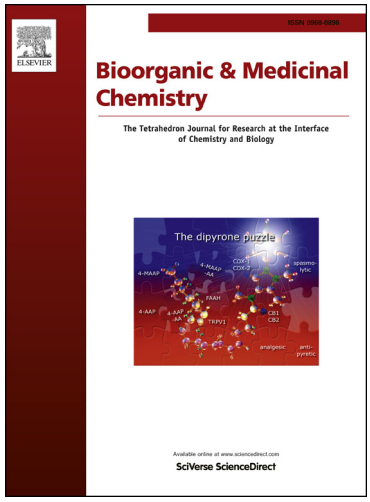

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Synthesis and biochemical evaluation of benzoylbenzophenone thiosemicarbazone analogues as potent and selective inhibitors of cathepsin L

Erica N. Parker[a], Jiangli Song[a], G. D. Kishore Kumar[a], Samuel O. Odutola[b], Gustavo E. Chavarria[a], Amanda K. Charlton-Sevcik[a], Tracy E. Strecker[a], Ashleigh L. Barnes[a], Dhivya R. Sudhan[c], Thomas R. Wittenborn[e], Dietmar W. Siemann[c,d], Michael R. Horsman[e], David J. Chaplin[f], Mary Lynn Trawick[a,b,*], Kevin G. Pinney[a,b,*]

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Abbreviations used

HUVECs, human umbilical vein endothelial cells; ECM, extracellular matrix; TSC, thiosemicarbazide; SAR, structure-activity relationship; Z-FR-AMC, N-carbobenzyloxy-L-Phe-L-Arg-7-amino-4-methylcoumarin; FBS, fetal bovine serum; SRB, sulforhodamine B.

ABSTRACT

Upregulation of cathepsin L in a variety of tumors and its ability to promote cancer cell invasion and migration through degradation of the extracellular matrix suggest that cathepsin L is a promising biological target for the development of anti-metastatic agents. Based on encouraging results from studies on benzophenone thiosemicarbazone cathepsin inhibitors, a series of fourteen benzoylbenzophenone thiosemicarbazone analogues were designed, synthesized, and evaluated for their inhibitory activity against cathepsins L and B. Thiosemicarbazone inhibitors 3-benzoylbenzophenone thiosemicarbazone **1**, 1,3-bis(4-fluorobenzoyl)benzene thiosemicarbazone **8**, and 1,3-bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone **32** displayed the greatest potency against cathepsin L with low IC_{50} values of 9.85 nM, 14.4 nM, and 8.12 nM, respectively. The benzoylbenzophenone thiosemicarbazone analogues evaluated were selective in their inhibition of cathepsin L compared to cathepsin B. Thiosemicarbazone analogue **32** inhibited invasion through Matrigel of MDA-MB-231 breast cancer cells by 70% at 10 μM. Thiosemicarbazone analogue **8** significantly inhibited the invasive potential of PC-3ML prostate cancer cells by 92% at 5 μM. The most active cathepsin L inhibitors from this benzoylbenzophenone thiosemicarbazone series (**1**, **8**, and **32**) displayed low cytotoxicity toward normal primary cells [in this case human umbilical vein endothelial cells (HUVECs)]. In an initial *in vivo* study, 3-benzoylbenzophenone thiosemicarbazone (**1**) was well-tolerated in a CDF1 mouse model bearing an implanted C3H mammary carcinoma, and showed efficacy in tumor growth delay. Low cytotoxicity, inhibition of cell invasion, and in vivo tolerability are desirable characteristics for anti-metastatic agents functioning through an inhibition of cathepsin L. Active members of this structurally diverse group of benzoylbenzophenone thiosemicarbazone cathepsin L inhibitors show promise as potential anti-metastatic, pre-clinical drug candidates.

Keywords

Small-molecule synthesis, thiosemicarbazone warhead, cathepsin L inhibitors, inhibition of cancer cell invasion and migration, anti-metastatic agents

1. Introduction

In various human cancers cathepsins L, B, K, H, S, and X display increased activity and play significant mechanistic roles in the invasion and migration of malignant cells.[1-5] Cysteine protease cathepsins degrade proteolytic targets predominately within lysosomes under normal physiological conditions.[1-5] In cancer, cathepsins promote metastasis through phagocytosis as well as extracellulary through multiple pathways including direct proteolysis of E-cadherin[6] and components in the extracellular matrix (ECM) such as fibronectin,[7-10] laminin,[8-10] collagen,[9-15] and indirectly through the activation of other proteases[16] resulting in the degradation of the ECM (Figure 1).

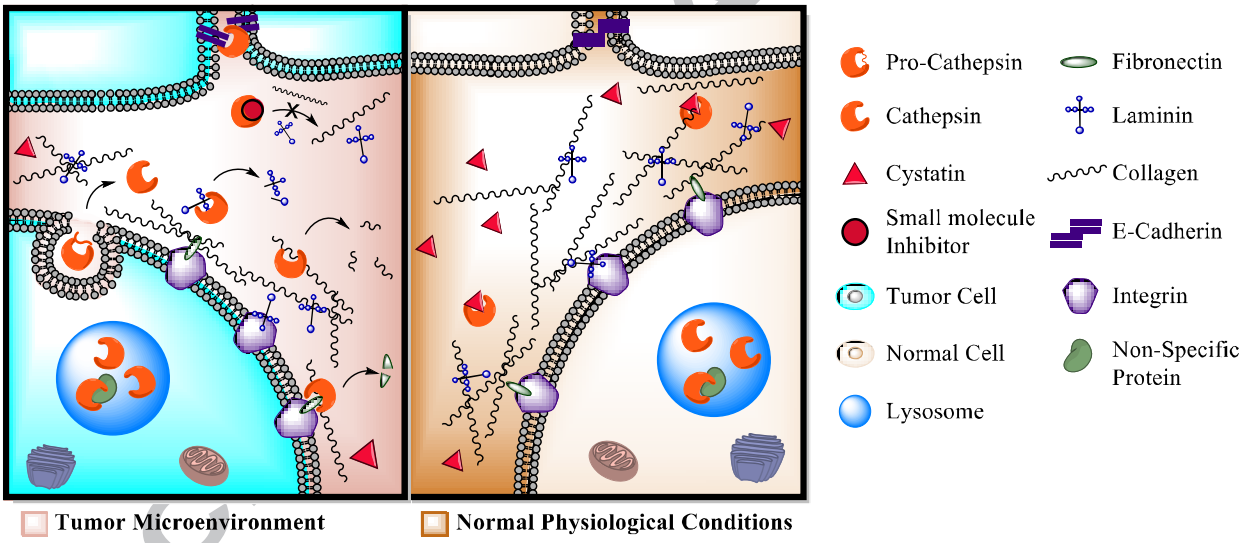


Figure 1. Overview of cysteine cathepsins in invasion and metastasis**.** Compared to normal physiological conditions, certain cathepsins are upregulated in the tumor microenvironment. Additionally, changes occur within the tumor microenvironment such as downregulation of endogenous inhibitors including the cystatins, extracellular acidification increasing the proteolytic capability of cathepsins, and extracellular secretion of cathepsins. Degradation of the extracellular matrix through the direct proteolysis of matrix and cell-adhesion proteins such as laminin, fibronectin, collagen, E-cadherin facilitates tumor cell invasion from the primary tumor siteand ultimately migration to a secondary tumor site. Small-molecule cathepsin inhibitors have the potential to inhibit the invasive nature of malignant cells and may provide increased effectiveness in combination with known chemotherapeutics.[17]

Elevated levels of cathepsin L, a ubiquitous endopeptidase, and cathepsin B, an endopeptidase as well as a carboxypeptidase, are associated with poor prognosis in breast cancer, colorectal cancer, lung cancer, brain tumors, melanoma, and head and neck cancer.[18, 19] Cathepsin K, an endopeptidase involved in bone resorption, shows increased activity in breast cancer, cervical cancer, and lung cancer.[20] The cathepsin K inhibitor Odanacatib (MK-0822) displayed promise in a recent clinical trial by reducing bone resorption in individuals with metastatic bone disease.[21-22]

In addition to metastasis, cathepsins are involved in other pathological processes. Drug candidates currently in the pipeline of pharmaceutical companies include the cathepsin K inhibitors Odanacatib (Merck)[21-24] and MIV-711 (Medivir)[25] for the treatment of osteoporosis, the cathepsin B inhibitor VBY-376 (Virobay)[26-27] and the pan cysteine protease inhibitor VBY-825[28-29] for the treatment of liver fibrosis, the cathepsin C inhibitor GSK2793660 (GlaxoSmithKline) for the treatment of bronchiectasis,[30-31] and the cathepsin S inhibitor MIV-247 (Medivir)[32] for the treatment of neuropathic pain. Although selective inhibitors of cathepsin L have not yet reached clinical trials, several have been described in the literature (Figure 2).

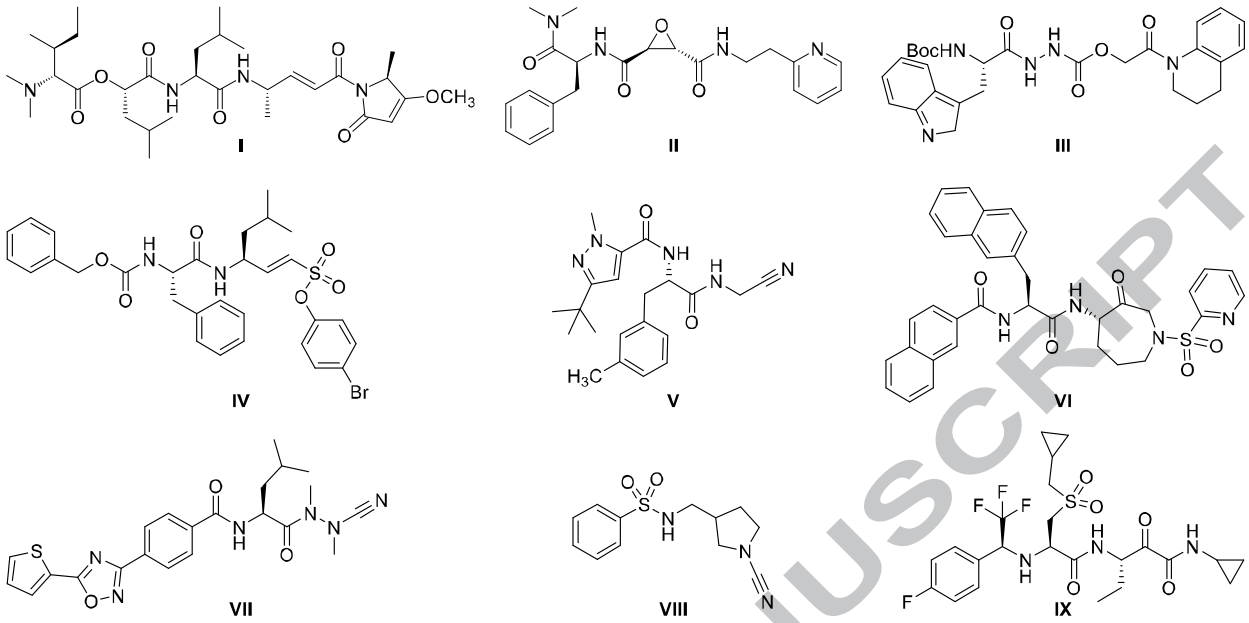


Figure 2. Representative potent inhibitors of cathepsin L utilizing various warheads. Notable selective inhibitors of cathepsin L include the natural product gallinamide A **I**,[33] the epoxysuccinamide inhibitor Clik 148 **II**,[34] the oxocarbazate **III**,[35] the vinyl sulfonate **IV**,[36] the dipeptidyl nitrile **V**,[37] and the azepanone **VI**.[38] Potent but non-selective inhibitors of cathepsin L include the nonpeptidic cyanamide **VII**,[39] the azadipeptide nitrile **VIII**,[40] and the diketone VBY-825 **IX**.[28-29]

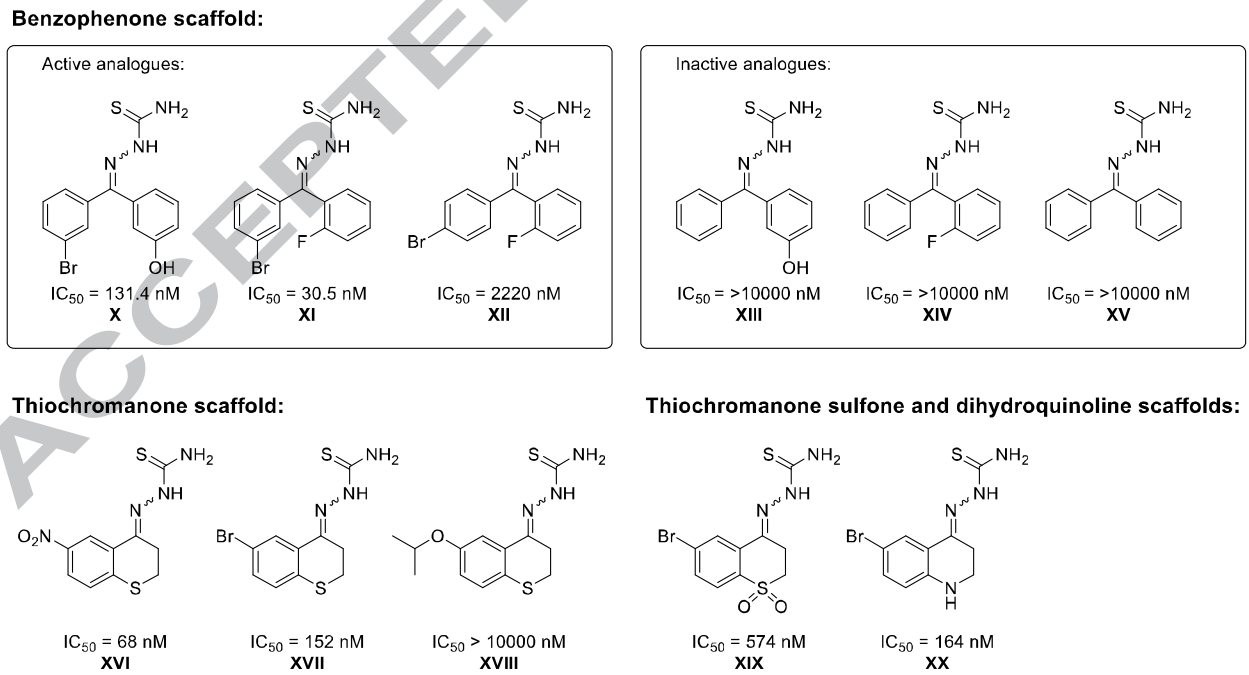
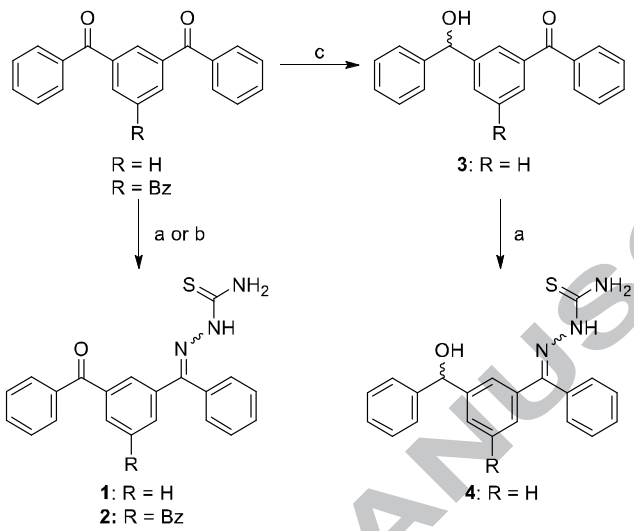


Figure 3. Activity of selected benzophenone thiosemicarbazone analogues against cathepsin L

Historically, molecules incorporating the thiosemicarbazone functionality have been used as antiviral and antibacterial agents in the treatment of smallpox[41-43] and tuberculosis.[44] In addition to the inhibition of cathepsins,[45-51, 52] incorporation of the thiosemicarbazone moiety as an electrophilic warhead has proved useful in the identification of potent small-molecule inhibitors of cysteine proteases found in *Trypanosoma cruzi* and *Trypanosoma brucei*,[53-57] *Plasmodium falciparum*,[57-59] *Leishmania Mexicana*,[60] and *Eimeria tenella*.[61] In the investigation of thiosemicarbazone inhibitors in our group[45-50] several cathepsin L inhibitors based on the benzophenone, thiochromanone, thiochromanone sulfone, and dihydroquinoline scaffolds including (3-bromo-3'-hydroxybenzophenone) thiosemicarbazone **X**,[46-48] (3-bromo-2'-fluorobenzophenone) thiosemicarbazone **XI**,[45] and 6-nitrothio-4-chromanone thiosemicarbazone **XVI**[49] emerged as promising lead compounds (Figure 3).

2. Results and discussion

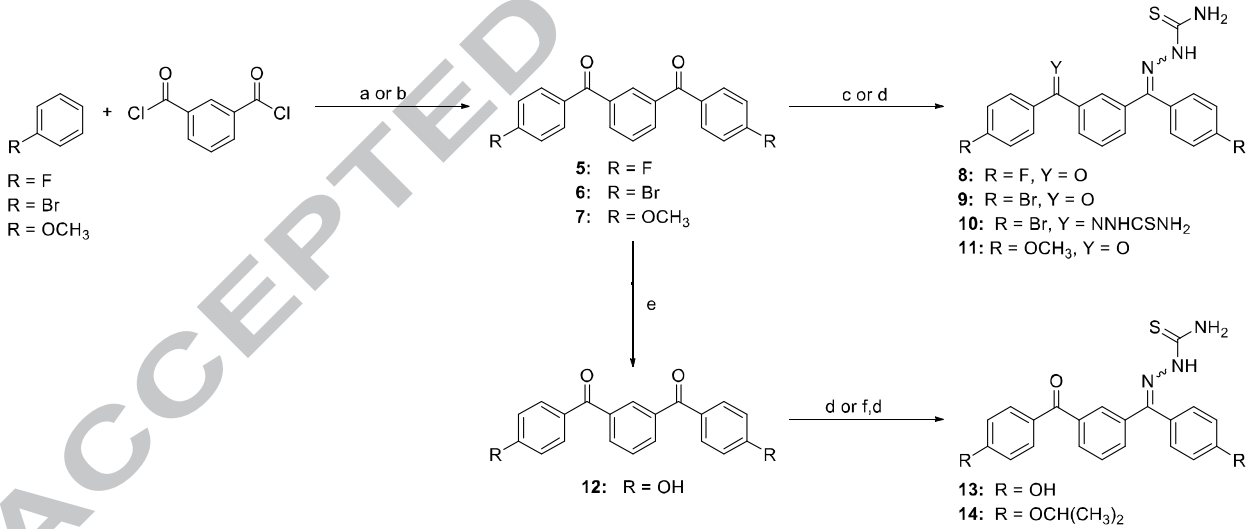
2.1 Design and synthetic chemistry



Scheme 1. Synthesis of benzoylbenzophenone thiosemicarbazone analogues. Reagents and conditions: (a) Thiosemicarbazide (TSC), TsOH, THF, reflux; (b) TSC, TsOH, MeOH, reflux; (c) EtOH, $NaBH_4$, 0 ^oC.

