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Functionalized benzophenone, thiophene, pyridine, and fluorene thiosemicarbazone derivatives as inhibitors of cathepsin L

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ABSTRACT

A series of thiosemicarbazone analogs based on the benzophenone, thiophene, pyridine, and fluorene molecular frameworks has been prepared by chemical synthesis and evaluated as small-molecule inhibitors of the cysteine proteases cathepsin L and cathepsin B. The two most potent inhibitors of cathepsin L in this series (IC_{50} <135 nM) are brominated-benzophenone thiosemicarbazone analogs that are further functionalized with a phenolic moiety (**2** and **6**). In addition, a bromo-benzophenone thiosemicarbazone acetyl derivative (**3**) is also strongly inhibitory against cathepsin L (IC_{50} = 150.8 nM). Bromine substitution in the thiophene series results in compounds that demonstrate only moderate inhibition of cathepsin L. The two most active analogs in the benzophenone thiosemicarbazone series are highly selective for their inhibition of cathepsin L versus cathepsin B.

Cathepsins L and B belong to the papain family of lysosomal cysteine proteases including cathepsins C, F, H, K, O, S, V, W, and X.[1] Recent studies have shown that these two specific cysteine proteases participate in tumor progression, hyperproliferation, apoptosis, angiogenesis, and metastasis by malignant cells.[2] They have a broad tissue distribution and are overexpressed in several types of human cancer including breast, prostate, lung, gastrointestinal, and epidermal.[3] Therefore, cysteine proteases, such as cathepsins L and B, are of special interest as targets for the development of novel chemotherapeutic agents.[4]

The thiosemicarbazone moiety has been utilized as a key functional group in a variety of anticancer agents.[5] Earlier studies have reported that benzophenone thiosemicarbazone analogs have potential application in the treatment of Chagas' disease, sleeping sickness, and malaria.[6]

More recently, we have designed and prepared by chemical synthesis a variety of benzophenone thiosemicarbazone analogs (e.g., I and II {IC_{50} = 30.5 and 46.5 nM, respectively}) that are potent inhibitors of cathepsin L (Fig. 1).[7] In addition other small molecules including further benzophenone analogs,[8] thiophene (III),[8] oxadiazole (IV),[9] pyrazole (V),[10] oxocarbazate (VI),[11] aldehyde (VII),[12] cyanopyrrolidine (VIII),[13] epoxide (IX),[14] and sulfonamide (X)[15] have been identified as inhibitors of cathepsin L (Fig. 1).

Presumably, the thiosemicarbazone moiety mimics the hydrolytic cleavage of peptide bonds that takes place in the active site of cathepsin L (Fig. 2).[16] Inspired by our previous success with benzophenone-based inhibitors,[7] we have extended the known SAR to include various functionalized thiophene, pyridine, fluorene, and additional benzophenone analogs bearing the thiosemicarbazone moiety.

Synthesis of certain benzophenone thiosemicarbazone derivatives utilized 3-hydroxybenzaldehyde as a starting material (Scheme 1). The appropriate hydroxy protected benzaldehyde was reacted with 3-bromophenylmagnesiumbromide to afford a secondary alcohol that was oxidized to its corresponding ketone. After formation of the thiosemicarbazone moiety, the silyl group was deprotected using TBAF to afford analogs **2** and **6**. Interestingly, attempts to form the thiosemicarbazone group through a condensation reaction with 3,3′-dibromo-2′-*tert*-butyldimethylsilyloxy benzophenone were not successful. The desired analog **10** was prepared by deprotection of the TBS group prior to formation of the thiosemicarbazone moiety (not shown in Scheme 1).

An alternative synthetic route, utilizing the appropriate Weinreb amide, was employed for the synthesis of benzophenone thiosemicarbazone analog **8** (Scheme 2).

