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Synthesis and Biochemical Evaluation of Thiochromanone Thiosemicarbazone Analogues as Inhibitors of Cathepsin L

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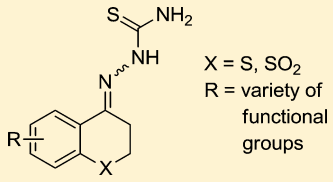
Supporting Information

ABSTRACT: A series of 36 thiosemicarbazone analogues containing the thiochromanone molecular scaffold functionalized primarily at the C-6 position were prepared by chemical synthesis and evaluated as inhibitors of cathepsins L and B. The most promising inhibitors from this group are selective for cathepsin L and demonstrate IC_{50} values in the low nanomolar range. In nearly all cases, the thiochromanone sulfide analogues show superior inhibition of cathepsin L as compared to their corresponding thiochromanone sulfone derivatives. Without exception, the compounds evaluated were inactive (IC_{50} > 10000 nM) against cathepsin B. The most potent inhibitor (IC_{50} = 46 nM) of cathepsin L proved to be the 6,7-difluoro analogue **4**. This small library of compounds significantly expands the structure−activity relationship known for small molecule, nonpeptidic inhibitors of cathepsin L.



KEYWORDS: *thiochromanone, thiosemicarbazone, cathepsin L, cathepsin B, inhibitor*

There are five classes of proteases including matrix metalloproteases (MMPs), cysteine proteases, serine proteases, aspartic proteases, and threonine proteases, which catalyze the hydrolysis of peptide bonds.[1,2] Because of their function in many disease states, including cancer metastasis and cardiovascular disease, proteases have become well-investigated therapeutic targets.[3−6] Cysteine protease cathepsins, members of the papain family, have recently been validated as an important enzymatic class to target in cancer research.[1,2,5] In this family, there are 11 cathepsin enzymes known to date in humans: B, C, F, H, K, L, O, S, V, W, and X.[5,7−9] Odanacatib, an inhibitor of cathepsin K developed by Merck, is currently in phase III clinical trials for the treatment of osteoporosis.[10] Cathepsins are found in the highest concentration in cellular lysosomes, and during cancer progression, they are secreted at an increased rate and degrade the extracellular matrix and basement membrane, which aid in cancer metastasis.[5] Small molecule inhibitors of cathepsin L have been previously identified (Figure 1), including azapenone (I),[11] cyanamide derivative (II),[12] and a purine nitrile analogue (III),[13] as well as amino acid-based molecules including an oxocarbazate analogue (IV)[14] and an epoxide derivative (V).[15]

Although structurally similar, the active sites of the individual cathepsins have small, but significant, differences in size and shape.[16] Taking advantage of these differences facilitates the design of selective inhibitors.[17,18] Previous research from our group has focused on inhibitors of the cysteine protease, cruzain, which has implications in the treatment of Chagas' disease.[19−21] As a preliminary assay to evaluate selectivity for cruzain inhibition, a series of compounds was also screened against the structurally similar human cysteine protease cathepsin L. Several compounds, based primarily on the benzophenone or thiochromanone thiosemicarbazone molecular structure, were identified as lead compound inhibitors of cathepsin L.[20,22−24] These original findings provided the foundational support for the current studies involving functionalized thiochromanone thiosemicarbazone inhibitors of cathepsin L. To the best of our knowledge, we are the first to

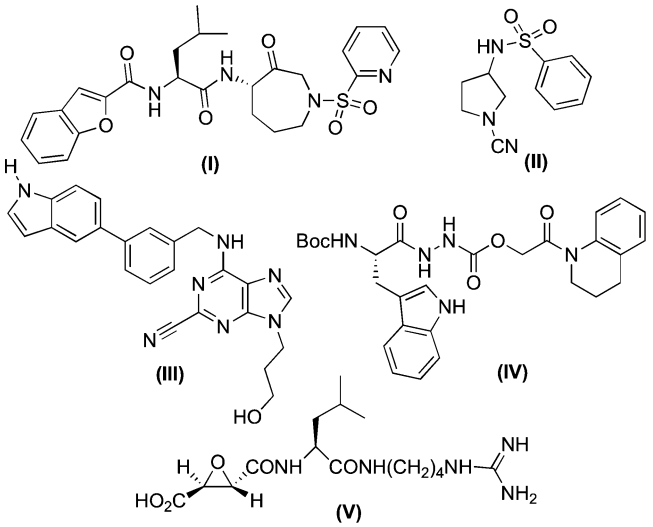


Figure 1. Representative inhibitors of cathepsin L.