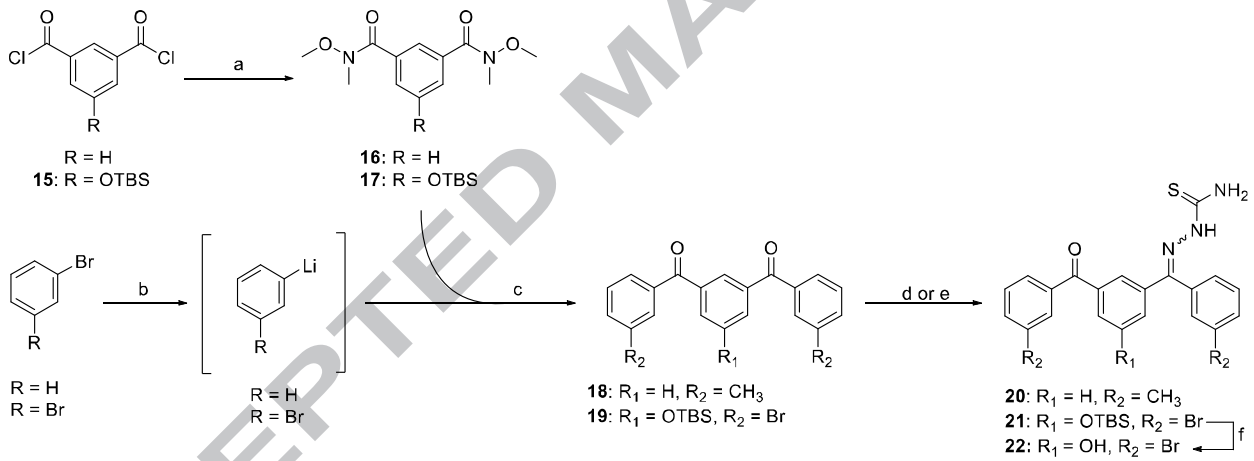
Functional group considerations based on our initial benzophenone thiosemicarbazone inhibitors included the incorporation of a benzoyl moiety which, in this study, led to the discovery that appropriately functionalized benzoylbenzophenone thiosemicarbazone analogues demonstrated potent inhibition of cathepsin L. In addition to incorporation of a benzoyl group, structural motifs previously identified in potent benzophenone thiosemicarbazone inhibitors were integrated into the design of this new series of benzoylbenzophenone thiosemicarbazone analogues.[51] Previously,[45-46] incorporation of the 3-bromo functionality in benzophenone thiosemicarbazone analogues proved critical in providing analogues which displayed potent inhibitory activity against cathepsin L as demonstrated by the large differences in IC_{50} values found for **X** and **XI** compared to the non-brominated analogues **XIII** and **XIV**, respectively

(Figure 3). Additionally, the 4-bromo analogue **XII** displayed reduced activity towards cathepsin L compared to the 3-bromo analogue **XI** further emphasizing the importance of the 3-bromo functionality. Drawing on previous SAR studies based on the benzophenone scaffold,[45-46] extension of key structural components of the potent cathepsin L benzophenone thiosemicarbazone inhibitors **X** and **XI** led to the design of benzoylbenzophenone thiosemicarbazone inhibitors **31** and **32** which incorporated the 3-bromo functionality in the central aromatic ring. In order to avoid potential complications that could arise due to the difficulty of separating thiosemicarbazone regioisomers, only symmetrical diketones were utilized in the synthesis of the new target compounds. Various synthetic methodologies (Schemes 1-4) were employed to assemble each benzoylbenzophenone molecular scaffold. Benzoylbenzophenone thiosemicarbazones **1, 2** and benzoylbenzhydrol thiosemicarbazone **4** were prepared from commercially available diketones as illustrated in Scheme 1.



Scheme 2. Synthesis of benzoylbenzophenone thiosemicarbazone analogues utilizing Friedel-Crafts acylation. Reagents and conditions: (a) $AlCl_3$ (neat), reflux; (b) $AlCl_3$, CH_2Cl_2, reflux; (c) TSC, TsOH, THF, microwave irradiation; (d) TSC, TsOH, THF, reflux; (e) $BF_3 \cdot SMe_2$, CH_2Cl_2, rt; (f) isopropyl bromide, K_2CO_3, DMF, 90 ^oC.

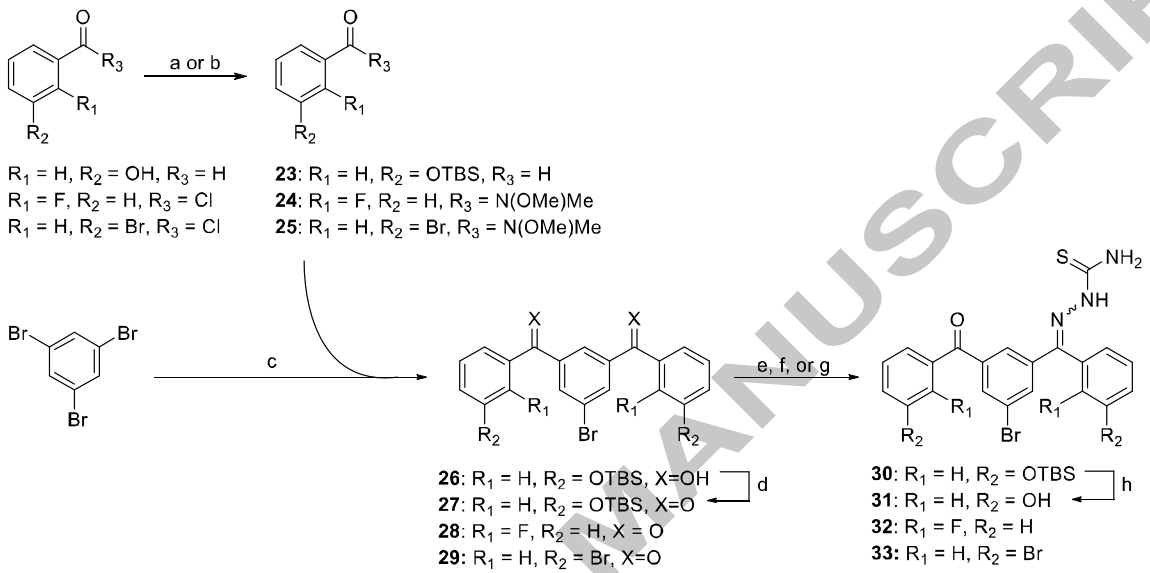
Utilization of Friedel-Crafts acylation chemistry facilitated the synthesis of *para* substituted benzoylbenzophenone analogues as depicted in Scheme 2. Reaction of isophthaloyl dichloride and the appropriately substituted benzene ring with aluminum chloride afforded *para* substituted 1,3-dibenzoylbenzene analogues **5-7** which underwent condensation with thiosemicarbazide to yield target thiosemicarbazone analogues **8-11.** Demethylation of 1,3-bis(4-methoxybenzoyl)benzene **7** followed by condensation with thiosemicarbazide afforded thiosemicarbazone **13** and reaction of diketone **12** with isopropyl bromide followed by condensation with thiosemicarbazide afforded the *p*-isopropoxy derivative **14.**



Scheme 3. Synthesis of benzoylbenzophenone thiosemicarbazone analogues utilizing isophthaloyl chloride. Reagents and conditions: (a) Me(OMe)NH ·HCl, NEt_3, CH_2Cl_2, 0 ^oC -rt; (b) n-BuLi, THF, -78 ^oC; (c) THF, -78 ^oC; (d) TSC, TsOH, MeOH, reflux; (e) TSC, TsOH, THF, microwave irradiation, 90 ^oC; (f) TBAF, THF, rt.

In order to incorporate *meta* substituents in the outermost rings of the benzoylbenzophenone scaffold (Scheme 3), isophthaloyl dichloride starting materials were used as precursors to Weinreb amides **16** and **17**. Diketones **18** and **19** were synthesized from an intermediate organolithium reagent and Weinreb amides **16** and **17** (separately)**.** Condensation of

the resulting *meta* substituted 1,3-dibenzoylbenzene analogues with thiosemicarbazide and removal of any protecting groups afforded benzoylbenzophenone thiosemicarbazone analogues **20** and **22**.



Scheme 4. Synthesis of benzoylbenzophenone thiosemicarbazone analogues utilizing 1,3,5-tribromobenzene. Reagents and conditions: (a) Me(OMe)NH ·HCl, NEt_3, CH_2Cl_2, 0 ^oC -rt; (b) TBSCl, DMF, imidazole, 0 ^oC -rt; (c) (i) t-BuLi, ether, -78 ^oC; (ii) 23, 24, or 25 in ether, -78 ^oC; (d) PCC, celite, CH_2Cl_2, 0 ^oC -rt; (e) TSC, TsOH, THF, microwave irradiation, 90 ^oC; (f) TSC, TsOH, THF, reflux; (g) TSC, $Ti(OiPr)_4$, THF, reflux (h) TBAF, THF, rt.

In our previous studies, benzophenone thiosemicarbazone analogues[45-48] containing a *meta*-bromo substituent were among those with the highest inhibitory activity against cathepsin L. Incorporation of *meta*-bromo substituents on the central aromatic ring in the benzoylbenzophenone scaffold was achieved by utilizing 1,3,5-tribromobenzene as a starting material. Halogen-metal exchange of 1,3,5-tribromobenzene with *t*-BuLi followed by the addition of two equivalents of either aldehyde **23** or Weinreb amides **24** and **25** (separately) allowed for the incorporation of a *meta*-bromo substituent on the central ring of diketone analogues **27-29** (Scheme 4). Subsequent condensation of the diketones **27-29** with

thiosemicarbazide yielded the benzoylbenzophenone thiosemicarbazone analogues **30, 32-33**, and deprotection of **30** afforded the *m*-hydroxy substituted analogue **31**.

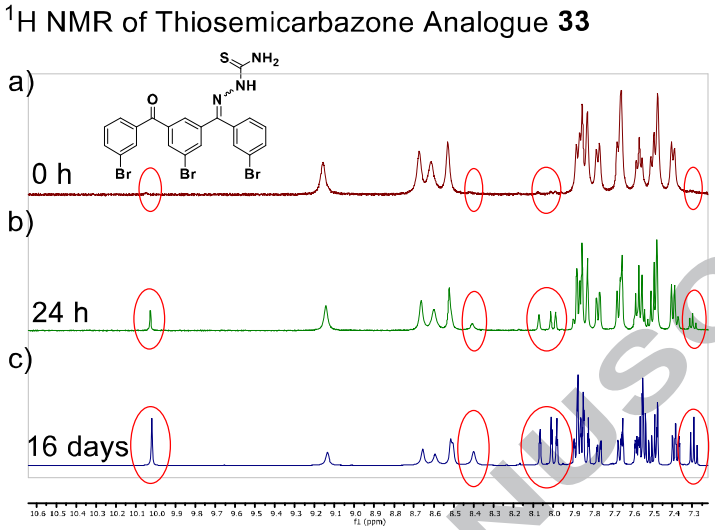


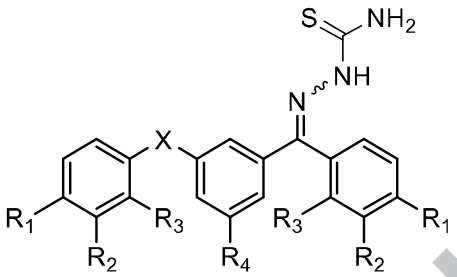
Figure 4. Isomerization of thiosemicarbazone analogue **33** in DMSO-d_6**.** The red ellipses indicate regions in the ^{1}H NMR spectra where additional peaks emerge due to the presence of the other geometrical isomer. (a) ^{1}H NMR of compound **33** after 0 hours in DMSO-d_6. (b) ^{1}H NMR of compound **33** after 24 hours in DMSO-d_6. (c) ^{1}H NMR of compound **33** after 16 days in DMSO-d_6.

Moderate to low yields were observed for the condensation reaction to form the mono thiosemicarbazone analogues due to the competing formation of the bis-thiosemicarbazone products. For some of the desired mono-thiosemicarbazone products a lower equivalency of thiosemicarbazide proved effective at minimizing formation of the bis derivative and maximizing yield, but not in all cases. With the exception of 1,3-bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone **32**, which was isolated as the Z isomer and did not isomerize after standing in acetone for one week, the final products exist as an inseparable mixture of *E/Z* isomers in solution (As evidenced by ^{1}H NMR). Imines including thiosemicarbazones are well-known for their propensity to isomerize in solution under catalyzed[62-63] and non-catalyzed[64-65] conditions or from heating while in the solid state.[66] As an example, benzoylbenzophenone thiosemicarbazone **33**, after purification and drying, was isolated as a single isomer; however,

the compound slowly isomerized in solution (Figure 4). After 16 days of standing in DMSO at room temperature, thiosemicarbazone **33** isomerized to a 1:1 mixture of *E/Z* isomers.

2.2 Cathepsin Inhibition Studies

Table 1. Inhibitory Activity of Benzoylbenzophenone Thiosemicarbazone Analogues



Cmpd	R_1	R_2	R_3	R_4	X	IC_{50}[a] Values (nM) Cat L	Cat B
1	H	H	H	H	C=O	9.9	>10000
2	H	H	H	Bz	C=O	56.0	>10000
4	H	H	H	H	CH(OH)	23.8	>10000
8	F	H	H	H	C=O	14.4	>10000
9	Br	H	H	H	C=O	1522	>10000
10	Br	H	H	H	$C=NNHC(S)NH_2$	>10000	>10000
11	OCH_3	H	H	H	C=O	5117	>10000
13	OH	H	H	H	C=O	340	>10000
14	$OCH(CH_3)_2$	H	H	H	C=O	>10000	>10000
20	H	CH_3	H	H	C=O	654	>10000
22	H	Br	H	OH	C=O	~10000[b]	>10000
31	H	OH	H	Br	C=O	71.6	>10000
32	H	H	F	Br	C=O	8.1	>10000
33	H	Br	H	Br	C=O	10347	>10000

[a]These values are averages of a minimum of a triplicate of experiments. Each assay utilized 2% DMSO with a 5 min pre-incubation period. Standard error values can be found in the Supplementary data.
[b] Compound **22** inhibited cathepsin L activity by 56.9% at 10000 nM.

The thiosemicarbazone derivative of commercially available 1,3-dibenzoylbenzene (3-benzoylbenzophenone thiosemicarbazone **1**) displayed pronounced inhibitory activity against cathepsin L with an IC_{50} value of 9.85 nM (Table 1). Interestingly, the analogous benzophenone thiosemicarbazone **XV** (Figure 3) was inactive (IC_{50} >10000 nM) against cathepsin L.[46] Since thiosemicarbazone **1** displayed pronounced activity against cathepsin L, several analogues were synthesized including compounds which incorporated previously reported molecular scaffolds (Figure 3) known to be effective in terms of providing compounds with strong inhibitory activity against cathepsin L.[45-48] In addition, certain structural modifications of the unsubstituted analogue **1** such as the incorporation of a structurally demanding benzoyl substituent on the central aromatic ring, exemplified by tribenzoylbenzophenone thiosemicarbazone analogue **2** (IC_{50} = 56.0 nM), and the incorporation of a secondary alcohol in place of the carbonyl, exemplified by 3-benzoylbenzhydrol thiosemicarbazone **4** (IC_{50} = 23.8 nM), were well tolerated**.** Various *para* substituted analogues were evaluated against cathepsin L. The *p*-fluoro analogue **8** had comparable activity to the extremely potent unsubstituted analogue **1.** The activity against cathepsin L diminished with increasing steric hindrance in the *para*-substituted series (*p*-hydroxy **13**, *p*-methoxy **11**, *p*-isopropoxy **14**). Thiosemicarbazone analogues **9, 22,** and **33** each with *p*-bromo or *m*-bromo substituents on the outermost rings of the benzoylbenzophenone molecular template, although still active, showed a significant reduction in regard to inhibitory activity against cathepsin L compared to the other compounds. Additionally, the *p*-bromo substituted bis-thiosemicabazone analogue **10** showed no activity against cathepsin L at a concentration of 10,000 nM. Compared to the parent benzophenone thiosemicarbazone **XI** (Figure 3) with an IC_{50} value of 30.5 nM, 1,3-bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone **32** exhibited a greater than three fold increase in activity against cathepsin L with an IC_{50} value of

8.12 nM. Incorporation of the benzophenone thiosemicarbazone **X** (Figure 3) molecular design into the benzoylbenzophenone scaffold led to two thiosemicarbazone analogues; compound **31** with *meta*-hydroxy substituents on the outermost rings and compound **22** with *meta*-bromo substituents on the outermost rings. Thiosemicarbazone **31** showed a nearly two fold increase in activity against cathepsin L with an IC_{50} value of 71.6 nM compared to the parent benzophenone thiosemicarbazone **X** (IC_{50} = 131.4 nM) while analogue **22** showed a significant decrease in activity against cathepsin L with an IC_{50} value of ~10000 nM. Overall, increasing steric bulk on the outermost rings likely resulted in benzoylbenzophenone analogues with decreased inhibitory activity. Interestingly, all active benzoylbenzophenone thiosemicarbazone analogues were selective against cathepsin L compared to cathepsin B and thiosemicarbazone analogues (**31** and **32**) of the parent benzophenone thiosemicarbazones **X** and **XI** (which do not have bulky groups on the outermost rings) displayed increased activity against cathepsin L. These studies have demonstrated that the benzoylbenzophenone scaffold coupled with the thiosemicarbazone electrophilic moiety represents an effective molecular design leading to low nM inhibitors of cathepsin L and additionally allows for a variety of structural modifications in the core scaffold while maintaining activity against cathepsin L.

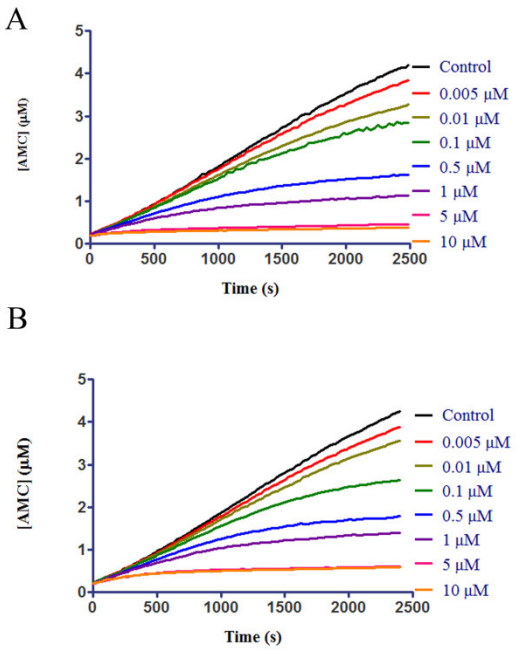


Figure 5. Representative progress curves of cathepsin L (1 nM) activity using Z-FR-AMC (10 μM) as substrate and increasing concentrations of (A) inhibitor **1** and (B) inhibitor **32** (0–10 μM). Reactions were initiated by the addition of enzyme with no pre-incubation with compounds. Release of the fluorescent product AMC was followed as a function of time.

Kinetic analysis of the closely related thiosemicarbazone analogue **X** (Figure 3) showed that it was a time-dependent and slowly reversible inhibitor of cathepsin L.[47] The reaction progress curves for benzoylbenzophenone thiosemicarbazone analogues **1** and **32** (Figure 5) were comparable to those previously observed for (3-bromo-3'-hydroxybenzophenone) thiosemicarbazone **X**. The cathepsin L catalyzed reaction was carried out with N-carbobenzyloxy-L-Phe-L-Arg-7-amino-4-methylcoumarin (Z-FR-AMC) as substrate in the presence of varying concentrations (0–10 μM) of thiosemicarbazone analogues **1** and **32**. The progress curves (Figure 5) demonstrated that these compounds are not classical, readily reversible inhibitors. The increase in inhibition as a function of time, as observed from a reduction in the slopes of the curves over time compared to untreated control, indicated that both compounds are time-dependent inhibitors of cathepsin L. Separate experiments demonstrated

that analogues **1** and **32** were slowly reversible inhibitors of cathepsin L (see Supplementary data).