In the non-symmetrical thiosemicarbazone analogs, *E*/*Z* double bond isomers were detectable by HPLC, however they were inseparable by chromatography. These ratios are reported in Table 1 although it is not possible to assign an individual HPLC peak to either the *E* or *Z* isomer. In addition, for certain thiosemicarbazone

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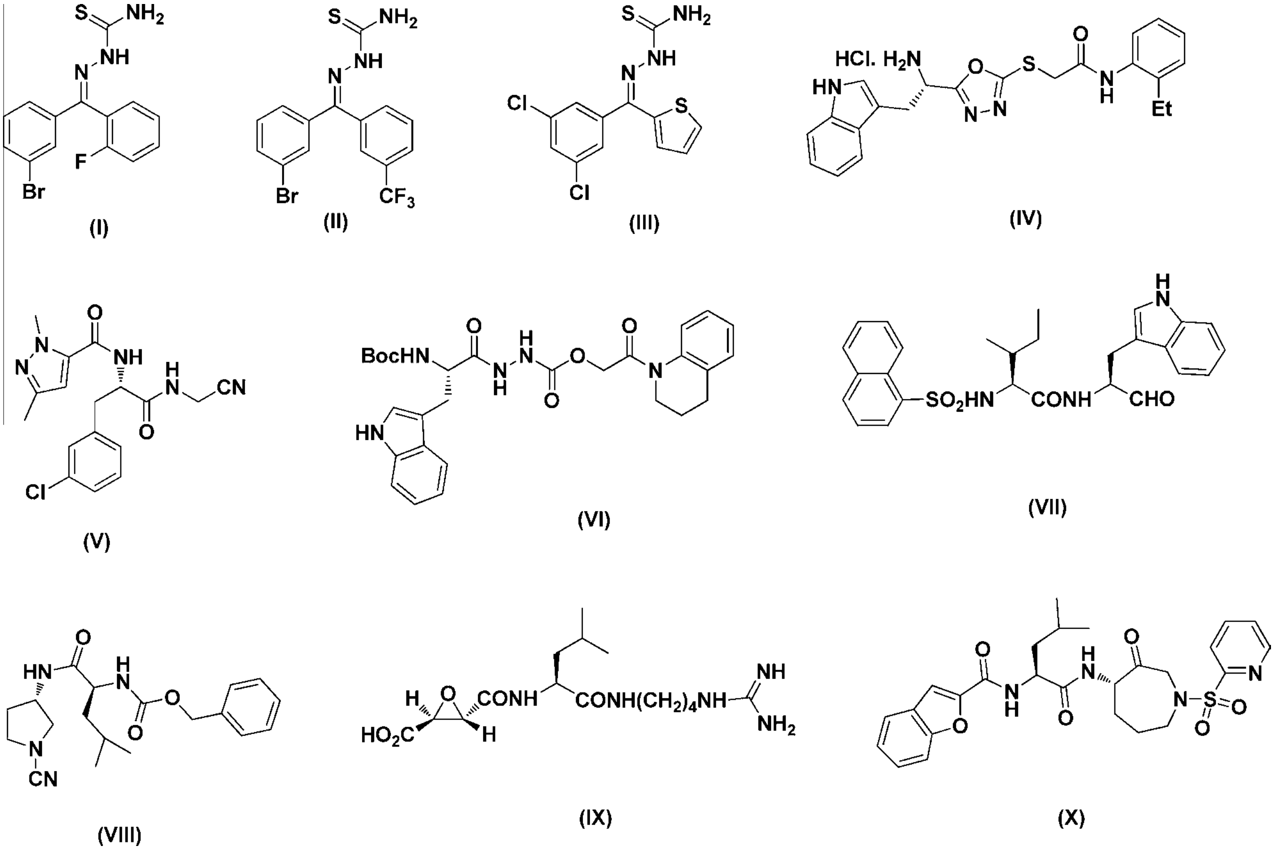


Figure 1. Representative small-molecule inhibitors of cathepsin L.