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report[20,24] thiochromanone thiosemicarbazones and their corresponding sulfone analogues as molecular scaffolds that inhibit cathepsin L. A sampling of previously reported inhibitors (Figure 2) containing the thiosemicarbazone moiety includes **VI**,[22] **VII**,[23] **VIII**,[22] and **IX**.[25]

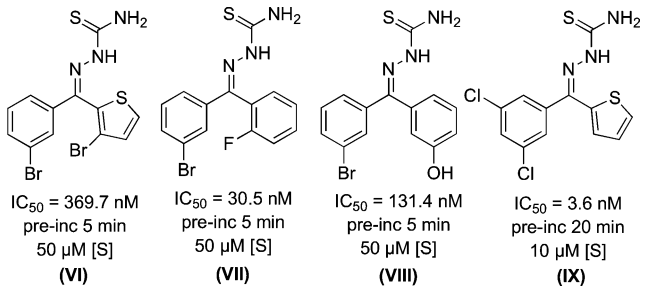
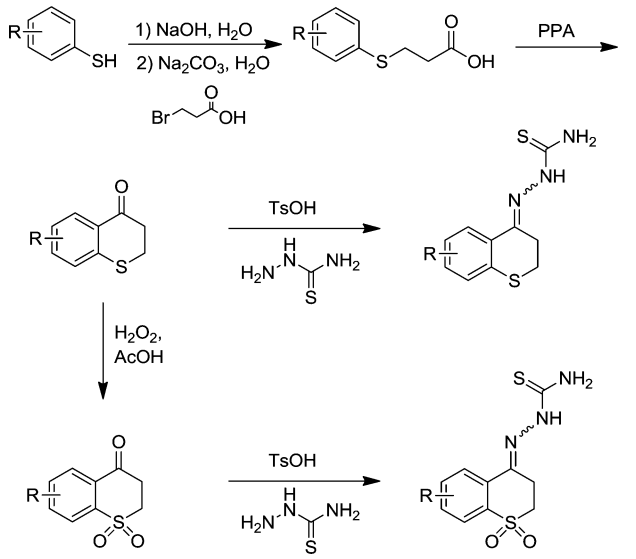


Figure 2. Representative thiosemicarbazone inhibitors of cathepsin L (IC_{50} values for inhibition of cathepsin L, preincubation time, and substrate concentration are indicated).

To incorporate structural diversity within the aryl portion of the target compounds, as shown in Scheme 1, 3-bromopropionic acid was reacted with various commercially available thiophenols appropriately functionalized on the aromatic ring. The resulting carboxylic acid intermediates underwent a ring-closing reaction upon treatment with polyphosphoric acid (PPA) to produce the functionalized thiochromanone analogues. Condensation of the resultant ketone moiety with thiosemicarbazide afforded the thiosemicarbazone derivatives in good yields. The corresponding 1,1-dioxo analogues were prepared in an analogous fashion by oxidation to obtain the sulfone derivatives, prior to the condensation reaction. Additional synthetic steps were necessary for the preparation of analogues **3**, **16**, **18**, **19**, **23**, **34**, and **36** (Table 1 and Supporting Information). Bromination of unsubstituted, commercially available thiochromanone afforded 6-bromothiochromanone,[19] which served as a starting material for the synthesis of analogues **3** and **23**. The preparation of the hydroxy analogues **16** and **34** involved demethylation of 6-methoxythiochromanone. The reduction of 6-nitrothiochromanone introduced the amine functionality in analogue **18**, while subsequent acylation provided the amide analogues **19** and **36**.

Scheme 1. General Synthetic Route toward Thiochromanone Thiosemicarbazone Analogues



While geometrical isomers (*E* and *Z*) are possible for nonsymmetrical thiosemicarbazone analogues, it is interesting to note that with the exception of two compounds (**18** and **34**), evidence for such isomeric mixtures was not observable by NMR or HPLC for the compounds in this study. Furthermore, nuclear Overhauser effect spectroscopy (NOESY) studies (carried out for compounds **4**, **13**, **16**, **17**, **27**–**29**, **31**, and **32**) suggested the presence of only the *E*-isomer in each of these compounds. In addition, a single-crystal X-ray diffraction study of compound **27** indicated the *E*-isomer. Details regarding the NOESY and X-ray studies are located in the Supporting Information.