2.3 Molecular modeling studies

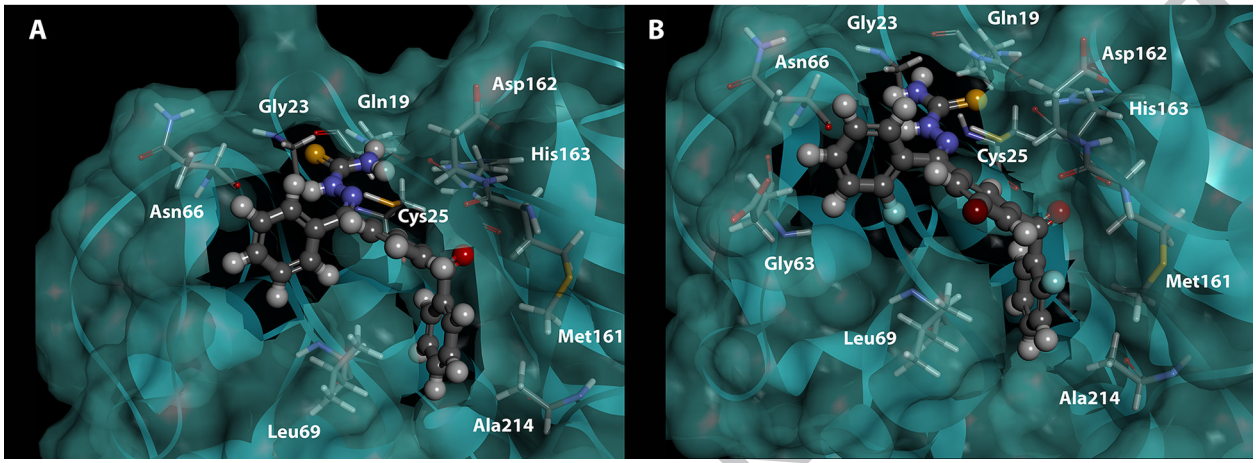


Figure 6. Molecular docking of analogues **1** and **32** within the active site of cathepsin L**.** (A) Analogue **1** and (B) analogue **32** are shown in ball and stick mode (C, gray; H, white; O, red; N, blue; S, yellow; F, light blue; Br, brown). Cathepsin L is shown in ribbon mode with a transparent molecular surface (Green) and enzyme active site amino acid residues are labeled and shown in stick mode (C, gray; H, white; O, red; N, blue; S, yellow).

Thiosemicarbazone analogues **1** and **32** which displayed the most pronounced activity against cathepsin L were selected for molecular docking studies. The co-crystal structure of cathepsin L with a nitrile inhibitor (PDB: 2XU1)[67] within the active site of cathepsin L was chosen as a starting model for the docking studies which were performed with Discovery Studio 4.1 (Accelrys). A number of different binding modes with similar interaction energies were obtained from docking analogues **1** and **32**. In each case, several of the top binding orientations placed the thiocarbonyl carbon atom of the inhibitor in close proximity to the Cys25 thiolate in a similar manner to that observed with benzophenone thiosemicarbazones **X**[47] and **XI**.[45] One of the top binding orientations for each of benzoylbenzophenone thiosemicarbazone analogues **1** and **32** which positions the Cys25 thiolate within 5 Å of the thiocarbonyl carbon atom is illustrated in

Figure 6. For analogue **1**, the benzophenone fragment of the inhibitor resides deep within the S2 pocket of cathepsin L which is considered an important subsite for binding among the papain family of cysteine proteases.[67-68] The remaining outermost ring of analogue **1** is oriented towards the S3 subsite. A hydrogen bond between the carbonyl oxygen of the inhibitor and the backbone NH of His163 and a hydrogen bond between the terminal NH_2 of the thiosemicarbazone and the backbone carbonyl of Asp162 is observed for analogue **1**. Molecular docking studies for analogue **32** orient the aryl rings of the inhibitor within the active site of cathepsin L in a similar fashion to analogue **1** with the benzophenone fragment occupying the S2 pocket and the opposing ring oriented toward the S3 subsite. For comparison, molecular docking studies were carried out with analogue **14,** which was inactive against cathepsin L. Molecular modeling indicated that analogue **14** did not bind in a manner that would facilitate formation of a covalent bond. The benzophenone portion resides in the S2 pocket of cathepsin L in a similar fashion observed for active cathepsin L inhibitors **1** and **32**. However, the remaining outermost ring resides in the S1 subsite instead of being oriented towards the S3 subsite and the thiosemicarbazone moiety is oriented towards the solvent instead of residing in the S1 subsite. In each of the top binding orientations, the thiocarbonyl carbon atom of analogue **14** was not in close proximity to the Cys25 thiolate ion of cathepsin L. Active cathepsin L inhibitors **1** and **32** bind in such a manner to facilitate formation of a transient covalent bond between the enzyme and inhibitor. This comparison emphasizes that formation of a transient covalent bond is necessary for activity against cathepsin L in this series of compounds (see Supplementary data).

2.4 Invasion and migration studies

A hallmark of invasive cancer cells is their ability to invade through the ECM. Cell invasion through Matrigel as a model for the ECM[69] of the highly metastatic prostate cancer cell line PC-3ML[48,70] and the highly invasive breast cancer cell line MDA-MB-231[71-72] was inhibited by selected benzoylbenzophenone thiosemicarbazone inhibitors. Additionally, selected thiosemicarbazone analogues were evaluated for their ability to inhibit cell migration. To separate anti-tumorigenic from anti-metastatic effects, non-cytotoxic doses of evaluated thiosemicarbazones were used. The results showed that treatment with thiosemicarbazone analogue **8** significantly impaired the invasive capacities of prostate PC-3ML cancer cells by 59% and 92% at 1 µM and 5µM, respectively (Figure 7). The unsubstituted analogue **1** inhibited cell invasion of PC-3ML prostate cancer cells by 37% and 53% at 10 µM and 25 µM, respectively.

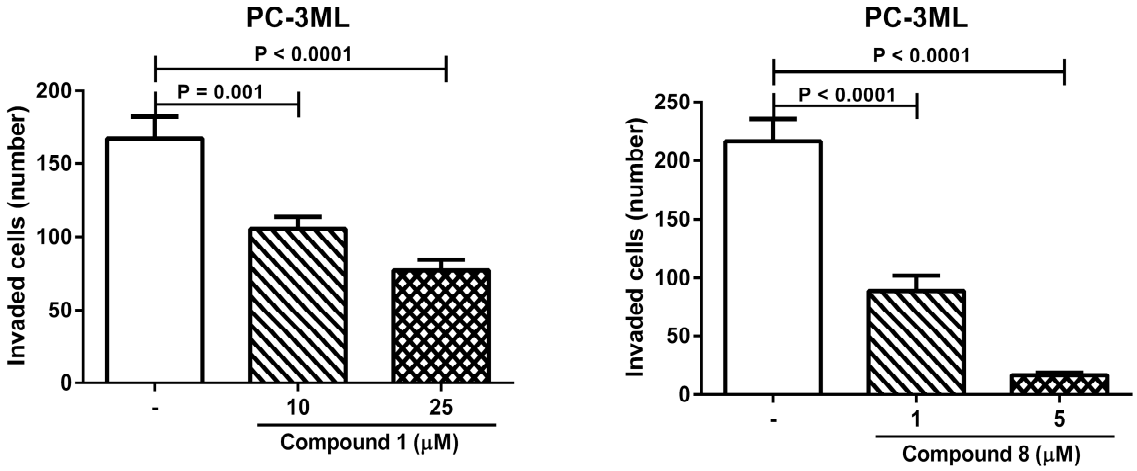


Figure 7. Effect of cathepsin L inhibitors on tumor cell invasion. PC-3ML prostate cancer cell invasion in the presence of cathepsin inhibitors compound **1** (A) or compound **8** (B). Data are mean and standard error values of 3-5 independent experiments.

Thiosemicarbazone **32** displayed the greatest effect on inhibition of invasion of MDA-MB-231 breast cancer cells with 70 % inhibition at 10 µM, slightly better than 60% inhibition

found for the irreversible pan cysteine protease inhibitor E64[73] which was used as a positive control for direct comparison of the effectiveness of the inhibitors (Figure 8). A significant change in inhibition of cell invasion was not observed for analogue **32** at 25 µM compared to 10 µM. However, the *p*-fluoro analogue **8** demonstrated an increase from 15% to 60% inhibition of invasion of MDA-MB-231 cells at 10 µM compared to 25 µM. The unsubstituted analogue **1**, which inhibited cell invasion by 30% at 25 µM, did not impact invasion of MDA-MB-231 cells as significantly as analogues **8** and **32**.

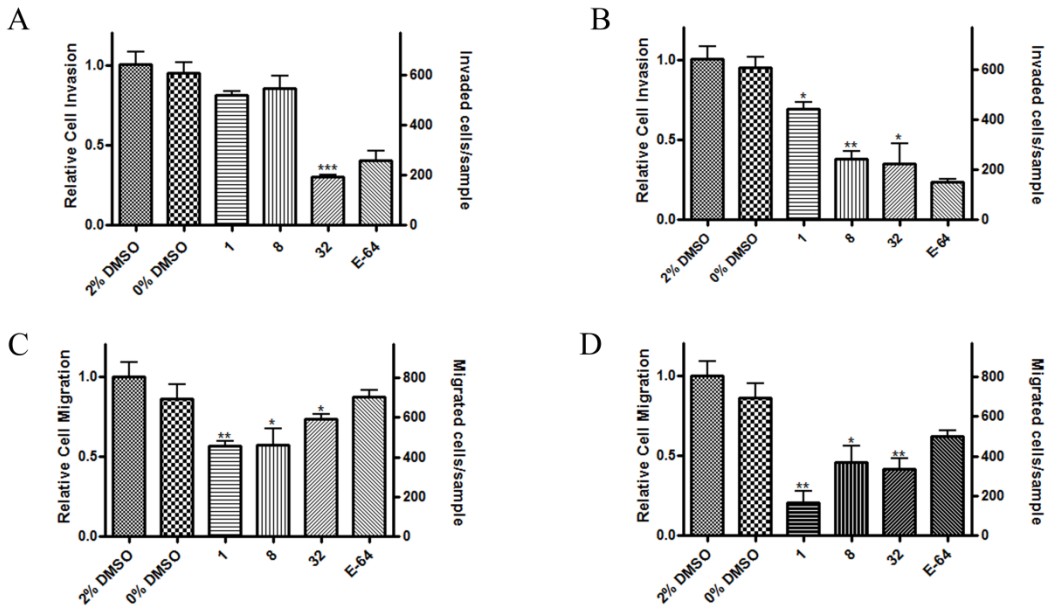


Figure 8. Inhibition of invasion and migration of MDA-MB-231 breast cancer cells by thiosemicarbazone analogues **1**, **8**, and **32**. (a) Inhibition of MDA-MB-231 breast cancer cell invasion through Matrigel at 10 µM concentration of inhibitor. (b) Inhibition of MDA-MB-231 breast cancer cell invasion through Matrigel at 25 µM concentration of inhibitor. (c) Inhibition of MDA-MB-231 breast cancer cell migration at 10 µM concentration of inhibitor. (d) Inhibition of MDA-MB-231 breast cancer cell migration at 25 µM concentration of inhibitor. All values are normalized to 2% DMSO vehicle and are from a minimum of a triplicate of experiments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

All three benzoylbenzophenone analogues when evaluated at 10 μM and 25 μM concentrations, inhibited MDA-MB-231 cell migration; 3-benzoylbenzophenone thiosemicarbazone **1** showed the greatest effect with 80% inhibition of migration at 25 μM which represented a greater than 2-fold increase in inhibition compared with the positive control E-64 (Figure 8). In addition, 1,3-bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone **32** and 1,3-bis(4-fluorobenzoyl)benzene thiosemicarbazone **8** also inhibited migration greater than 50% at 25 μM.

2.5 Growth Inhibition of Mammary Carcinoma

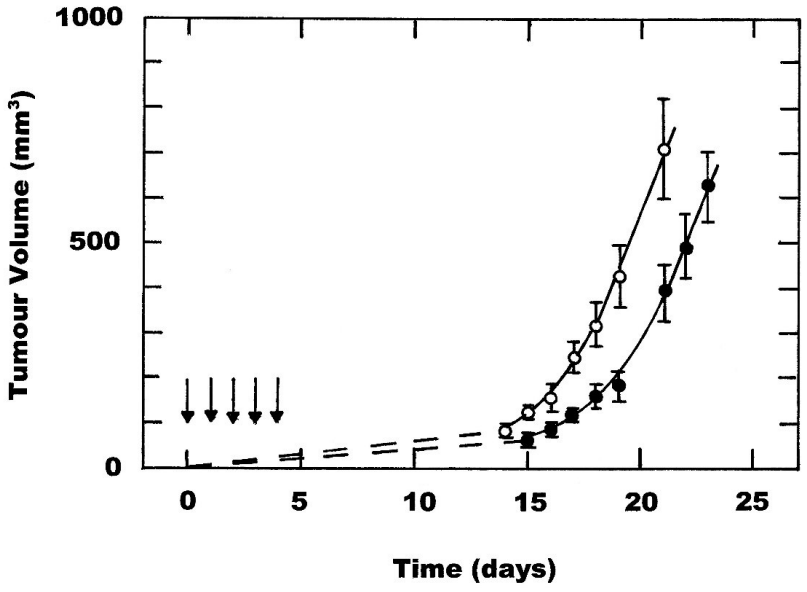


Figure 9. 3-Benzoylbenzophenone thiosemicarbazone (**1**) effects on the growth of C3H mammary carcinomas implanted in the right rear foot of female CDF1 mice. Mice were given 5 daily intraperitoneal injections from the day of tumour implant (as indicated by the arrows). Results show means ± 1 S.E. from 5-8 mice/group. [Legend: O Control (10% Tween80) ● Analogue **1** (20 mg/kg)]

To investigate the effect of 3-benzoylbenzophenone thiosemicarbazone (**1**) (20 mg/kg) on tumor initiation, animals were observed on a daily basis and tumor volume determined, as described in Figure 9, when tumors were measureable. From these data the endpoint of TGT_{500} (time taken in days for tumors to reach 500 mm^3) was determined. For control animals injected with vehicle (10% Tween80) for 5 days from the time of tumor implant the mean TGT_{500} ($\pm$ 1 S.E.) was found to be 20.1 days ($\pm$ 0.6 days) and this was significantly (Student's T-test; significance level of $p<0.05$) increased to 22.1 days ($\pm$ 0.5 days) when mice were given 5 daily treatments with 3-benzoylbenzophenone thiosemicarbazone (**1**). This initial *in vivo* study demonstrated that analogue **1** was well tolerated in this mouse model and showed modest, statistically relevant efficacy in tumor growth delay. Cathepsin L inhibitors, functioning as anti-metastatic agents, are not anticipated to impart dramatic decreases in tumor growth when utilized as single agents; however, it is anticipated that their full value will be realized in combination therapy with standard chemotherapy and/or radiation.[17]

2.6 Cytotoxicity

Table 2. Evaluation of Cytotoxicity Against HUVECs

Compound	Doxorubicin	Paclitaxel	**1**	**8**	**31**	**32**
Cytotoxicity GI_{50} (μM)	0.0268	0.00148	53.3	>126	13.3	>85.9

Benzoylbenzophenone thiosemicarbazone analogues **1**, **8**, **31**, and **32** were evaluated for cytotoxicity against normal endothelial cells (HUVECs). Compared to known chemotherapeutics, doxorubicin and paclitaxel, none of the compounds displayed significant cytotoxicity against HUVECs. Compounds **8** and **32** were the least toxic against HUVECs with

GI_{50} values greater than 126 μM and 85.9 μM, respectively. Low cytotoxicity to normal cells (HUVECs in this case) is a desirable feature since the development of cathepsin L inhibitors as anti-metastatic agents would likely involve chronic dosing to achieve efficacy.

3. Conclusions

Several benzoylbenzophenone thiosemicarbazone analogues were synthesized and evaluated for their inhibitory activity against cathepsins L and B. Thiosemicarbazone inhibitors **1**, **8** and **32** displayed the highest activity against cathepsin L with low IC_{50} values of 9.85 nM, 14.4 nM and 8.12 nM, respectively. The benzoylbenzophenone thiosemicarbazone analogues represent a class of potent inhibitors that can tolerate structural changes and maintain activity against cathepsin L. The high versatility of the core molecular scaffold offers potential for optimization of the benzoylbenzophenone thiosemicarbazone analogues as inhibitors of cathepsin L. Both thiosemicarbazone analogues **1** and **32** demonstrated time-dependent inhibition of cathepsin L. Molecular docking studies of analogues **1** and **32** revealed that the benzophenone fragment of the benzoylbenzophenone thiosemicarbazone inhibitors is capable of filling the lipophilic S2 pocket of cathepsin L thus placing the thiosemicarbazone moiety in close proximity to the Cys25 thiolate. In contrast, molecular modeling of analogue **14** (inactive against cathepsin L) indicated that it did not bind to the enzyme in a conformation that would promote formation of a covalent bond. Kinetic studies combined with molecular docking studies indicate that the formation of a covalent bond between the enzyme and the most potent of this subset of inhibitors represents an important contribution to their efficacy. The most potent inhibitor of cathepsin L from this series, 1,3-bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone **32** significantly inhibited the ability of MDA-MB-231 cells to invade through Matrigel by 70%.

Additionally, the *p*-fluoro analogue **8** inhibited cell invasion of PC-3ML cells by 92% at 5 μM. 3-Benzoylbenzophenone thiosemicarbazone (**1**) delayed growth *in vivo* of recently implanted tumors in a C3H mouse mammary carcinoma model. Cathepsin L inhibitors **1**, **8**, **31**, and **32** demonstrated very low cytotoxicity toward HUVECs. The high potency against cathepsin L and ability to inhibit the invasion of tumor cells *in vitro* coupled with the desirable property of low cytotoxicity to normal cells positions these compounds as viable pre-clinical candidates for further development.

4. Experimental Section

4.1 Chemistry

4.1.1 General Synthetic Protocols.

All reactions were carried out under an inert atmosphere of nitrogen unless otherwise noted. Anhydrous solvents were used for carrying out reactions. Unless otherwise noted all reagents and solvents were purchased from commercial suppliers and used without further purification. Hexanes used for column chromatography were distilled from 4 Å molecular sieves prior to use. Anhydrous tetrahydrofuran and dichloromethane were purchased from commercial suppliers or dried using a VAC (Vacuum Atmospheres Co.) solvent purification system. Reactions were monitored by SiliaPlate™ silica gel thin layer chromatography (TLC) plates (250 μm, F-254, 60 Å). Manual flash chromatography and automated flash chromatography was carried out with silica gel purchased from either Silicycle Inc (230-400 mesh) or Biotage (40-65 microns). Proton (^{1}H), Carbon (^{13}C), and Fluorine (^{19}F) NMR spectra were recorded using a Varian Inova 500

MHz NMR system, Bruker Avance III HD 600 MHz NMR system, or a Bruker Avance III HD 400 MHz NMR system. Chemical shifts are reported in ppm (δ), coupling constants (*J*) are reported in hertz (Hz), and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). High resolution mass spectra (HRMS) were obtained in the Baylor University Mass Spectrometry Core Facility on a Thermo Scientific LTQ Orbitrap Discovery using electrospray ionization (ESI). Purity of final compounds was analyzed using an Agilent Technologies 1200 series HPLC system with a Diode Array and Multiple Wavelength Detector SL, equipped with a Zorbax reliance cartridge guard-column; Agilent Eclipse XDB-C18 column (4.6 mm ID X 250 mm, 5 µm particle size, 80 Å pore size); T = 25 ^{o}C; flow rate 1.0 mL/min; injection volume 20 µL; monitored at 254 nm, 300 nm and 320 nm. Two HPLC methods were used in the purity analysis of final compounds: Method A: water:acetonitrile, gradient 50:50 to 10:90 from 0-25 min and isocratic 10:90 from 25-30 min Method B: water:acetonitrile, gradient 70:30 to 10:90 from 0-25 min and isocratic 10:90 from 25-30 min.