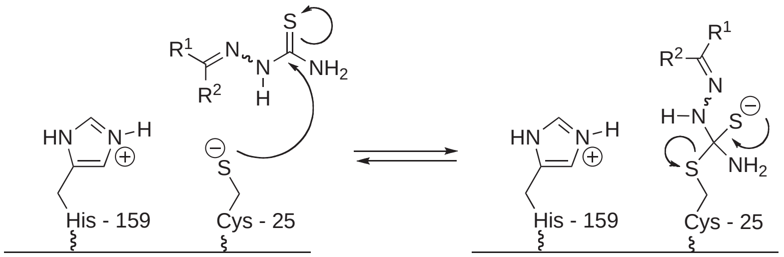
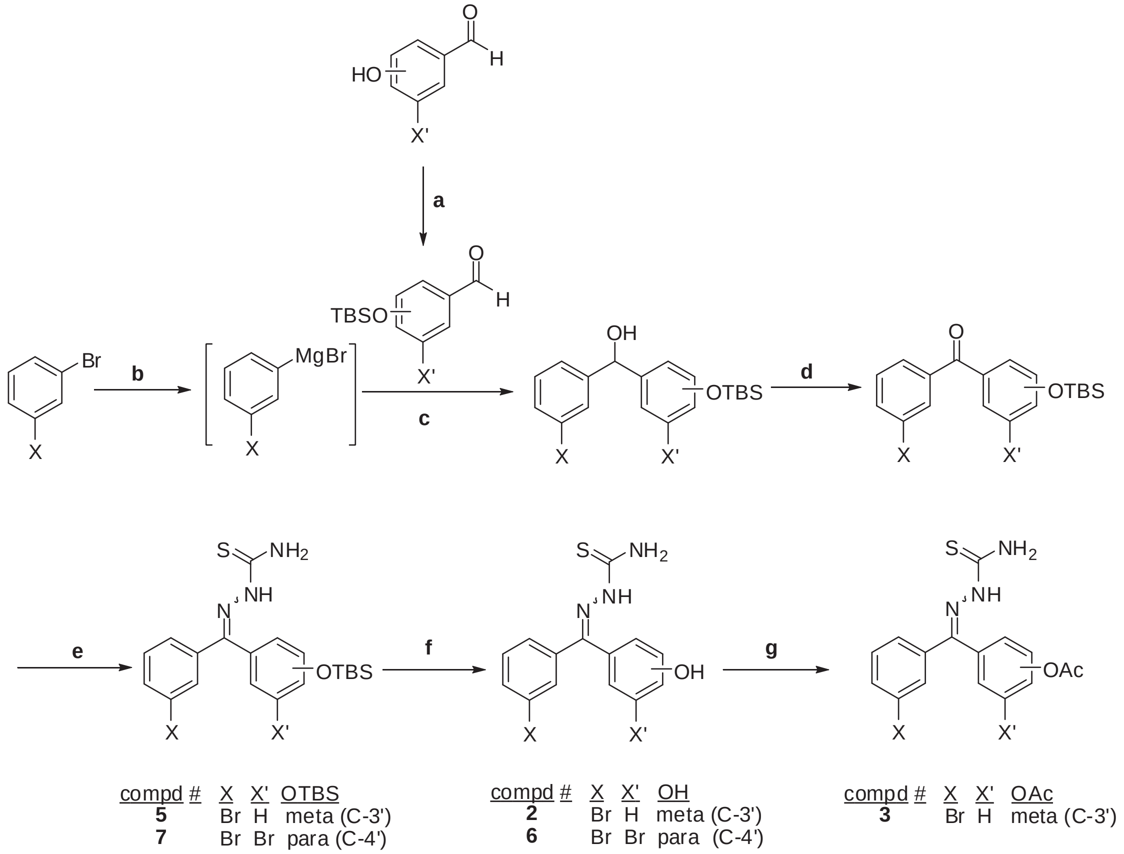


Figure 2. Proposed mechanism delineating the inhibition of cathepsin L with thiosemicarbazone analogs.

derivatives that resulted from the 1,3-dibromobenzene Grignard chemistry, a small impurity (H vs Br)[7] proved inseparable (Table 1).

