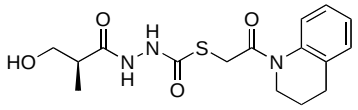
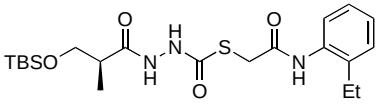
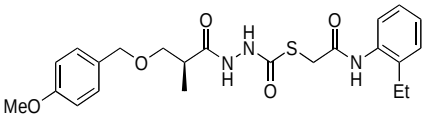
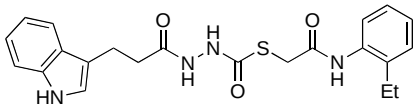
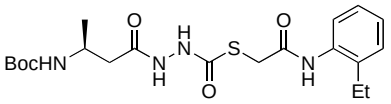
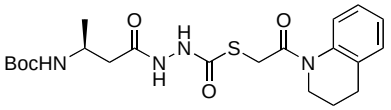
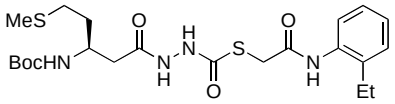
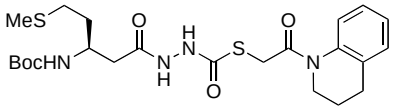
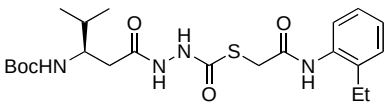
(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
47		[M+Na]+ 565.2097 565.2097 0	13.2	1.51	2.64
48		[M+Na]+ 553.2087 553.2097 1.80	>25.0	15.4	4.42
49		[M+Na]+ 565.2088 565.2097 1.26	>25.0	17.9	5.89
50		[M+Na]+ 565.2083 565.2097 1.23	9.28	0.37	0.95
51		[M+Na]+ 553.2082 553.2097 2.7	>25.0	0.51	0.65
52		[M+H]+ 609.1385 609.1382 0.49	>25.0	0.58	0.91
53		[M+Na]+ 592.2198 592.2206 1.16	>25.0	0.38	0.92
54		[M+Na]+ 553.2094 553.2097 0.54	13.7	1.85	3.00
55		[M+Na]+ 570.1649 570.1635 1.05	5.07	0.70	1.44
56		[M+Na]+ 597.1998 597.1995 0.50	>25.0	0.43	1.64
57		[M+H]+ 628.2802 628.2805 0.50	4.35	>25.0	5.15
58		[M+H]+ 616.2786 616.2805 3.1	14.3	1.27	2.15

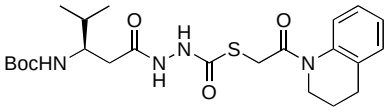
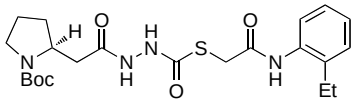
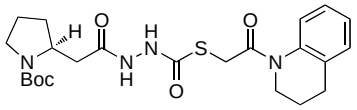
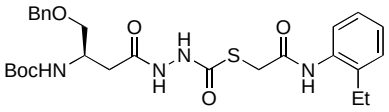
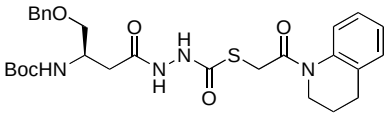
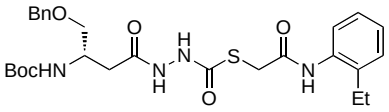
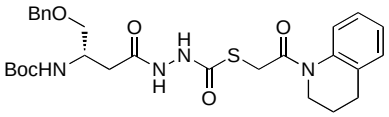
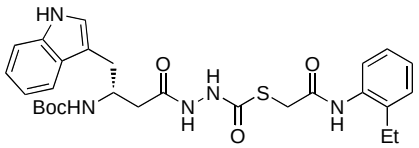
(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
59		[M+H]+ 662.2664 662.2648 0.60	>25.0	>25.0	>25.0
60		[M+H]+ 674.2639 674.2648 1.33	>25.0	>25.0	>25.0
61		[M+H]+ 662.2669 662.2648 3.17	>25.0	>25.0	>25.0
62		[M+Na]+ 577.2145 577.2169 4.16	>25.0	3.67	2.15
63		[M+H]+ 565.2214 565.2193 3.71	19.7	>25.0	3.07
64		[M+Na]+ 433.1513 433.1522 2.08	>25.0	>25.0	>25.0
65		[M+H]+ 491.2057 491.2077 4.07	13.7	>25.0	>25.0
66		[M+H]+ 503.2071 503.2077 1.19	11.6	>25.0	>25.0

(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
67		[M+Na]+ 362.1144 362.1150 1.66	>25.0	>25.0	>25.0
68		[M+Na]+ 374.1152 374.1150 0.53	>25.0	>25.0	>25.0
69		[M+Na]+ 362.1144 362.1150 1.70	>25.0	>25.0	>25.0
70		[M+Na]+ 374.1152 374.1150 0.53	>25.0	>25.0	>25.0
71		[M+Na]+ 476.2003 476.2015 2.52	>25.0	>25.0	>25.0
72		[M+Na]+ 482.1727 428.1726 0.21	>25.0	>25.0	>25.0
73		[M+H]+ 447.1485 447.1467 4.92	>25.0	>25.0	>25.0
74		[M+H]+ 439.2004 439.2015 2.5	>25.0	>25.0	>25.0
75		[M+H]+ 451.2012 451.2015 0.67	>25.0	>25.0	>25.0
76		[M+H]+ 499.2047 499.2049 0.40	>25.0	5.52	2.41
77		[M+H]+ 511.2044 511.2049 0.98	>25.0	6.95	3.01
78		[M+Na]+ 489.2125 489.2148 4.70	11.5	7.52	>25.0

(Table 2) contd…..