4.1.2 3-Benzoylbenzophenone thiosemicarbazone (1).[51] *p*-Toluenesulfonic acid monohydrate (0.026 g, 0.136 mmol) was added to a solution of 1,3-dibenzoylbenzene (2.00 g, 6.98 mmol) in anhydrous methanol (20 mL). After stirring at reflux for 10 min, thiosemicarbazide (0.476 g, 5.235 mmol) was added to the flask. After 26 h, methanol was removed under reduced pressure and water (50 mL) was then added. The products were extracted with ethyl acetate (2 x 50 mL) and the combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient 90:10 to 52:48) afforded 3-benzoylbenzophenone thiosemicarbazone (0.829 g, 5.24 mmol, 44% yield) as a light yellow solid. ^{1}H NMR (500 MHz,

$CDCl_3$): δ 8.71 (1H, s), 8.00-7.99 (1H, m), 7.85-7.83 (1H, m), 7.77-7.67 (3H, m), 7.60-7.29 (10H, m), 6.61-6.57 (1H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.97, 195.29, 179.04, 178.99, 149.85, 149.70, 139.13, 137.95, 136.99, 136.94, 136.70, 136.01, 132.97, 132.81, 132.28, 131.84, 131.47, 131.45, 131.34, 130.68, 130.56, 130.48, 130.11, 130.09, 130.05, 128.86, 128.62, 128.58, 128.39, 128.34, 127.76. HRMS (ESI) calculated for $C_{21}H_{17}N_3OSH^+$ $(M+H)^+$ 360.11651, found 360.11667. HPLC retention time (Method A): 7.50 min. (Obtained as a mixture of E/Z isomers)

4.1.3 1,3,5-Trisbenzoylbenzene thiosemicarbazone (2).[51] 1,3,5–Tribenzoylbenzene (2.34 g, 6.00 mmol) (purified by flash chromatography before using) and *p*-toluenesulfonic acid monohydrate (11.4 mg, 0.06 mmol) were dissolved in anhydrous THF (20 mL) and heated to reflux. Thiosemicarbazide (546 mg, 6.00 mmol) was then added to the flask and the reaction mixture was stirred at reflux for 35 h. The reaction mixture was concentrated under reduced pressure and the products were extracted from water (100 mL) with ethyl acetate (2 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes: dichloromethane: ethyl acetate, gradient 80:10:10 to 40:30:30) afforded 1,3,5-trisbenzoylbenzene thiosemicarbazone (0.961 g, 2.07 mmol, 35% yield) as a yellow solid. ^1H NMR (500 MHz, Acetone-d_6): δ 9.40 (0.2H, s), 8.72 (0.8H, s), 8.33 (.2H, t, J = 1.5 Hz), 8.28 (1.6H, d, J = 1.5 Hz), 8.21 (0.8H, s), 8.13 (0.2H, s), 8.07 (0.8H, t, J = 1.5 Hz), 8.05 (0.4H, d, J = 1.5 Hz), 7.96-7.95 (0.8H, m), 7.85-7.84 (3.2H, m), 7.78 (0.8H, s), 7.73-7.52 (10.4 H, m), 7.43-7.37 (0.8H, m). ^{13}C NMR (125 MHz, Acetone-d_6): δ 194.47, 194.33, 180.08, 179.66, 147.62, 147.43, 139.25, 138.02, 137.94, 136.77, 136.74, 136.68, 133.68, 133.01, 132.99, 132.59, 131.83, 131.62, 131.37, 130.94, 130.47, 130.06, 130.05, 130.00, 129.99, 129.90, 128.67, 128.62, 128.53,

128.42, 127.85. X-Ray crystallographic data obtained for 1,3,5-trisbenzoylbenzene thiosemicarbazone has been deposited in the Cambridge Crystallographic Data Centre and was allocated the deposition number CDCC 1042567. HRMS (ESI) calculated for $C_{28}H_{21}O_2N_3SH^+$ $(M+H)^+$ 464.14272, found 464.14280. HPLC retention time (Method A): 10.53, 10.81 min. (Obtained as a mixture of E/Z isomers)

[Note: The use of 0.8H and 0.2H for ^{1}H NMR integral values relate to isomer ratios. This system is use throughout.]

4.1.4 3-benzoylbenzyhdrol (3).[51,74] 1,3-Dibenzoylbenzene (2.00 g, 6.98 mmol) was dissolved in anhydrous ethanol (20 mL). The reaction mixture was cooled to 0 ^{o}C followed by the addition of sodium borohydride (0.090g, 2.094 mmol). The reaction mixture was stirred for 4 h and quenched by the addition of a small amount of water. The reaction mixture was concentrated under reduced pressure and the products were extracted from water with ethyl acetate (2 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes: ethyl acetate, gradient 93:7 to 30:70) afforded 3-benzoylbenzhydrol (0.601 g, 2.084 mmol, 30% yield). ^{1}H NMR (500 MHz, Acetone-d_6): δ 7.90 (1H, t, J = 1.7 Hz), 7.77-7.74 (2H, m), 7.72-7.69 (1H, m), 7.67-7.62 (2H, m), 7.55-5.53 (2H, m), 7.51-7.48 (1H, m), 7.46-7.44 (2H, m), 7.34-7.30 (2H, m), 7.25-7.21 (1H, m), 5.95 (1H, s), 5.04 (1H, brs). ^{13}C NMR (125 MHz, Acetone-d_6): δ 196.47, 146.94, 146.03, 138.59, 139.40, 133.24, 131.36, 130.58, 129.24, 129.23, 129.11, 128.54, 127.96, 127.36, 75.71. HRMS (ESI) calculated for $C_{20}H_{16}O_2H^+$ $(M+H)^+$ 289.12231, found 289.12266.

4.1.5 3-Benzoylbenzhydrol thiosemicarbazone (4).[51] *p*-Toluenesulfonic acid monohydrate (0.007 g, 0.03 mmol) was added to a solution of 3-benzoylbenzyhdrol (0.396 g, 1.09 mmol) in anhydrous tetrahydrofuran (10 mL). After stirring at reflux for 10 min, thiosemicarbazide (0.199 g, 2.19 mmol) was added to the reaction mixture. After 2 days, tetrahydrofuran was removed under reduced pressure and water (50 mL) was added. The products were extracted with ethyl acetate (2 x 20 mL) and the combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient 88:12 to 0:100) afforded 3-benzoylbenzhydrol thiosemicarbazone (0.228 g, 0.631 mmol, 57% yield) as a white solid. ^{1}H NMR (500 MHz, Acetone-d_6): δ 8.51 (0.5H, brs), 8.50 (0.5H, brs), 8.08 (0.5H, brs), 8.00 (0.5H, brs), 7.82 (0.5H, t, *J* = 1.7 Hz), 7.74-7.61 (4.5H m), 7.49-7.19 (10H, m), 5.97 (0.5H, d, *J* = 3.9 Hz), 5.82 (0.5H, d, *J* = 3.9 Hz), 5.10 (0.5H, d, *J* = 3.9 Hz), 4.87 (0.5H, d, *J* = 3.9 Hz). ^{13}C NMR (125 MHz, Acetone-d_6): δ 179.44, 179.41, 149.48, 149.42, 147.57, 145.86, 145.21, 144.96, 136.76, 136.61, 131.66, 131.35, 130.05, 129.81, 129.72, 129.70, 128.46, 128.29, 128.28, 128.19, 128.07, 127.96, 127.61, 127.07, 126.89, 126.55, 126.39, 126.35, 126.24, 125.55, 74.99, 74.48. HRMS (ESI) calculated for $C_{21}H_{19}N_3OSNa^+$ $(M+Na)^+$ 384.11410, found 384.11447. HPLC retention time (Method B): 11.73, 12.32. (Obtained as a mixture of E/Z isomers)

4.1.6 1,3-Bis(4-fluorobenzoyl)benzene (5).[51,75] Aluminum trichloride (6.53 g, 49.5 mmol) was added to a solution of isophthaloyl dichloride (5.00 g, 24.8 mmol) in dichloromethane (100 mL). After heating at reflux for 30 min, fluorobenzene (3.41 g, 37.3 mmol) was added. After stirring for 12 h, the reaction mixture was poured onto crushed ice, neutralized with 10% NaOH (100 mL), and extracted with dichloromethane (3 × 100 mL). The combined organic layers were

washed with water, followed by brine and dried over anhydrous sodium sulfate. After the organic layer was concentrated under reduced pressure, purification using flash chromatography (silica gel, hexanes: ethyl acetate, 60:40) afforded 1,3-bis-(4-fluorobenzoyl)benzene (3.37 g, 10.5 mmol, 56% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (1H, t, *J* = 1.7 Hz), 8.00 (2H, dd, *J* = 7.7 Hz, 1.7 Hz), 7.87 (4H, dd, *J* = 8.9 Hz, 5.4 Hz), 7.64 (1H, t, *J* = 7.7 Hz), 7.18 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.24, 165.65 (d, *J* = 255 Hz), 137.81, 133.32, 133.17 (d, *J* = 3.2 Hz), 132.71 (d, *J* = 9.3 Hz), 130.79, 128.66, 155.75 (d, *J* = 22 Hz). ^{19}F NMR (470 MHz, $CDCl_3$): δ 104.91 (1F, tt, *J* = 8.3 Hz, 5.4 Hz). HRMS (ESI) calculated for $C_{20}H_{12}F_2O_2H^+$ $(M+H)^+$ 323.08781, found 323.08817.

4.1.7 1,3-Bis(4-bromobenzoyl)benzene (6).[51,76] To a flask containing aluminum trichloride (1.36 g, 10.2 mmol) under nitrogen was added bromobenzene (15 mL, 142.8 mmol). Isophthalylchloride (1.00 g, 4.88 mmol) was dissolved in a minimal amount of bromobenzene and added to the reaction flask. The reaction stirred at reflux for 6 h, after which it was stirred at room temperature for 12 h, followed by another 3 h at reflux. The reaction was quenched with water (20 mL). The resulting mixture was then added to 1M HCl (50 mL) cooled in an ice bath. The mixture was extracted with ethyl acetate (3 × 10 mL) and the combined organic layers were washed sequentially with deionized water, dilute HCl, deionized water, and brine. Crude product crashed out of the organic layers as a white solid and was collected via filtration. Recrystallization of the solid from ethyl acetate afforded 1,3-bis(4-bromobenzoyl)benzene (0.671 g, 1.51 mmol, 31% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.04 (2H, dd, *J* = 7.8 Hz, 1.7 Hz), 7.98 (t, 1H, *J* = 1.7 Hz), 7.82-7.78 (4H, d, *J* = 8.6 Hz), 7.77 (t, 1H, *J* = 7.7 Hz), 7.74-7.71 (4H, d, *J* = 8.6 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 194.08, 136.78, 135.52, 133.51,

131.75, 131.72, 130.44, 129.24, 121.10. HRMS (ESI) calculated for $C_{20}H_{12}Br_2O_2H^+$ $(M+H)^+$ 442.92768, found 442.92792.

4.1.8 1,3-Bis(4-methoxybenzoyl)benzene (7).[51,77] Aluminum trichloride (5.60 g, 42.0 mmol) was added to a solution of isophthaloyl dichloride (4.00 g, 19.7 mmol) in dichloromethane (50 mL) and heated to reflux for 30 min. Anisole (2.58 mL, 23.7 mmol) was added and the reaction mixture was refluxed for 20 h. The reaction mixture was poured onto the crushed ice. The resulting solution was neutralized with 10% NaOH (100 mL), and extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with water, followed by brine, and dried over anhydrous sodium sulfate. After the organic layer was concentrated under reduced pressure, the purification using flash chromatography (silica gel, hexanes: ethyl acetate, 60:40) afforded 1,3-bis-(4-methoxy benzoyl)benzene (2.60 g, 11.9 mmol, 63% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09 (1H, td, *J* = 1.7 Hz, 0.4Hz), 7.95 (2H, dd, *J* = 7.7 Hz, 1.7 Hz), 7.84 (4H, d, *J* = 8.9 Hz), 7.61 (1H, td, *J* = 7.7 Hz, 0.4 Hz), 6.97 (4H, d, *J* = 8.9 Hz), 3.88 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.71, 163.50, 138.36, 132.72, 132.60, 130.61, 129.62, 128.38, 113.74, 55.54. HRMS (ESI) calculated for $C_{22}H_{18}O_4H^+$ $(M+H)^+$ 347.12779, found 347.12817.

4.1.9 1,3-Bis(4-fluorobenzoyl)benzene thiosemicarbazone (8).[51] 1,3-Bis-(4-fluorobenzoyl)benzene (0.347 g, 1.08 mmol) was dissolved in anhydrous methanol (50 mL). The solution was heated to reflux for 15 min followed by the addition of thiosemicarbazide (0.049 g, 0.54 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.020 g, 0.11 mmol). After 10 h at reflux, the solution was concentrated under reduced pressure.

Purification using flash chromatography (silica gel, hexanes: ethyl acetate, 70:30) afforded the desired product (0.110 g, 0. 278 mmol, 52% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.66 (1H, brs), 7.99-7.97 (0.5H, m), 7.95 (0.5H, m), 7.90 (1H, dd, *J* = 8.9 Hz, 5.4 Hz), 7.83 (1H, dd, *J* = 8.9 Hz, 5.4 Hz), 7.77-7.73 (1H, m), 7.69-7.66 (1H, m), 7.54-7.52 (0.5H, m), 7.51-7.46 (1.5H, m), 7.38 (1H, brs), 7.32-7.30 (2H, m), 7.23-7.20 (1H, m), 7.18-7.14 (1H, m), 7.07-7.04 (1H, m), 6.35 (1H, brs). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.44, 193.71, 179.16, 179.08, 165.74 (d, *J* = 256 Hz), 165.63 (d, *J* = 255 Hz), 164.16 (d, *J* = 252 Hz), 163.74 (d, *J* = 253 Hz), 148.67, 139.19, 138.02, 137.00, 133.31 (d, *J* = 3.0 Hz), 132.90 (d, *J* = 3.0 Hz), 132.81 (d, *J* = 9.2 Hz), 132.71 (d, *J* = 9.1 Hz), 132.28, 132.19 (d, *J* = 3.2 Hz), 131.83, 131.37, 131.26, 130.73 (d, *J* = 8.5 Hz), 130.34, 129.82, 129.75 (d, *J* = 8.6 Hz), 128.59, 128.49, 126.44 (d, *J* = 3.7 Hz), 117.56 (d, *J* = 22 Hz), 115.84 (d, *J* = 22 Hz), 115.81 (d, *J* = 22 Hz), 115.65 (d, *J* = 22 Hz). ^{19}F NMR (565 MHz, $CDCl_3$): δ -104.48 (0.5 F, tt, *J* = 8.3 Hz, 5.4 Hz), -104.80 (0.5 F, tt, *J* = 8.3 Hz, 5.4 Hz), -108.02 (0.5 F, tt, *J* = 8.0 Hz, 5.5 Hz), -109.13 (0.5 F, tt, *J* = 8.2 Hz, 5.4 Hz). HRMS (ESI) calculated for $C_{21}H_{15}F_2N_3OSH^+$ $(M\text{-}H)^+$ 396.09767, found 396.09741. HPLC retention time (Method A): 8.09, 8.37 min. (Obtained as a mixture of E/Z isomers)

4.1.10 1,3-Bis(4-bromobenzoyl)benzene thiosemicarbazone (9). 1,3-Bis(4-bromobenzoyl)benzene (106 mg, 0.238 mmol) was dissolved in anhydrous ethanol (2 mL) followed by the addition of *p*-toluenesulfonic acid monohydrate (1.0 mg, 0.005 mmol) and thiosemicarbazide (21.7 mg, 0.238 mmol). The reaction was carried out at 100 ^{o}C for 30 min under microwave irradiation. The solvent was removed under reduced pressure and the crude reaction mixture was purified using flash chromatography (silica gel, hexanes: dichloromethane: ethyl acetate, gradient, 50:50:0 to 40:50:10) to afford 1,3-bis(4-bromobenzoyl)benzene

thiosemicarbazone (17.5 mg, 0.034 mmol, 14% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.45 (0.5H, s), 9.11 (0.5H, s), 8.58 (0.5H, s), 8.56 (0.5H, s), 8.37 (0.5H, s), 8.32 (0.5H, s), 8.05 (0.5H, d, *J* = 8.1 Hz), 7.91 (0.5H, m), 7.82-7.69 (5H, m), 7.66-7.53 (5H, m), 7.34-7.31 (1H, m). ^{13}C NMR (125 MHz, DMSO-d_6): δ 194.37, 194.28, 146.81, 146.80, 137.78, 136.86, 136.82, 135.70, 135.68, 135.58, 132.98, 132.69, 131.90, 131.72, 131.66, 131.64, 131.51, 131.32, 131.28, 130.91, 130.76, 130.56, 130.45, 130.12, 130.00, 129.58, 128.70, 128.48, 127.04, 126.97, 123.52, 123.29. HRMS (ESI) calculated for $C_{21}H_{15}Br_2N_3OSNa^+$ $(M+Na)^+$ 537.91948, found 537.91980. HPLC retention time (Method A): 13.78, 14.50 min. The purity for the mono thiosemicarbazone product (**9**) is less than 95%. The major impurity is the bis-thiosemicarbazone product (**10**) which was inactive against cathepsin L and B. The combined mono and bis thiosemicarbazone products are 94.8% at 254 nm and greater than 95% at 320 nm (254 nm: mono thiosemicarbazone (**9**) 88.21% and bis thiosemicarbazone (**10**) 6.56%. 320 nm: mono thiosemicarbazone (**9**) 93.99% and bis thiosemicarbazone (**10**) 5.63%). (Obtained as a mixture of E/Z isomers)

4.1.11 1,3-Bis(4-bromobenzoyl)benzene bis-thiosemicarbazone (10).[51] 1,3-Bis(4-bromobenzoyl)benzene (0.550 g, 1.21 mmol) was dissolved in dry THF (20 mL). The solution was heated at reflux for 15 min, and thiosemicarbazide (0.220 g, 2.42 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.023 g, 0.121 mmol) were added to the reaction mixture. After 24 h at reflux, the result solution was concentrated under reduced pressure and the residue was dissolved in dichloromethane (25 mL). The organic layer was washed with deionized water (15 mL), dried over anhydrous sodium sulfate, and concentrated. The crude product was purified via column chromatography to give the double condensation product in

<1% isolated yield (6.7 mg, 11.3 μmol). The ^{1}H NMR for the major isomer is reported. The ^{13}C NMR for the isomer mixture is reported. Three isomers exist for the bis thiosemicarbazones.^{1}H NMR (500 MHz, DMSO-d_6): δ 9.87 (2H, s), 8.46 (2H, s), 8.29 (2H, s), 7.84 (1H, t, *J* = 7.5 Hz), 7.64 (4H, d, *J* = 8.5 Hz), 7.56 (4H, d, *J* = 7.5 Hz), 7.48 (2H, dd, *J* = 7.5 Hz, 1.5 Hz), 7.20 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6): δ 178.73, 147.42, 146.67, 146.45, 137.67, 136.03, 135.55, 133.37, 132.76, 132.36, 131.25, 131.17, 130.90, 130.76, 130.25, 129.93, 129.86, 129.56, 129.50, 129.43, 129.38, 129.32, 126.59, 123.53, 123.27, 123.09. X-Ray crystallographic data obtained for 1,3,5-trisbenzoylbenzene thiosemicarbazone has been deposited in the Cambridge Crystallographic Data Centre and was allocated the deposition number CDCC 1046061. HRMS (ESI) calculated for $C_{22}H_{18}Br_2N_6S_2Na^+$ $(M+Na)^+$ 610.92933, found 610.92822. HPLC retention time (Method A): 10.19, 11.76, 12.16 min. (Obtained as a mixture of isomers)