Each of the synthesized thiosemicarbazone analogs was evaluated in terms of its ability to inhibit cathepsin L (Table 1). For comparison, these compounds were also evaluated for their inhibitory activity against the widely expressed enzyme cathepsin B. Three of the five benzophenone analogs (**2**, **6**, and **8**) that incorporate the phenolic functionality were active inhibitors of cathepsin L (IC_{50} <250 nM). This is potentially significant in terms of drug design and development since the phenolic moiety provides a molecular handle on which to incorporate prodrug moieties to improve bioavailability. Importantly, a comparison of the cathepsin L activity between benzophenone thiosemicarbazone analogs **1** and **2** confirms the importance of a *meta*-bromo substituent in the 'A-ring' in many of these compounds.[7a] This similar trend is seen in a comparison of brominated analog **17** (IC_{50} = 1000 nM) bearing a 'B-ring' pyridine functionality versus the non-brominated corresponding compound **16** (IC_{50} >10,000 nM). The bromo-benzophenone acetyl derivative (**3**) is also strongly inhibitory against cathepsin L (IC_{50} = 150.8 nM). The two fluorene analogs **24** and **25** are inactive as inhibitors of cathepsin L (IC_{50} >10,000 nM). In this preliminary grouping of compounds, only benzophenone analogs **3** and **11** showed any detectable activity against cathepsin B (IC_{50} = 1600 nM and 1424 nM, respectively). These results confirm the importance of a benzophenone scaffold in thiosemicarbazone inhibitors of cathepsin L reported in our previous study.[7a] The best inhibitors in our previous study[7a] all contained a *meta*-bromo substituent in the A-ring and that is the case with the current results. A number of additional SAR considerations have emerged. The most potent inhibitor in our current study (compound **6**, IC_{50} = 126.1 nM) features a *meta*-bromo, *para*-hydroxy substitution pattern in the B-ring, thus extending the SAR with functional group diversity at these positions while maintaining strong inhibition against cathepsin L. The extension of this work to now include a *meta*-hydroxy substituent in the B-ring (compound **2**) in place of a corresponding fluorine atom

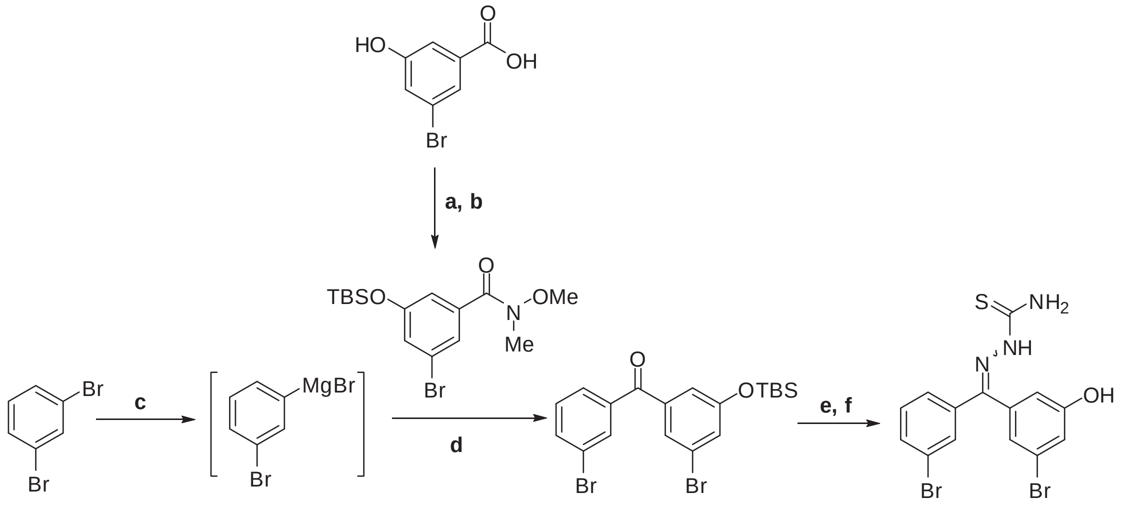


compd #	X	X'	OTBS
5	Br	H	meta (C-3')
7	Br	Br	para (C-4')

compd #	X	X'	OH
2	Br	H	meta (C-3')
6	Br	Br	para (C-4')

compd #	X	X'	OAc
3	Br	H	meta (C-3')

Scheme 1. Synthesis of various benzophenone-based thiosemicarbazone derivatives. Reagents and conditions: (a) *t*-butyldimethylsilylchloride (TBSCl), imidazole, DMF, rt, 6–12 h; (b) Mg, Et_2O, reflux, 1–2 h; (c) Et_2O, rt, 6–12 h; (d) pyridiniumchlorochromate (PCC), Celite, CH_2Cl_2, rt, 3–8 h; (e) $NH_2NHCSNH_2$, *para*-toluenesulfonic acid, MeOH, reflux, 6–12 h; (f) tetrabutylammonium fluoride (TBAF), THF, rt, 3–4 h; (g) Ac_2O, pyridine, CH_2Cl_2, rt, 4 h.



