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Synthesis and Biological Evaluation of Analogues of a Novel Inhibitor of β-Amyloid Secretion

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A drug library of 17200 compounds was screened to select small molecules that inhibit the secretion of amyloid β peptide (Aβ), the major component of Alzheimer disease senile plaques, from a human neuronal cell line. Twenty-nine hits were validated that decreased Aβ secretion by >40% at 10 μM, for a 0.17% hit rate. A lead hit was selected for further study based on its activity and low cytotoxicity, and it was found to inhibit Aβ secretion through activation of the α-secretase pathway. Twenty-four commercially available and 53 synthesized analogues were analyzed for activity. Selected analogues were evaluated for biological stability by incubation with hepatoma cells and for transcellular permeability using Caco-2 cell monolayers. The analogue with the best permeability was evaluated in 2-month old amyloid precursor protein transgenic mice and found to acutely reduce cerebral Aβ levels by 40% after a single iv administration.

Introduction

Alzheimer disease (AD[a]) is the most common form of senile dementia, and it is characterized pathologically by decreased brain mass, extracellular senile plaques, and intracellular neurofibrillary tangles.[1] The major risk factor for AD is age, and it affects more than a third of all people that reach 85 years of age.[1] Although the pathogenesis of AD is still controversial, rare human mutations that lead to familial early onset AD are found in genes that lead to increased expression or processing of the amyloid precursor protein (APP) into amyloid β peptide (Aβ), which is the major component of senile plaques.[2−4] The so-called "amyloid hypothesis" for AD pathogenesis is supported by transgenic mouse models that overexpress mutant APP and genes involved in APP processing, which lead to the production of senile plaques and cognitive impairment.[5,6]

We took an unbiased approach to discover small molecule inhibitors of Aβ release from cultured human cells. Here we describe the selection of a lead hit, it is mechanism of action, and biological tests of commercially available and synthesized analogues. Ultimately, our lead drug was found to be effective in reducing cerebral Aβ levels in APP transgenic mice.

Results

Drug Library Screen and Lead Hit Selection. We screened 17200 small drug-like molecules obtained from ChemBridge (San Diego, CA) for their ability to inhibit Aβ secretion into media conditioned by stably transfected H4βAPP695wt human CNS derived cells.[7] The initial screen was performed in 96-well plates with drugs distributed by the hanging drop method yielding estimated drug levels of 10−25 μM. Total Aβ levels in the 24 h conditioned media were measured by a sandwich ELISA assay, and cytotoxicity was assessed by methylene blue staining of the cells in the wells, as previously described.[8] After assay validation,[8] we screened each compound in a single well and identified 80 compounds that both reduced Aβ levels in the conditioned media by at least 40%, and did not reduce methylene blue staining by 25% or more. Each of the 80 drugs was rescreened in duplicate, and 29 positive hits were individually ordered and validated in dose response studies, as previously described,[8] yielding a confirmed hit rate of 0.17% of the screened compounds. We selected **1** (Figure 1A, ChemBridge no. 5538506) as the lead hit for three reasons: (1) It displayed Aβ lowering activity at 1 μM in H4βAPP695wt cells (Figure 1B), (2) it did not have significant cytotoxicity, measured by total cell protein content, at 10 μM (Figure 1B), and (3) it had the lowest miLogP value of 5.165, an indicator of hydrophobicity associated with blood−brain barrier penetration, of the hits with similar potency. **1** was also effective in reducing secreted Aβ levels in cultured primary neurons derived from APP transgenic mice (Figure 1C).

Mechanism of Action of Lead Hit. The alternate processing of holoAPP by either the β-secretase (a precursor to Aβ production) or α-secretase (which clips in the middle of the Aβ region) is shown in Figure 2A. **1** induced decrease in total Aβ secretion may be due to its effect on APP processing or due to decreased production of its precursor APP. To probe its mechanism, H4βAPP695wt cells were treated with 10 μM **1** for 24 h. Using Western blot assays, we measured secreted APPα (sAPPα) and APPβ (sAPPβ) in the conditioned media, and full-length holo-APP and the 83 residue long α-C-terminal fragment (α-CTF) in cell lysate. **1** caused an increase in sAPPα and α-CTF level without altering sAPPβ or cellular

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[a]Abbreviations: Aβ, amyloid β peptide; AD, Alzheimer disease; APP, amyloid precursor protein; sAPPα, secreted form of amyloid precursor protein after α-secretase cleavage; sAPPβ, secreted form of amyloid precursor protein after β-secretase cleavage; α-CTF, the amyloid precursor protein C-terminal fragment after α-secretase cleavage; PKC, protein kinase C; β-BMEA, β-benzylmercaptoethylamine, ADAM, a disintegrin and metalloproteinase.

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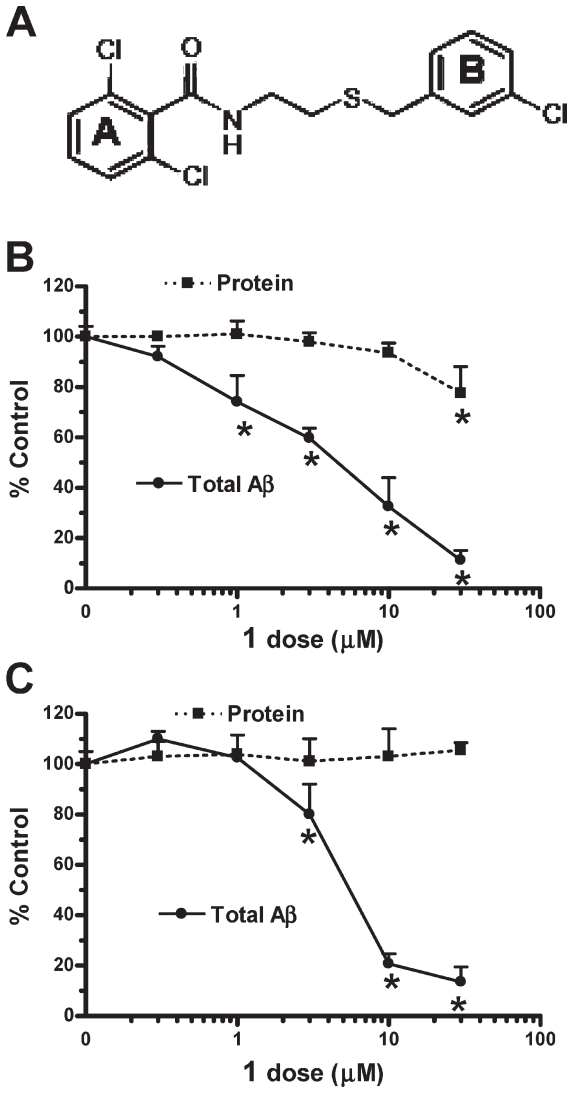


Figure 1. Compound **1** inhibits Aβ secretion. (A) Structure of **1**, with the labeling of the two phenyl rings. (B,C) Dose response of **1** on Aβ secretion and total cellular protein after 24 h incubation with H4βAPP695wt cells (B) or primary neurons from APP/PS1 transgenic mice (C). Data show mean ± SD for quadruplicate wells; *, $p < 0.05$ vs 0 dose control by ANOVA with Dunnett's multiple comparison test.

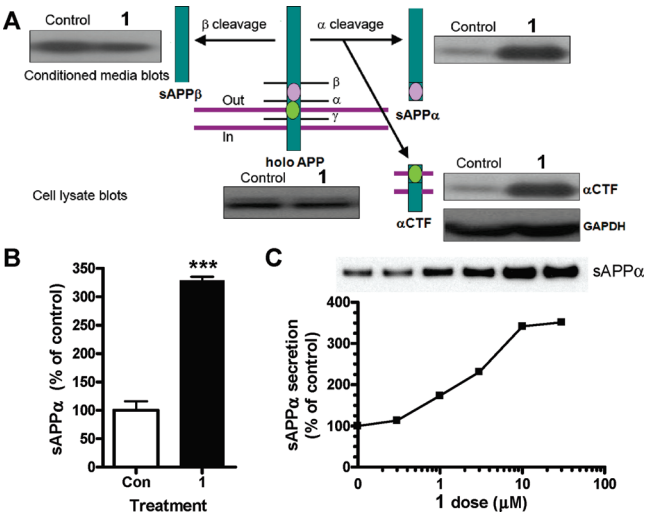


Figure 2. Compound **1** activates α-secretase processing of APP. (A) Scheme of APP processing and Western blot bands from conditioned media (top) and cell lysate (bottom) after 24 h treatment with 10 μM **1** or vehicle control. APP is shown in turquoise, with the Aβ region shown in the green plus purple ovals. The cleavage sites of the α, β, and γ secretases are shown. **1** treatment leads to increased sAPPα and αCTF without altering holoAPP levels, indicating increased α-secretase cleavage of APP. (B) Quantification of sAPPα induction by 10 μM **1** treatment ($N = 3$ each ± SD; ***, $p < 0.0001$). (C) Dose response of sAPPα induction by **1** showing Western blot band (top) and quantification (bottom).

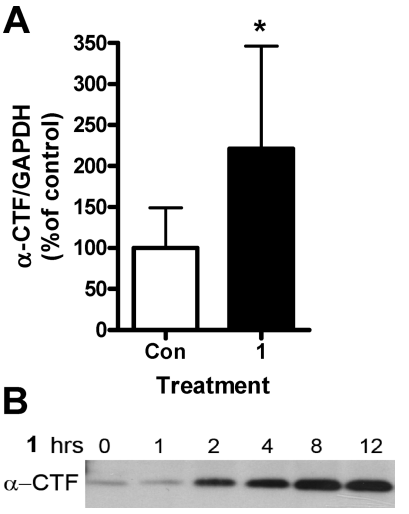


Figure 3. αCTF induction by **1**. (A) Quantification of αCTF induction by 24 h treatment with 10 μM **1** or vehicle control ($N = 7$ each ± SD; *, $p < 0.05$). (B) Time course of αCTF induction by 10 μM **1**.

full length APP (Figure 2A). **1** treatment significantly increased sAPPα levels by over 3-fold, with a dose response similar to that observed for inhibition of Aβ secretion (Figure 2B,C). **1** also raised cellular α-CTF levels by over 2-fold (Figure 3A), an effect that was observed in as little as 2 h after addition of **1**, and reached maximal levels by 8 h (Figure 3B). The effect of a 4 h treatment with **1** on the increase in cellular of α-CTF was not blocked by the protein synthesis inhibitor cyclohexamide, indicating that new protein synthesis was not required for this effect (data not shown). Thus, **1** appears to decrease Aβ secretion by activating the α-secretase pathway, a favorable pathway as the large secreted sAPPα protein is reported to have neurotrophic and neuroprotective activities.[9]

We measured the activity of α-secretase using a fluorogenic peptide substrate in lysates of H4βAPP695wt cells. Pretreatment of the cells with 10 μM **1** for 24 h led to a significant 20% increase in α-secretase activity (Figure 4A), with a dose response similar to that observed for inhibition of Aβ secretion (Figure 4B). However, addition of **1** directly to the cell lysates, rather than to the live cells, had no effect on α-secretase activity (data not shown), implying an indirect cellular activation of this pathway rather than direct activation of the α-secretase protease by **1**.

The tumor promoter phorbal myristate acetate is an activator of protein kinase C (PKC) that has been reported to activate α-secretase activity.[10,11] Thus, we wanted to determine if protein kinase C inhibitors could block the effect of **1** on Aβ secretion. The PKC inhibitors Bisindolylmaleimide I and Calphostin C had no effect on basal or **1** inhibited Aβ secretion, while the PKC inhibitor Rottlerin itself reduced Aβ secretion but did not significantly alter the effect of **1** (Figure 5).

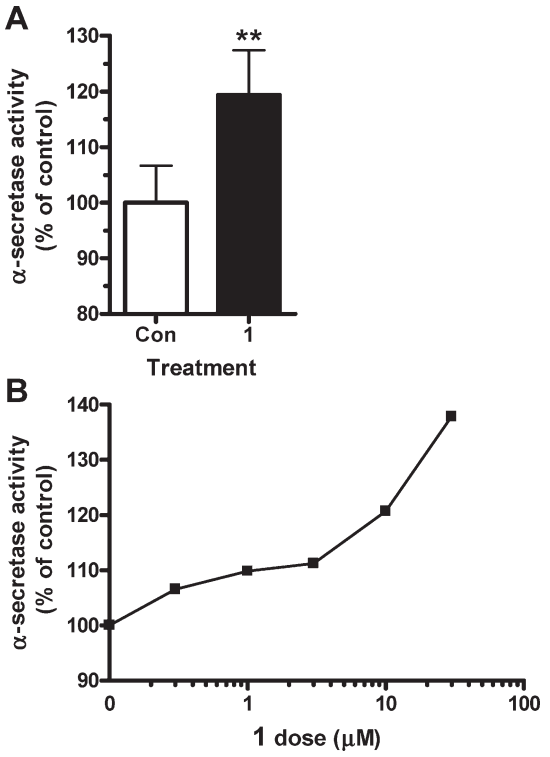


Figure 4. α-Secretase activity measured in vitro is induced by treatment of cells with **1**. (A) α-Secretase in cell lysates from cells treated for 24 h with 10 μM **1** or vehicle control (N = 6 each ± SD, **, p = 0.001). (B) Dose−response of α-secretase activity induction by **1**.

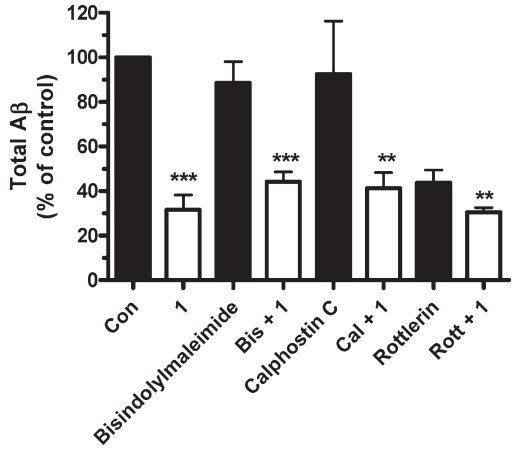


Figure 5. Effect of **1** on Aβ secretion is not blocked by PKC inhibitors. Results show secreted Aβ levels compared to the respective controls with **1** treated cells shown in open bars (N = 4 each, + SD; **, $p < 0.01$; and ***, $p < 0.001$ for effect of **1** vs the respective control for each treatment in the filled bar).