4.1.12 1,3-Bis(4-methoxybenzoyl)benzene thiosemicarbazone (11). 1,3-Bis(4-methoxybenzoyl)benzene (0.346 g, 1.00 mmol), thiosemicarbazide (0.091 g, 1.00 mmol) and *p*-toluenesulfonic acid monohydrate (0.010 g, 0.050 mmol) were dissolved in tetrahydrofuran (10 mL) and the reaction was carried out at 90 ^{o}C for 45 min under microwave irradiation. The solvent was removed under reduced pressure and the crude reaction mixture was purified using flash chromatography (silica gel, hexanes: ethyl acetate, gradient, 88:12 to 40:60) to afford 1,3-bis(4-methoxybenzoyl)benzene thiosemicarbazone (0.189 mg, 0.410 mmol, 45% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.807 (0.6H, s), 8.667 (0.4H, s), 7.973-7.952 (1H, m), 7.869 (0.8H, d *J* = 8.8 Hz), 7.813 (1.2H, d, *J* = 8.9 Hz), 7.741-7.700 (1H, m), 7.677-7.654 (1H, m), 7.501 (0.4H, dt, *J* = 7.7 Hz, 1.5 Hz), 7.460-7.393 (2.4H, m), 7.245 (1.2H, d, *J* = 8.8 Hz), 7.082 (1.2H, d, *J* = 8.8 Hz), 7.008 (0.8H, *J* = 8.9 Hz), 6.958 (1.2H, d, *J* = 8.9 Hz), 6.864 (0.8H, d, *J* = 8.9 Hz),

6.353 (1H, s), 3.897 (1.8H, s), 3.894 (3H, s), 3.827 (1.2H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.85, 194.11, 178.87, 178.69, 163.64, 163.50, 161.55, 161.11, 150.18, 149.93, 139.83, 138.65, 137.24, 132.68, 132.61, 131.80, 131.49, 131.33, 131.15, 131.10, 130.07, 130.02, 129.78, 129.61, 129.39, 129.31, 128.71, 128.56, 128.20, 122.39, 115.48, 114.00, 113.93, 113.65, 55.58, 55.54, 55.50, 55.43. HRMS (ESI) calculated for $C_{23}H_{21}N_3O_3SNa^+$ $(M+Na)^+$ 442.11958, found 442.11960. HPLC retention time (Method A): 7.21, 7.62 min (Obtained as a mixture of E/Z isomers)

4.1.13 1,3-Bis(4-hydroxybenzoyl)benzene (12).[51,78] To a well-stirred solution of 1,3-bis-(4-methoxybenzoyl)benzene (1.80 g, 5.20 mmol) in dichoromethane (85 mL) was added boron trifluoride dimethyl sulfide complex ($BF_3 \cdot SMe_2$, 15 mL). The mixture was stirred for 27 h. After the reaction was quenched by water, the mixture was extracted with ethyl acetate (3 × 80 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The resulting solid was further purified using flash chromatography (silica gel, hexanes: ethyl acetate, 50:50) to afford the pure product (0.620 g, 1.95 mmol, 38% yield) as a white solid. ^{1}H NMR (500 MHz, Acetone-d_6): δ 9.28 (2H, s), 8.04 (1H, td, J = 1.7 Hz, 0.4 Hz), 7.98 (2H, dd, J = 7.7 Hz, 1.7 Hz), 7.80 (4H, m), 7.73 (1H, td, J = 7.7 Hz, 0.4 Hz), 7.00 (4H, m). ^{13}C NMR (125 MHz, Acetone-d_6): δ 194.46, 162.74, 139.45, 133.49, 133.13, 130.96, 129.71, 128.38, 116.11. HRMS (ESI) calculated for $C_{20}H_{14}O_4H^+$ $(M+H)^+$ 319.09649, found 319.09671.

4.1.14 1,3-Bis(4-hydroxybenzoyl)benzene thiosemicarbazone (13). 1,3-Bis(4-hydroxybenzoyl)benzene (0.318 g, 1.00 mmol) was dissolved in anhydrous ethanol (10 mL)

followed by the addition of *p*-toluenesulfonic acid monohydrate (0.005 g, 0.025 mmol) and thiosemicarbazide (0.910 g, 1.00 mmol). The reaction was carried out at 100 ^{o}C for 45 min under microwave irradiation. The solvent was removed under reduced pressure and the crude reaction mixture was purified using flash chromatography (silica gel, dichloromethane: ethyl acetate, gradient, 90:10 to 60:40) to afford 1,3-bis(4-hydroxybenzoyl)benzene thiosemicarbazone (0.160 mg, 0.410 mmol, 50% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.49 (0.4H, s), 10.48 (0.6H, s), 10.08 (0.6H, s), 9.90 (0.4H, s), 9.03 (0.4H, s), 8.62 (0.6H, s), 8.56 (0.6H, s), 8.45 (0.4H, s), 8.40 (0.6H, s), 8.17 (0.4H, s), 8.06 (0.6H, d, *J* = 7.9 Hz), 7.85 (0.4H, dt, *J* = 7.8 Hz, 1.4 Hz), 7.78-7.74 (1.8H, m), 7.65-7.63 (1.8H, m), 7.56-7.51 (1H, m), 7.49-7.46 (1.2H, m), 7.20 (1.2H, d, *J* = 8.5 Hz), 7.00 (1.2H, d, *J* = 8.5 Hz), 6.91 (0.8H, d, *J* = 8.7 Hz), 6.87 (1.2H, d, *J* = 8.7 Hz), 6.75 (0.8H, d, *J* = 8.8 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 193.87, 193.65, 177.88, 177.72, 162.23, 162.19, 159.27, 158.78, 148.80, 148.71, 139.04, 138.27, 137.11, 132.83, 132.66, 131.96, 130.50, 130.22, 130.10, 130.03, 129.84, 129.50, 129.33, 128.40, 128.27, 127.60, 127.59, 127.31, 120.89, 116.57, 115.39, 115.31, 115.2. HRMS (ESI) calculated for $C_{21}H_{17}N_3O_3SNa^+$ $(M+Na)^+$ 414.08828, found 414.08841. HPLC retention time (Method B): 6.33, 6.75 min. Analysis by ^{1}H NMR shows that compound **13** contains 1.42 wt% ethyl acetate (6 mol% ethyl acetate). Ethyl acetate was found to have an IC_{50} value >>10000 nM. (Obtained as a mixture of E/Z isomers)

4.1.15 1,3-Bis(4-isopropoxybenzoyl)benzene (intermediate for compound 14).[51] Reactions were conducted using a commercially available microwave reactor (Biotage). In a microwave vial, 1,3-bis-(4-hydroxybenzoyl)benzene (0.310 g, 0.975 mmol), isopropyl bromide (0.851 g, 6.92 mmol) and potassium carbonate (0.955 g, 6.91 mmol) were added to DMF (10 mL), with a magnetic stir bar. The vial was capped tightly and the reaction mixture was heated from r.t. to 90

^{o}C for 2 h. After the reaction mixture was cooled to room temperature, the vial was opened and the mixture was transferred to a round bottom flask. The mixture was quenched with water (50 mL) and extracted with ether (2 × 50 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified using flash chromatography (silica gel, hexanes: ethyl acetate, 50:50) to afford the pure product (0.23 g, 0.57 mmol, 59% yield) as a white solid. ^{1}H NMR (500 MHz, Acetone-d_6): δ 8.07 (1H, s), 7.99 (2H, dd, *J* = 7.6 Hz, 1.4 Hz), 7.84 (4H, d, *J* = 8.7 Hz), 7.71 (1H, t, *J* = 7.6 Hz), 7.05 (4H, d, *J* = 8.7), 4.76 (2H, Septet, *J* = 6.0 Hz), 1.35 (12H, d, *J* = 6.0 Hz). ^{13}C NMR (125 MHz, Acetone-d_6): δ 193.51, 162.03, 138.41, 132.40, 130.25, 129.22, 128.57, 115.07, 69.96, 21.33. HRMS (ESI) calculated for $C_{26}H_{26}O_4H^+$ $(M+H)^+$ 403.19039, found 403.19055.

4.1.16 1,3-Bis(4-isopropoxybenzoyl)benzene thiosemicarbazone (14).[51] 1,3-Bis-(4-isopropoxybenzoyl)benzene (0.14 g, 0.39 mmol) was dissolved in anhydrous methanol (20 mL). The solution was heated at reflux for 15 min followed by the addition of thiosemicarbazide (0.043 g, 0.47 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate. After 10 h at reflux, the solvent was removed under vacuum and the resulting solid was further purified using flash chromatography (silica gel, hexanes: ethyl acetate, 50:50) to afford the desired product as a white solid (0.050 g, 0. 105 mmol, 27% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (0.6H, brs), 8.66 (0.4H, brs), 7.96-7.94 (1H, m), 7.84 (0.8H, d, *J* = 8.9 Hz), 7.78 (1.2H, d, *J* = 8.9 Hz), 7.74-7.65 (2H, m), 7.49 (0.4H, m), 7.45-7.40 (2.4H, m), 7.21 (1.2H, d, *J* = 8.8 Hz), 7.03 (1.2H, d, *J* = 8.8 Hz), 6.98-6.95 (0.8H, d, *J* = 8.9 Hz), 6.91 (1.2H, d, *J* = 8.9 Hz), 6.83 (0.8H, d, *J* = 8.9 Hz), 6.46 (1H, brs), 4.69-4.54 (2H, m), 1.40 (3.6H, d, *J* = 6.1 Hz), 1.38-1.37 (6H, m), 1.33 (2.4H, d, *J* = 6.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.80, 194.08, 178.87, 178.66, 162.19,

162.04, 159.98, 159.59, 150.32, 150.04, 139.91, 138.71, 137.28, 132.74, 132.66, 131.72, 131.40, 131.34, 131.09, 131.03, 130.09, 129.97, 129.77, 129.41, 129.15, 128.84, 128.79, 128.17, 128.16, 121.94, 116.79, 115.53, 115.263, 114.98, 70.21, 70.19, 70.17, 70.03, 22.01, 21.91. HRMS (ESI) calculated for $C_{27}H_{29}N_3O_3SH^+$ $(M+H)^+$ 476.20024, found 476.20059. HPLC retention time (Method A): 14.82, 15.31 min (Obtained as a mixture of E/Z isomers)

4.1.17 5-(*tert*-butyldimethylsilyloxy)isophthalic acid (intermediate for compound 15).[79] 3-hydroxyisophthalic acid (5.46 g, 30.0 mmol), *tert*-butyldimethylchlorosilane (22.50 g, 150.0 mmol), and imidazole (12.24 g, 180.0 mmol) were dissolved in *N,N*-dimethylformamide (150 mL) and the reaction mixture was stirred for 11 h between 50-60 ^oC. The reaction mixture was diluted with water (150 mL) and extracted with hexanes (4 x 100 mL). The organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The crude product was dissolved in tetrahydrofuran (30 mL) followed by the addition of glacial acetic acid (90 mL), and distilled water (30 mL). After stirring for 3 h at room temperature, the reaction mixture was diluted with distilled water (60 mL) and cooled to 0 ^oC. The precipitated product was filtered. The filtrate was concentrated and cooled again to 0 ^oC. The precipitated product was filtered and the combined solids were dried to yield *5-(tert*-butyldimethylsilyloxy)isophthalic acid (6.55 g, 22.1 mmol ,74% yield). ^1H NMR (600 MHz, DMSO-d_6): δ 13.34 (2H, s), 8.10 (1H, t, J = 1.5 Hz), 7.56 (2H, d, J = 1.5 Hz), 0.96 (9H, s), 0.22 (6H, s). ^{13}C NMR (150 MHz, DMSO-d_6): δ 166.28, 155.35, 132.80, 124.46, 123.21, 25.49, 17.98, -4.60. HRMS (ESI) calculated for $C_{14}H_{20}O_5SiNa^+$ $(M+Na)^+$ 319.09722, found 319.09741.

4.1.18 5-*(tert*-butyldimethylsilyloxy)isophthaloyl chloride (15). [79] Oxalyl chloride (2.04 mL, 27.8 mmol) was added dropwise to a solution of *5-(tert*-butyldimethylsilyloxy*)*isophthalic acid (2.82 g, 9.51 mmol) in dichloromethane (90 mL). *N,N*-Dimethylformamide (0.073 mL, 0.095 mmol) was added dropwise. The reaction mixture was allowed to stir until the solution was transparent. After 2 h, the solvent was removed under reduced pressure. The product was dissolved in anhydrous dichloromethane (20 mL) and the solvent was removed under reduced pressure. This was repeated two times to afford *5-(tert*-butyldimethylsilyloxy)isophthaloyl chloride as yellow solid. The product was used immediately without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46 (1H, t, *J* = 1.6 Hz), 7.82 (2H, d, *J* = 1.6 Hz), 1.02 (9H, s), 0.28 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.21, 156.95, 135.55, 128.34, 126.72, 25.67, 18.39, -4.30.

4.1.19 N^1,N^3-dimethoxy-N^1,N^3-dimethyl isophthalamide (16).[51] Triethylamine (5.61 mL, 40.0 mmol) was added dropwise to a solution of *N,O*-dimethylhydroxylamine hydrochloride (2.93 g, 30.0 mmol) in anhydrous dichloromethane (35 mL) at 0 ^{o}C. After stirring for 10 min, a solution of isophthaloyl dichloride (2.03 g, 10 mmol) dissolved in anhydrous dichloromethane (6 mL) was added dropwise. The reaction mixture was returned to room temperature and stirred for 5 h. The reaction was quenched with water (50 mL) and the products were extracted with dichloromethane (2 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, dichloromethane:methanol, 95:5) afforded N^1,N^3-dimethoxy-N^1,N^3-dimethyl isophthalamide (2.47 g, 9.77 mmol, 97% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.98 (1H, s), 7.76 (2H, dd, *J* = 7.8 Hz, 1.6 Hz), 7.44 (1H, t, *J* = 7.8 Hz), 3.53 (6H, s),

3.35 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.15, 133.96, 130.55, 128.20, 128.05, 61.27, 33.79. HRMS (ESI) calculated for $C_{12}H_{16}N_2O_4H^+$ $(M+H)^+$ 253.11828, found 253.11859.

4.1.20 5-((*tert*-butyldimethylsilyl)oxy)-*N*^{1},*N*^{3}-dimethoxy-*N*^{1},*N*^{3}-dimethyl isophthalamide (17). Triethylamine (5.35 mL, 38.1 mmol) was added dropwise to a solution of *N,O*-dimethylhydroxylamine hydrochloride (2.78 g, 28.5 mmol) in dichloromethane (50 mL) at 0 °C. A solution of *5-(tert*-butyldimethylsilyloxy)isophthaloyl chloride in dichloromethane (10 mL) was added dropwise and the reaction was allowed to return to room temperature. After 6 h, the reaction was quenched with water (90 mL) and extracted with dichloromethane (3 x 60 mL). The combined organic phases were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, dichloromethane:methanol, gradient, 100:0 to 90:10) afforded *5-((tert*-butyldimethylsilyl)oxy)-*N*^{1},*N*^{3}-dimethoxy-*N*^{1},*N*^{3}-dimethyl isophthalamide as a white solid (3.40 g, 8.89 mmol, 93% yield over two steps)**.** ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.38 (1H, t, *J* = 1.5 Hz), 7.15 (2H, d, *J* = 1.5 Hz), 3.52 (6 H, s), 3.26 (6H, s), 0.96 (9H, s), 0.21 (6H, s). ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.52, 154.16, 135.50, 120.98, 120.26, 60.80, 25.45, 17.94, -4.66. HRMS (ESI) calculated for $C_{18}H_{30}N_2O_5SiH^+$ $(M+H)^+$ 383.19968, found 383.20117.

4.1.21 1,3-Bis(3-methylbenzoyl)benzene (18).[51,80] 3-Bromotoluene (6.684 g, 39.08 mmol) was dissolved in anhydrous tetrahydrofuran (30 mL). The solution was stirred for 5 min and cooled to -78 °C followed by the dropwise addition of n-butyllithium in hexanes (2.5 M, 13.6 mL). After stirring for 3.5 h, a solution of *N*^{1},*N*^{3}-dimethoxy-*N*^{1},*N*^{3}-dimethyl isophthalamide tetrahydrofuran (5 mL) was added dropwise and the solution was stirred for 1 h at -78 °C. The reaction mixture

was quenched with 1 M HCl (40 mL) and the products were extracted with dichloromethane (2 x 50 mL). The combined organic phases were washed with aqueous saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient, 95:5 to 70:30) afforded 1,3-bis(3-methylbenzoyl)benzene (2.47 g, 9.77 mmol, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.17-8.15 (1H, m), 8.02 (2H, dd, *J* = 7.7 Hz, 1.7 Hz), 7.66-7.61 (3H, m), 7.59 (2H, d, *J* = 7.5 Hz), 7.42 (2H, d, *J* = 7.5 Hz), 7.37 (2H, t, *J* = 7.5 Hz), 2.43 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 192.22, 138.55, 138.06, 137.20, 133.79, 133.54, 131.30, 130.59, 128.63, 128.40, 127.55, 21.53. HRMS (ESI) calculated for $C_{22}H_{18}O_2H^+$ $(M+H)^+$ 315.13796, found 315.13833.

4.1.22 1,3-Bis(3-bromobenzoyl)-5-*tert*-butyldimethylsilyloxybenzene (19). n-Butyllithium in hexanes (2.5M, 4.0 mL, 10 mmol) was added dropwise to a solution of 1,3-dibromobenzne (2.53 mL, 20.9 mmol) in tetrahydrofuran (50 mL) cooled to -78 ^{o}C. After 30 min, a solution of *5-((tert*-butyldimethylsilyl)oxy*)-N*^{1},*N*^{3}-dimethoxy-*N*^{1},*N*^{3}-dimethyl isophthalamide (2.00g, 5.23 mmol) in tetrahydrofuran (10 mL) was added dropwise to the reaction mixture. After stirring for 2 h, the reaction mixture was quenched with 1 M HCl (30 mL). The product was extracted with ethyl acetate (3 x 50 mL). The combined organic phases were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexane:ethyl acetate, gradient, 98:2 to 90:10) afforded 1,3-bis(3-bromobenzoyl)-5-*tert*-butyldimethylsilyloxybenzene as a yellow oil (1.63 g, 2.84 mmol, 57% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (2H, t, *J* = 1.7 Hz), 7.75-7.70 (4H, m), 7.68 (1H, t, *J* = 1.5 Hz), 7.45 (2H, d, *J* = 1.5 Hz), 7.38 (2H, t, *J* = 7.8 Hz), 1.0 (9H, s), 0.26 (6H, s). ^{13}C NMR

(125 MHz, $CDCl_3$): δ 193.76, 156.14, 138.75, 138.57, 135.77, 132.76, 130.04, 128.50, 125.17, 124.18, 122.79, 25.80, 18.24, -4.35. HRMS (ESI) calculated for $C_{26}H_{26}Br_2O_3SiH^+$ $(M+H)^+$ 573.00907, found 573.00909.