Thiocarbazate	Structure	HRMS (*m/z*) Observed Theoretical Error (ppm)	IC_{50} (μM) Cat B	IC_{50} (μM) Cat L	IC_{50} (μM) Cat S
79		[M+H]+ 479.2319 479.2328 1.88	15.8	>25.0	>25.0
80		[M+H]+ 465.2172 465.2172 0	>25.0	>25.0	>25.0
81		[M+H]+ 477.2177 477.2172 1.05	>25.0	>25.0	>25.0
82		[M+Na]+ 567.2276 567.2253 4.05	>25.0	>25.0	4.47
83		[M+H]+ 557.2435 557.2434 0.18	>25.0	2.12	5.95
84		[M+H]+ 545.2438 545.2434 0.73	>25.0	>25.0	15.2
85		[M+H]+ 557.2454 557.2434 3.59	>25.0	>25.0	>25.0
86		[M+Na]+ 576.2249 576.2257 1.39	>25.0	>25.0	>25.0

CHARACTERIZATION OF THIOCARBAZATE LIBRARY

As neither thiocarbazates nor their activity as protease inhibitors have been described prior to our publication, we profiled a subset of twenty-two compounds at a concentration of 10 μM for inhibitory activity against 75 different proteases [29] (Fig. **2**). The proteases chosen covered a broad spectrum of classes including serine proteases, metallo-proteases, aspartyl proteases and cysteine proteases. The aim of this study was to determine quickly whether thiocarbazates as a class displayed selectivity towards different families of proteases, or displayed broad protease inhibition properties.

The heatmap illustrated in Fig. (**2**) allows us to draw several broad conclusions. First, the thiocarbazates as a class exhibit selectivity towards cysteine proteases. No significant activity was detected against any other protease family member (e.g., serine, aspartyl, metallo-). Second, even among the cysteine protease family, a preference for the papain family is observed, as only modest activity against representatives of the calpain or caspase families is seen. Finally, several thiocarbazates exhibit potent activity (>95% inhibition at 10 μM) against cathepsins L, S, V, K, and papain.

Based on this study, we focused further attention on a more thorough biological characterization of this library against cathepsins B, L and S. This choice was based on the potential for potent inhibition, as well as selectivity. In addition, all three cathepsins represent important targets for drug discovery efforts. Towards this end, IC_{50} values were generated for all 82 members of the thiocarbazate library (Table **1**) against cathepsin B, L and S.