Several members of the ADAM (a disintegrin and metalloproteinase) family, ADAM9, ADAM10, and ADAM17, have been reported to have α-secretase activity.[12] These proteases are made in inactive pro forms and must be proteolytically cleaved into their active forms.[13,14] Using antibodies specific for these three ADAM proteases, we assessed the levels of the pro and active forms in cells cultured with increasing concentrations of **1**. We observed a dose dependent increase in active ADAM9 and ADAM10 but did not observe much effect on the active form of ADAM17 (Figure 6). At the 10 μM dose of **1**, we observed a 31% increase in the ratio of active/pro ADAM9 ($p < 0.05$),

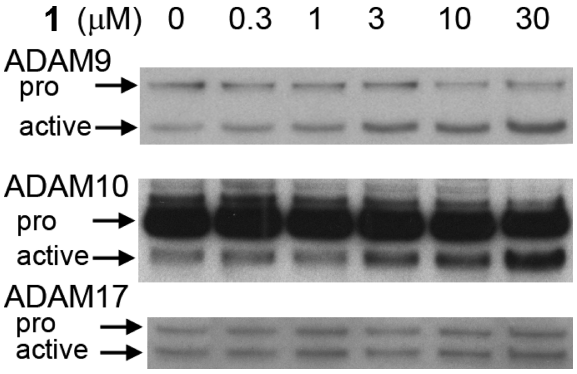


Figure 6. Compound **1** promotes cellular processing of ADAM9 and ADAM10. **1** at increasing doses was incubated on H4βAPP695wt cells for 24 h. Cell lysates were prepared for Western blots. Arrows point to the pro and active forms of ADAM9, ADAM10, and ADAM17.

N = 3 each control and **1**) and a 42% increase in the ratio active/pro ADAM10 ($p < 0.05$). Although the direct target for **1** has not been identified, it seems likely that its mechanism is via the increased ADAM 9 and ADAM10 processing into active α-secretase, which cleaves APP in a manner that precludes Aβ production.

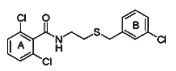
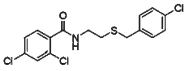
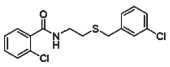
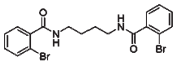
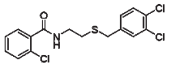
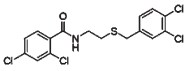
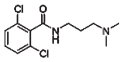
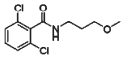
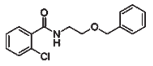
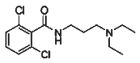
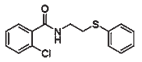
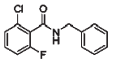
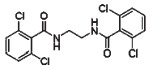
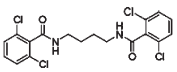
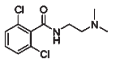
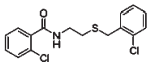
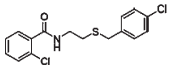
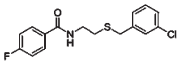
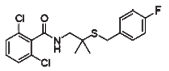
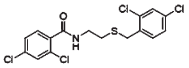
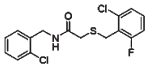
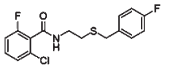
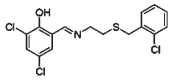
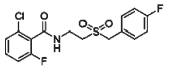
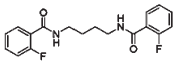
Commercial Analogue Evaluation. We searched for compounds having at least 60% similarity to **1** using the ChemBridge Hit2lead.com and PubChem Web sites. We were able to obtain 24 compounds (**2**−**25**) commercially which were tested for their ability to decrease Aβ secretion from H4βAPP695wt cells. Each drug, along with **1**, was tested in 1−4 independent experiments, each in quadruplicate, at the 10 μM dose. Table 1 shows the chemical structure of **1** and the 24 analogues, their source, product number, molecular weight, and the mean % reduction in Aβ secretion compared to the 0.02% DMSO vehicle control. In these experiments, 10 μM **1** yielded a 75.3 ± 11.6% decrease in Aβ secretion (N = 4 ± SD). None of the 24 analogues were as effective as **1**. Visual inspection of these compounds revealed that the two *ortho*-chlorines on aryl region A are critical, removing one of these chlorines in **3** led to a large loss in activity.

Design, Synthesis, and Evaluation of Novel Analogues. Figty-three novel analogues (**26**−**78**) were synthesized to examine their efficacy in reducing Aβ secretion. In examining **1**, the molecule can be parsed into three regions: aryl region A, linker region, and aryl region B. These regions along with our synthetic approach to constructing this small library are shown in Scheme 1. We then took the strategy of iterative modification of the three regions starting from commercially available benzyl halides (**III**). Conversion of **III** to the corresponding β-benzylmercaptoethylamine (β-BMEA) derivatives (**II**) gave derivatives that could further be reacted with substituted benzoyl chlorides, giving the desired amides (**I**).

In general, various β-BMEA derivatives were synthesized from the corresponding benzyl halides using our newly developed procedure.[15,16] The commercially unavailable benzyl halides were synthesized from the corresponding benzyl alcohols or toluene derivatives (Scheme 2),[17,18] whereas the commercially unavailable 2,6-disubstituted benzoyl chlorides were synthesized from the corresponding acid derivatives (Scheme 2). In particular, 2,6-dibromobenzoic acid was synthesized from 2,6-dibromotoulene using a $KMNO_4$ mediated oxidation strategy.[19]

By replacing 2,6-dichlorobezoyl moiety of aryl region A, we synthesized seven new analogues (**36**, **54**, **56**, **63**, **67**, **74**, and **75**), whereas the replacement of 3-chloro moiety of aryl

Table 1. Commercial Analogues Inhibition of Aβ Secretion[a]

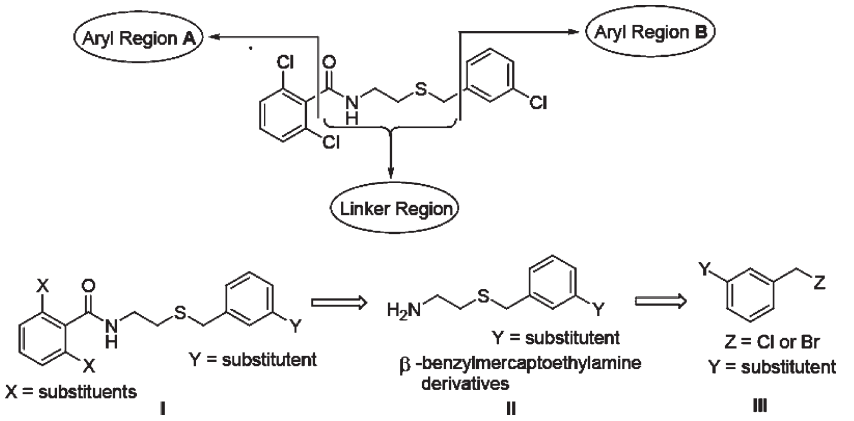
Analogue	Structure	MW	% Aβ Decrease*	Vendor**	Product #
1		375	75.3	C	5538506
2		375	5.3	C	6460634
3		340	4.7	C	6478444
4		454	7.9	C	5333392
5		375	0.7	C	6757682
6		409	4.3	C	6842391
7		275	15.8	C	5736522
8		262	6.9	C	5730201
9		290	10.3	C	5924186
10		303	5.1	C	5739287
11		292	-4.4	C	6465442
12		264	3.8	C	7272827
13		406	7.6	C	7243118
14		434	4.0	C	7287552
15		261	6.6	C	7478133
16		340	4.0	C	6399827
17		340	-12.0	C	6408972
18		324	6.4	C	7739577
19		386	37.0	R	9M-709
20		409	39.8	C	6698964
21		358	47.8	C	7991391
22		342	27.3	M	SEW06618
23		375	5.3	M	SEW06549
24		374	39.2	M	SEW06625
25		332	0.2	C	5328293

[a] *All drugs tested at 10 μM, % decrease relative to vehicle treated control cells. **Vendors: C, ChemBridge; M, Maybridge; R, Ryan Scientific.

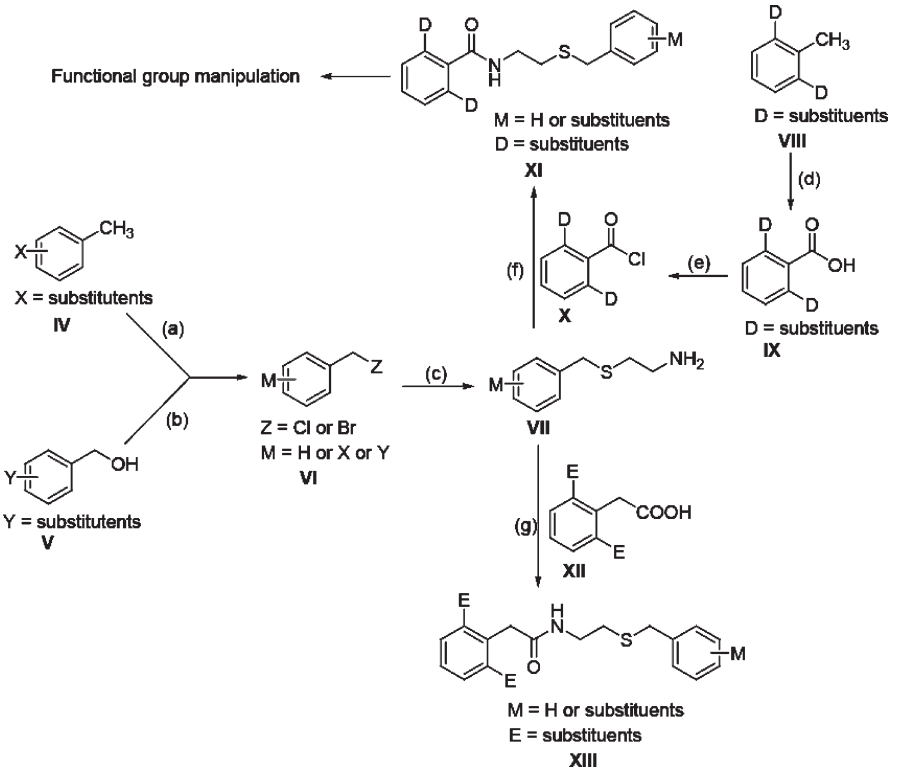
region B gave us a library of 30 new analogues (**26**−**29**, **38**−**41**, **43**−**45**, **47**−**53**, **58**−**62**, **65**, **68**−**70**, **73**, **76**, and **77**). Modification at the linker region yielded five new analogues (**57**, **66**, **71**, **72**, and **78**). Finally, on the basis of initial findings in conjunction with the above chemistry, several analogues were synthesized whereby more than one region was diversified, yielding an additional 11 analogues (**30**−**35**, **37**, **42**, **46**, **55**, and **64**). Although the majority of the molecules synthesized followed the route outlined in Scheme 2, several required specific manipulations. Detailed schemes for these transformations can be found in Supporting Information Schemes 1−7.

The structure of these compounds and their effects on reducing Aβ secretion and total protein levels (cytotoxicity) are shown in Table 2. Thirteen compounds (**27**, **29**, **40**, **48**, **50**, **51**, **52**, **53**, **54**, **55**, **65**, **73**, and **76**) at a 10 μM dose had roughly similar efficacy as **1** (> 50% inhibition of Aβ secretion), but five of these (**40**, **50**, **51**, **54**, and **55**) displayed cytotoxicity with a decrease in cell protein > 20% at the 30 μM dose. Inspection of these 13 active analogues reveals some flexibility of substitutions on aryl region B. Replacing the *meta*-chorine with *meta*-methyl ether (**27**), hydroxyl (**29**), nitrate (**40**), methyl (**48**), carbon trifluoride (**50**), bromine (**51**), or iodine (**53**) all yielded good activity. Other substitutions on aryl region B meta position did not yield good activity such as cyanate (**38**), amine (**41**), carboxylate (**44**), fluorine (**47**), formyl (**49**), and other more bulky substitutions (**39**, **43**, **45**, **68**, **69**, **70**). Removing the aryl region B *meta*-chlorine (**65**) retained activity, as did substituting an *ortho*-chlorine (**76**); however, substitutions with *para*-chlorine (**77**), *para*-methyl ether (**26**), or *para*-hydroxyl (**28**) led to loss of activity. While an aryl region B with two substituents could be tolerated, such as *meta*-chlorine *meta*-methyl ether (**73**), *meta*−*meta*-methyl ether (**60**), *ortho*−*meta*-methyl ether (**52**), and *ortho*-amide *meta*-chlorine (**61**), other compounds with two substitutions such as *meta*−*meta*-hydroxyl (**59**) and *ortho*−*meta*-hydroxyl (**58**) lost activity. On aryl region A, substitutions for one or both of the two chlorines in the ortho-positions, or their removal, were not well tolerated (**34**, **35**, **36**, **37**, **56**, **63**, **67**, **74**, and **75**), with the exception of bromine substitution, which

Scheme 1. Synthetic Analysis for Defining a Structure Activity Relationship for **1**



Scheme 2. General Synthetic Scheme for the Preparation of **1** Analogues[a]



[a] Reagents: (a) NBS, BPO, CCl_4, reflux; (b) $SOCl_2$, Et_3N, DCM, 50 °C; (c) LiOH-H_2O-EtOH, $HSCH_2CH_2NH_2 \cdot HCl$, 35 °C, 40 min; (d) $KMnO_4$, t-BuOH-H_2O, celite, reflux, conc HCl; (e) $SOCl_2$, reflux; (f) Et_3N, DCM, 0 °C to RT; (g) DCC, Et_3N, DCM, RT.

maintained activity (**54** and **55**). No tested substitutions in the linker region were well tolerated (**30**, **31**, **32**, **33**, **57**, **66**, **71**, **72**, and **78**).

To screen for resistance to metabolism, **1** and eight noncytotoxic analogues with > 50% Aβ reducing activity (**27**, **29**, **48**, **52**, **53**, **65**, **73**, and **76**) were incubated at 20 μM for 24 h with or without THLE-3 human hepatoma cells. This media was diluted 1:1 with fresh media (drug concentration diluted to 10 μM) and used to treat H4βAPP695wt cells for 24 h to assay inhibition of Aβ secretion. Table 3 shows that **1** and most of the active analogues maintained ~50% of their Aβ inhibiting activity during the 24 h incubation with the hepatoma cells, with the exception of **52**, which lost virtually all of its activity. The three analogues with the most net activity after incubation with the hepatoma cells were **76**, **53**, and **48** with 49%, 40%, and 38% inhibition of Aβ secretion, respectively.