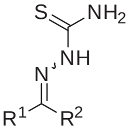
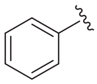
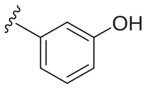
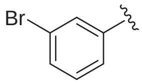
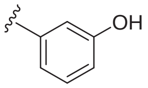
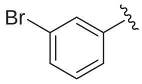
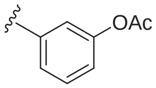
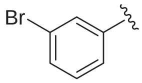
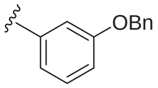
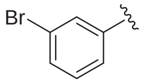
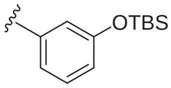
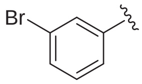
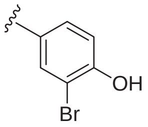
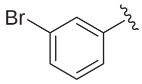
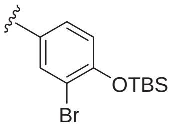
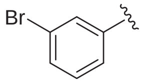
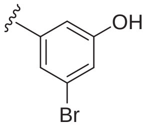
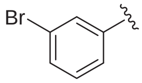
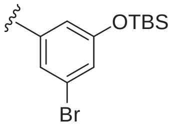
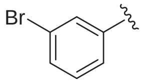
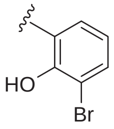
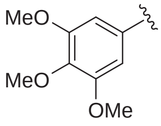
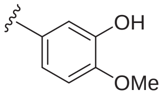
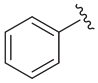
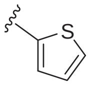
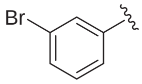
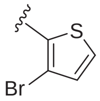
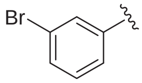
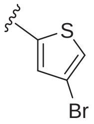
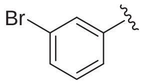
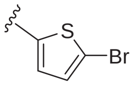
Analog **8**

Scheme 2. Synthesis of benzophenone-based thiosemicarbazone analog **8**. Reagents and conditions: (a) *t*-butyldimethylsilylchloride (TBSCl), imidazole, DMF, rt, 18–24 h; (b) 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), *N*-methylmorpholine (NMM), HCl·NH(OMe)Me, THF, rt, 24 h; (c) Mg, Et_2O, reflux, 1–2 h; (d) Et_2O, rt, 3–12 h; (e) $NH_2NHCSNH_2$, *para*-toluenesulfonic acid, MeOH, reflux, 6–12 h; (f) tetrabutylammonium fluoride (TBAF), THF, rt, 1–3 h.

(compound **5** from our previous study)[7a] actually improves the inhibitory activity towards cathepsin L (IC_{50} = 131.4 nM vs 250 nM, respectively).

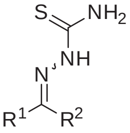
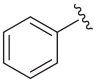
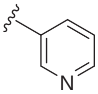
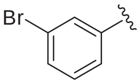
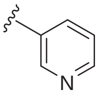
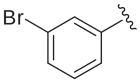
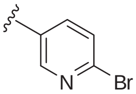
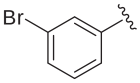
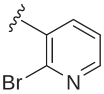
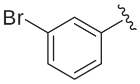
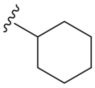
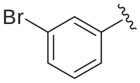
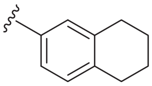
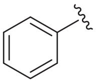
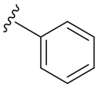
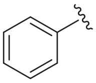
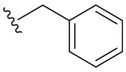
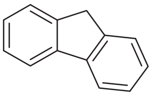
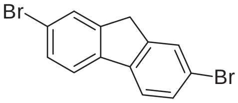
The most active inhibitor of cathepsin L in this series was evaluated for its cytotoxicity (Table 2). Consistent with the proposed mechanism of action (with cathepsin L), this compound