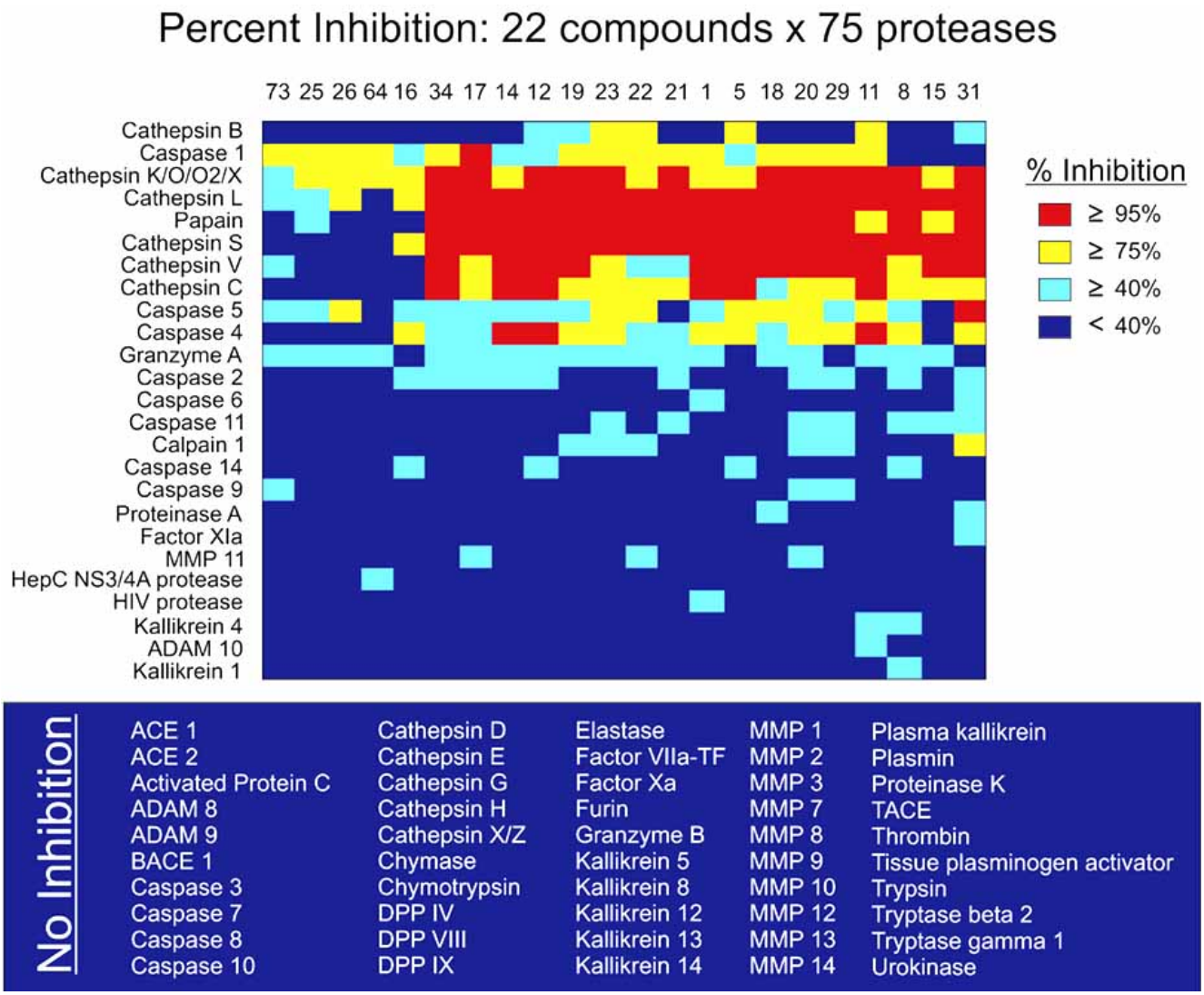


Fig. (2). Protease profiling heatmap of 22 thiocarbazates at 10 μM against 75 proteases.

Based on these data, we offer several generalizations for broad cathepsin inhibition. First, the substitution patterns displayed at positions A and B are key determinants for inhibitory activity. Alpha amino acid derived thiocarbazates are the preferred substituents, while those prepared from beta amino acid derivatives (**74**-**86**) and acid precursors (**67**-**73**) are devoid of activity. Among the thiocarbazates that incorporate alpha amino acids, the size of substituent B had a profound effect on potency. Thiocarbazates containing large groups (such as 3-indolemethylene, benzyl, 4-benzyloxybenzyl) or medium sized substituents (such as isopropyl, methyl thioethyl) were more potent than those incorporating smaller groups [e.g., hydrogen (**64**), methyl (**25**)]. Incorporation of the specific amino acids proline (**32**, **33**, **80**, **81**) or glutamic acid (**27**, **28**) proved detrimental to activity. Based on our earlier modeling studies on a prototypical thiocarbazate (**1**) [21], we presume the B substituent occupies the S2 site of the active site binding pocket. The structure activity relationships (SAR) trends for the B position are consistent with substrate specificity profiling studies on cysteine proteases that indicate a preference for hydrophobic amino acids at the P2 position [30]. Stereochemistry preferences at position B were also explored through the preparation of key enantiomeric pairs such as **1** and **16**; **46** and **48**; and **47** and **49**. In those examples, the thiocarbazate derived from the L-amino acid was more potent than that derived from the D isomer. Structural requirements for activity at position C involved a strong preference for a carbonyl group (**6** *vs* **5**; **30** *vs* **29**; **35** *vs* **34**). In all cases where the direct comparison could be made, removal of the carbonyl group significantly diminishes the inhibitory activity against all three cathepsins. In contrast, the specific nature of the amine side chain influenced activity far less.

CATHEPSIN B

Inhibitors of cathepsin B have been proposed for the treatment of cancer, osteoporosis, arthritis, and viruses [2]. As such, there is an interest in identifying potent and selective inhibitors of this member of the papain family. As a class, a number of thiocarbazates effectively inhibit

cathepsin B with potencies between 500nM and 10 μM, a range lower than the potencies associate with cathepsin L and S inhibition. The B position generally tolerated a wider array of substitutents (aliphatic, aromatic, and histidine) than the other cathepsins. This observation is consistent with substrate profiling studies indicating a broader tolerance at the P2 position [30]. Unlike other cathepsins, cathepsin B contains a glutamic acid lining the S2 pocket which may explain its unique tolerance of an imidazole moiety occupying the S2 site and the selectivity observed in compounds **65** and **66**. The most potent thiocarbazates, with IC_{50} values in the sub-micromolar range, incorporate leucine in the A/B area (e.g., **21**, **22**, **23**) and a variety of substituents at C, but are non-selective.

CATHEPSIN L

The role of cathepsin L in bone and cartilage remodeling, as well as in infectivity by certain infectious organisms, has focused interest on identifying inhibitors of this protease. A number of potent inhibitors of cathepsin L were identified among the thiocarbazate library. The most potent (<100 nM) and selective (≥10-fold over cathepsin B and S) examples (e.g., **1**, **5**, **7**, **8**, **9**, **12**, **39**) contained an aromatic group at position B, with a preference for 3-indolemethyl over benzyl and substituted benzyl analogs. The high potency afforded when an aromatic moiety can occupy the S2 site is consistent with substrate specificity studies showing cathepsin L's strong preference for aromatic amino acid residues over aliphatic residues at the P2 position. This preference has been attributed to a small residue (Ala205) at the bottom of the S2 site, which results in a relatively large and open-ended pocket compared to the analogous larger residues, and therefore small pockets, found in cathepsin B and S [31]. There is a tolerance for a broad array of C substituents with anilides (**1**, **8**, **9**), constrained anilides (**5**), alkyl amides (**7**, **12**), amines (**39**) and esters (**15**) exhibiting potent inhibition. This position, which according to our previous modeling studies [21] likely occupies the S1' site, does not appear to be a strong determinant of selectivity, an observation consistent with the high similarity of the cathepsin B, S and L S1' binding pockets [32].