To screen for bioavailability of **1** and seven active noncytotoxic analogues (**27**, **48**, **52**, **53**, **65**, **73**, and **76**), we assessed their transcellular transport through confluent monolayers of Caco-2 cells grown on transwell inserts. Monolayer integrity was confirmed by high levels of transcellular electrical resistance (> 160 ohms/cm^2) and by transcellular impermeability to the fluorescent dye lucifer yellow. The compounds were placed in the upper chamber, and 4 h later the lower chamber media was withdrawn and used to treat H4βAPP695wt cells for 24 h to assay inhibition of Aβ secretion. The concentration of each compound added to the upper chamber would lead to 10 μM in the lower chamber if equilibration was complete, and thus the activity of the lower chamber media was compared to the activity of 10 μM drug added directly to the H4βAPP695wt cells. Table 4 shows the result of this study. **1** crossed the monolayer and the bottom chamber media led to a 27%

Table 2. Newly Synthesized Analogues and Inhibition of Aβ Secretion[a]

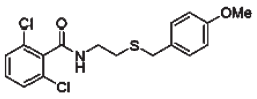
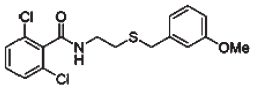
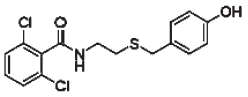
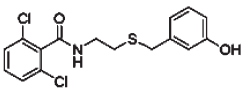
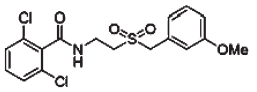
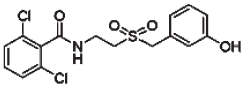
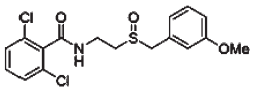
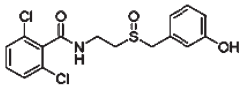
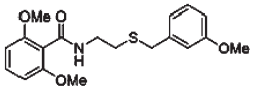
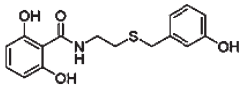
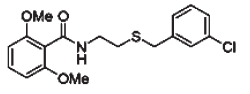
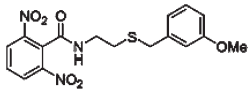
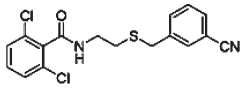
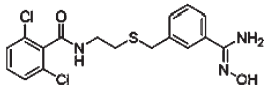
				Total Aβ (% decrease)				Cell protein (% decrease)			
				10 μM		30 μM		10 μM		30 μM	
Compound	Structure	MW (MP)	miLogP	Mean	SD	Mean	SD	Mean	SD	Mean	SD
26		370 (80)	4.57	1.6	14.0	1.1	1	6.7	10.1	12.7	1.1
27		370 (60)	4.54	60.6	5.0	73.9	3.7	8.4	8.8	13.2	8.3
28		356 (138)	4.03	13.1	4.0	56.7	4.1	8.7	5.5	4.4	4.1
29		356 (108)	4.01	56.4	4.5	67.7	6.8	6.3	1.6	6.6	6.4
30		402 (172)	3.42	5.3	21.5	4.9	9.8	1.2	8.0	8.9	4.9
31		388 (139)	2.88	-10.1	22.5	9.6	8.2	7.7	19.1	4.4	7.4
32		386 (144)	3.02	-13.6	11.0	-15.8	23	1.6	7.7	9.2	4.9
33		372 (165)	2.48	-23.7	27.8	15.9	11	2.8	3.7	4.4	7.4
34		361 (41)	3.30	6.2	27.1	-0.6	12	0.5	16.7	3.9	6.7
35		319 (60)	2.63	-22.5	17.9	-25.2	23	10.5	9.6	14.3	5.6
36		366 (90)	3.92	-13.1	18.6	11.2	7.3	7.1	5.2	-0.6	6.3
37		391 (69)	3.11	-14.1	7.0	-2.8	12	3.7	7.7	-3.8	10.8
38		365 (123)	4.24	20	8.9	38.7	6.7	-4.8	4.8	4.3	12.3
39		398 (126)	2.80	-1.5	13.8	1.6	12	9.2	13.6	5.9	15.1

Table 2. Continued

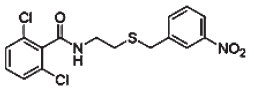
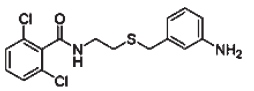
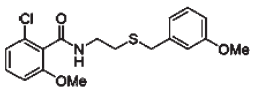
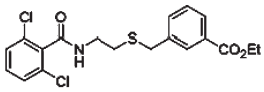
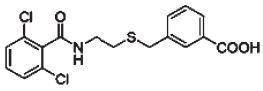
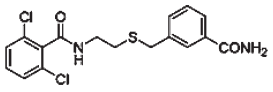
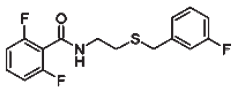
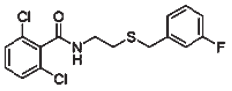
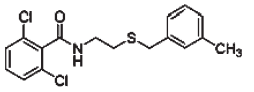
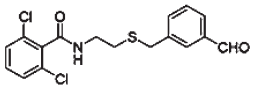
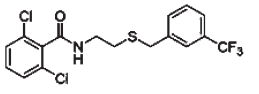
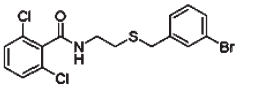
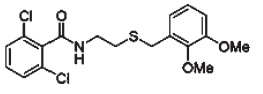
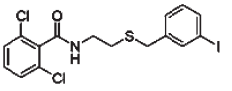
Compound	Structure	MW (MP)	miLogP	Total Aβ (% decrease) 10 μM Mean	10 μM SD	30 μM Mean	30 μM SD	Cell protein (% decrease) 10 μM Mean	10 μM SD	30 μM Mean	30 μM SD
40		385 (76)	4.45	58.5	6.9	72.5	4.5	7.5	14.0	24.6	7.1
41		355 (55)	3.56	0.1	7.8	17	11	15.3	9.7	13.1	12.1
42		366 (58)	3.92	-2.9	16.0	-4.7	16	1.9	21.4	9.3	9.5
43		412 (43)	5.03	3	16.0	10.1	13	5.8	17.4	5.4	9
44		384 (165)	4.40	-11.4	22.2	-3.8	15	7.1	15.6	3.2	13.7
45		383 (148)	3.31	6.2	15.1	32.3	8.3	-2.8	19.7	-8.6	15.2
46		325 (39)	3.62	-16.8	12.9	-15.5	8.6	0.1	12.1	1.8	7.7
47		358 (70)	4.65	10.2	30.1	36.1	8.6	2.6	9.2	3.7	6.6
48		354 (56)	4.94	70.5	4.3	85.8	4.2	11.7	5.6	16.6	8.9
49		368 (63)	3.83	19.9	12.0	36.4	11	1.5	12.8	-2.1	11.5
50		408 (54)	5.59	73.9	4.7	93.4	2.9	19	6.5	47.8	15.4
51		419 (74)	5.30	75	3.6	90.1	3.1	12.3	9.2	38.6	12
52		400 (108)	4.43	57.8	16.3	72.8	8.3	-0.1	10.3	8.7	5.8
53		466 (75)	5.57	84.4	5.3	88.3	6.4	9.2	7.5	19	7.7

Table 2. Continued

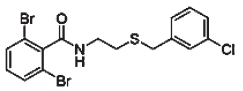
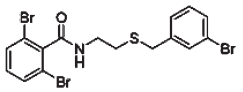
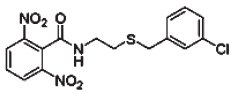
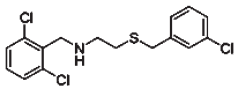
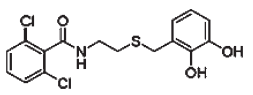
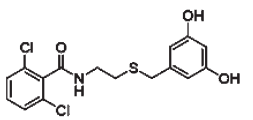
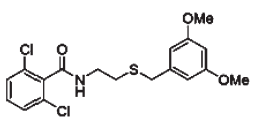
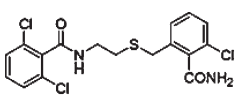
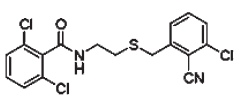
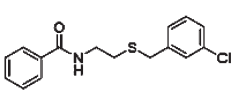
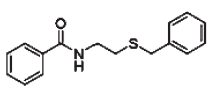
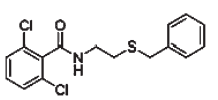
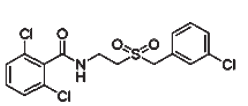
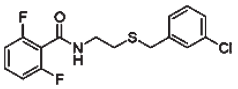
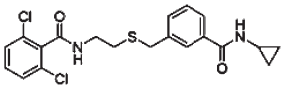
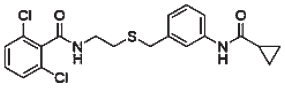
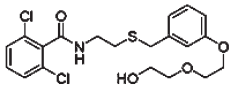
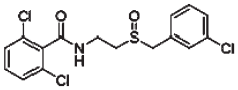
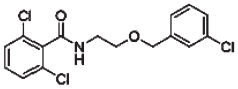
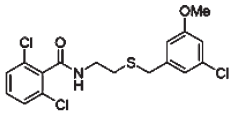
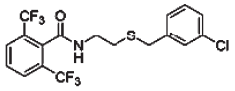
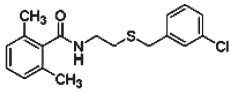
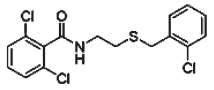
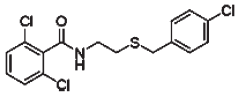
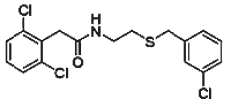
Compound	Structure	MW (MP)	miLogP	Total Aβ (% decrease) 10 μM Mean	10 μM SD	30 μM Mean	30 μM SD	Cell protein (% decrease) 10 μM Mean	10 μM SD	30 μM Mean	30 μM SD
54		463 (55)	5.43	74.2	11.8	83.4	6.7	6.7	8.3	20.8	5.3
55		508 (70)	5.56	78.6	6.4	87.3	5.9	9.8	7.1	23.9	6.6
56		395 (139)	3.73	28.8	18.2	52.9	15	0.1	9.3	17.8	7.6
57		361 (liq)	5.53	45.2	15.6	45.6	21	-3.6	11.3	6.6	10.8
58		372 (liq)	3.75	-4.3	22.1	33.1	8.2	-4.8	21.0	14	9.8
59		372 (55)	3.48	3.1	13.0	17.2	13	-0.4	12.9	-0.7	10.4
60		400 (46)	4.55	47.9	6.3	80.4	4.1	2	9.8	-8.7	22.6
61		417 (64)	3.91	44.7	10.6	49.7	6.1	2.5	12.8	-5.6	16.2
62		400 (166)	5.06	48.7	6.8	52.3	14	3.5	9.7	1.3	10.7
63		306 (75)	3.91	0.3	17.6	10.7	11	2.4	10.8	-4.5	15.4
64		271 (65)	3.25	-5.7	16.4	5.5	11	0.7	9.7	-4.1	11.2
65		340 (96)	4.51	51.6	11.7	58.6	12	-1.1	12.9	-0.7	10.2
66		407 (156)	4.04	8.9	14.0	9.3	16	-2.2	10.2	-3.8	12.2

Table 2. Continued

Compound	Structure	MW (MP)	miLogP	Total Aβ (% decrease) 10 μM Mean	10 μM SD	30 μM Mean	30 μM SD	Cell protein (% decrease) 10 μM Mean	10 μM SD	30 μM Mean	30 μM SD
67		342 (41)	4.14	4.9	9.9	21.6	7.7	5.1	8.8	8.1	8.6
68		423 (42)	4.05	1.9	7.2	11	9.9	0	4.8	-1	9
69		423 (46)	4.54	-21.4	34.7	-2	31	3	11.7	3.2	20.3
70		444 (liq)	3.71	17.4	16.8	24.3	9.8	-7.3	20.5	6.1	9.8
71		391 (174)	3.64	4	21.7	-16.5	11	-4.9	10.2	7.3	2.7
72		359 (70)	4.62	10.5	18.7	-5.1	14	-9.9	18.4	4.2	4.4
73		405 (83)	5.17	67.7	5.4	81	1.8	0.9	10.3	0.4	12.1
74		442 (93)	6.02	6.2	13.6	13.5	12	-4.2	18.6	-0.4	9.8
75		339 (53)	4.71	29.5	5.0	51.6	14	0.9	9.3	-9.4	22.9
76		375 (110)	5.14	75.6	4.3	80	4	-0.5	11.1	-11	29.6
77		375 (104)	5.19	21.8	9.0	36.5	13	-6.4	17.6	3	14.9
78		389 (116)	5.26	6.7	9.8	13.5	24	-1.3	4.9	-0.7	16.5

[a] MP, melting point given in °C; liq denotes a viscous liquid at room temperature. 30 and 66 were recrystallized from ethanol.

reduction in Aβ production, yielding 38% of the theoretical activity. The compound with the most activity in the lower chamber was **48**, which decreased Aβ production by 50%, with 71% of its theoretical activity. The lower chamber media from the other two compounds that were highly active after incubation with hepatocytes, **76** and **53**, decreased Aβ secretion by 32% and 1%, respectively. On the basis of the combined activity data from these last two studies, **48** was selected as the lead drug for in vivo study.

In Vivo Activity of Lead Drug. In APP transgenic mice, cerebral Aβ turns over rapidly prior to the onset of plaque formation with a half-life of ~2 h,[20] allowing one to test Aβ

Table 3. Drug Activity Persistence after Incubation with Hepatoma Cells

	hepatoma incubation[a]		
compd	no total Aβ (% decrease)	yes total Aβ (% decrease)	% remaining activity[b]
1	69.4	34.6	50
27	60.6	22.2	37
29	56.4	23.1	41
48	70.5	38.3	54
52	57.8	1.9	3
53	84.4	40.0	47
65	51.6	27.0	52
73	67.7	33.5	49
76	75.6	48.8	65

[a] Drugs incubated with H4 cells at 10 μM, with or without preincubation with hepatoma cells for 24 h. [b] % of activity surviving after hepatoma cell incubation.

Table 4. Epithelial Transcellular Transport of Drug Activity

	transcellular transport[a]		
compd	no total Aβ (% decrease)	yes total Aβ (% decrease)	% theoretical activity[b]
1	69.4	26.7	38.4
27	60.6	17.9	29.5
48	70.5	49.9	70.8
52	57.8	26.5	45.9
53	84.4	1.1	1.3
65	51.6	31.0	60.1
73	67.7	2.7	4.0
76	75.6	31.6	41.8

[a] Drugs alone or after incubation with Caco-2 cells to yield 10 μM if in equilibrium. [b] % of activity passing through Caco-2 monolayer.