4.1.23 1,3-Bis(3-methylbenzoyl)benzene thisosemicarbazone (20).[51] *p*-Toluenesulfonic acid monohydrate (0.001 g, 0.01 mmol) was added to a solution of 1,3-bis(3-methylbenzoyl)benzene (0.154 g, 0.480 mmol) in anhydrous methanol (10 mL). After stirring at reflux for 10 min, thiosemicarbazide (0.022 g, 0.24 mmol) was added to the reaction mixture and stirred for 7 h under an inert atmosphere of nitrogen gas. After 7 h, methanol was removed under reduced pressure and 10 mL of water was then added. The products were extracted with ethyl acetate and the combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient 90:10 to 40:60) afforded 1,3-bis(3-methylbenzoyl)benzene thisosemicarbazone (0.057 g, 0.015 mmol, 64% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.17 (0.4H, s), 8.66 (0.6H, s), 8.55 (0.4H, s), 8.48 (0.6H, s), 8.43 (0.6H, s), 8.27 (0.4H, s), 8.21 (0.6H, d, *J* = 7.8 Hz), 7.93 (0.4H, dt, *J* = 7.8 Hz, 1.5 Hz), 7.81 (0.4H, t, *J* = 7.6 Hz), 7.74-7.71 (1.2 H, m), 7.64-7.39 (7H, m), 7.33-7.30 (0.4 H, m), 7.27-7.17 (2H, m), 2.40 (1.8 H, s), 2.38 (1.2H, s), 2.36 (1.8H, s), 2.31 (1.2H, s). ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.32, 195.31, 148.25, 148.14, 139.47, 138.18, 138.08, 137.98, 137.63, 137.27, 136.74, 136.70, 136.59, 136.34, 133.58, 133.51, 132.61, 131.91, 130.93, 130.78, 130.76, 130.64, 130.60, 130.46, 130.02, 129.99, 129.92, 129.75, 129.73, 128.66, 128.62, 128.50, 128.37, 128.21, 127.83, 127.18, 127.01, 125.27, 125.16, 20.97, 20.93, 20.88, 20.82. HRMS (ESI) calculated for $C_{23}H_{21}N_3OSNa^+$

$(M+Na)^+$ 410.12975, found 410.13013. HPLC retention time (Method A): 11.38 min. (Obtained as a mixture of E/Z isomers)

4.1.24 1,3-Bis(3-bromobenzoyl)-5-*tert*-butyldimethylsilyloxybenzene thiosemicarbazone (21). 1,3-Bis(3-bromobenzoyl)-5-*tert*-butyldimethylsilyloxybenzene (1.39 g, 2.43 mmol), thiosemicarbazide (0.166 g, 1.82 mmol), and *p*-toluenesulfonic acid monohydrate (0.010 g, 0.05 mmol) were dissolved in tetrahydrofuran (15 mL). The reaction was carried out at 90 ^oC for 30 min under microwave irradiation. The solvent was removed under reduced pressure and the crude reaction mixture was purified using flash chromatography (silica gel, hexanes:ethyl acetate, gradient, 93:7 to 50:50) to afford 1,3-bis(3-bromobenzoyl)-5-*tert*-butyldimethylsilyloxybenzene thiosemicarbazone (0.418 g, 0.650 mmol, 36% yield). ^1H NMR (500 MHz, DMSO-d_6): δ 9.35 (0.7H, s), 9.12 (0.3H, s), 8.57 (1H, m), 8.44 (0.7H, s), 8.34 (0.3H, s), 8.04 (0.7H, t, *J* = 1.7 Hz), 7.94 (0.7H, t, *J* = 1.7 Hz), 7.89-7.85 (1H, m), 7.83-7.80 (1H, m), 7.78 (0.3H, ddd, *J* = 8Hz, 2Hz, 1Hz), 7.70-7.68 (0.3H, m), 7.62-7.60 (0.3H, t, *J* = 1.7 Hz), 7.59-7.57 (1H, m), 7.56-7.48 (1.3H, m), 7.47-7.44 (0.7H, m), 7.39 (0.3H, dt, *J* = 7.6 Hz, 1.2 Hz), 7.33-7.29 (1.7H, m), 7.20 (0.7H, t, *J* = 1.5 Hz), 7.13-7.11 (1H, m), 0.96 (6H, s), 0.91 (3H, s), 0.24 (4H, s), 0.15(2H, s). ^{13}C NMR (125 MHz, DMSO-d_6): δ 193.42, 193.38, 178.53, 155.99, 155.02, 146.05, 145.55, 139.32, 138.75, 138.66, 138.65, 138.44, 138.02, 135.57, 135.51, 133.66, 132.97, 132.90, 132.28, 132.02, 131.85, 131.69, 131.26, 130.80, 130.72, 130.36, 129.48, 128.90, 128.68, 127.68, 126.89, 124.47, 123.04, 122.84, 122.75, 122.12, 122.07, 121.85, 121.82, 121.80, 121.48, 25.52, 25.51, 18.00, 17.96, -4.59. HRMS (ESI) calculated for $C_{27}H_{29}Br_2N_3O_2SSiH^+$ $(M+H)^+$ 646.01893, found 646.01995.

4.1.25 1,3-Bis(3-bromobenzoyl)-5-hydroxybenzene thiosemicarbazone (22). 1,3-Bis(3-bromobenzoyl)-5-hydroxybenzene (0.388 g, 0.600 mmol) was dissolved in tetrahydrofuran (5 mL) followed by the dropwise addition of a 1M solution of tetra-butylammonium fluoride in tetrahydrofuran (1.20 mL, 1.20 mmol) at room temperature. After 1 h, the reaction mixture was diluted with ethyl acetate (30 mL) and washed with brine (15 mL). The combined organic phases were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, gradient, hexane:ethyl aceate: methanol, 77.5:20:2.5 to 57.5:40:2.5) afforded 1,3-bis(3-bromobenzoyl)-5-hydroxybenzene thiosemicarbazone as a yellow solid (0.277 g, 0.520 mmol, 87% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.39 (0.6H, s), 9.98 (0.4H, s), 9.24 (0.6H, s), 9.07 (0.4H, s), 8.62-8.54 (1H, m), 8.46 (0.6H, s), 8.28 (0.4H, s), 8.09 (0.6H, s), 7.94 (0.6H, t, *J* = 1.8 Hz), 7.88-7.84 (1H, m), 7.83-7.79 (1H, m), 7.77 (0.4H, ddd, *J* = 8.1 Hz, 2.0 Hz, 1.0 Hz), 7.69-7.66 (0.4H, m), 7.61-7.47 (2.4 H, m), 7.44 (0.6H, d, *J* = 7.8 Hz), 7.39-7.35 (0.8H, m), 7.34-7.27 (1.6H, m), 7.12 (0.4H, dd, *J* = 2.2 Hz, 1.0 Hz), 7.00-6.99 (0.6H, m), 6.97 (0.6H, dd, *J* = 2.2 Hz, 1.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 193.74, 193.73, 178.41, 158.46, 157.38, 146.50, 146.10, 139.12, 138.98, 138.91, 138.58, 138.16, 137.65, 135.43, 135.33, 133.81, 132.85, 132.59, 132.32, 131.91, 131.72, 132.71, 131.17, 130.75, 130.65, 130.40, 129.36, 128.86, 128.66, 127.71, 127.04, 122.82, 122.17, 121.87, 121.82, 120.40, 119.89, 119.52, 118.49, 117.60, 117.29. HRMS (ESI) calculated for $C_{21}H_{15}Br_2N_3O_2SNa^+$ $(M+Na)^+$ 553.91439, found 553.91436. HPLC retention time (Method B): 15.75, 16.88 min. (Obtained as a mixture of E/Z isomers)

4.1.26 3-(*tert*-butyldimethylsilyloxy)benzaldehyde (23).[81] 3-Hydroxybenzaldehyde (2.00 g, 16.4 mmol) was dissolved in *N,N*-dimethylformamide (50 mL) followed by the addition of

imidazole (2.23 g, 32.8 mmol). The reaction mixture was cooled to 0 ^{o}C and *tert*-butyldimethylchlorosilane (3.69 g, 24.6 mmol) was added. The reaction mixture was returned to room temperature and stirred for 3 h. After reaction completion, the reaction mixture was quenched with saturated aqueous sodium bicarbonate (50 mL) and the product was extracted with hexanes (2 X 50 mL). The organic extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude mixture was purified using flash chromatography (silica gel, hexanes:ethyl acetate, gradient, 100:00 to 90:10) to afford 3-(*tert*-butyldimethylsilyloxy)benzaldehyde (3.57, 15.1 mmol , 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.95 (1H, s), 7.47 (1H, dt, *J* = 7.3 Hz, 1.2 Hz), 7.40 (1H, t, *J* = 7.8 Hz), 7.34-7.32 (1H, m), 7.12-7.09 (1H, m), 1.0 (9H, m), 0.23 (6H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 192.23, 156.54, 138.07, 130.21, 126.68, 123.69, 120.01, 25.76, 18.34, -4.28. HRMS (ESI) calculated for $C_{13}H_{20}O_2SiH^+$ $(M+H)^+$ 237.13053, found 237.13066.

4.1.27 2-Fluoro-*N*-methoxy-*N*-methyl-benzamide (24).[51,82] Triethylamine (5.32 mL, 37.8 mmol) was added dropwise to a solution of *N,O*-dimethylhydroxylamine hydrochloride (2.77 g, 28.4 mmol) in anhydrous dichloromethane (45 mL) at 0 ^{o}C. After stirring for 10 min, 2-flurobenzoyl chloride (0.303 mL, 2.52 mmol) in anhydrous dichloromethane (15 mL) was added dropwise. The reaction mixture was returned to room temperature and stirred for 5 h. The reaction mixture was quenched with water (60 mL) and the products were extracted with dichloromethane (2 x 60 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient 93:7 to 60:40) afforded 2-Fluoro-*N*-methoxy-*N*-methyl-benzamide (3.12 g, 17.0 mmol, 90% yield). ^{1}H NMR (500 MHz,

$CDCl_3$): δ 7.44-7.38 (2H, m), 7.19 (1H, t, *J* = 7.5 Hz), 7.10 (1H, t, *J* = 8.9 Hz), 3.55 (3H, brs), 3.35 (3H, brs). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.40, 158.66, (d, *J* = 249 Hz), 131.50, 128.90, 124.11, 123.52 (d, *J* = 17 Hz), 115.69 (d, *J* = 21 Hz), 61.21, 32.31. ^{19}F NMR (470 MHz, $CDCl_3$): δ -114.04 (1F, s). HRMS (ESI) calculated for $C_9H_{10}FNO_2H^+$ $(M+H)^+$ 184.07683, found 184.07702.

4.1.28 3-Bromo-*N*-methoxy-*N*-methyl-benzamide (25).[83] To a well stirred suspension of *N,O*-dimethylhydroxylamine hydrochloride (5.33 g, 54.7 mmol) in dichloromethane (120 mL) was added triethylamine (10.2 mL, 72.9 mmol) dropwise at 0 °C. After stirring for a few minutes, 3-bromobenzoyl chloride (4.81 mL, 36.4 mmol) in dichloromethane (20 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was quenched with water (150 mL) and extracted with dichloromethane (3 x 100 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated. Purification using flash chromatography (silica gel, hexane: ethyl acetate, gradient, 90:10 to 60:40) afforded 3-bromo-*N*-methoxy-*N*-methyl-benzamide as a colorless oil (8.60 g, 35.2 mmol, 97% yield). ^1H NMR (500 MHz, $CDCl_3$): 7.83 (1H, t, *J* = 1.8 Hz), 7.61 (1H, dt, *J* = 7.7 Hz, 1.3 Hz), 7.59 (1H, ddd, *J* = 8.0 Hz, 2.1 Hz, 1.1 Hz), 7.28 (1H, m), 3.35 (3H, s), 3.36 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 168.32, 136.06, 133.69, 131.36, 129.74, 126.93, 122.14, 61.33, 33.71. HRMS (ESI) calculated for $C_9H_{10}BrNO_2H^+$ $(M+H)^+$ 243.99677, found 243.99704.

4.1.29 1,3-Bis[(3-*tert*-butyldimethylsilyloxyphenyl)hydroxymethyl]-5-bromobenzene (26). *Tert*-butyllithium in pentane (1.7M, 11.76 mL) was added dropwise to a solution of 1,3,5 tribromobenzene (1.57 g, 5.00 mmol) in anhydrous diethyl ether (20 mL) cooled to -78 °C. The

mixture was sonicated at every 30 min interval. After 2 h, a solution of 3-(*tert*-butyldimethylsilyloxy)benzaldehyde (2.37 g, 10.0 mmol) in diethyl ether (10 mL) was added dropwise and the reaction mixture was allowed to slowly warm to room temperature over a period of 19 h. The reaction was quenched with 1 M HCl (30 mL) and extracted with diethyl ether (3 X 30 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate, followed by brine and dried over anhydrous sodium sulfate. After removing the solvent under reduced pressure, the crude mixture was purified using flash chromatography (silica gel, hexanes:ethyl acetate, gradient, 100:00 to 40:60) to afford 1,3-bis[(3-*tert*-butyldimethylsilyloxyphenyl)hydroxymethyl]-5-bromobenzene (1.16 g, 1.85 mmol, 37% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.411-7.390 (2H, m), 7.357 (.5H, s), 7.327 (.5H, s), 7.206-7.169 (2H, m), 6.905-6.881 (2H, m), 6.836-6.881 (2H, m), 6.768-6.741 (2H, m), 5.707-5.704 (2H, m), 2.196 (2H, s), 0.964 (9H, s), 0.963 (9H, s), 0.168 (6H, s), 0.166 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 155.89, 145.94, 145.89, 144.59, 144.56, 129.68, 129.67, 128.62, 128.51, 123.21, 122.71, 122.65 119.58, 119.56, 119.45, 119.43, 118.27, 118.23, 75.39, 25.68, 18.21, -4.40. HRMS (ESI) calculated for $C_{32}H_{45}BrO_4Si_2Na^+$ $(M+Na)^+$ 651.19320, found 651.19128.

4.1.30 1,3-Bis[(3-*tert*-butyldimethylsilyloxy)benzoyl]-5-bromobenzene (27). 1,3-Bis[(3-*tert*-butyldimethylsilyloxyphenyl)hydroxymethyl]-5-bromobenzene (1.10 g, 1.75 mmol) in dichloromethane (5 mL) was added dropwise to a solution of pyridinium chlorochromate (1.13 g, 5.24 mmol), celite (1.00 g), and dichloromethane (5 mL) at 0 ^{o}C. The reaction mixture was returned to room temperature. After 4.5 h, the reaction mixture was filtered over a pad of celite. The celite was rinsed several times with dichloromethane and the filtrate was concentrated. The solvent was removed under reduced pressure and the crude reaction mixture was purified using

flash chromatography (silica gel, hexanes:ethyl acetate, gradient, 90:08 to 20:80) to afford 1,3-bis[(3-*tert*-butyldimethylsilyloxy)benzoyl]-5-bromobenzene (1.02 g, 1.63 mmol, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (2H, d, *J* = 1.5 Hz), 8.08 (1H, t, *J* = 1.5 Hz), 7.38-7.34 (4H, m), 7.26-7.25 (2H, m), 7.11-7.08 (2H, m), 0.98 (18H, s), 0.21 (12H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 193.91, 155.94, 139.51, 137.70, 135.96, 129.68, 129.51, 125.21, 123.25, 122.65, 121.14, 25.63, 18.22, -4.39. HRMS (ESI) calculated for $C_{32}H_{41}BrO_4Si_2H^+$ $(M+H)^+$ 625.17995, found 625.17969.

4.1.31 1,3-Bis(2-fluorobenzoyl)-5-bromobenzene (28).[49] *Tert*-butyllithium in pentane (1.6M, 7.72 mL) was added dropwise to a solution of 1,3,5-tribromobenzene (0.972 g, 3.09 mmol) in anhydrous ether (30 mL) at -78 ^{o}C under a flow of nitrogen gas. After 2 h, 2-fluoro-*N*-methoxy-*N*-methyl-benzamide (1.132 g, 6.18 mmol) dissolved in anhydrous ether (5 mL) was added dropwise and the reaction mixture was allowed to slowly come to room temperature and stirred for 24 h. After 24 h, the reaction mixture was quenched with water (30 mL) and the products were extracted with ether (2 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient 95:5 to 60:40) afforded 1,3-bis(3-fluorobenzoyl)-5-bromobenzene (0.617 g, 6.18 mmol, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (2H, m), 8.12 (1H, pentet, *J* = 1.4 Hz), 7.61-7.56 (4H, m), 7.30 (2H, td, *J* = 7.5 Hz, 1.0Hz), 7.20-7.16 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.23, 160.40 (d, *J* = 254 Hz), 139.67, 133.61, 134.29 (d, *J* = 8.6), 131.12 (d, *J* = 1.9 Hz), 129.31, 125.79 (d, *J* = 13.8 Hz), 124.79 (d, *J* = 3.8 Hz), 123.18, 116.70 (d, *J* = 21 Hz). ^{19}F NMR (470 MHz, $CDCl_3$):

δ -109.73 - -109.78 (1F, m). HRMS (ESI) calculated for $C_{20}H_{11}BrF_2O_2H^+$ $(M+H)^+$ 400.99833, found 400.99857.

4.1.32 1,3-Bis(3-bromobenzoyl)-5-bromobenzene (29). Tert-butyllithium (1.7 M, 11.8 mL) was added dropwise to a solution of 1,3,5-tribromobenzene in diethyl ether (25 mL) at -78° C. The mixture was sonicated for 0.5 min at 20 min intervals to help dissolve the starting material. After 1 h, a solution of 3-bromo-*N*-methoxy-*N*-methyl-benzamide (2.44 g, 10.0 mmol) in tetrahydrofuran (15 mL) was added to the reaction mixture and allowed to slowly come to room temperature overnight. The reaction was quenched with 1 M HCl (30 mL) and extracted with diethyl ether (3 x 30 mL). The combined organic phases were washed with saturated aqueous sodium bicarbonate and concentrated. Crystallization of the crude mixture in acetone followed by recrystallization in ethanol afforded 1,3-bis(3-bromobenzoyl)-5-bromobenzene as a white solid (1.77 g, 0.338 mmol, 68% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.17 (2H, d, *J* = 1.5 Hz), 7.96 (2H, t, *J* = 1.7 Hz), 7.92-7.98 (3H, m), 7.81-7.79 (2H, m), 7.55 (2H, t, *J* = 7.9 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 192.28, 138.60, 138.18, 135.88, 135.54, 131.98, 130.86, 129.61, 128.93, 122.31, 122.04. HRMS (ESI) calculated for $C_{20}H_{11}Br_3O_2H^+$ $(M+H)^+$ 520.83819, found 520.83820.

4.1.33 1,3-Bis[(3-*tert*-butyldimethylsilyloxy)benzoyl]-5-bromobenzene thiosemicarbazone (30). 1,3-Bis[(3-*tert*-butyldimethylsilyloxy)benzoyl]-5-bromobenzene (0.890 g, 1.42 mmol), thiosemicarbazide (0.970 g, 1.06 mmol) and *p*-toluenesulfonic acid monohydrate (0.135 g, 0.0710 mmol) were dissolved in tetrahydrofuran (10 mL) and the reaction was carried out at 90 °C for 30 min under microwave irradiation. The solvent was removed under reduced pressure

and the crude reaction mixture was purified using flash chromatography (silica gel, hexanes:ethyl acetate, gradient, 90:10 to 20:80) to afford 1,3-bis[(3-*tert*-butyldimethylsilyloxy)benzoyl]-5-bromobenzene thiosemicarbazone as a yellow solid (0.329 g, 0.471 mmol , 33% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.77 (1H, s), 7.87 (2H, d, *J* = 1.7 Hz), 7.83 (1H, t, *J* = 1.7 Hz), 7.48-7.44 (1H, m), 7.37-7.32 (2H, m), 7.30 (1H, dt, *J* = 7.6 Hz, 1.5 Hz), 7.23-7.22 (1H, m), 7.08 (1H, ddd, *J* = 7.8 Hz, 2.5 Hz, 1.5 Hz), 7.02 (1H, ddd, *J* = 8.3 Hz, 2.5 Hz, 1.0 Hz), 6.86 (1H, dt, *J* = 7.6 Hz, 1.0 Hz), 6.71-6.70 (1H, m), 6.44 (1H, s), 0.99 (9H, s), 0.98 (9H, s), 0.23 (6H, s), .22 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.25, 179.26, 157.34, 156.02, 148.09, 139.76, 138.89, 137.90, 134.01, 133.88, 131.74, 131.25, 129.70, 127.55, 125.27, 123.35, 122.80, 122.70, 121.28, 120.99, 119.99, 29.84, 25.77, 18.35, -4.17, -4.24. HRMS (ESI) calculated for $C_{33}H_{44}BrN_3O_3SSi_2H^+$ $(M+H)^+$ 698.18981, found 698.18915.