All of the thiochromanone thiosemicarbazone analogues were evaluated for their ability to inhibit cathepsins L and B in separate assays. In general, the sulfide series of analogues was more active, in terms of cathepsin L inhibition, than their corresponding sulfone derivatives with individual IC_{50} values either much more active or equivalent. The most active inhibitors of cathepsin L in this study were the 6,7-difluoro analogue **4** and the 6-nitro analogue **17** (IC_{50} values of 46 and 68 nM, respectively). The lower IC_{50} values of compounds **5**–**7**, **15**, and **25**–**27** suggested that substitution at the C-8 position is not productive. Incorporation of a bromine atom at the C-6 position (compound **3**) resulted in improved inhibition of cathepsin L as compared to fluorine and chlorine atom replacement (compounds **1** and **2**). An increase in aliphatic substituent size at the C-6 position (compare compounds **8**/**13**, **9**, **10**) reflected a dramatic decrease in inhibition of cathepsin L. Compounds **3**, **4**, **17**, and **35** were strong inhibitors of cathepsin L. It is interesting to note that incorporation of a slightly electron-donating methyl group at C-6 (compound **8**) and a strongly electron-withdrawing trifluoromethyl group at C-6 (compound **13**) in each case led to excellent inhibitors of cathepsin L. Incorporation of hydroxyl, amino, methoxy, thiomethyl, and acetamide functionalities (compounds **16**, **18**, **11**, **12**, and **19**) at C-6 all led to loss of cathepsin L inhibition. The 6-bromo and 6-nitro substituted sulfones (compounds **23** and **35**) demonstrated significant inhibition of cathepsin L although less active than their sulfide counterparts (compounds **3** and **17**). The C-6 trifluoromethyl sulfone (**32**) was comparable in activity to its corresponding sulfide (**13**). All of the compounds evaluated in this study were inactive against cathepsin B (IC_{50} > 10000 nM). This reflects a selectivity ratio (cathepsin L/cathepsin B) of <0.0046 for the most active cathepsin L inhibitor (**4**). The inhibition of cathepsin L assay is very sensitive to experimental parameters such as preincubation time and substrate concentration.[18] Therefore, it is difficult to compare IC_{50} values, in an absolute sense, obtained for small molecule inhibitors of cathepsin L between separate laboratories under varying experimental conditions. With this perspective in mind, it is encouraging to note that the most potent thiochromanone thiosemicarbazone inhibitors of cathepsin L described in this study (**4** and **17**) demonstrated activity comparable to an exemplary benzophenone thiosemicarbarzone inhibitor (**VII**, Figure 2) assayed under comparable conditions.

In summary, from a focused small library of 36 analogues with a diverse functionality, nine were found to be potent inhibitors of cathepsin L (IC_{50} < 300 nM). The two most active analogues were the 6,7-difluorothiochromanone thiosemicarba-

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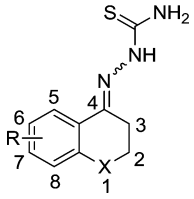
Table 1. Inhibitory Activity of Thiosemicarbazone Derivatives against Cathepsins L and B



			IC_{50} (nM)[a]					IC_{50} (nM)[a]	
compd	R	X	Cat L	Cat B	compd	R	X	Cat L	Cat B
1	6-F	S	741	>10000	**19**	6-NHAc	S	>10000	>10000
2	6-Cl	S	228	>10000	**20**	fused 5,6-phenyl	S	>10000	>10000
3	6-Br	S	152	>10000	**21**	6-F	SO_2	>10000	>10000
4	6,7-F	S	46	>10000	**22**	6-Cl	SO_2	>10000	>10000
5	6,8-F	S	1500	>10000	**23**	6-Br	SO_2	574	>10000
6	6-F,8-Br	S	>10000	>10000	**24**	6,7-F	SO_2	3650	>10000
7	6-Br,8-F	S	434	>10000	**25**	6,8-F	SO_2	>10000	>10000
8	6-CH_3	S	214	>10000	**26**	6-F,8-Br	SO_2	>10000	>10000
9	6-CH_2CH_3	S	2,720	>10000	**27**	6-Br,8-F	SO_2	1117	>10000
10	6-*i*-Pr	S	>10000	>10000	**28**	6-CH_3	SO_2	>10000	>10000
11	6-OCH_3	S	>10000	>10000	**29**	6-CH_2CH_3	SO_2	6521	>10000
12	6-SCH_3	S	>10000	>10000	**30**	6-*i*-Pr	SO_2	>10000	>10000
13	6-CF_3	S	284	>10000	**31**	6-OCH_3	SO_2	>10000	>10000
14	6-OCF_3	S	256	>10000	**32**	6-CF_3	SO_2	260	>10000
15	8-OCF_3	S	>10000	>10000	**33**	6-OCF_3	SO_2	3960	>10000
16	6-OH	S	>10000	>10000	**34**	6-OH	SO_2	>10000	>10000
17	6-NO_2	S	68	>10000	**35**	6-NO_2	SO_2	112	>10000
18	6-NH_2	S	>10000	>10000	**36**	6-NHAc	SO_2	>10000	>10000

[a]Each assay utilized 2% DMSO with a 5 min preincubation and 50 μM substrate (cat. L) or 60 μM substrate (cat. B).

zone **4** and the 6-nitro derivative **17** (IC_{50} = 46 and 68 nM, respectively).

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization, molecular modeling and X-ray crystallography, and details regarding biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s):The corresponding author (KGP) discloses a potential conflict of interest in regard to a paid consulting relationship with Oxigene Inc.

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