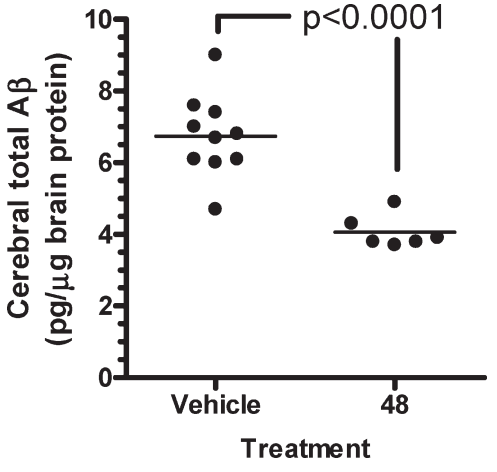


Figure 7. Compound **48** decreases cerebral Aβ levels in mice. APP/PS1 transgenic mice were injected iv with 100 to 125 mg/kg **48** or vehicle control. Six hours later, the mice were sacrificed and cerebral Aβ levels were measured by an ELISA assay and normalized to cerebral protein. Compound **48** led to a 39.6% reduction in cerebral Aβ levels ($p < 0.0001$).

lowering drugs acutely after a single administration. Thus, we administered a single high dose of our lead drug **48** (100 mg/kg) or vehicle iv to female APP transgenic mice and sacrificed the mice 6 h later for assay of cerebral Aβ levels. **48** led to a 39.6% decrease in cerebral Aβ levels (Figure 7, $p < 0.0001$).

Discussion

Alzheimer disease is a progressive, debilitating, and ultimately fatal disease of the elderly affecting memory and cognition. Although there may be several distinct processes involved in AD pathogenesis, the "amyloid hypothesis" is supported by the presence of high levels of Aβ containing senile plaques, the genetics of early onset familial AD, and transgenic mouse models.[21] Age is the major AD risk factor, with AD affecting over 3% of those 65−74 years of age, 19% of those 75−84 years of age, and 47% of those over 85 years of age.[22] Thus, we postulate that the inhibition of Aβ production in middle age subjects, prior to Aβ plaque deposition, could prevent or significantly delay the onset of AD. Delaying the age of AD onset by just two years is predicted to decrease AD incidence by 25%, while delaying the age of onset by five years would eliminate half of AD incidence.[23]

In the current study, we undertook an unbiased chemical library screen to find compounds that would decrease Aβ secretion by neuronal cells. We selected a lead hit, **1**, and characterized its mechanism of action. Aβ is produced by sequential β- and γ-secretase cleavage of APP, while the alternative APP cleavage by α-secretase destroys the Aβ peptide. γ-Secretase inhibitors that inhibit Aβ production have been developed, but γ-secretase also processes other cellular proteins such as Notch-1, which may lead to detrimental side effects.[24] Peptide analogue inhibitors for β-secretase activity, mediated by the BACE1 protein, have also been developed, but they have been hampered by poor cellular and blood−brain barrier penetration.[25] We found that **1** upregulated sAPPα secretion and the cellular accumulation of CTF-α, thus we concluded that **1** upregulates α-secretase activity. Because sAPPα has neurotrophic and neuroprotective activities, α-secretase activation is thought to be an attractive candidate for the prevention of AD.[26]

Phorbol esters that activate PKC have been shown to inhibit Aβ production through activation of α-secretase activity, although the full pathway for this activation is not well understood.[11,12,27] However, phorbol esters are tumor promoters and are thus not likely to be used as human therapeutics. Other PKC activators such as bryostatin, which does not have tumor promoter activity, also activate α-secretase and have been shown to decrease cerebral Aβ levels after chronic treatment in mutant APP/PS1 transgenic mice.[28,29] Additionally, muscarinic agonists and the neuropeptide PACAP activate α-secretase via their specific G protein-coupled receptors that also signal through PKC.[12] Thus, we sought to determine if the effect of **1** was also mediated through PKC. Through the use of inhibitors of various PKC isoforms, we conclude that **1** did not induce α-secretase via PKC activation.

Three proteins that belong to a disintegrin and metalloproteinase (ADAM) family have been found to have α-secretase activity, ADAM9, ADAM10, and ADAM17.[12] These are zinc dependent membrane bound proteins with "sheddase" activity, which cleave other membrane proteins to release soluble ectodomain protein fragments. Gene knockout mice have not been conclusive about the relative role of these three ADAM proteins in α-secretase activity in vivo, and ADAM10 and 17 knockout mice are embryonic and perinatal lethal, respectively.[12] ADAM9 and ADAM10 are expressed highly in neurons, while cerebral ADAM17 is expressed primarily in endothelial cells and astrocytes.[30,31] ADAM proteases are expressed as precursors and require proteolytic removal of the prodomain in order to become active; thus, proprotein convertases which cleave and activate ADAM proteases may regulate cellular ADAM activity.[13,14] Our results demonstrate that **1** treatment of cells led to increased α-secretase activity in cell lysates and was accompanied by

increases in the active forms of both ADAM9 and ADAM10. However, because **1** did not activate α-secretase activity when added directly to cell lysates, we conclude that **1** may work through either activation of proprotein convertases or through effects on cell trafficking so that the ADAMs and their convertases intersect in the same cellular compartment. Additional studies will need to be done to test these hypotheses.

A major hurdle for AD drug development is the ability of test compounds to cross the blood−brain barrier. We used Caco-2 cells, an epithelial cell monolayer with tight junctions, as a surrogate to help predict which analogues might best penetrate the blood−brain barrier.[32] On the basis of these results, we selected **48** for our in vivo screen. These studies employed a very high dose of **48**, but we were able to observe a significant ∼40% decrease in cerebral Aβ levels in the drug treated mice vs the control mice after a single iv administration. Whether this analogue is potent enough to be useful for human studies still needs to be determined; however, we did not discover any analogues thus far with efficacy at nM doses. Nevertheless, drugs that work via α-secretase activation may prove to be a useful method for significantly delaying the onset of AD, and thus preventing the majority of AD incidence.

Conclusions

We performed a drug library screen to identify compounds that inhibit Aβ secretion by cultured neuronal cells. We determined that the lead hit lowered Aβ secretion by activation of the α-secretase processing of its precursor protein, APP. We made a series of analogues of the lead hit and screened these for activity along with some commercially available analogues. We then tested the best analogues in cell based systems to predict which might be most effective in vivo. This lead drug was effective in lowering cerebral Aβ levels in a transgenic mouse model of AD-like pathology. Further studies are required to determine the target of this drug, the mechanism by which it activates α-secretase activity and its ability to delay the onset of amyloid plaque formation in a mouse model via chronic treatment.

Experimental Section

Antibodies and Biological Reagents. Mouse anti-Aβ/APP monoclonal antibodies 6E10 (SIG-39320) and 4G8-biotin (SIG-39240), and rabbit anti sAPPβ (SIG-39138) were obtained from Signet (now part of Covance), rabbit polyclonal anti-APP C-terminal antibody (A8717) was obtained from Sigma-Aldrich, rabbit monoclonal anti ADAM-9 (4151) was obtained from Cell Signaling, rabbit polyclonal anti-ADAM-10 (2051) was obtained from ProSci, rabbit polyclonal anti mouse ADAM-17 (AB19026) was obtained from Chemicon, and rabbit anti-GAPDH (ab9485) was obtained from Abcam. Other cell treatment reagents were purchased from Sigma-Aldrich except for the PKC inhibitors (Calbiochem).

Cell Culture and Drug Treatment. H4βAPP695wt cells, a human neuroblastoma cell line stably transfected with a wtβAPP695 expression vector construct under the control of a CMV promoter (kindly provided by Dr. Chris Eckman from Mayo Clinic, Florida) were cultured as previously described.[7] Twenty-four hours before drug treatment, the media was replaced with serum-free media. Cells were then incubated with drugs (dissolved in DMSO) or vehicle for 24 h for collection of conditioned media and cell lysates. For the PKC inhibitors experiment, three inhibitors were treated 30 min prior to drug challenge. Primary cortical neurons were prepared from embryonic day 18 APP and presenilin 1 (APPswe, PSEN1dE9) transgenic mice on the C57BL/6 genetic background (Jackson Laboratories, strain no. 5864) as previously described.[33] THLE-3 human hepatoma cells (from ATCC) were plated in 24-well plates that were precoated with 10 μg/mL bovine serum albumin, 10 μg/mL fibronectin, and 30 μg/mL bovine type I collagen. The cells were grown in BEGM media (Clonetics) until confluent and treated with drugs at 20 μM for 24 h. This conditioned media was harvested, diluted 1:1 in serum-free medium, and incubated with H4βAPP695wt cells for 24 h prior to analysis of secreted Aβ, which was compared to the effect of 10 μM drug treatments without prior incubation with the THLE-3 cells. Caco-2 cells (ATCC) were plated in 12-well plates with Transwell inserts (Costar) at 70000 cells per well and grown for 21 days. The media volume of the apical and basal chambers was 0.5 and 1.5 mL, respectively. Transepithelial resistance was measured in each well and found to be $>160\ \Omega/cm^2$. Drugs at 40 μM and 20 μg/mL of lucifer yellow were added in serum-free medium to the apical chamber. Four hours later, the basal chamber media was harvested and used to measure lucifer yellow fluorescence to confirm monolayer integrity as well as for treatment of H4βAPP695wt cells for 24 h prior to analysis of secreted Aβ, which was compared to the effect of direct addition of 10 μM of the corresponding drug.

Assays for Secreted Aβ and Processed APP. Conditioned medium was collected, protease inhibitor cocktail was added, and cell debris was cleared by centrifugation prior to assays for secreted total Aβ by ELISA and sAPPα and sAPPβ by Western blot. The total Aβ ELISA, normalized to total cell lysate protein by BCA assay, was preformed as previously described[8] using the capture antibody 6E10, which recognizes residues 1−16 of human Aβ, a region present in sAPPα but absent in sAPPβ, the long processed forms of secreted APP, and 4G8 for the detection antibody, which is absent in both sAPPα and sAPPβ. For Western blot analysis of sAPPα and sAPPβ, the volume of conditioned media was adjusted by the protein level in the cell lysate prior to SDS gel electrophoresis on 4−12% Bis-Tris SDS gels (Invitrogen). Protein was transferred to PVDF membranes (Invitrogen), blocked with casein blocker (Pierce), and incubated with primary antibodies 6E10, which recognizes sAPPα but not sAPPβ, or with an antibody specific for sAPPβ (both at 1:1000). After washing and incubation with HRP conjugated secondary antibodies, the signals were detected using ECL Western Blotting Detection kit (Pierce).

Assays for Cell Lysate Proteins. Cells were washed with cold phosphate buffered saline (PBS) and lysed on ice by vigorous mixing over 15 min with RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM sodium chloride, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS, supplemented with 10% protease inhibitor cocktail and 2 mM PMSF . After centrifugation at 14000*g* for 30 min to remove nuclei and other debris at 4 °C, the protein content of the supernatants was measured using the BCA assay kit (Pierce). Twenty μg of cell protein was mixed with 4× Nupage loading buffer and with reducing agent (Invitrogen), heated at 70 °C for 10 min, and loaded onto a 4−20% Bis-Tris gel with MES gel running buffer. Blots were prepared and probed with anti APP C-terminus, which binds to both holoAPP, detected at ∼110 KDa, and αCTF detected at ∼10 KDa. An anti GAPDH antibody was also added to detect GAPDH at 38 KDa, which was used as a loading control for normalization. Although this gel system did not routinely detect βCTF, preliminary studies using urea gels demonstrated that the βCTF fragment could be separated from αCTF but is a minor band compared to the αCTF band. For analysis of ADAM9, ADAM10, and ADAM17, the blots were probed with the appropriate antibodies and processed as described above.

α-Secretase Activity Assay. H4βAPP695wt were treated with vehicle or **1** for 24 h, washed with ice cold PBS, and lysed in extraction buffer provided with the α-secretase assay kit (R&D Systems) for 10 min on ice and centrifuged at 10000*g* for 1 min to

remove nuclei. Protein content of the supernatant was determined by the BCA assay, and 50 μg of lysate protein were added to wells of a 96-well assay plate and the volume was adjusted to 100 μL using the lysis buffer. 100 μL of reaction buffer and 10 μL of the fluorogenic substrate were added, and the plates were incubated at 37 °C in the dark with gentle agitation for 2 h. Activity was detected in a fluorescence plate reader using an excitation of 355 nm and an emission of 510 nm after subtraction of fluorescence in the absence of cell lysate.

In Vivo Drug Administration and Cerebral Aβ Assay. Transgenic mice expressing humanized mutant versions of APP and presenilin 1 (APPswe, PSEN1dE9) on the C57BL/6 genetic background were obtained from Jackson Laboratories (strain no. 5864) and bred to wild type C57BL/6 mice in order to obtain ~50% transgenic that were used in these studies. These mice develop Aβ senile plaques by 6 months of age.[34] To prepare the drug stock, 17.5 mg of compound **48** was dissolved in 40 μL of DMSO, which was mixed sequentially with 110 μL of Cremophor EL (Sigma) and 900 μL of sterile saline. Two month old female transgenic mice weighing 20−25 g were anesthetized with ketamine/xylazine and injected iv via the retroorbital plexus with vehicle control or with 2.5 mg of compound **48** in 150 μL of the drug stock (yielding a dose of ~100 to 125 mg/kg). Six h later, the mice were euthanized by CO_2 inhalation, the brains were removed, and the cerebellum discarded. One brain hemisphere was homogenized for 45 s on ice using a tissue grinder in 1 mL of solution containing 5 M guanadine HCl, 50 mM Tris (final pH adjusted to 8.3), and 5% protease inhibitor cocktail. Any debris was removed by centrifugation for 10 min at 2000*g*. The supernatant was diluted 1:10 in 20% casein blocker in PBS with 5% protease inhibitor cocktail. The AB1-40 standards were diluted to have an equal concentration of homogenization buffer and other reagents. The protein concentration of the brain homogenate was determined with the BCA assay, and the total Aβ levels were normalized to brain protein levels.

Statistical Analyses. Western blot band intensities were quantified by densitometric analyses using a BioRad GelDoc 2000 with Quantity One software. Data are presented as the mean ± SD. Statistical analyses were carried out using a two-tailed Student's *t* test using GraphPad Prism software, with $p < 0.05$ regarded as statistically significant.