Table 1
Inhibition of human cathepsins L and B for various functionalized benzophenone, thiophene, pyridine, and fluorene thiosemicarbazones

	R^1	R^2	Isomer ratio estimated by HPLC[a]	H replaced by Br (% determined by HPLC)	IC_{50} (nM)[b]	
					Cat L	Cat B
1[c]			1:0.4	NA[d]	>10,000	>10,000
2			1:0.1	0.1	131.4	>10,000
3			1:1	1.0	150.8	1600
4[f]			ND[e]	3.0	>10,000	>10,000
5[f]			1:0.1	0.8	>10,000	>10,000
6[f]			1:0.6	0.1	126.1	9493
7[f]			1:0.9	0.3	>10,000	>10,000
8[g]			1:0.2	0.4	232.4	>10,000
9[g]			1:0.7	0.8	>10,000	>10,000
10[f]			ND[e]	0.2	>10,000	>10,000
11[c]			1:0.7	NA[d]	3600	1424
12[c]			ND[e]	NA[d]	>10,000	>10,000
13[f]			1:0.2	0.4	369.7	>10,000
14[f]			1:0.1	0.1	814.5	>10,000
15[f]			ND[e]	1.8	931.3	>10,000

(continued on next page)

Table 1 (*continued*)

	R^1	R^2	Isomer ratio estimated by HPLC[a]	H replaced by Br (% determined by HPLC)	IC_{50} (nM)[b]	
					Cat L	Cat B
16[c]			ND[e]	NA[d]	>10,000	>10,000
17[f]			ND[e]	4.0	1000	>10,000
18[g]			ND[e]	1.5	>10,000	>10,000
19[g]			ND[e]	0.5	>10,000	>10,000
20[h]			ND[e]	0.3	>10,000	>10,000
21[g]			1:0.5	1.4	9650	>10,000
22[c]			ND[e]	NA[d]	>10,000	>10,000
23[c]			ND[e]	NA[d]	5410	>10,000
24[c]			ND[e]	NA[d]	>10,000	>10,000
25[c]			ND[e]	NA[d]	>10,000	>10,000

[a] *E*/*Z* isomer not assigned.

[b] 2% DMSO.

[c] Synthesis begins with commercially available ketone.

[d] NA = not applicable.

[e] ND = not detected

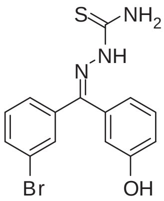
[f] Synthesis begins with aldehyde derivatives (see Scheme 1).

[g] Synthesis begins with carboxylic acid derivatives (see Scheme 2).

[h] Synthesis begins with acid chloride (see Ref. 7a).

was not particularly cytotoxic against these selected human cancer cell lines.

Table 2
Cytotoxicity against selected human cancer cell lines for analog **2**

	Cytotoxicity GI_{50} (μM)	
	NCI-H460	DU-145
2	23.3	21.6

In summary, this study has identified three new bromo-benzophenone thiosemicarbazone analogs **2**, **6**, and **8** each bearing a phenolic moiety that are potent inhibitors of cathepsin L. Future studies will center on additional synthetic manipulations along with further biochemical and biological evaluation of these important new lead compounds and related analogs.

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NMR spectrometer (Award CHE-0420802). The authors thank Ms. Ashleigh Locke and Ms. Erica N. Parker for valuable assistance with portions of the chemical synthesis, Dr. Alejandro Ramirez (Mass Spectrometry Core Facility, Baylor University) for HRMS analysis, and Dr. James Karban and Dr. Michelle Nemec (Director) for use of the shared Molecular Biosciences Center. The authors are grateful to Dr. Michael P. Hay (Auckland Cancer Society Research Centre) for valuable consultation regarding certain aspects of this project.

Supplementary data

Supplementary data (detailed experimental syntheses and details regarding biological assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.026.

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16. Portions of this work have been reported in the following dissertation: Arispe, W. M. Inhibitors of human cathepsin L and cruzain as therapeutic agents. Ph.D. Dissertation, Baylor University, Waco, TX, 2008.