CATHEPSIN S

Inhibitors of cathepsin S have garnered significant attention as potential treatment for several autoimmune diseases such as psoriasis, rheumatoid arthritis, multiple sclerosis and asthma. Among the thiocarbazate library, a number of sub-micromolar inhibitors have been identified. Unlike cathepsins B and L, cathepsin S shows a broad tolerance for the nature of the substituent at B. Strong inhibition (≤ 1 μM) was observed among thiocarbazates containing aromatic (**7**, **12**, **15**), branched alkyl (**24**), thioethers (**17**, **18**, **19**, **20**), and benzyl ethers (**51**, **52**, **53**). This hydrophobic preference was observed in substrate specificity profiling [30]. Similar to the cathepsin B and L SAR, tolerances for diverse size and functionalities at position C were observed. The most potent cathepsin S inhibitors typically were even more potent cathepsin L inhibitors (e.g., **7**, **9**, **12**). However certain trends suggest that selectivity over cathepsin L is possible. For example, several alkyl thioether (e.g., **17**, **18**) and benzyl ether (e.g., **44**, **45**, **48**, **49**) analogs exhibit >2-fold selectivity over cathepsin L. These suggestions of selectivity may find their basis on the presence of a hydrophobic phenylalanine residue at the S2 subsite, rather than the smaller alanine in cathepsin L which prefers larger residues.

CATHEPSIN S DOCKING STUDIES

The publicly available x-ray crystal structure of cathepsin S complexed to a small molecule inhibitor (2hh5.pdb) was selected to predict the protein/ligand binding interactions of thiocarbazates **69** and **20**. Both inhibitors are presumed to bind within the catalytic triad binding site of cathepsin S. These two compounds were selected for docking studies given their differences in inhibitory activity: **69** has an IC_{50} of only 25 μM, whereas **20** has an IC_{50} of 310 nM. Each compound was docked independently in the binding site of Cathepsin S (Fig. **3**) [33]. As expected, a significant difference between the total energy scores for **69** and **20** was calculated. The total energy score for the potent inhibitor **20** was –6.13 kcal/mol, while the score for the weakly active **69** was only –1.54 kcal/mol. It is presumed that each of the two compounds binds covalently to the active site Cys25 thiolate in the protein with the electrophilic carbon adjacent to the sulfur in the thiocarbazates. The distance between the Cys25 sulfur and the electrophilic carbon of the inhibitors is in the range of 2.9-3.3 Å for both compounds, further supporting covalent binding between the inhibitor and the protein.

Examination of the non-covalent interactions between **20** and the catalytic binding site residues of cathepsin S reveals strong hydrophobic interactions within the S2 subsite, specifically between Phe 211 of cathepsin S and the indole of **20**. In the S1' subsite, there is also a strong hydrophobic pocket formed by the interaction of the O-*tert*-butyl group of **20** and Trp 186. Non-covalent binding interactions of the weakly active inhibitor were also noted. However, docking studies reveal that **69** does not provide any side-chain hydrophobic group for interaction within the S2 subsite (Fig. **3**), as seen with **20**. This observation could explain the lack of potency of **69**. The overlay of **69** and **20** in the binding site of cathepsin S illustrates this void in the S1' pocket around Trp 186 for **69**.

CONCLUSIONS

Based on our earlier finding that thiocarbazates can act as inhibitors of proteases, we designed and synthesized a library with structural modifications at three different positions. The library was designed to incorporate diverse size, shapes and functionalities in order to profile thiocarbazates as a class of inhibitors against a series of representative proteases. Preliminary profiling indicated that thiocarbazates are selective for the papain-like cysteine protease; exhibiting limited or no activity against serine, aspartyl, metallo-proteases, nor members of the calpain or caspase families of cysteine proteases. Complete IC_{50} characterization against cathepsins B, L and S identified a number of potent and selective inhibitors of cathepsin L. In addition, trends to direct the design of new thiocarbazates exhibiting potent and selective inhibitory activity against cathepsins B and S were also developed. Many of these SAR trends are consistent with previous studies detailing the

substrate specificity of cathepsins B, L and S. and can be rationalized on the basis of residues in the enzyme binding pocket. Further characterization of the cathepsin L inhibitors disclosed, as well as the development of improved inhibitors of Cathepsin B and L will be reported in due course.

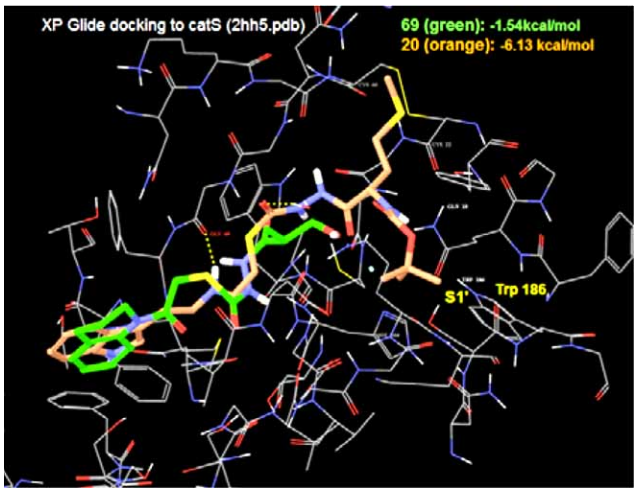


Fig. (3). Docking poses of **20** and **69** into cathepsin S Model.