Chemistry. Flash chromatography as performed on silica gel (60 Å, 230−400 mesh) purchased from Dynamics Adsorbents (Atlanta, GA). Purity of synthesized compounds was assessed using an Agilent 1200 HPLC equipped with an Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm) monitoring absorbance at 254 nM. All final products were >95% pure unless otherwise noted in the experimental details. Purities were confirmed by NMR. All purchased compounds tested were at least 95% pure as judged by TLC analysis via densitometry using either anisaldehyde, 2,4-dinitrophenylhydrazine, or iodine as a stain. TLC was done on hard layer, organic binder TLC-plates with a fluorescent indicator and visualized by UV light (254 nm). Solvents and reagents were of commercially available analytical grade quality and used as received without any further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova spectrometer (at the Department of Chemistry, CWRU) operating at 400 and 100 MHz for the ^{1}H and ^{13}C NMR spectra, respectively. The internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.2) for ^{1}H and ^{13}C spectra, respectively. NMR data are presented in the following order: chemical shift, peak multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, bs = broad singlet), coupling constant, proton number. Mass spectra were obtained on a Kratos MS 25 mass spectrometer (at the Department of Chemistry, Case Western Reserve University) using FAB ionization method in *m*-nitrobenzyl alcohol or glycerol matrices. Melting points were determined with a MEL-TEMP capillary apparatus and are uncorrected. β-Benzylmercaptoethylamine (β-BMEA) derivatives were prepared following our own procedure.[15,16] 1-Chloromethyl-3-iodo-benzene, 2-bromomethyl-6-chlorobenzonitrile, 2-benzyloxy-ethylamine, and 2,6-dibromobenzoic acid were prepared by following literature procedures.[17−19,35−37] All other chemicals were purchased from commercial sources (Aldrich, Acros, A. K. Scientific, Matrix Scientific, Milestone Pharm Tech, and Wako) and used without any further purification.

Typical Procedure for the Preparation of β-BMEA Derivatives (VII of Supporting Information Scheme 7). LiOH (0.245 g, 10.2 mmol) was dissolved in 5 mL of water, and 15 mL of ethanol was added. The resulting solution was added to a flask containing cysteamine hydrochloride (0.568 g, 5 mmol), followed by the dropwise addition of benzyl halides (5 mmol) with continuous stirring. The reaction mixture was stirred for 40 min at 35 °C, and ethanol was removed in vacuo. Subsequently, 20 mL of water was added, and the mixture was extracted with dichloromethane (3 × 30 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified via column chromatography over silica gel) using a mobile phase consisting of a suitable mixture of DCM−methanol to afford the chromatographically pure desired β-benzylmercaptoethylamine derivatives.

Typical Procedure for the Coupling of β-BMEA Derivatives (VII of Scheme 2) and 2,6-Disubstituted Benzoyl Chlorides. (Synthesis of 2,6-dichloro-*N*-[2-(3-methoxy-benzylsulfanyl)-ethyl]-benzamide, **27** as representative example.) To a solution of 2-(3-methoxy-benzylsulfanyl)-ethylamine (0.394 g, 2 mmol) in dry DCM (15 mL) and Et_3N (0.505 g 5 mmol) was added 2,6-dichloro-benzoyl chloride (0.420 g, 2 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was further stirred for ~1 h, during which time it was allowed to warm to room temperature. Solvent evaporation in vacuo gave the crude product, which was purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane−ethyl acetate (gradient from 15% v/v ethyl acetate/hexane to 35% v/v ethyl acetate/hexane) to afford the chromatographically pure desired amidated product, **27** in 92% yield (0.68 g), 90% pure by HPLC: mp = 60−62 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.65 (t, J = 6.4 Hz, 2H), 3.54 (q, J = 6.4 Hz, 2H), 3.68 (s, 2H), 3.76 (s, 3H), 6.36 (t, J = 5.6 Hz, 1H), 6.72−6.74 (m, 1H), 6.84−6.88 (m, 2H), 7.15−7.25 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.67, 35.82, 38.59, 55.21, 112.78, 114.24, 121.17, 127.98, 129.58, 130.62, 132.13, 135.79, 139.57, 159.71, 164.50. HRMS calculated for $C_{17}H_{18}Cl_2NO_2S$ $(M + H)^+$ 370.04353, found 370.04491.

Typical Procedure for Demethylation of Methoxy Derivatives. (Synthesis of 2,6-dichloro-*N*-[2-(3-hydroxy-benzylsulfanyl)-ethyl]-benzamide, **29** as representative example.) 2,6-Dichloro-*N*-[2-(3-methoxy-benzylsulfanyl)-ethyl]-benzamide, **27** (370 mg, 1 mmol), was dissolved in anhydrous dichloromethane (10 mL) under a nitrogen atmosphere, and the solution was subsequently cooled to −78 °C. A solution of BBr_3 in hexane (2.5 mL of a 1 M solution, 2.5 mmol) was added dropwise. The reaction mixture was stirred for 30 min at −78 °C, warmed slowly to room temperature, and stirred an additional 3 h at room temperature. The reaction was quenched by dropwise addition of 1 M HCl (5 mL) at 0 °C followed by the addition of 15 mL of room temperature water, the layers were separated, and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water followed by brine, dried over Na_2SO_4, and then concentrated in vacuo. The crude product was purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 20% v/v ethyl acetate/hexane to 45% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure desired **29** as off-white solid (0.3 g, 85%): mp = 108−110 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.63 (t, J = 6.4 Hz, 2H), 3.55 (q, J = 6.4 Hz, 2H), 3.61 (s, 2H), 6.43 (t, J = 6.0 Hz, 1H), 6.63−6.66 (m, 1H), 6.78−6.8 (m, 2H), 7.07 (t, J = 8 Hz, 1H), 7.19−7.29 (m, 3H),

7.31 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.25, 35.58, 38.97, 114.45, 116.07, 120.64, 128.17, 129.89, 130.99, 132.24, 135.43, 139.52, 156.36, 165.29. HRMS calculated for $C_{16}H_{16}Cl_2NO_2S$ $(M + H)^+$ 356.02788, found 356.02903.

Typical Procedure for the Preparation of Sulfone Derivatives. (Synthesis of 2,6-dichloro-*N*-[2-(3-methoxy-phenylmethanesulfonyl)-ethyl]-benzamide, **30** as representative example.) To a solution of 2,6-dichloro-*N*-[2-(3-methoxy-benzylsulfanyl)-ethyl]-benzamide, **27** (370 mg, 1 mmol), in glacial acetic acid (6 mL) was added hydrogen peroxide (2 mL, 30% solution). After 24 h stirring at room temperature, the acetic acid was removed under vacuum and the crude sulfone was purified by recrystallization from ethanol (0.3 g, 75%): mp = 172−173 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.19−3.22 (m, 2H), 3.83 (s, 3H), 3.91−3.96 (m, 2H), 4.27 (s, 2H), 6.55 (bs, 1H), 6.93−6.99 (m, 3H), 7.23−7.35 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 32.98, 50.72, 55.59, 60.81, 115.19, 116.35, 123.04, 128.30, 128.93, 130.51, 131.14, 132.39, 135.45, 160.31, 164.95. LRMS calculated for $C_{17}H_{18}Cl_2NO_4S$ $(M + H)^+$ 402.0, found 402.1.

Typical Procedure for the Preparation of Sulfoxide Derivatives. (Synthesis of 2,6-dichloro-*N*-[2-(3-methoxy-phenylmethanesulfinyl)-ethyl]-benzamide, **32** as representative example.) To a solution of 2,6-dichloro-*N*-[2-(3-methoxy-benzylsulfanyl)-ethyl]-benzamide, **27** (370 mg, 1 mmol), in chloroform at −10 °C was added *m*-chloroperbenzoic acid (1 mmol) and the resulting mixture was stirred at −10 °C for 12 h. Subsequently, a saturated solution of $NaHCO_3$ (15 mL) was added, the reaction was stirred for 5 min, followed by extracting with chloroform (2 × 20 mL). The organic layer was washed with water, dried over Na_2SO_4, concentrated in vacuo, and the crude product was purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 20% v/v ethyl acetate/hexane to 50% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure desired product, **32** as white solid (0.247 g, 64%): mp = 144−146 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.71−2.76 (m, 1H), 3.03−3.09 (m, 1H), 3.81 (s, 3H), 3.82−3.89 (m, 1H), 3.92 (s, 2H), 3.94−4.01 (m, 1H), 6.77 (t, J = 2.0 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.87−6.89 (m, 1H), 7.21−7.30 (m, 4H), 7.35 (t, J = 5.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 34.56, 49.55, 55.53, 58.46, 114.32, 115.84, 122.51, 128.20, 130.35, 130.49, 130.88, 132.45, 135.96, 160.19, 165.15. HRMS calculated for $C_{17}H_{18}Cl_2NO_3S$ $(M + H)^+$ 386.03844, found 386.03650.

Typical Procedure for Amidation of 2,6-Disubstituted Benzoic Acids through Acid Chloride Formation. (Synthesis of *N*-[2-(3-chloro-benzylsulfanyl)-ethyl]-2,6-dimethyl-benzamide, **75** as representative example.).One drop of DMF was added to a solution of 2,6-dimethylbenzoic acid (0.3 g, 2 mmol) in thionyl chloride (2 mL), and the mixture was refluxed for 2 h. It was then cooled to room temperature and the excess thionyl chloride was removed in vacuo. The solid residue was dissolved in dry DCM (5 mL) and cooled to 0 °C. A mixture of β-(3-chlorobenzyl)mercaptoethylamine (0.404 g, 2 mmol) and triethylamine (1 mL, 7 mmol) in dry DCM (5 mL) was added in dropwise manner at 0 °C under nitrogen atmosphere, and the mixture was allowed to stir at room temperature for 1.5 h. After the completion of reaction (as judged by TLC) the solvent was removed in vacuo and the solid residue was dissolved in DCM (30 mL). The organic phase was washed with brine (15 mL) and water (15 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude residue was purified by silica gel column chromatography using a mixture of hexane/ethyl acetate (gradient from 15% v/v ethyl acetate/hexane to 35% v/v ethyl acetate/hexane) as eluent to obtain the desired amidated product, **75** (0.507 g, 76%): mp = 53−55 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.27 (s, 6H), 2.65 (t, J = 6.4 Hz, 2H), 3.57 (q, J = 6.4 Hz, 2H), 3.69 (s, 2H), 6.06 (bs, 1H), 6.99 (d, J = 7.6 Hz, 2H), 7.14 (t, J = 7.6 Hz, 1H), 7.19−7.23 (m, 3H), 7.32 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.41, 31.42, 35.46, 38.11, 127.27, 127.65, 127.71, 128.96, 129.12, 130.07, 134.39, 134.65, 137.61, 140.32, 170.61. HRMS calculated for $C_{18}H_{21}ClNOS$ $(M + H)^+$ 334.10324, found 334.10221.

Procedure for DCC-Mediated Coupling of Carboxylic Acids. (Synthesis of *N*-[2-(3-chloro-benzylsulfanyl)-ethyl]-2-(2,6-dichloro-phenyl)-acetamide, **78** as representative example.) To a solution of 2,6-dichlorophenylacetic acid (0.41 g, 2 mmol) in dry DCM (10 mL) was added DCC (0.435 g, 2.1 mmol) and resultant mixture was stirred under N_2 for 30 min. A mixture of β-(3-chlorobenzyl)mercaptoethylamine (0.404 g, 2 mmol) and triethylamine (0.252 g, 2.5 mmol) in dry DCM (4 mL) was added and the resultant mixture was left for stirring at room temperature under N_2 for 1 h. The reaction mixture was then filtered to remove the precipitated dicyclohexylurea (DHU). The filtrate was concentrated in vacuo and the crude product was dissolved with cold EtOAc to effect the precipitation of more DHU, which was removed by further filtration. The organic layer was concentrated in vacuo and the crude product was purified by silica gel column chromatography using a mixture of hexane/ethyl acetate (gradient from 15% v/v ethyl acetate/hexane to 35% v/v ethyl acetate/hexane) as eluent to obtain the desired amidated product, **78** (0.62 g, 80%): mp = 116−118 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.54 (bs, 2H), 3.38−3.39 (m, 2H), 3.63 (s, 2H), 3.92 (s, 2H), 5.88 (bs, 1H), 7.16−7.36 (m, 7H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.35, 35.49, 38.39, 39.06, 127.24, 127.59, 128.64, 129.09, 129.44, 130.04, 131.78, 134.59, 136.52, 140.36, 168.53. HRMS calculated for $C_{17}H_{17}Cl_3NOS$ $(M + H)^+$ 388.00964, found 388.00983.

Specific Procedures for Reactions Deviating from Typical Conditions. 2,6-Dichloro-*N*-(2-[3-(*N*-hydroxycarbamimidoyl)-benzylsulfanyl]-ethyl)-benzamide (39). 2,6-Dichloro-*N*-[2-(3-cyano-benzylsulfanyl)-ethyl]-benzamide, **38** (0.365 g, 1 mmol), hydroxylamine hydrochloride (0.278 g, 4 mmol) and K_2CO_3 (0.276 g, 2 mmol) were dissolved in a mixture of water (6 mL) and ethanol (9 mL). The solution was gently stirred for 10 min and then was heated under reflux for 5 h. The resulting solution was cooled, concentrated in vacuo, and the residue was partitioned between water and DCM. The organic extracts were dried over Na_2SO_4, evaporated in vacuo, and the crude product was purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 25% v/v ethyl acetate/hexane to 80% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure desired, **39** (0.165 g, 42%) as a white solid: mp = 126−127 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.54 (t, J = 6.8 Hz, 2H), 3.39 (q, J = 6.4 Hz, 2H), 3.78 (s, 2H), 5.75 (bs, 2H), 7.27−7.52 (m, 6H), 7.63 (s, 1H), 8.82 (t, J = 5.2 Hz, 1H), 9.59 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 30.38, 35.39, 39.36, 124.62, 126.60, 128.74, 128.81, 130.10, 131.65, 131.77, 134.16, 137.07, 139.06, 151.32, 164.24. HRMS calculated for $C_{17}H_{18}Cl_2N_3O_2S$ $(M + H)^+$ 398.04968, found 398.04691.