4.1.34 1,3-Bis(3-hydroxybenzoyl)-5-bromobenzene thiosemicarbazone (31). 1,3-Bis[(3-*tert*-butyldimethylsilyloxy)benzoyl]-5-bromobenzene thiosemicarbazone (0.325 g, 0.465 mmol) was dissolved in tetrahydrofuran (10 mL) followed by the addition of tetra-butylammonium fluoride trihydrate (0.604 g, 1.91 mmol) at room temperature. After 1.5 h, the reaction mixture was diluted with ethyl acetate (50 mL), washed with brine (50 mL), and dried over anhydrous sodium sulfate. After concentrating the reaction mixture under reduced pressure, the crude product was purified using flash chromatography (silica gel, hexanes: dichloromethane, gradient, 50:50 to 0:100 followed by dichloromethane: ethyl acetate, gradient 100:0 to 60:40) to afford 1,3-bis(3-hydroxy-benzoyl)-5-bromobenzene thiosemicarbazone as a yellow solid (0.158 mg, 0.336 mmol, 72% yield). ^{1}H NMR (500 MHz, DMSO-d_6) : δ 9.99 (0.8H, s), 9.92 (0.2H, s), 9.88 (0.2H, s), 9.86 (0.8H, s), 9.47 (0.2H, s), 8.72 (0.8H, s), 8.70 (0.8H, s), 8.53 (0.8H, s), 8.48 (0.8H, s), 8.45

(0.2H, s), 8.09 (0.2H,s), 7.98 (0.2H, t, *J* = 1.6 Hz), 7.81-7.79 (1H, m), 7.62 (0.8H, *J* = 1.5 Hz), 7.47-7.44 (1H, m), 7.37 (0.2H, t, *J* = 7.8 Hz), 7.34-7.31 (0.8H, m), 7.27-7.24 (0.4H, m), 7.17 (0.2H, t, *J* = 7.9 Hz), 7.13-7.04 (2.8H, m), 6.98 (0.8H, ddd, *J* = 8.3 Hz, 2.4 Hz, .65 Hz), 6.89 (0.2H, t, *J* = 2.0 Hz), 6.80 (0.2H, ddd, *J* = 7.9 Hz, 2.4 Hz, 1.1 Hz), 6.78-6.76 (0.8H, m), 6.72-6.70 (0.8H, m). ^{13}C NMR (125 MHz, DMSO-d_6): δ 193.79, 193.70, 177.89, 158.50, 157.55, 157.48, 157.23, 146.51, 146.47, 140.20, 139.30, 138.72, 137.60, 137.38, 137.16, 134.93, 134.80, 132.63, 132.49, 132.46, 131.38, 131.25, 129.77, 129.70, 129.37, 128.84, 127.44, 122.68, 122.10, 121.38, 120.38, 120.50, 120.46, 188.48, 117.36, 116.81, 115.84, 115.79, 114.55. HRMS (ESI) calculated for $C_{21}H_{16}BrN_3O_3SNa^+$ $(M+Na)^+$ 491.99880, found 491.99903. HPLC retention time (Method B): 9.97, 10.34 min. (Obtained as a mixture of E/Z isomers)

4.1.35 1,3-Bis(2-fluorobenzoyl)-5-bromobenzene thiosemicarbazone (32).[51] *p*-Toluenesulfonic acid monohydrate (0.006 g, 0.03 mmol) was added to a solution of 1,3-bis(3-fluorobenzoyl)-5-bromobenzene (0.190 g, 0.473 mmol) in anhydrous tetrahydrofuran (15 mL). After stirring at reflux for 10 min, thiosemicarbazide (0.088g, 0.97 mmol) was added to the reaction mixture and stirred for 28 h under an inert atmosphere of nitrogen gas. After 28 h, tetrahydrofuran was removed under reduced pressure and 10 mL of water was then added. The products were extracted with ethyl acetate (2 x 50 mL) and the combined organic phases were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification using flash chromatography (silica gel, hexanes:ethyl acetate, gradient 90:11 to 30:70) afforded 1,3-bis(3-fluorobenzoyl)-5-bromobenzene thiosemicarbazone (0.068 g, 0.143 mmol, 30% yield). ^{1}H NMR (500 MHz, Acetone-d_6): δ 8.92 (1H, brs), 8.44 (1H, t, *J* = 1.8 Hz), 8.33 (1H, brs), 7.92 (1H, q, *J* = 1.5 Hz), 7.86 (1H, brs), 7.74-7.64 (3H, m), 7.59

(1H, td, *J* = 7.4 Hz, 1.8 Hz), 7.54-7.48 (2H, m), 7.46-7.41 (1H, m), 7.36 (1H, td, *J* = 7.5 Hz, 1.0 Hz), 7.27 (1H, ddd, *J* = 10 Hz, 8.4 Hz, 1.0 Hz). ^{1}H NMR (400 MHz, Acetone-d_6, ^{19}F decoupled) 8.92 (1H, brs), 8.44 (1H, t, *J* = 1.8 Hz), 8.33 (1H, brs), 7.92 (1H, t, *J* = 1.7 Hz), 7.85 (1H, brs), 7.74-7.64 (3H, m), 7.59 (1H, dd, *J* = 7.7 Hz, 1.8 Hz), 7.57-7.47 (2H, m), 7.43 (1H, d, *J* = 8.4 Hz), 7.36 (1H, td, *J* = 7.5 Hz, 1.0 Hz), 7.27 (1H, dd, *J* = 8.4 Hz, 1.0 Hz). ^{13}C NMR (125 MHz, Acetone-d_6): δ 191.49, 180.88, 160.87 (d, *J* = 251 Hz), 160.34 (d, *J* = 248), 141.81, 140.39, 140.01, 134.98 (d, *J* = 8.6), 134.13 (d, *J* = 8.3), 134.01, 133.79 (d, *J* = 1.4 Hz), 131.69 (d, *J* = 2.5 Hz), 131.59 (d, *J* = 3.1 Hz), 127.98 (d, *J* = 0.83 Hz), 126.83 (d, *J* = 3.5 Hz), 126.65 (d, *J* = 14 Hz), 125.59 (d, *J* = 3.5), 123.57, 118.89 (d, *J* = 17 Hz), 117.82 (d, *J* = 21 Hz), 117.11 (d, *J* = 22 Hz).). ^{19}F NMR (470 MHz, Acetone-d_6): δ ¯112.74 - ¯112.79 (1F, m), ¯113.57 - ¯113.67 (1F, m). HRMS (ESI) calculated for $C_{21}H_{14}BrF_2N_3OSH^+$ $(M+H)^+$ 474.00818, found 474.00845. HPLC retention time (Method A): 10.19 min.

4.1.36 1,3-Bis(3-bromobenzoyl)-5-bromobenzene thiosemicarbazone (33). 1,3-Bis(3-bromobenzoyl)-5-bromobenzene (0.523 g, 1.00 mmol) was dissolved in tetrahydrofuran (10 mL) followed by the dropwise addition of titanium isopropoxide (1.18 mL, 4.00 mmol). The reaction was stirred for 10 min followed by the addition of thiosemicarbazide (0.182 g, 2.00 mmol). After refluxing for 2 h, the reaction mixture was quenched with 1 M HCl (20 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic phases were washed saturated aqueous sodium bicarbonate (20 mL) and dried over anhydrous sodium sulfate. Purification using flash chromatography (silica gel, hexane: ethyl acetate, gradient, 90:10 to 20:80) afforded 1,3-bis(3-bromobenzoyl)-5-bromobenzene thiosemicarbazone (0.140 g, mmol, 0.234 mmol, 23% yield). The product isomerizes in DMSO. The ^{1}H NMR and ^{13}C NMR for the isomer mixture is

reported. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.02 (0.5H, s), 9.14 (0.5H, s), 8.66 (0.5H, s), 8.60 (0.5H, s), 8.55-8.48 (1H, m), 8.40 (0.5H, s), 8.07 (0.5H, t, *J* = 1.7 Hz), 8.01 (0.5H, t, *J* = 1.7 Hz), 7.98 (0.5H, t, *J* = 1.7Hz), 7.91-7.84 (2.5H, m), 7.83 (0.5H, t, *J* = 1.7 Hz), 7.70 (0.5H, ddd, *J* = 8.01 Hz, 2.0 Hz, 1.0 Hz), 7.67 (0.5H, ddd, *J* = 7.7 Hz, 1.6 Hz, 1.0 Hz), 7.65 (0.5H, t, *J* = 1.7 Hz), 7.61-7.47 (3H, m), 7.42-7.36 (1H, m), 7.33-7.27 (0.5H, t, *J* = 8.0 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 192.38, 178.49, 144.58, 138.87, 138.30, 138.14, 135.71, 133.08, 132.97, 132.92, 132.58, 131.83, 131.23, 130.71, 128.82, 127.85, 127.74, 122.96, 122.42, 121.94. X-Ray crystallographic data obtained for 1,3-bis(3-bromobenzoyl)-5-bromobenzene thiosemicarbazone has been deposited in the Cambridge Crystallographic Data Centre and was allocated the deposition number CDCC 1042568. HRMS (ESI) calculated for $C_{21}H_{14}Br_3N_3OSH^+$ $(M+H)^+$ 593.84805, found 593.84797. HPLC retention time (Method A): 17.56. (Exist as a mixture of E/Z isomers in solution)

4.2 Biology

4.2.1 Cathepsin L Inhibition Assay

Enzyme assays for cathepsins L and B were modified from procedures originally described by Barrett and Kirschke.[84] Assays to determine the effects of inhibitors on human liver cathepsin L (Sigma Aldrich) activity were carried out using a fluorogenic peptide substrate N-carbobenzyloxy-L-Phe-L-Arg-7-amino-4-methylcoumarin (Z-FR-AMC) (BACHEM). It should be noted that IC_{50} values were only determined for compounds that, at a concentration of 10 μM, demonstrated greater than 50% inhibition of enzyme activity. Cathepsin L was pre- incubated with inhibitors at various concentrations (or with vehicle) for 5 min at 25˚C. The assay was initiated by the addition of substrate Z-FR-AMC. The final assay conditions were 100 mM

NaOAc buffer, pH 5.5, 1 mM EDTA, 3 mM DTT, 0.01% Brij 35 (Sigma), 1 nM cathepsin L, 2% DMSO (Sigma) and 50 µM of Z-FR-AMC, in a total reaction volume of 200 µl. Inhibitors were diluted to include a final concentration range of 10 µM to 10 pM. The release of AMC from the substrate was monitored fluorometrically at 15 second intervals for 5 min at 25 ^{o}C using black 96 well Corning 3686 assay microplates with a Thermo Fluoroskan Ascent FL microplate reader at excitation and emission filter wavelengths of 355 nm and 460 nm, respectively. Data were analyzed and IC_{50} values calculated utilizing GraphPad Prism 5.0 software. IC_{50} $\pm$S.E (see Supplementary data) values represent the average results from a minimum of three separate experiments.

4.2.2 Cathepsin B Inhibition Assay

Assays to determine the effects of inhibitors on cathepsin B activity were carried out using the fluorogenic peptide substrate N-carbobenzyloxy-L-Arg-L-Arg-7-amino-4-methylcoumarin (Z-RR-AMC), with IC_{50} values determined for compounds that demonstrated greater than 50% inhibition of enzyme activity at 10 µM of inhibitor concentration. Human liver Cathepsin B (EMD Millipore) was pre-incubated with inhibitors at various concentrations for 5 min at 37 ^{o}C. The assay was initiated by the addition of substrate Z-RR-AMC (EMD Millipore) and the final assay conditions were 1 nM cathepsin B, 60 µM Z-RR-AMC, 120 mM sodium potassium phosphate buffer pH 6.0, 1 mM EDTA, 3 mM DTT, 0.01% Brij 35, and 2% DMSO. Inhibitors were diluted to include a final concentration range of 10 µM to 10 pM. The reaction was monitored fluorometrically for 5 min at 37 ^{o}C using black 96 well Corning 3686 assay microplates with a Thermo Fluoroskan Ascent FL microplate reader at excitation and emission

filter wavelengths of 355 nm and 460 nm, respectively. Data were analyzed and IC_{50} values calculated utilizing GraphPad Prism 5.0 software. Experiments were performed in triplicate.

4.2.3 Progress Curves

Various concentrations of compounds (final concentrations were 10, 5, 1, 0.5, 0.1, 0.01 and 0.005 μM) were mixed with Z-FR-AMC solution (final concentration 10 μM). Reactions were initiated by the addition of cathepsin L in assay buffer with no pre-incubation time with inhibitor. Readings were taken every 30 sec for 50 min using a fluorescence microplate reader as described above.

4.2.4 Cell Culture

Human umbilical vein endothelial cell culture conditions:

Human umbilical vein endothelial cells (HUVECs) from pooled donors (Invitrogen) were grown on rat tail collagen-1 coated flasks (CELLCOAT®) in M200 medium (Invitrogen) supplemented with 1% gentamycin sulfate (Teknova), 1% amphotericin B (Corning) and low serum growth factor supplement kit (Invitrogen). HUVECs were passaged using Trypsin EDTA/Trypsin Neutralizer solutions (Invitrogen) as per manufacturer recommendations. Cells were maintained at 37 ^{o}C in a humidified atmosphere of 5% CO_2. HUVECs were not used beyond passage 5 in these experiments.

PC-3ML cell culture conditions:

PC-3ML are highly metastatic sublines isolated from PC-3 cells.[70] Cell were cultured in appropriate media supplemented with 10% FBS at 37 ^{o}C in a humidified atmosphere of 5% CO_2 in air.

MDA-MB-231 cell culture conditions:

MDA-MB-231 cells (ATCC) were cultured in DMEM (Corning) supplemented with 10% fetal bovine serum (Gibco One Shot®) obtained from Invitrogen and 1% gentamycin sulfate, and passaged using trypsin solution (Mediatech) in T-75 culture flasks (Corning).

4.2.5 Cell Invasion Assay

PC-3ML

Invasion assays were performed using Matrigel coated invasion inserts from BD Biosciences (354480). Cells suspended in serum free media were seeded into the inserts. Media supplemented with 10% FBS was added to the bottom chamber. The cells were incubated under desired conditions and 24h later, cells that invaded to the underside of the membrane were stained and counted under a microscope.

MDA-MB-231

The effects of compounds **1, 8,** and **32** on the invasive ability of MDA-MB-231 cells were analyzed using a cell invasion assay with BD Bioscience Matrigel™ invasion kits. MDA-MB-231 cells were cultured and passaged as previously described. Cells were trypsinized and removed from the culture flasks when they were 80% confluent. Cell density was determined with a Beckman Coulter Z-Coulter cell counter. Stock solutions of compounds **1, 8,** and **32** in DMSO were prepared in addition to E-64, a known generic cysteine protease inhibitor which was used as a positive control. BD BioCoat Matrigel® invasion chambers, which contained 8 micron pore size PET membranes with a layer of Matrigel, were used for the experiment. The experiment was initiated by adding DMEM supplemented with 10% FBS (which functions as a chemoattractant) and gentamicin to a 24-well microplate (i.e. lower chamber). Then, the inserts were carefully placed on top of the wells containing the chemoattractant. Cell and compound

solutions were added such that the final conditions per well were: 2% DMSO, 50,000 cells in DMEM and 25 or 10 μM of the compounds. Final conditions for untreated (controls) cells were: 2% DMSO and 50,000 cells. The 24-well plates containing the invasion chambers were placed in an incubator with a 5% CO_2 environment for 24 hours at 37 ^{o}C. The experiments were terminated, invaded cells were fixed with methanol, stained using a Diff staining kit (IMEB Inc), and rinsed with deionized water. Samples were air-dried and membranes were removed and placed on glass slides. Each sample was observed with a Zeiss Axiovert 40 CFL inverted microscope to perform manual cell counting (ten fields were observed under a 40x objective). Experiments were performed in triplicate.

4.2.6 Cell Migration Assay

Inhibition of MDA-MB-231 cell migration was determined with an assay similar to that described for the cell invasion assay. However, the membrane inserts had 8μm pores but no Matrigel layer. Experiments were performed in triplicate.

4.2.7 Growth Inhibition of C3H Mammary Carcinoma

Experiments were performed using 10-14-week-old female CDF1 mice, in which a C3H mammary carcinoma was implanted in the right rear foot. This tumor model is an anaplastic adenocarcinoma that arose spontaneously in a C3H mouse at Aarhus University Hospital and was originally designated as HB;[85] the name was changed to C3H mammary carcinoma when it was grown in the more stable CDF1 mouse variant.[86] C3H mammary carcinomas do not grow in culture, thus experimental tumors were produced following sterile dissection of large flank tumors as previously described.[87] Basically, macroscopically viable tumor tissue was minced

with scissors and 5-10 µl of this material implanted into the foot. Treatments were started at the time of tumor implantation; tumor volume was determined by the formula D1 x D2 x D3 x π/6 (where the D values represent three orthogonal diameters). All animal studies were conducted according to the animal welfare policy of Aarhus University (http://dyrefaciliteter.au.dk), with the Danish Animal Experiments Inspectorate's approval. 3-Benzoylbenzophenone thiosemicarbazone (**1**) was supplied by Baylor University (Waco, Texas, USA). It was freshly prepared before each experiment by dissolving in Tween80 and then diluted to 10% using saline before injection. Stock solutions were kept cold and protected from light. 3-Benzoylbenzophenone thiosemicarbazone (**1**) was injected intraperitoneally (i.p.) in a volume of 0.02 ml/g mouse body weight for 5 consecutive days starting either on the day of tumor implantation or when tumors had reached 200 mm^3.

4.2.7 Cytotoxicity Assay

The sulforhodamine B (SRB) assay was used to assess inhibition of human cell line growth as previously described.[88-90] Briefly, HUVECs were passaged using normal conditions and plated at 9,000 cells/well in 96-well plates (Corning) and incubated for 24 h. Ten-fold serial dilutions of the compounds to be tested were then added to the wells. After 48 h, treated and control cells were fixed with 10% trichloroacetic acid, stained with 0.4% sulforhodamine B (Acid Red 52) (TKI) for 30 minutes, and subsequently washed 4 times with 1% acetic acid in water. The plates were air dried, and the SRB dye was solubilized with 200 µL of 10 mM Tris base and read at 540 nm with an automated Biotek Elx800 plate reader (Biotek). Values were normalized to 630 nm to account for background absorbance.[88] A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in net protein absorbance relative to controls) was

calculated from a minimum of two replicates and averaged for a minimum of three experiments with Excel software.

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Supplementary data

Supplementary data including X-ray crystallography, ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, HRMS, HPLC analysis, molecular modeling studies, enzyme kinetic studies, and Lipinski rule of five analysis associated with this article can be found, in the online version, at

References

1. Fonović, M.; Turk, B. *Biochim. Biophys. Acta* **2014**, *1840*, 2560-2570.
2. Mohamed, M. M.; Sloane, B.F. *Nature Reviews Cancer* **2006**, *6*, 764-775.