EXPERIMENTAL SECTION

General Procedure for the Synthesis of Thiocarbazates

To a solution of acid (10.0 mmol) in THF (15 mL) was added NEt_3 (1.45 mL). After stirring at room temperature for 5 min, the mixture was cooled to -10ºC followed by the addition of the solution of ethyl chloroformate (1.0 mL) in THF (15 mL). After stirring 10 min, the mixture was filtered and washed with THF (2 x 5 mL). The combined filtrate was slowly added to a solution of hydrazine (2 mL) in methanol (26 mL) at 0ºC. The mixture was allowed to warm to room temperature and was concentrated after stirring 1 h. The residue was diluted with ethyl acetate (80 mL) and washed successively with saturated $NaHCO_3$ (12 mL) and brine (12 mL). The organic layer was dried over Na_2SO_4 and filtered. After removal of volatile solvents, the residue was used without further purification.

The hydrazide (1.0 mmol, 1.0 equiv.) was dissolved into a solution of KOH in 95% EtOH (0.25 M, 4.4 mL, 1.1 equiv.) in a 25 mL round bottom flask at room temperature. After stirring for 5 min, a balloon of carbonyl sulfide gas was attached to the flask. The flask was purged with the gas (5 s) and a full balloon was reattached. The reaction mixture was stirred for 15 h at 23°C, followed by the addition of a bromide (1.1 equiv.). The reaction was monitored by LC-MS, and the alkylating agent was typically consumed within 20 to 60 min. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by preparative reverse phase HPLC and identified by HRMS.

Using this procedure compounds **1**, **5-15** and **73** were prepared from L-N-Boc trytophan; **16** from D-N-Boc-tryptophan; **17-20** and **76-77** from N-Boc methionine; **21-24** from N-Boc valine; **25-26** and **78-79** from N-Boc alanine; **27-28** from N-Boc-Asp(OBzl); **29-31** from N-Boc-leucine;**32-33** and **80-81**from N-Boc-proline; **34** from N-Boc-phenylalanine; **36-43** from N-Boc-Try(Bzl); **44-45** from N-Boc-Thr(Bzl); **46-47**, **50-56** and **74-75** from N-Boc-serine; **48-49** from D-N-Boc-serine; **57-58** from N-Boc-Lys(Z); **59-61** from N-Boc-Glu(Xan); **62-63** from N-Boc-Arg(NO2); **64** from N-Boc-glycine; **65-66** from N-Boc-histine; **62-72** from 2-methyl-3-hydroxypropionate.

Synthesis of Thiocarbazates Derived from 2-Methyl-3-Hydroxypropionate

Synthesis of **67-70**: To a solution of 2-methyl-3-hydroxypropionate (1 mL, 7.94 mmol) in THF (10 mL) was added hydrazine hydrate (1.16 mL, 11.91 mmol). The mixture was stirred at room temperature overnight and concentrated to give hydrazide (820 mg, 88%). The hydroxyl thiocarbazates **67-70** were obtained following the procedure described above.

Synthesis of **71**: To the solution of **69** (34 mg, 0.1 mmol) in CH_2Cl_2 (8 mL) was added imidazole (13.6 mg, 0.20 mmol) and TBSCl (17 mg, 0.11 mmol) at 0º C. The resulting mixture was warmed and stirred at room temperature for 48h. The mixture was washed with 1M HCl, saturated $NaHCO_3$ and brine solution and extracted with CH_2Cl_2. The combined organic layers were dried and concentrated. The residue was purified by HPLC to give **71** (22mg, 50%).

Synthesis of **72**: To the solution of **69** (34 mg, 0.1 mmol) in CH_2Cl_2 (7 mL) was added 2-(4-methoxylbenzyoxy)-3-nitropyridine (39 mg, 0.15 mmol) and CSA (5 mg). The resulting mixture was stirred at room temperature for 3 h. The mixture was washed with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried and concentrated. The residue was purified by HPLC to give **72** (7 mg, 16%).

NMR of Representative Thiocarbazates

Compound 1: ^{1}H NMR (500 MHz, DMSO-d_6, VT-350K) δ 10.61 (br s, 1H), 10.04 (br s, 1H), 9.88 (br s, 1H), 9.14 (br s, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 7.4 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.21 (d, *J* = 7.3 Hz, 1H), 7.16-7.11 (m, 3H), 7.05 (t, *J* = 7.1 Hz, 1H), 6.97 (t, *J* = 7.4 Hz, 1H), 6.47 (br s, 1H), 4.31 (br s, 1H), 3.73 (br s, 2H), 3.17 (dd, *J* = 14.7, 4.1 Hz, 2H), 2.96 (m, 2H), 2.59 (q, *J* = 7.5 Hz, 2H), 1.29 (br s, 9H), 1.13 (t, *J* = 7.5 Hz, 3H).

Compound 5: ^{1}H NMR (500 MHz, DMSO-d_6,) δ 9.79-10.80 (m, 3H), 7.67 (m, 1H), 7.46 (m, 1H), 7.33 (m, 1H), 6.93-7.18 (m, 5H), 6.78 & 6.31-6.40 (br s, 1H), 4.23 (br s, 1H), 4.04 (br, s, 1 H), 3.87 (br s, 1H), 3.70 (br s, 2H), 3.12 (m, 1H), 2.90 (m, 1H), 2.69 (m, 2H), 1.88 (m, 2H), 1.29 & 1.13 (br s, 9H).