***N*-[2-(3-Amino-benzylsulfanyl)-ethyl]-2,6-dichloro-benzamide (41).** 2,6-Dichloro-*N*-[2-(3-nitro-benzylsulfanyl)-ethyl]-benzamide, **40** (0.385 g, 1 mmol), and sodium acetate (0.41 g, 5 mmol) were added to 15 mL of EtOH, and the mixture was heated to 75 °C until the solid dissolved completely. $SnCl_2 \cdot 2H_2O$ (1.12 g, 5 mmol) was added, and the reaction mixture was subsequently stirred at 75 °C for 40 min. After removal of solvent in vacuo, the residue was dissolved in 40 mL EtOAc and washed with 45% K_2CO_3 (25 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 25% v/v ethyl acetate/hexane to 70% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure **41** (0.265 g, 75%): mp = 55−56 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 6.4 Hz, 2H), 3.57 (q, J = 6.4 Hz, 2H), 3.62 (s, 2H), 3.69 (bs, 2H), 6.29 (bs, 1H), 6.49−6.52 (m, 1H), 6.64−6.68 (m, 2H), 7.03 (t, J = 7.6 Hz, 1H), 7.21−7.31 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.82, 35.99, 38.83, 114.22, 115.67, 119.18, 128.25, 129.74, 130.87, 132.42, 136.09, 139.27, 146.97, 164.73. LRMS calculated for $C_{16}H_{17}Cl_2N_2OS$ $(M + H)^+$ 355.0, found 355.2.

3-[2-(2,6-Dichloro-benzoylamino)-ethylsulfanylmethyl]-benzoic Acid (44). To a solution of 3-[2-(2,6-dichloro-benzoylamino)-ethylsulfanylmethyl]-benzoic acid methyl ester, **43** (0.398 g, 1 mmol), in THF (7 mL) was added 5 mL of aqueous LiOH (0.072 g, 3 mmol) solution and the resulting mixture was stirred at 60 °C (~2 h) until the ester starting material was consumed completely (as judged by TLC). The mixture was then allowed to cool to room temperature, and solvent was removed in vacuo followed by dilution of the residue with water. The resulting aqueous mixture was extracted once with EtOAc. The pH of the aqueous phase was adjusted to approximately 1 via the addition of 1 M HCl. The precipitate was filtered off, washed with water, and dried, yielding **44** (0.29 g, 75%) as a white solid, 90% pure by HPLC: mp = 165−166 °C. ^{1}H NMR (acetone-d_6, 400 MHz): δ 2.69−2.73 (m, 2H), 3.57−3.62 (m, 2H), 3.92 (s, 2H), 7.37−7.47 (m, 4H), 7.65−7.67 (m, 1H), 7.89−7.92 (m, 2H), 8.05 (t, J = 2.0 Hz, 1H). ^{13}C NMR (acetone-d_6, 100 MHz): δ 30.48, 35.09, 39.17, 128.21, 128.37, 128.79, 130.32, 130.95, 131.06, 132.00, 133.79, 136.97, 139.69, 164.05, 166.87. HRMS calculated for $C_{17}H_{16}Cl_2NO_3S$ $(M + H)^+$ 384.02279, found 384.02352.

***N*-[2-(3-Carbamoyl-benzylsulfanyl)-ethyl]-2,6-dichloro-benzamide (45).** To a stirred solution of 2,6-dichloro-*N*-[2-(3-cyano-benzylsulfanyl)-ethyl]-benzamide, **38** (0.365 g, 1 mmol), in DMSO (1.5 mL) at 0 °C was added 30% H_2O_2 (0.12 mL) and anhydrous K_2CO_3 (0.03 g). The reaction mixture was allowed to warm to room temperature and stirred for an additional 10 min. Then 30 mL of water was added to the reaction mixture followed by stirring for an additional 10 min. The aqueous phase was extracted with EtOAc (3 × 20 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and the crude product was purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 25% v/v ethyl acetate/hexane to 75% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure **45** (0.25 g, 65%) as a white solid: mp = 148−150 °C. ^{1}H NMR (CD_3OD, 400 MHz): δ 2.61−2.65 (m, 2H), 3.50−3.53 (m, 2H), 3.84 (s, 2H), 7.32−7.43 (m, 4H), 7.55 −7.57 (m, 1H), 7.73−7.75 (m, 1H), 7.86 −7.87 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 29.59, 34.94, 39.06, 126.23, 128.02, 128.12, 128.61, 130.95, 132.01, 132.47, 133.98, 136.08, 139.39, 165.98, 171.03. HRMS calculated for $C_{17}H_{17}Cl_2N_2O_2S$ $(M + H)^+$ 383.03878, found 383.03804.

2,6-Dichloro-*N*-[2-(3-formyl-benzylsulfanyl)-ethyl]-benzamide (49). To a stirred solution of 2,6-dichloro-*N*-[2-(3-cyano-benzylsulfanyl)-ethyl]-benzamide, **38** (0.365 g, 1 mmol), in dry THF (10 mL) at −78 °C under nitrogen atmosphere was added 1 M solution of DIBAL-H in toluene (1.2 mL, 1.2 mmol) dropwise. The reaction mixture was stirred and gradually warmed from −78 °C to room temperature over 2 h. The reaction mixture was then quenched by addition of a mixture of saturated aqueous NH_4Cl solution and 6 M aqueous HCl (5:1, v/v) at 0 °C. The organic solvent was removed in vacuo, and the residue was partitioned between ethyl acetate and brine. The organic solvent was dried over anhydrous Na_2SO_4, concentrated in vacuo, and the crude product was purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 20% v/v ethyl acetate/hexane to 45% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure **49** as white solid (0.19 g, 52%): mp = 63−65 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.71 (t, J = 6.4 Hz, 2H), 3.63 (q, J = 6.4 Hz, 2H), 3.83 (s, 2H), 6.28 (bs, 1H), 7.23−7.31 (m, 3H), 7.49 (t, J = 7.6 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.85 (s, 1H), 9.99 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.03, 35.56, 38.76, 128.29, 128.86, 129.61, 130.19, 130.96, 132.39, 135.19, 135.94, 136.92, 139.51, 164.78, 192.38. HRMS calculated for $C_{17}H_{16}Cl_2NO_2S$ $(M + H)^+$ 368.02788, found 368.02880.

[2-(3-Chloro-benzylsulfanyl)-ethyl]-(2,6-dichloro-benzyl)-amine (27). To a stirred solution of β-(3-chlorobenzyl)mercapto-ethylamine (0.202 g, 1 mmol) and K_2CO_3 (0.276 g, 2 mmol) in 5 mL of dry DMF was added 2,6-dichlorobenzyl bromide (0.24 g, 1 mmol) in a dropwise manner. The resulting mixture was allowed to stir for 18 h at room temperature. The reaction mixture was poured into 35 mL of water and stirred for 10 min. The aqueous layer was extracted with DCM (2 × 25 mL), the combined organic extracts was dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 25% v/v ethyl acetate/hexane to 80% v/v ethyl acetate/hexane) to yield chromatographically pure **57** as colorless liquid (0.216 g, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.96 (s, 1H), 2.59 (t, J = 6.4 Hz, 2H), 2.77 (t, J = 6.4 Hz, 2H), 3.60 (s, 2H), 4.08 (s, 2H), 7.12−7.22 (m, 4H), 7.27 (s, 1H), 7.31 (d, J = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.95, 35.63, 47.09, 48.18, 127.19, 127.44, 128.63, 129.12, 129.19, 129.94, 134.50, 135.81, 136.16, 140.63. HRMS calculated for $C_{16}H_{17}Cl_3NS$ $(M + H)^+$ 360.01473, found 360.01422.

2,6-Dichloro-*N*-(2-[3-(cyclopropanecarbonyl-amino)-benzylsulfanyl]-ethyl)-benzamide (69). To a solution of *N*-[2-(3-amino-benzylsulfanyl)-ethyl]-2,6-dichloro-benzamide, **41** (0.423 g, 1 mmol), and Et_3N (0.303 g, 3 mmol) in dry CH_2Cl_2 (10.0 mL) was added cyclopropanecarbonyl chloride (0.105 g, 1 mmol) at room temperature. The resulting solution was refluxed for 12 h, cooled to room temperature, and the solvent was removed in vacuo to afford a thick oily residue which was purified by column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 25% v/v ethyl acetate/hexane to 50% v/v ethyl acetate/hexane) as eluent to obtain chromatographically pure **69** (0.21 g, 50%) as a white solid: mp = 46−47 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.63−0.65 (m, 2H), 0.74 (bs, 2H), 1.47−1.49 (m, 1H), 2.61 (t, J = 6.8 Hz, 2H), 3.44 (q, J = 6.8 Hz, 2H), 3.64 (s, 2H), 6.98 (d, J = 7.6 Hz, 1H), 7.16−7.27 (m, 6H), 7.57 (s, 1H), 8.57 (s,1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 8.05, 15.46, 30.32, 35.83, 38.97, 118.88, 121.24, 124.42, 128.15, 129.47, 130.76, 132.33, 136.09, 138.49, 138.79, 165.16, 172.95. HRMS calculated for $C_{20}H_{21}Cl_2N_2O_2S$ $(M + H)^+$ 423.07008, found 423.07069.

2,6-Dichloro-*N*-(2-(3-[2-(2-hydroxy-ethoxy)-ethoxy]-benzylsulfanyl)-ethyl)-benzamide (70). To a stirred solution of 2,6-dichloro-*N*-[2-(3-hydroxy-benzylsulfanyl)-ethyl]-benzamide, **29** (0.356 g, 1 mmol) and K_2CO_3 (0.414 g, 3 mmol) in 4 mL of dry DMF was added ethylene glycol mono-2-chloroethyl ether (0.13 g, 1.05 mmol). The resulting mixture was allowed to stir for 12 h at 100 °C. After cooling to room temperature, water was added (25 mL) and the reaction mixture was extracted with DCM (2 × 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified via column chromatography over silica gel using a mobile phase consisting of a mixture of hexane/ethyl acetate (gradient from 25% v/v ethyl acetate/hexane to 80% v/v ethyl acetate/hexane) to yield chromatographically pure **70** as colorless liquid (0.230 g, 52%), 86% pure by HPLC. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.68 (t, J = 6.4 Hz, 2H), 3.54−3.63 (m, 4H), 3.66−3.70 (m, 4H), 3.82−3.84 (m, 2H), 4.11−4.13 (m, 2H), 6.76−6.83 (m, 2H), 6.90−6.92 (m, 2H), 7.17−7.29 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.73, 35.97, 38.90, 61.77, 67.55, 69.81, 72.77, 113.59, 115.26, 121.73, 128.14, 129.81, 130.76, 132.34, 136.17, 139.88, 159.02, 164.79. HRMS calculated for $C_{20}H_{24}Cl_2NO_4S$ $(M + H)^+$ 444.08031, found 444.07924.

2,6-Dichloro-*N*-[2-(4-methoxy-benzylsulfanyl)-ethyl]-benzamide (26). The compound was prepared using 2-(4-methoxy-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 80−81 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.68 (t, J = 6.4 Hz, 2H), 3.60 (q, J = 6.0 Hz, 2H), 3.69 (s, 3H), 3.78 (s, 3H), 6.13 (bs, 1H), 6.80−6.83 (m, 2H), 7.22−7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.75, 35.27, 38.56, 55.38, 114.13, 128.15, 129.91, 130.05, 130.77, 132.33, 135.96, 158.84, 164.59. HRMS calculated for $C_{17}H_{18}Cl_2NO_2S$ $(M + H)^+$ 370.04353, found 370.04217.

2,6-Dichloro-*N*-[2-(4-hydroxy-benzylsulfanyl)-ethyl]-benzamide (28). The compound was prepared from **26** using the procedure described for **29**: mp = 138−140 °C. ^{1}H NMR (acetone-d_6,

400 MHz): δ 2.66−2.69 (m, 2H), 3.54−3.59 (m, 2H), 3.74 (s, 2H), 6.76−6.79 (m, 2H), 7.19−7.23 (m, 2H), 7.36−7.43 (m, 3H), 7.85 (bs, 1H), 8.32 (s, 1H). ^{13}C NMR (acetone-d_6, 100 MHz): δ 30.15, 34.93, 39.26, 115.39, 128.22, 129.43, 130.36, 130.95, 132.01, 137.01, 156.64, 164.03. HRMS calculated for $C_{16}H_{16}Cl_2NO_2S$ $(M + H)^+$ 356.02788, found 356.02820.

2,6-Dichloro-*N*-[2-(3-hydroxy-phenylmethanesulfonyl)-ethyl]-benzamide (31). The compound was prepared from **30** using the procedure described for **29**: mp = 139−141 °C. ^{1}H NMR (acetone-d_6, 400 MHz): δ 3.34−3.38 (m, 2H), 3.82−3.87 (m, 2H), 4.43 (s, 2H), 6.85−6.88 (m, 1H), 6.99 (d, J = 7.6 Hz, 2H), 7.22 (t, J = 7.6 Hz, 1H), 7.41−7.43 (m, 3H), 7.98 (bs, 1H), 8.56 (s, 1H). ^{13}C NMR (acetone-d_6, 100 MHz): δ 33.38, 50.63, 59.29, 115.87, 118.21, 122.40, 128.28, 129.88, 130.21, 131.25, 131.98, 136.45, 157.76, 164.46. LRMS calculated for $C_{16}H_{16}Cl_2NO_4S$ $(M + H)^+$ 388.0, found 388.2.

2,6-Dichloro-*N*-[2-(3-hydroxy-phenylmethanesulfinyl)-ethyl]-benzamide (33). The compound was prepared from **29** using the procedure described for **32**: mp = 165−166 °C. ^{1}H NMR (CD_3OD, 400 MHz): δ 2.91−2.95 (m, 1H), 3.13−3.20 (m, 1H), 3.68−3.75 (m, 1H), 3.81−3.85 (m, 1H), 4.06−4.16 (m, 2H), 6.76−6.84 (m, 3H), 7.19 (t, J = 8.0 Hz, 1H), 7.37−7.42 (m, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 33.77, 50.96, 58.53, 115.70, 117.42, 121.16, 128.14, 129.99, 130.84, 131.73, 132.17, 136.66, 158.00, 164.89. HRMS calculated for $C_{16}H_{16}Cl_2NO_3S$ $(M + H)^+$ 372.02279, found 372.02187.

2,6-Dimethoxy-*N*-[2-(3-methoxy-benzylsulfanyl)-ethyl]-benzamide (34). The compound was prepared using 2,6-dimethoxybenzoyl chloride as acid chloride and following the procedure described for **27**: mp = 41−43 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (t, J = 6.4 Hz, 2H), 3.60 (q, J = 6.4 Hz, 2H), 3.73 (s, 2H), 3.79 (s, 9H), 6.13 (bs, 1H), 6.55 (d, J = 8.4 Hz, 2H), 6.78 (dd, J = 1.6 Hz, J = 7.6 Hz, 1H), 6.89−6.92 (m, 2H), 7.19−7.29 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.33, 35.99, 38.78, 55.45, 56.18, 104.22, 113.01, 114.49, 115.89, 121.46, 129.79, 130.96, 139.97, 157.66, 159.99, 166.17. HRMS calculated for $C_{19}H_{24}NO_4S$ $(M + H)^+$ 362.14260, found 362.14429.