3. Gocheva, V.; Joyce, J. A. *Cell Cycle* **2007**, *6*, 60-64.
4. Lankelma, J. M.; Vorrend, D. M.; Barwari, T.; Koetsveld, J.; Van der Spek, A. H.; De Porto, A. P.; Van Rooijen, G.;Van Noorden, C. J. *Life Sci.* **2010**, *86*, 225-233.
5. Brömme, D.; Wilson, S. Role of Cysteine Cathepsins in Extracellular Proteolysis. In Extracellular Matrix Degradation; Parks, W. C.; Mecham, R. P., Eds.; Biology of Extracellular Matrix 2; Springer Berlin Heidelberg, 2011; pp 23-51.
6. Gocheva, V.; Zeng, W.; Ke, D.; Klimstra, D.; Reinheckel, T.; Peters, C.; Hanahan, D.; Joyce, J. A. *Genes Dev.* **2006**, 543-546.
7. Blondeau, X.; Vidmar, S. L.; Emod, I.; Pagano, M.; Turk, V.; Keil-Dloua, V. *Biol. Chem. Hoppe-Seyler* **1993**, *374,* 651-656.
8. Ishidoh, K.; Kominami, E. *Biochem. Biophys. Res. Commun*. **1995**, *217*, 624-631.
9. Guinec, N.; Dalet-Fumeron, V.; Pagano, M. *Biol. Chem. Hoppe-Seyler* **1993**, *374*, 1135-1146.
10. Buck, M. R.; Karustis, D. G.; Day, N. A.; Honn, K.V.; Sloane, B. F. *Biochem J.* **1992**, *282*, 273-278.
11. Kirschke, H.; Kembhavi, A. A.; Bohley, P.; Barret, A. *J. Biochem. J.* **1982**, *201*, 367-372.
12. Maciewicz, R. A.; Wotton, S. F.; Etherington, D. J.; Duance, V. C. *FEBS Lett.* **1990**, *269*, 189-193.
13. Garnero, P.; Borel, O.; Byrjalsen, I.; Ferreras, M.; Drake, F. H.; McQueney, M. S.; Foged, N. T.; Delmas, P. D.; Delaissé, J.-M. *J. Biol. Chem.* **1998**, *273*, 32347-32352.
14. Sameni, M.; Dosescu, J.; Moin, K.; Sloane, B. F. *Mol. Imaging* **2003**, *2*, 159-175.

15. Rothberg, J. M.; Bailey, K. M.; Wojtkowiak, J. W.; Ben-Nun, Y.; Bogyo, M.; Weber, E.; Moin, K.; Blum, G.; Mattingly, R. R.; Gillies, R. J.; Sloane, B. F. *Neoplasia* **2013**, *15*, 1125-1137.

16. Manson, S. D.; Joyce, J. A. *Trends in Cell Biol.* **2011,** *21,* 228-237.

17. Bell-McGuinn, K. M.; Garfall, A. L.; Bogyo, M.; Hanahan, D.; Joyce, J. A. *Can. Res.* **2007**, *65*, 7378-7385.

18. Berdowska, I. *Clin. Chim. Acta* **2004**, *342*, 41-69

19. Ullah, M. F.; Aatif, M. *Cancer Treat. Rev.* **2009**, *35*, 193-200

20. Chen, B.; Platt, M. O. *J. Transl. Med.* **2011**, *9*, 109-120.

21. Jensen, A. B.; Wynne, C.; Ramirez, G.; He, W.; Song, Y.; Berd, Y.; Wang, H.; Mehta, A.; Lombardi, A. *Clin. Breast Cancer* **2010**, *10*, 452-458.

22. Clezardin, P. *Breast Cancer Res.* **2011**, *13*, 207.

23. Stoch, S. A.; Zajic, S.; Stone, J. A.; Miller, D. L.; van Bortel, L.; Lasseter, K. C.; Pramanik, B.; Cilissen, C. ; Liu, Q.; Liu, L.; Boyd, B. S.; Paneblanco, D.; Ding, Yu, Gottesdiener, K.; Wagner, J. A. *Br. J. Clin. Pharmacol.* **2012**, 75, 1240-1254.

24. Gauthier, J. Y.; Chauret, N.; Cromlish, W.; Desmarais, S.; Duong, L. T.; Falgueyret, J.-P.; Kimmel, D. B.; Lamontagne, S.; Léger, S.; LeRiche, T.; Li, C. S.; Massé, F.; McKay, D. J.; Nicoll-Griffith, D. A.; Oballa, R. M.; Palmer, J. T.; Percival, D.; Riendeau, D.; Robichaud, J.; Rodan, G. A.; Rodan, S. B.; Seto, C.; Thérien, M.; Truong, V.-L.; Venuti, M. C.; Wesolowski, G.; Young, R. N.; Zamboni, R.; Black, W. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 923-928.

25. Lindstrom, E.; Grabowska, U.; Jerling, M.; Edenius, C.; MIV-711, A Highly Selective Cathepsin K Inhibitor, Reduces Biomarkers of Bone Resorption and Cartilage

Degradation in Healthy Subjects. Proceedings of the 2014 OASRI World Congress, Paris, France, April 24-27, 2014. Osteoarthritis and Cartilage, **2014**, S197.

26. About Cathepsin B and Liver Disease. http://www.virobayinc.com/liver-fibrosis.php (Accessed on March 20, 2014).
27. Evaluation of Safety and Pharmacokinetics of Single Doses of VBY-376 in Healthy Adults. http://clinicaltrials.gov/ct2/show/NCT00557583 (Accessed on October 1, 2014).
28. Pan cathepsin inhibitor: VBY-825. http://www.virobayinc.com/VBY-825.php (Accessed on October 1, 2014)
29. Elie, B. T.; Gocheva, V.; Shree, T.; Darlrymple, S. A.; Holsinger, L. J.; Joyce, J. A. *Biochimie* **2010**, *92*, 1618-1624.
30. GSK Product development pipeline February 2014. http://www.gsk.com/media/280387/product-pipeline-2014.pdf (Accessed on September, 8, 2014)
31. A Study to Evaluate the Safety, Tolerability, Pharmacokinetics (PK), Pharmacodynamics (PD) and Food Effect of Single or Repeat Doses of GSK2793660 in Healthy Subjects. https://clinicaltrials.gov/ct2/show/NCT02058407 (Accessed on September, 8, 2014).
32. Rizoska, B; Edenius, C.; Göhlin, K.; Hewitt, E.; Lindström, E.; Malcangio, M.; Pitcher, T.; Tunblad, K. MIV-247 A Cathepsin Protease Inhibitor Developed as a Novel Therapy for Neuropathic Pain. Proceedings of the 15th World Congress on Pain 2014, Buenos Aires, Argentina, October 6-11, 2014.
33. Miller, B.; Friedman, A. J.; Choi, H.; Hogan, J.; McCammon, J. A.; Hook, V.; Gerwick, W. H. *J. Nat. Prod*. **2014**, *77*, 92-99

34. Katunuma, N.; Murata, E.; Kakegawa, H.; Matsui, A.; Tsuzuki, H.; Tsuge, H.; Turk, D.; Turk, V.; Fukushima, M.; Yada, Y.; Asao, T. *FEBS Lett.* **1999**, *458*, 6-10.

35. Shah, P. P.; Wang, T.; Kaletsky, R. L.; Myers, M. C.; Purvis, J. E.; Jing, H.; Huryn, D. M.; Greenbaum, D. C.; Smith III, A. B.; Bates, P.; Diamond, S. L. *Mol. Pharmacol.* **2010**, *78*, 319-324.

36. Dana, D.; De, S.; Rathod, P.; Davalos, A. R.; Novoa, D. A.; Paroly, S.; Torres, V. M. Afzal, N.; Lankalapalli, R. S.; Rotenberg, S. A.; Chang, E. J. Subramaniam, G.; Kumar, S. *Chem. Commun.* **2014**, *50*, 10875-10878.

37. Assad, N.; Bethel, P. A.; Coulson, M. D.; Dawson, J. E.; Ford, S. J.; Gerhardt, S.; Grist, M.; Hamlin, G. A.; James, M. J.; Jones, E. V.; Karoutchi, G. I.; Kenny, P. W.; Morley, A. D.; Oldham, K.; Rankine, N.; Ryan, D.; Wells, S. L.; Wood, L.; Augustin, M.; Krapp, S.; Simader, H.; Steinbacher, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4280-4283.

38. Marquis, R. W.; James, I.; Zeng, J.; Trout, R. E. L.; Thompson, S.; Rahman, A.; Yamashita, D. S.; Xie, R.; Ru, Y.; Gress, C. J.; Blake, S.; Lark, M. A.; Hwang, S.-M.; Tomaszek, T.; Offen, P.; Head, M. S.; Cummings, M. D.; Veber, D. F. *J. Med. Chem.* **2005**, 48, 6870-6878.

39. Falgueyret, J.-P.; Oballa, R. M.; Okamoto, O.; Wesolowski, G.; Aubin, Y.; Rydzewski, R. M.; Prasit, P.; Riendeau, D.; Rodan, S. B.; Percival M. D. *J. Med. Chem.* **2001**, *44*, 94-104.

40. Frizler, M.; Lohr, F.; Furtmann, N.; Kläs, J.; Gütschow, M. *J. Med. Chem.* **2011**, *54*, 396-400.

41. Bauer, D. J. Clinical experience with the antiviral drug Marboran® (1-methylisatin 3-thiosemicarbazone) *Ann. N. Y. Acad. Sci*. 1965, *130*, 110–117.

42. Bauer D. J.; St Vincent, L.; Kempe, C.; Young, P. A.; Downie, A.W. *Am. J. Epidemiol.I* **1969**, *90*, 130-145.

43. Weiss, M. M.; Weiss, P. D.; Mathisen, G.; Guze, P. *Clin. Infect. Dis.* **2004**, *39*, 1668-1673.

44. Caminero, J. A.; Sotgiu, G.; Zumla, A.; Migliori, G. B. *Lancet Infect. Dis.* **2010**, *10*, 621-629.

45. Kishore Kumar, G. D.; Chavarria, G. E.; Charlton-Sevcik, A. K.; Arispe, W. M.; MacDonough, M. T.; Strecker, T. E.; Chen, S.-E.; Siim, B. G.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. Bioorg. *Med. Chem. Lett.* **2010**, *20*, 1415–1419.

46. Kishore Kumar, G. D.; Chavarria, G. E.; Charlton-Sevcik, A. K.; Yoo, G. K.; Song, J.; Strecker, T. E.; Siim, B. G.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6610–6615.

47. Chavarria, G. E.; Horsman, M. R.; Arispe, W. M.; Kishore Kumar, G. D; Chen, S.-E.; Strecker, T. E.; Parker, E. N.; Chaplin, D. J.; Pinney, K.G.; Trawick, M.L. *Eur. J. Med. Chem.* **2012,** *58*, 568-572.

48. Sudhan, D. R.; Siemann, D. W. *Clin. Exp. Metastasis* **2013**, *30*, 891-902.

49. Song, J.; Jones, L. M.; Kishore Kumar, G. D.; Conner, Liela Bayeh; E. S.; Chavarria, G. E.; Charlton-Sevcik, A. K.; Shen-En Chen; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *ACS Med. Chem. Lett*. **2012**, *3*,450-453.

50. Song, J.; Jones, L. M.; Chavarria, G. E.; Charlton-Sevcik, A.K.; Jantz, A.; Johansen, A.; Bayeh, L.; Soeung, V.; Snyder, L.K.; Lade, S.D.; Chaplin, J.; Trawick, M.L.; Pinney, K.G. *Bioorganic and Medicinal Chemistry Letters* **2013**, *23,* 2801-2807.

51. Chaplin, D. J.; Gaddale Devanna, K.K; Parker, E.; Pinney, K. G.; Jiangli, S.; Trawick, M. L. PCT Int. Appl., WO 2013142038 A2 2013092, September 26, 2013.

52. Raghav, N.; Kaur, R. *Med. Chem. Res.* **2014**, *23*, 4669-4679.

53. Siles, R.; Chen. S.-E.; Zhou, M.; Pinney, K. G.; Trawick, M. L. *Bioorg. Med. Chem. Lett.* **2006,** *16*, 4405-4409.

54. Du, X.; Guo, C.; Hansell, E.; Doyle, P. S.; Caffey, C. R.; Holler, T. P.; McKerrow, J. H.; *J. Med. Chem.* **2002**, *45*, 2695-2707

55. Mallari, J. P.; Shelat, A.; Kosinski, A.; Caffrey, C. R.; Connelly, M.; Zhu, F.; McKerrow, J. H.; Guy, R. K. *Bioorg. Med. Chem Lett.* **2008**, *18*, 2883-2885.

56. Caputto, M. E.; Fabian, L. E.; Benítez, D.; Merlino, A.; Ríos, N.; Cerecetto, H.; Moltrasio, G. Y.; Moglioni, A. G.; González, M.; Finkielsztein, L. M. *Bioorg. Med. Chem.* **2011**, *19*, 6818-6826.

57. Greenbaum, D. C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C. R.; Lehrman, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *J. Med. Chem.* **2004**, 47, 3212-3219.

58. Chiyanzu, I.; Clarkson, C.; Smith, P. J.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem.* **2005**, *13*, 3249-3261.

59. Liu, Y.; Cui, K.; Lu, W.; Luo, W.; Wang, J.; Huang, J.; Guo, C. *Molecules* **2011**, *16*, 4527-4538.

60. Schröder, J.; Noack, S.; Marhöfer, R. J.; Mottram. J. C.; Coombs, G. H.; Selzer, P. M. *PLoS ONE* [Online] **2013**, *8*, e77460. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0077460 (Accessed on September, 8, 2014)

61. Schaeffer, M.; Schroeder, J.; Heckeroth, A. R.; Noack, S.; Gassel, M.; Mottram, J. C.; Selzer, P. M.; Coombs, G. H. *Antimicrob. Agents Chemother.* **2012**, *56*, 1190-1201.

62. Johnson, J. E.; Morales, N. M.; Gorczyca, A. M.; Dolliver, D. D.; McAllister, M. A. *J. Org. Chem.* **2001**, *66*, 7979-7985.

63. Karabatsos, G. J.; Vane, F. M.; Taller, R. A.; Hsi, N. *J. Am. Chem. Soc.* **1964**, *86*, 3351-3357.

64. Kerek, F.; Ostrogovich, G.; Simon, Z. *J. Chem. Soc. B* **1971**, 541-544.

65. Ostrogovich, G.; Simon, Z.; Kerek, F. *Revue Tomaine de Chimie*, **1970**, *15*, 1453-1459.

66. Curtin, D. Y.; Grubbs, E. J.; McCarty, C. G. *J. Am. Chem. Soc.* **1966**, *88*, 2775-2786.

67. Hardegger, L. A.; Kuhn, B.; Spinnler, B.; Anselm, L.; Ecabert, R.; Stihle, M.; Gsell, B.; Thoma, R.; Diez, J.; Benz, J.; Plancher, J.-M.; Hartmann, G.; Banner, D. W.; Happ, W.; Diderich, F. *Angew. Chem. Int. Ed.* **2011**, *50*, 314-318.

68. Choe, Y.; Leonetti, F.; Greenbaum, D. C.; Lecaille, F.; Bogyo, M.; Bromme, D.; Ellman, J. A.; Craik, C. S. *J. Biol. Chem.* **2006**, *281*, 12824–12832.

69. Albini, A.; Iwamoto, Y.; Kleinman, H. K; Martin, G. R.; Aaronson, S. A.; Kozlowski, J. M.; McEwan, R. N. *Cancer Res.* **1987**, *47*, 3239-3245.

70. Wang, M.; Stearns, M. E. *Differentiation* **1991**, *48*, 115-125.

71. Neve, R. M,; Chin, K.; Fridlyand, J.; Yeh, J.; Baehner, F. L.; Fevr, T.; Clark, L.; Bayani, N.; Coppe, J.; Tong, F.; Speed, T.; Spellman, P. T.; DeVries, S.; Lapuk, A.; Wang, N.J.; Kuo, W.-L.; Stilwell, J. L.; Pinkel, D.; Albertson, D. G.; Waldman, F. M.; McCormick, F.; Dickson, R. B.; Johnson, M. D.; Lippman, M.; Ethier, S.; Gazdar, A.; Gray, J. W.. *Cancer Cell* **2006**, *10*, 515-527.

72. Zajc, I.; Sever, N.; Bervar, A.; Lah, T. T. *Cancer. Lett*. **2002**, *187*, 185-190.

73. Tamai, M.; Yokoo, C.; Murata, M.; Oguma, K.; Sota, K.; Sato, E.; Kanaoka, Y. *Chem. Pharm. Bull.* **1987**, *35*, 1098-1104.

74. Mitchell, D.; Lukeman, M.; Lehnherr, D.; Wan, P. Formal intramolecular photoredox chemistry of meta-substituted benzophenones. *Org. Lett.* **2005**, *7*, 3387-3389.

75. Zhao, W.; Carreira, E. M. *Org. Lett.* **2006**, *8*, 99-102.

76. Ye S.; Chen, J.; Di, C.; Liu, Y.; Lu, K.; Wu, W.; Du, C.; Liu, Y.; Shuai, Z.; Yu, G. *J. Mater. Chem.* **2010**, *20*, 3186-3194.

77. Sharghi, H.; Jokar, M.; Doroodmand, M. M.; Khalifeh, R. *Adv. Synth. Catal.* **2010**, *352*, 3031-3044.

78. Blick, F. F.; Patelski, R. A. *J. Am. Chem. Soc.* **1938**, *60*, 2283-2285.

79. Miller, T. M.; Kwock, E. W.; Neenan, T. X. *Macromolecules* **1992**, *25*, 3143-3148.

80. Teki, Y.; Takui, T.; Itoh, K.; Iwamura, H.; Kobayashi, K. *J. Am. Chem. Soc.* **1986**, *108*, 2147-2156.

81. Ronald, S.; Michael, S. PCT Int. Appl. WO 9907336 A1 19990218, February 18, 1999.

82. Knudsen, S. M.; Pettersson, I.; Lau, J.; Behrens, C.; Petersen, A. K.; Garibay, P. W. PCT Int. Appl. WO 2006003096 A1 20060112, January 12, 2006.

83. Chang, L. L. PCT Int. Appl. WO 9822108 A1 19980528, May 28, 1998.

84. Barrett, A. J.; Kirschke, H. *Method. Enzymol.* **1981**, *80*, Pt C, 535-561.

85. Overgaard, K.; Overgaard, J. *Eur. J. Cancer* **1972**, *8*, 65-78.

86. Overgaard, J. *Int. J. Radiat. Oncol. Biol. Phys.* **1980**, *6*, 1507-1515.

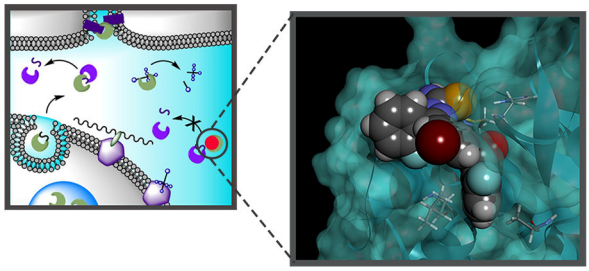
87. Horsman, M.R.; Murata, R.; Breidahl, T.; Nielsen, F.U.; Maxwell, R.J.; Stødkilde-Jørgensen, H.; Overgaard, *J. Adv. Exp. Med. Biol.* **2000**, *476*, 311-323.

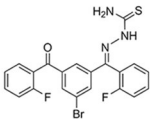
88. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757-766.

89. Vichai, V.; Kirtikara, K. *Nat. Protoc*. **2006**, *1*, 1112-1116.

90. MacDonough, M. T.; Strecker, T. E.; Hamel, E.; Hall, J. J.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2013**, *21*, 6831-6843.

91. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.





Compound **32**

Cat L IC_{50} = 8.12 nM