Compound 14: ^{1}H NMR (500 MHz, DMSO-d_6,) δ9.93-10.87 (m, 3H), 7.65 (m, 2H), 7.31 (m, 2H), 7.13 (m, 2H), 7.04 (m, 2H), 6.87(m, 1H), 4.28 (m, 1H), 3.86 (m, 2H), 3.12 (m, 1H), 2.90 (m, 1H), 2.69 (m, 2H), 2.10 (s, 3 H), 2.15-1.81 (m, 2H), 1.28 & 1.14 (br s, 9H).

Compound 19: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (br s, 1H), 7.21-7.11 (m, 5H), 5.33 (br s, 1H), 4.72 (m, 2H), 4.42 (d. *J* = 5.4 Hz, 1H), 3.93 (br s, 1H), 3.91 (br s, 1H), 3.82 (t, *J* = 5.8 Hz, 1H), 3.75 (t, *J* = 5.9 Hz, 1H), 2.94 (t. *J* = 5.8 Hz, 1H), 2.85 (t, *J* = 5.9 Hz, 1H), 2.63-2.58 (m, 2H), 2.12 (br s, 1H), 1.96 (ddd, *J* = 14.2, 7.1, 7.1 Hz, 1H), 1.43 (s, 9H).

Compound 24: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (br s, 1H), 8.35 (br s, 1H), 7.21(s, 1H), 7.18(d, *J* = 8.3 Hz, 2H), 6.96 (d, *J* 8.3 Hz, 2H), 5.15 (br s, 1H), 4.07-4.01 (m, 1H), 3.62 (br s, 2H), 2.70 (s, 2H), 2.69 (s, 2H), 2.05 (br s, 1H), 1.75 (t, *J* = 3.0 Hz, 4H), 1.41 (s, 9H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.97 (d. *J* = 6.7 Hz, 3H).

Compound 37: ^{1}H NMR (500 MHz, $CDCl_3$) δ7.41 (d, *J* = 6.9 Hz, 2H), 7.38-7.35 (m, 2H), 7.32-7.30 (m, 1H), 7.20-7.17 (m, 2H), 7.16-7.11 (m, 4H), 6.89 (dd, *J* = 8.6, 3.2 Hz, 2H), 5.06 (br s, 1H), 5.01 (s, 2H), 4.70 (d, *J* = 14.5 Hz, 2H), 4.44 (br s, 1H), 3.90 (d, *J* = 9.5 Hz, 2H),3.81 (t, *J* = 6.0 Hz, 1H), 3.73 (t, *J* = 6.0 Hz, 1H), 3.10 (d, *J* = 14.1, 6.3 Hz, 1H), 2.97 (t, *J* = 6.9 Hz, 1H), 2.93 (t, *J* = 5.8 Hz, 1H), 2.84 (t, *J* = 5.8 Hz, 1H), 1.37 & 1.37 (s, 9H).

Compound 38: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, *J* = 8.6 Hz, 2H), 8.61 (br s, 1H), 8.15 (d, *J* = 8.4 Hz, 2H), 7.61 (t, *J* = 8.1 Hz, 1H), 7.40-7.36 (m, 4H), 7.32-7.29(m, 1H), 7.16 (t, *J* = 7.9 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 5.15 (br s, 1H), 4.99 (s, 2H), 4.45 (br, d, *J* = 6.0 Hz, 1H), 3.77 (br s, 2H), 3.11-3.08 (m, 1H), 2.97 (br s, 1H), 1.37 (s, 9H).

Compound 72: ^{1}H NMR (500 MHz, DMSO-d_6,) δ 9.37-10.16 (m, 3H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.11 (m, 5H), 6.89 (d, *J* = 8.0 Hz, 2H), 4.48 & 4.38 (br s, 2H), 3.80 (m, 1H), 3.73 (s, 3H), 3.69 (s, 1H), 3.56 (m, 2H), 2.58 (m, 3H), 1.11 (t, *J* = 7.5 Hz, 3H), 1.03 (m, 3H).

Compound 85: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.27 (m, 5H), 7.20-7.05 (m, 4H), 5.37 (br s, 1H), 4.52 (s, 2H), 4.14-4.11 (m, 1H), 3.92 (br s, 2H), 3.80 (t, *J* = 6.4 Hz, 2H), 3.63-3.56 (m, 1H), 3.54-3.51(m, 1H), 2.74-2.58 (m, 2H), 2.66-2.53(m, 2H), 1.99-1.91 (m, 2H), 1.42 (s, 9H).

IC_{50} Determinations

IC_{50} determinations were conducted with the following assay buffer: 20 mM sodium acetate, 1 mM EDTA, and 5 mM cysteine, pH 5.5. Compounds were serially diluted in DMSO and transferred into a 96-well Corning 3686 assay microplate to give a 16-point two-fold serial dilution dose response ranging from 25 μM to 760 pM. Human liver cathepsin L (Calbiochem 219402) was activated by incubating with assay buffer for 30 min. Upon activation, cathepsin L (300 pM, 8.7 ng/mL) was incubated with 1 μM Z-Phe-Arg-AMC substrate (Sigma C9521) and test compound in 100 mL of assay buffer for 1 h at room temperature. Fluorescence of AMC released by enzyme-catalyzed hydrolysis of Z-Phe-Arg-AMC was read on a PerkinElmer Envision microplate reader (excitation 355 nm, emission 460 nm). Data were scaled using internal controls and fitted to a four-parameter logistic model (IDBS XLfit equation 205) to obtain IC_{50} values in triplicate.