2,6-Dihydroxy-*N*-[2-(3-hydroxy-benzylsulfanyl)-ethyl]-benzamide (35). The compound was prepared from **34** using the procedure described for **29**: mp = 61−62 °C. ^{1}H NMR (acetone-d_6, 400 MHz): δ 2.77 (t, J = 6.4 Hz, 2H), 3.84 (q, J = 6.4 Hz, 2H), 4.11 (s, 2H), 6.68−6.75 (m, 3H), 6.82 (d, J = 8.8 Hz, 1H), 6.93 (s, 1H), 7.02 (t, J = 8.0 Hz, 1H), 7.67 (t, J = 8.4 Hz, 1H), 10.49 (bs, 1H). ^{13}C NMR (acetone-d_6, 100 MHz): δ 35.39, 41.24, 57.24, 103.83, 112.16, 114.30, 116.14, 119.87, 129.47, 139.11, 139.71, 157.87, 159.86, 165.52. LRMS calculated for $C_{16}H_{18}NO_4S$ $(M + Na)^+$ 342.1, found 342.2.

***N*-[2-(3-Chloro-benzylsulfanyl)-ethyl]-2,6-dimethoxy-benzamide (36).** The compound was prepared following the procedure described for **27**. 2,6-Dimethoxybenzoyl chloride was used as acid chloride and 2-(3-chloro-benzylsulfanyl)-ethylamine as the amine component: mp = 90−91 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.68 (t, J = 6.4 Hz, 2H), 3.60 (q, J = 6.4 Hz, 2H), 3.72 (s, 2H), 3.79 (s, 6H), 6.13 (bs, 1H), 6.55 (d, J = 8.4 Hz, 2H), 7.19−7.29 (m, 4H), 7.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.38, 35.51, 38.81, 56.19, 104.23, 115.81, 127.34, 127.55, 129.15, 130.05, 130.99, 134.59, 140.55, 157.65, 166.18. HRMS calculated for $C_{18}H_{21}ClNO_3S$ $(M + H)^+$ 366.09307, found 366.09277.

***N*-[2-(3-Methoxy-benzylsulfanyl)-ethyl]-2,6-dinitro-benzamide (37).** The compound was prepared using 2,6-dinitrobenzoyl chloride as acid chloride and following the procedure described for **27**: mp = 69−71 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.71 (t, J = 6.4 Hz, 2H), 3.54 (q, J = 6.0 Hz, 2H), 3.69 (s, 2H), 3.77 (s, 3H), 6.45 (bs, 1H), 6.71 (d, J = 7.2 Hz, 1H), 6.85−6.89 (m, 2H), 7.17 (t, J = 7.6 Hz, 1H), 7.73 (t, J = 8.4 Hz, 1H), 8.33 (d, J = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.61, 36.07, 39.15, 55.44, 113.09, 114.39, 121.39, 128.01, 129.68, 129.81, 130.91, 139.79, 147.53, 159.96, 161.90. LRMS calculated for $C_{17}H_{18}N_3O_6S$ $(M + H)^+$ 392.1, found 392.3.

2,6-Dichloro-*N*-[2-(3-cyano-benzylsulfanyl)-ethyl]-benzamide (38). The compound was prepared using 3-(2-amino-ethylsulfanylmethyl)-benzonitrile as the amine component and following the procedure described for **27**: mp = 123−125 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (t, J = 6.4 Hz, 2H), 3.63 (q, J = 6.4 Hz, 2H), 3.78 (s, 2H), 6.26 (bs, 1H), 7.24−7.32 (m, 3H), 7.43 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.65 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.05, 35.33, 38.74, 112.94, 118.81, 128.31, 129.67, 131.00, 131.15, 132.39, 132.55, 133.67, 135.89, 139.91, 164.82. HRMS calculated for $C_{17}H_{15}Cl_2N_2OS$ $(M + H)^+$ 365.02821, found 365.02794.

2,6-Dichloro-*N*-[2-(3-nitro-benzylsulfanyl)-ethyl]-benzamide (40). The compound was prepared using 2-(3-nitro-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 76−78 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.72 (t, J = 6.4 Hz, 2H), 3.64 (q, J = 6.4 Hz, 2H), 3.86 (s, 2H), 6.27 (bs, 1H), 7.23−7.32 (m, 3H), 7.50 (t, J = 7.6 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.22 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.15, 35.45, 38.79, 122.53, 123.92, 128.30, 129.80, 130.99, 132.38, 135.29, 135.87, 140.51, 148.63, 164.80. HRMS calculated for $C_{16}H_{15}Cl_2N_2O_3S$ $(M + H)^+$ 385.01804, found 385.02043.

2-Chloro-6-methoxy-*N*-[2-(3-methoxy-benzylsulfanyl)-ethyl]-benzamide (42). The compound was prepared using 2-chloro-6-methoxy-benzoyl chloride as the acid chloride and following the procedure described for **27**: mp = 58−59 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.68 (t, J = 6.4 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.71 (s, 2H), 3.78 (s, 3H), 3.79 (s, 3H), 6.17 (s, 1H), 6.75−6.81 (m, 2H), 6.88−6.91 (m, 2H), 6.97 (d, J = 8.0 Hz, 1H), 7.18−7.27 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.13, 36.00, 38.74, 55.74, 56.30, 109.67, 113.02, 114.86, 121.41, 121.86, 126.38, 129.80, 130.89, 132.11, 139.85, 157.43, 159.97, 165.37. HRMS calculated for $C_{18}H_{21}ClNO_3S$ $(M + H)^+$ 366.09307, found 366.09276.

3-[2-(2,6-Dichloro-benzoylamino)-ethylsulfanylmethyl]-benzoic Acid Ethyl Ester (43). The compound was prepared using 3-(2-amino-ethylsulfanylmethyl)-benzoic acid ethyl ester as the amine component and following the procedure described for **27**: mp = 43−45 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.39 (t, J = 7.2 Hz, 3H), 2.71 (t, J = 6.4 Hz, 2H), 3.62 (q, J = 6.4 Hz, 2H), 3.79 (s, 2H), 4.37 (q, J = 7.2 Hz, 2H) 6.22 (bs, 1H), 7.22−7.31 (m, 3H), 7.38 (t, J = 7.6 Hz, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.55, 31.14, 35.77, 38.69, 61.33, 128.26, 128.67, 128.93, 130.04, 130.90, 131.09, 132.43, 133.50, 135.99, 138.61, 164.73, 166.56. HRMS calculated for $C_{19}H_{20}Cl_2NO_3S$ $(M + H)^+$ 412.05409, found 412.05431.

2,6-Difluoro-*N*-[2-(3-fluoro-benzylsulfanyl)-ethyl]-benzamide (46). The compound was prepared following the procedure described for **27**. 2,6-Difluorobenzoyl chloride was used as acid chloride and 2-(3-fluoro-benzylsulfanyl)-ethylamine as the amine component, 92% pure by HPLC: mp = 39−41 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.68 (t, J = 6.4 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.74 (s, 2H), 6.39 (bs, 1H), 6.92−6.96 (m, 3H), 7.06−7.11 (m, 2H), 7.24−7.29 (m, 1H), 7.33−7.39 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.09, 35.64, 38.77, 112.12, 112.17, 112.38, 114.33, 114.54, 115.79, 116.02, 124.73, 124.76, 130.24, 130.33, 131.88, 131.98, 132.08, 140.74, 140.82, 158.91, 158.97, 160.67, 161.42, 161.48, 161.89, 164.35. HRMS calculated for $C_{16}H_{15}F_3NOS$ $(M + H)^+$ 326.08264, found 326.08419.

2,6-Dichloro-*N*-[2-(3-fluoro-benzylsulfanyl)-ethyl]-benzamide (47). The compound was prepared using 2-(3-fluoro-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 70−72 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.70 (t, J = 6.4 Hz, 2H), 3.61 (q, J = 6.4 Hz, 2H), 3.74 (s, 2H), 6.18 (bs, 1H), 6.91−6.96 (m, 1H), 7.04−7.11 (m, 2H), 7.23−7.32 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.05, 35.61, 35.62, 38.67, 114.35, 114.57, 115.82, 116.03, 124.74, 124.77, 128.28, 130.27, 130.35, 130.92, 132.43, 135.98, 140.71, 140.79, 161.89, 164.34, 164.74. HRMS calculated for $C_{16}H_{15}Cl_2FNOS$ $(M + H)^+$ 358.02354, found 358.02334.

2,6-Dichloro-*N*-[2-(3-methyl-benzylsulfanyl)-ethyl]-benzamide (48). The compound was prepared using 2-(3-methyl-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 56−57 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.32 (s, 3H), 2.69 (t, J = 6.4 Hz, 2H), 3.59 (t, J = 6.4 Hz, 2H), 3.70 (s, 2H), 6.15 (bs, 1H), 7.02 (d, J = 7.2 Hz, 1H), 7.09−7.19 (m, 3H), 7.24−7.33 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.59, 31.02, 36.00, 38.70, 126.09, 128.22, 128.26, 128.74, 129.79, 130.87, 132.47, 136.09, 138.05, 138.59, 164.69. HRMS calculated for $C_{17}H_{18}Cl_2NOS$ $(M + H)^+$ 354.04861, found 354.04895.

2,6-Dichloro-*N*-[2-(3-trifluoromethyl-benzylsulfanyl)-ethyl]-benzamide (50). The compound was prepared using 2-(3-trifluoromethyl-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 54−55 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.70 (t, J = 6.4 Hz, 2H), 3.62 (q, J = 6.4 Hz, 2H), 3.80 (s, 2H), 6.23 (bs, 1H), 7.23−7.31 (m, 3H), 7.43 (t, J = 7.6 Hz, 1H), 7.49−7.54 (m, 2H), 7.59 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.00, 35.62, 38.76, 122.87, 124.23, 124.26, 125.58, 125.71, 125.75, 128.22, 129.31, 130.89, 130.96, 131.29, 132.34, 132.52, 135.92, 139.32, 164.83. HRMS calculated for $C_{17}H_{15}Cl_2F_3NOS$ $(M + H)^+$ 408.02035, found 408.01914.

***N*-[2-(3-Bromo-benzylsulfanyl)-ethyl]-2,6-dichloro-benzamide (51).** The compound was prepared using 2-(3-bromo-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 74−76 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (t, J = 6.4 Hz, 2H), 3.61 (q, J = 6.4 Hz, 2H), 3.71 (s, 2H), 6.19 (bs, 1H), 7.17 (t, J = 8.0 Hz, 1H), 7.23−7.38 (m, 5H), 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.07, 35.48, 38.67, 122.88, 127.75, 128.29, 130.37, 130.59, 130.93, 132.02, 132.43, 135.97, 140.55, 164.75. HRMS calculated for $C_{16}H_{15}BrCl_2NOS$ $(M + H)^+$ 417.94347, found 417.94308.

2,6-Dichloro-*N*-[2-(2,3-dimethoxy-benzylsulfanyl)-ethyl]-benzamide (52). The compound was prepared using 2-(2,3-dimethoxy-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 108−110 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.74 (t, J = 6.4 Hz, 2H), 3.66 (q, J = 6.4 Hz, 2H), 3.77 (s, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 6.28 (bs, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 6.99 (t, J = 8.0 Hz, 1H), 7.22−7.31 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.94, 31.09, 39.03, 55.93, 61.21, 111.68, 122.38, 124.32, 128.24, 130.84, 132.26, 132.47, 136.18, 147.27, 152.91, 164.69. HRMS calculated for $C_{18}H_{20}Cl_2NO_3S$ $(M + H)^+$ 400.05409, found 400.05331.

2,6-Dichloro-*N*-[2-(3-iodo-benzylsulfanyl)-ethyl]-benzamide (53). The compound was prepared using 2-(3-iodo-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 75−78 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (t, J = 6.4 Hz, 2H), 3.61 (q, J = 6.4 Hz, 2H), 3.67 (s, 2H), 6.20 (bs, 1H), 7.03 (t, J = 8.0 Hz, 1H), 7.03−7.32 (m, 4H), 7.56 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.11, 35.38, 38.69, 94.75, 128.29, 128.38, 130.51, 130.93, 132.44, 135.99, 136.53, 137.94, 140.59, 164.73. HRMS calculated for $C_{16}H_{15}Cl_2INOS$ $(M + H)^+$ 465.92961, found 465.92940.

2,6-Dibromo-*N*-[2-(3-chloro-benzylsulfanyl)-ethyl]-benzamide (54). The compound was prepared using 2,6-dibromobenzoic acid as the acid component and following the procedure described for **75**: mp = 55−57 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.72 (t, J = 6.4 Hz, 2H), 3.62 (q, J = 6.4 Hz, 2H), 3.72 (s, 2H), 6.15 (bs, 1H), 7.10 (t, J = 8.0 Hz, 1H), 7.22−7.23 (m, 3H), 7.34 (s, 1H), 7.51 (d, J = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.95, 35.54, 38.65, 120.66, 127.31, 127.68, 129.15, 130.10, 131.58, 131.97, 134.68, 139.75, 140.27, 166.57. HRMS calculated for $C_{16}H_{15}Br_2ClNOS$ $(M + H)^+$ 461.89296, found 461.88998.

2,6-Dibromo-*N*-[2-(3-bromo-benzylsulfanyl)-ethyl]-benzamide (55). The compound was prepared following the procedure described for **75**. 2,6-Dibromobenzoic acid and 2-(3-bromo-benzylsulfanyl)-ethylamine were used as acid and amine component respectively: mp = 70−72 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.68−2.73 (m, 2H), 3.57−3.62 (m, 2H), 3.71 (s, 2H), 6.23 (bs, 1H), 7.07−7.12 (m, 1H), 7.15−7.19 (m, 1H), 7.25−7.27 (m, 1H), 7.35−7.37 (m, 1H), 7.48−7.52 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.93, 35.49, 38.69, 120.65, 122.88, 127.78, 130.39, 130.58, 131.57, 131.96, 132.04, 139.74, 140.56, 166.57. HRMS calculated for $C_{16}H_{15}Br_3NOS$ $(M + H)^+$ 505.84244, found 505.83892.

***N*-[2-(3-Chloro-benzylsulfanyl)-ethyl]-2,6-dinitro-benzamide (56).** The compound was prepared following the procedure described for **27**. 2,6-Dinitrobenzoyl chloride was used as acid chloride and 2-(3-chloro-benzylsulfanyl)-ethylamine as the amine component: mp = 139−140 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.74 (t, J = 6.0 Hz, 2H), 3.61 (q, J = 6.4 Hz, 2H), 3.71 (s, 2H), 6.35 (bs, 1H), 7.19−7.25 (m, 3H), 7.33 (s, 1H), 7.77 (t, J = 8.0 Hz, 1H), 8.38 (d, J = 8.0 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 29.74, 34.54, 39.60, 127.50, 127.92, 128.29, 129.34, 130.09, 130.87, 132.01, 133.62, 141.97, 147.92, 161.90. HRMS calculated for $C_{16}H_{15}ClN_3O_5S$ $(M + H)^+$ 396.04209, found 396.04216.

2,6-Dichloro-*N*-[2-(2,3-dihydroxy-benzylsulfanyl)-ethyl]-benzamide (58). The compound was prepared from **52** using the procedure described for **29**. Highly viscous liquid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.66 (t, J = 6.4 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.76 (s, 2H), 6.48 (s, 1H), 6.58−6.72 (m, 4H), 7.04 (bs, 1H), 7.17−7.26 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.66, 39.15, 114.82, 120.82, 121.98, 124.30, 128.28, 131.15, 132.34, 135.43, 142.69, 144.86, 165.74. HRMS calculated for $C_{16}H_{16}Cl_2NO_3S$ $(M + H)^+$ 372.02279, found 372.02221.

2,6-Dichloro-*N*-[2-(3,5-dihydroxy-benzylsulfanyl)-ethyl]-benzamide (59). The compound was prepared from **60** using the procedure described for **29**: mp = 55−57 °C. ^{1}H NMR (acetone-d_6, 400 MHz): δ 2.68−2.72 (m, 2H), 3.55−3.59 (m, 2H), 3.65 (s, 2H), 6.23 (t, J = 2.4 Hz, 1H), 6.36 (d, J = 2.0 Hz, 2H), 7.35−7.42 (m, 3H), 7.93 (bs, 1H), 8.28 (s, 2H). ^{13}C NMR (acetone-d_6, 400 MHz): δ 30.44, 35.62, 39.27, 107.64, 128.22, 130.99, 132.01, 136.89, 141.02, 158.73, 164.22. HRMS calculated for $C_{16}H_{16}Cl_2NO_3S$ $(M + H)^+$ 372.02279, found 372.02313.

2,6-Dichloro-*N*-[2-(3,5-dimethoxy-benzylsulfanyl)-ethyl]-benzamide (60). The compound was prepared using 2-(3,5-dimethoxy-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 46−47 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.72 (t, J = 6.4 Hz, 2H), 3.61 (q, J = 6.4 Hz, 2H), 3.67 (s, 2H), 3.77 (s, 6H), 6.19 (bs, 1H), 6.31 (t, J = 2.0 Hz, 1H), 6.48 (d, J = 2.0 Hz, 2H), 7.22−7.31 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.07, 36.32, 38.74, 55.56, 99.51, 106.93, 128.25, 130.88, 132.41, 136.05, 140.53, 161.10, 164.69. HRMS calculated for $C_{18}H_{20}Cl_2NO_3S$ $(M + H)^+$ 400.05409, found 400.05415.

2-Chloro-6-((2-(2,6-dichlorophenyl)-2-oxoethylthio)methyl)-benzamide (61). The compound was prepared from **62** using the procedure described for **45**: mp = 64−66 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 7.2 Hz, 2H), 3.45 (q, J = 7.2 Hz, 2H), 3.77 (s, 2H), 6.29 (bs, 1H), 6.74 (s, 2H), 7.21−7.29 (m, 6H), 7.38−7.39 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.89, 33.39, 39.63, 128.22, 128.63, 130.44, 130.77, 130.89, 132.29, 135.88, 136.07, 137.65, 164.99, 169.11. HRMS calculated for $C_{17}H_{16}Cl_3N_2O_2S$ $(M + H)^+$ 416.99980, found 417.00006. 91% pure by HPLC.

2,6-Dichloro-*N*-[2-(3-chloro-2-cyano-benzylsulfanyl)-ethyl]-benzamide (62). The compound was prepared using 2-(2-aminoethylsulfanylmethyl)-6-chloro-benzonitrile as the amine component and following the procedure described for **27**: mp = 166−168 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.78 (t, J = 6.8 Hz, 2H), 3.71 (q, J = 6.8 Hz, 2H), 3.95 (s, 2H), 6.33 (bs, 1H), 7.25−7.33 (m, 3H), 7.41−7.49 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.39, 34.39, 38.92, 113.72, 115.11, 128.30, 128.51, 128.87, 130.99, 132.41, 133.73, 135.87, 137.86, 144.84, 164.89. HRMS calculated for $C_{17}H_{14}Cl_3N_2OS$ $(M + H)^+$ 398.98924, found 398.98777.

***N*-[2-(3-Chloro-benzylsulfanyl)-ethyl]-benzamide (63).** The compound was prepared following the procedure described for **27**. Benzoyl chloride was used as acid chloride and 2-(3-chloro-benzylsulfanyl)-ethylamine as the amine component: mp = 75−76 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 6.4 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.69 (s, 2H), 6.70 (bs, 1H), 7.18−7.26 (m, 3H), 7.33 (s, 1H), 7.39−7.43 (m, 2H), 7.47−7.51 (m, 1H), 7.74−7.76 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.43, 35.55, 38.64, 127.18, 127.26, 127.64, 128.82, 129.11, 130.09, 131.80, 134.53, 134.64, 140.42, 167.78. HRMS calculated for $C_{16}H_{17}ClNOS$ $(M + H)^+$ 306.07194, found 306.07133.

***N*-(2-Benzylsulfanyl-ethyl)-benzamide (64).** The compound was prepared following the procedure described for **27**. Benzoyl chloride was used as acid chloride and 2-benzylsulfanyl-ethylamine as the amine component: mp = 65−67 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 6.4 Hz, 2H), 3.58 (q, J = 6.4 Hz, 2H), 3.73 (s, 2H), 6.65 (bs, 1H), 7.21−7.25 (m, 1H), 7.28−7.33 (m, 4H), 7.39−7.45 (m, 2H), 7.46−7.50 (m, 1H), 7.73−7.75 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.33, 36.02, 38.59, 127.19, 127.44, 128.79, 128.88, 129.07, 131.75, 134.62, 138.29, 167.72. HRMS calculated for $C_{16}H_{18}NOS$ $(M + H)^+$ 272.11091, found 272.11101.

***N*-(2-Benzylsulfanyl-ethyl)-2,6-dichloro-benzamide (65).** The compound was prepared using 2-benzylsulfanyl-ethylamine as the amine component and following the procedure described for **27**: mp = 96−97 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (t, J = 6.4 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.74 (s, 2H), 6.16 (bs, 1H), 7.19−7.33 (m, 8H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.95, 36.04, 38.70, 127.45, 128.26, 128.87, 129.09, 130.89, 132.45, 136.07, 138.16, 164.71. HRMS calculated for $C_{16}H_{16}Cl_2NOS$ $(M + H)^+$ 340.03296, found 340.03318.

2,6-Dichloro-*N*-[2-(3-chloro-phenylmethanesulfonyl)-ethyl]-benzamide (66). The compound was prepared from **1** following the procedure described for **30**: mp = 156−158 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.29 (t, J = 6.8 Hz, 2H), 3.63 (q, J = 6.4 Hz, 2H), 4.62 (s, 2H), 7.37−7.49 (m, 7H), 8.99 (t, J = 5.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 33.14, 50.92, 58.15, 128.79, 129.17, 130.49, 131.06, 131.47, 131.77, 131.93, 133.69, 136.59, 164.64. HRMS calculated for $C_{16}H_{15}Cl_3NO_3S$ $(M + H)^+$ 405.98382, found 405.98338.

***N*-[2-(3-Chloro-benzylsulfanyl)-ethyl]-2,6-difluoro-benzamide (67).** The compound was prepared following the procedure described for **27**. 2,6-difluorobenzoyl chloride was used as acid chloride and 2-(3-chloro-benzylsulfanyl)-ethylamine as the amine component: mp = 41−43 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 6.4 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.71 (s, 2H), 6.44 (bs, 1H), 6.90−6.96 (m, 2H) 7.19−7.26 (m, 3H), 7.32−7.39 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.08, 35.34, 38.77, 112.11, 112.37, 114.27, 127.28, 127.64, 129.11, 130.06, 131.88, 131.99, 132.08, 134.64, 140.29, 158.88, 160.69, 161.40, 161.47. HRMS calculated for $C_{16}H_{15}ClF_2NOS$ $(M + H)^+$ 342.05309, found 342.05252.

2,6-Dichloro-*N*-[2-(3-cyclopropylcarbamoyl-benzylsulfanyl)-ethyl]-benzamide (68). The compound was prepared from **44** following the procedure described for **78**: mp = 42−43 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.35−0.38 (m, 2H), 0.47−0.49 (m, 2H), 2.48−2.52 (m, 2H), 2.71−2.73 (m, 1H), 3.58 (q, J = 6.4 Hz, 2H), 3.74 (s, 2H), 7.18−7.37 (m, 5H), 7.45 (d, J = 7.6 Hz, 1H), 7.67−7.75 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 5.80, 23.41, 28.25, 34.68, 39.41, 127.07, 127.69, 128.13, 129.35, 130.91, 132.03, 132.21, 134.36, 135.71, 138.21, 165.37, 169.05. HRMS calculated for $C_{20}H_{21}Cl_2N_2O_2S$ $(M + H)^+$ 423.07008, found 423.07114.

2,6-Dichloro-*N*-[2-(3-chloro-phenylmethanesulfinyl)-ethyl]-benzamide (71). The compound was prepared from **1** following the procedure described for **32**: mp = 174−176 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.74−2.79 (m, 1H), 2.97−3.00 (m, 1H), 3.52−3.55 (m, 1H), 3.59−3.61 (m, 1H), 4.07 (d, J = 12.8 Hz, 1H), 4.23 (d, J = 12.8 Hz, 1H), 7.27−7.29 (m, 1H), 7.38−7.41 (m, 4H), 7.45−7.47 (m, 2H), 8.98 (t, J = 5.6 Hz, 1H) . ^{13}C NMR (DMSO-d_6, 100 MHz): δ 33.60, 50.71, 56.49, 128.45, 128.77, 129.71, 130.69, 130.99, 131.78, 133.68, 134.44, 136.81, 164.55. HRMS calculated for $C_{16}H_{15}Cl_3NO_2S$ $(M + H)^+$ 389.98890, found 389.98985.

2,6-Dichloro-*N*-[2-(3-chloro-benzyloxy)-ethyl]-benzamide (72). The compound was prepared using 2-(3-chloro-benzyloxy)-ethylamine as the amine component and following the procedure described for **27**: mp = 70−72 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.66−3.73 (m, 4H), 4.50 (s, 2H), 6.26 (bs, 1H), 7.18−7.32 (m, 7H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 39.89, 69.18, 72.64, 125.94, 127.95, 128.17, 128.28, 129.98, 130.87, 132.43, 134.60, 136.14, 140.09, 164.75. HRMS calculated for $C_{16}H_{15}Cl_3NO_2$ $(M + H)^+$ 358.01684, found 358.01726.

2,6-Dichloro-*N*-[2-(3-chloro-5-methoxy-benzylsulfanyl)-ethyl]-benzamide (73). The compound was prepared using 2-(3-chloro-5-methoxy-benzyloxy)-ethylamine as the amine component and following the procedure described for **27**: mp = 83−84 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (bs, 2H), 3.59 (bs, 2H), 3.66 (s, 2H), 3.78 (s, 3H), 6.33 (bs, 1H), 6.76 (s, 2H), 6.91 (s, 1H), 7.27 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.05, 35.59, 38.61, 55.77, 113.27, 121.39, 128.15, 128.25, 130.90, 132.38, 135.16, 135.97, 141.15, 160.58, 164.76. HRMS calculated for $C_{17}H_{17}Cl_3NO_2S$ $(M + H)^+$ 404.00455, found 404.00305.

***N*-[2-(3-Chloro-benzylsulfanyl)-ethyl]-2,6-bis-trifluoromethyl-benzamide (74).** The compound was prepared following the procedure described for **27**. 2,6-Bis-trifluoromethylbenzoyl chloride was used as acid chloride and 2-(3-chloro-benzylsulfanyl)-ethylamine as the amine component. The reaction was carried out at refluxing condition. mp = 93−94 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.64 (t, J = 6.8 Hz, 2H), 3.58 (q, J = 6.8 Hz, 2H), 3.69 (s, 2H), 6.28 (bs, 1H), 7.19−7.22 (m, 3H), 7.32 (s, 1H), 7.66 (t, J = 8.4 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.44, 35.36, 38.86, 121.79, 124.53, 127.27, 127.65, 128.83, 129.08, 129.15, 129.47, 129.79, 130.02, 130.06, 130.12, 130.17, 134.02, 134.66, 140.18, 164.38. HRMS calculated for $C_{18}H_{15}ClF_6NOS$ $(M + H)^+$ 442.04670, found 442.04687.

2,6-Dichloro-*N*-[2-(2-chloro-benzylsulfanyl)-ethyl]-benzamide (76). The compound was prepared using 2-(2-chloro-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 110−112 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.75 (t, J = 6.4 Hz, 2H), 3.65 (q, J = 6.4 Hz, 2H), 3.87 (s, 2H), 6.28 (bs, 1H), 7.16−7.32 (m, 5H), 7.34−7.39 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.31, 33.53, 38.89, 127.22, 128.26, 128.91, 130.09, 130.90, 131.12, 132.44, 134.14, 135.93, 136.01, 164.73. HRMS calculated for $C_{16}H_{15}Cl_3NOS$ $(M + H)^+$ 373.99399, found 373.99368.

2,6-Dichloro-*N*-[2-(4-chloro-benzylsulfanyl)-ethyl]-benzamide (77). The compound was prepared using 2-(4-chloro-benzylsulfanyl)-ethylamine as the amine component and following the procedure described for **27**: mp = 104−106 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.67 (t, J = 6.8 Hz, 2H), 3.59 (q, J = 6.4 Hz, 2H), 3.71 (s, 2H), 6.25 (bs, 1H), 7.22−7.31 (m, 7H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.86, 35.29, 38.71, 128.28, 128.99, 130.44, 130.93, 132.39, 133.19, 135.90, 136.71, 169.75. HRMS calculated for $C_{16}H_{15}Cl_3NOS$ $(M + H)^+$ 373.99399, found 373.99380.

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Supporting Information Available: Supplemental synthetic schemes. This material is available free of charge via the Internet at http://pubs.acs.org.

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