Cathespin Assays

Human spleen cathepsin S (Calbiochem 219344, 40 ng/mL) was assayed using 15 μM Z-Phe-Arg-AMC substrate. Human liver cathepsin B (Calbiochem 219362, 65 ng/mL) was assayed using 15 μM Z-Arg-Arg-AMC substrate (Bachem I-1135). All reactions were performed in 20 mM sodium acetate buffer containing 1 mM EDTA and 5 mM cysteine, pH 5.5.

ACKNOWLEDGEMENTS

Financial support for this work was provided by the NIH (5U54HG003915). We thank Dr. G.T. Furst (NMR Facility) and Dr. R. Kohli (Mass Spectrometry Facility) at the University of Pennsylvania for assistance in obtaining NMR and high-resolution mass spectra.

REFERENCES

[1] Schirmeister, T.; Kaeppler, U. Non-peptidic inhibitors of cysteine proteases. *Mini Rev. Med. Chem.*, **2003**, *3*, 361.

[2] Vasiljevera, O.; Reinheckel, T.; Peters, C.; Turk, D.; Turk, V.; Turk, B. Emerging roles of cysteine cathepsins in disease and their potential as drug targets. *Curr. Pharm. Des.*, **2007**, *13*, 385.

[3] McKerrow, J.H.; Engel, J.C.; Caffrey, C.R. Cysteine protease inhibitors as chemotherapy for parasitic infections. *Bioorg. Med. Chem.*, **1999**, *7*, 639.

[4] Hernandez, A.H; Roush, W.R. Recent advances in the synthesis, design and selection of cysteine protease inhibitors. *Curr. Opin. Chem. Biol.*, **2002**, *6*, 459.

[5] a) Schoellmann, G.; Shaw, E. Direct evidence for the presence of histidine in the active center of chymotrypsin. *Biochemistry*, **1963**, *2*, 252; b) Rasnick, D. Synthesis of peptide fluoromethyl ketones and the inhibition of human cathepsin B. *Anal. Biochem.*, **1985**, *149*, 461; c) Rauber, P.; Angliker, H.; Walker, B.; Shaw, E. The synthesis of peptidylfluoromethanes and their properties as inhibitors of serine proteinases and cysteine proteinases. *Biochem. J.*, **1986**, *239*, 633.

[6] Crawford, C.; Mason, R.W.; Wikstrom, P.; Crawford, E.S. The design of peptidyldiazomethane inhibitors to distinguish between the cysteine proteinases calpain II, cathepsin L and cathepsin B. *Biochem. J.*, **1988**, *253*, 751.

[7] Yasuma, T.; Oi, S.; Choh, N.; Nomura, T.; Furuyama, N.; Nishimura, A.; Fujisawa, Y.; Sohda, T. Synthesis of peptide aldehyde derivatives as selective inhibitors of human cathepsin L and their inhibitory effect on bone resorption. T. *J. Med. Chem.*, **1998**, *41*, 4301.

[8] Smith, R.A.; Copp, L.J.; Coles, P.J.; Pauls, H.W.; Robinson, V.J.; Spencer, R.W.; Heard, S.B.; Krantz, A. New inhibitors of cysteine proteinases. Peptidyl acyloxymethyl ketones and the quiescent nucleofuge strategy. *J. Am. Chem. Soc.*, **1988**, *110*, 4429.

[9] Fujishima, A.; Imaia, Y.; Nomura, T.; Fujisawa, Y.; Yamamoto, Y.; Sugawara, T.; The crystal structure of human cathepsin L complexed with E-64. *FEBS Lett.*, **1997**, *407*, 47.

[10] Spaltenstein, A.; Leban, J.J.; Huang, J.J.; Reinhardt, K.R.; Viveros, O.H.; Sigafoos, J.; Crouch, R. Design and synthesis of novel protease inhibitors. Tripeptide α′,β′-epoxyketones as nanomolar inactivators of the proteasome. *Tetrahedron Lett.*, **1996**, *37*, 1343.

[11] Albeck, A.; Persky, R.; Kliper, S. Design of a new selective cysteine protease inactivator and its mechanistic implications. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1767.

[12] Lynas, J.F.; Hawthorne, S.J.; Walker, B. Development of peptidyl alpha-keto-beta-aldehydes as new inhibitors of cathepsin L--comparisons of potency and selectivity profiles with cathepsin B. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1771.

[13] Marquis, R.W.; James, I.; Zeng, J.; Trout, R.E.L.; Thompson, S.; Rahman, A.; Yamashita, D.; Cie, R.; Ru, Y.; Gress, C. J.; Blake, S.; Lark, M. A.; Hwang, S.; Tomaszek, T.; Offen, P.; Head, M.S.; Cummings, M.D.; Veber, D.V. Azepanone-based inhibitors of human cathepsin L. *J. Med. Chem.*, **2005**, *48*, 6870.

[14] Schirmeister, T. New peptidic cysteine protease inhibitors derived from the electrophilic alpha-amino acid aziridine-2,3-dicarboxylic acid. *J. Med. Chem.*, **1999**, *42*, 560.

[15] Radim, V.; Matthias, B.; Christoph, G.; Nikolaus, S.; Josef, S., Werner, S.; Franziska, S.; Mladenovic, M.; Bernd, E.; Matthias, L.; Knut, B.; Tanja, S. Aziridine-based inhibitors of cathepsin l: synthesis, inhibition activity, and docking studies. *Chem. Med. Chem.*, **2006**, *1*, 1126.

[16] Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Brömme, D. Vinyl sulfones as mechanism-based cysteine protease inhibitors. *J. Med. Chem.*, **1995**, *38*, 3193.

[17] Xing, R.; Hanzlik, R. P. Azapeptides as inhibitors and active site titrants for cysteine proteinases. *J. Med. Chem.*, **1998**, *41*, 1344.

[18] Myers, M.C.; Shah, P.P.; Diamond, S.L.; Huryn, D.M.; Smith, A.B., III. Identification and synthesis of a unique thiocarbazate cathepsin L inhibitor. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 210.

[19] Myers, M.C.; Shah, P.P.; Beavers, M.P.; Napper, A.D.; Diamond, S.L.; Smith, A.B., III; Huryn, D.M. Design, synthesis, and evaluation of inhibitors of cathepsin L: exploiting a unique thiocarbazate chemotype. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 3646.

[20] Shah, P.P.; Myers, M.C.; Beavers, M.P.; Purvis, J.E.; Jing, H.; Grieser, H.J.; Sharlow, E.R.; Napper, A.D.; Huryn, D.M.; Cooperman, B.S.; Smith, A.B., III; Diamond, S.L. Kinetic characterization and molecular docking of a novel, potent, and selective slow-binding inhibitor of human cathepsin L. *Mol. Pharmacol.*, **2008**, *74*, 34-41.

[21] Beavers, M.P., Myers, M.C.; Shah, P.P.; Purvis, J.E.; Diamond, S.L.; Copperman, B.S.; Huryn, D.M.; Smith, A.B., III Molecular docking of cathepsin L inhibitors in the binding site of papain. *J. Chem. Info. Mod.*, **2008**, *48*, 1464.

[22] Leadscope, Inc.

[23] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Del. Rev.*, **2001**, *46*, 3.

[24] Veber, D.F.; Johnson, S.R.; Cheng, H.Y.; Smith, B.R.; Ward, K.W.; Kopple, K.D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.*, **2002**, *45*, 2615.

[25] Chande, M.S.; Singh-Jathar, K. Carbon oxysulphide: A novel reagent for the synthesis of 4-aminoanilino-3-aryl/aryloxymethyl/ thiophenoxymethyl-1,2,4-triazolin-5-ones and 5-arylamino-2-mercapto-1,3,4-oxadiazoles. *Ind. J. Chem.*, **1998**, *37B*, 352.

[26] Patrick, P.; Walailak, S.; Filisaty, V. Enantioselective synthesis of the dioxabicyclo[3.2.1]octane core of the zaragozic acids *via* intramolecular Wacker-type cyclisation reactions. *Org. Biomol. Chem.*, **2004**, *2*, 2220.

[27] Nakano, M.; Kikuchi, W.; Matsuo, J.; Mukaiyama, T. An efficient method for the p-methoxybenzylation of hydroxy groups with 2-(4-methoxybenzyloxy)-3-nitropyridine. *Chem. Lett.*, **2001**, *30*, 424.

[28] Linder, M.R.; Steurer, S.; Podlech, J. (S)-3-(tert-Butyloxycarbonyl-amino)-4-Phenylbutanoic acid. *Org. Syn.*, **2002**, *79*, 154.

[29] Available at: www.reactionbiology.com.

[30] a) Choe, Y.; Leonetti, F.; Greenbaum, C.C.; Lecaille, F.; Bogyo, M.; Bromme, D.; Ellman, J.A.; Craik, C.S.J. Substrate profiling of cysteine proteases using a combinatorial peptide library identifies functionally unique specificities. *Biol. Chem.*, **2006**, *281*, 12824. b) Gosalia, D.N.; Salisbury, C.M.; Ellman, J.A.; Diamond, S.L. High throughput substrate specificity profiling of serine and cysteine proteases using solution-phase fluorogenic peptide microarrays. *Mol. Cell Proteomics*, **2005**, *4*, 626.

[31] Bromme, D.; Bonneau, P.R.; Lachance, P.; Storer, A.C. Engineering the S2 subsite specificity of human cathepsin S to a cathepsin L- and cathepsin B-like specificity. *J. Biol. Chem.*, **1994**, *269*, 30238.

[32] Pfizer, J.M.; Assfalg-Machleidt, I.; Machleidt, W.; Moroder, L.; Schaschke, N. Primed-site probing of papain-like cysteine proteases. *Int. J. Pept. Res. Ther.*, **2007**, *13*, 93.

[33] Tully, D.C.; Liu, H.; Chatterjee, A.K.; Alper, P.B.; Epple, R.; Williams, J.A.; Roberts, M.J.; Woodmansee, D.H.; Masick, B.T.; Tumanut, C.; Li, J.; Spraggon, G.; Hornsby, M.; Lesley, S.A.; Harris, J.L.; Karnewsky, D.S. Synthesis and SAR of arylaminoethyl amides as noncovalent inhibitors of cathepsin S: P3 cyclic ethers. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 5112.

Received: January 20, 2009 **Revised: June 17, 2009** **Accepted: July 21, 2009**