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Structure–Activity Studies of Bis-*O*-Arylglycolamides: Inhibitors of the Integrated Stress Response

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The integrated stress response comprises multiple signaling pathways for detecting and responding to cellular stress that converge at a single event—the phosphorylation of Ser51 on the α-subunit of eukaryotic translation initiation factor 2 (eIF2α). Phosphorylation of eIF2α (eIF2α-P) results in attenuation of global protein synthesis via the inhibitory effects of eIF2α-P on eIF2B, the guanine exchange factor (GEF) for eIF2. Herein we describe structure–activity relationship (SAR) studies of bis-*O*-arylglycolamides, first-in-class integrated stress response inhibitors (ISRIB). ISRIB analogues make cells insensitive to the effects of eIF2α-P by activating the GEF activity of eIF2B and allowing global protein synthesis to proceed with residual unphosphorylated eIF2α. The SAR studies described herein support the proposed pharmacology of ISRIB analogues as binding across a symmetrical protein–protein interface formed between protein subunits of the dimeric eIF2B heteropentamer.

Introduction

The accumulation of unfolded proteins in the endoplasmic reticulum (ER) induces an adaptive response known as the unfolded protein response (UPR).[1–3] Pharmacological modulation of the UPR has emerged as a promising new therapeutic approach in cancer and neurodegenerative disease.[4,5] Three molecular sensors of unfolded proteins underlie the UPR (Figure 1): inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK). Activation of PERK results in both the attenuation of global protein synthesis and the de-repression of activating transcription factor 4 (ATF4) mRNA translation via phosphorylation of Ser51 on the α-subunit of eukaryotic translation initiation factor 2 (eIF2α). In addition to PERK, eIF2α-Ser51 phosphorylation is catalyzed by three other kinases: protein kinase double-stranded RNA-dependent (PKR) in response to viral infection, general control non-derepressible-2 (GCN2) in response to amino acid starvation, and heme-regulated inhibitor (HRI) in response to heme deficiency, oxidative stress, heat shock, or osmotic shock.[6] These various stress-induced signaling pathways that converge on eIF2α-P are collectively known as the integrated stress response (ISR) (Figure 1).

A recent small-molecule phenotypic screen of the PERK pathway using an ATF4-luciferase reporter identified a novel class of small molecules that render cells insensitive to eIF2α phosphorylation, effectively releasing the brake on global protein synthesis inhibition in stressed cells.[7] Interestingly, these compounds were shown to act downstream of eIF2α phosphorylation and thus are effective antagonists of the ISR generally, not only when activated via the PERK pathway. Accordingly, this compound series was named ISRIB (for Integrated Stress Response InhiBitors). The progenitor compound in this series is a symmetrical bis-*O*-arylglycolamide (**1**, Figure 2). In mice, **1** was shown to improve memory consolidation in rodents[7] and conferred neuroprotection in a mouse model of prion disease, while avoiding the pancreatic toxicity associated with direct inhibition of PERK with kinase inhibitors.[8]

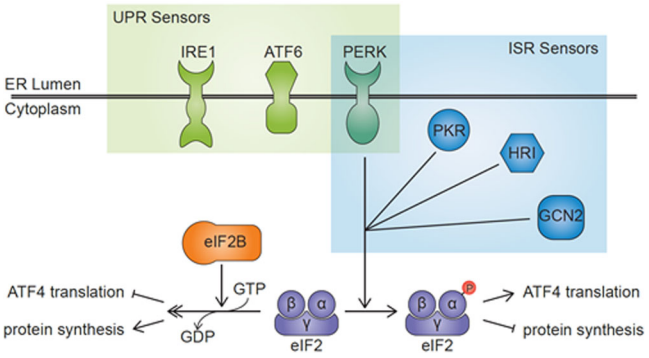

Figure 1. UPR and ISR sensors detect and respond to a variety of cellular stresses such as unfolded proteins in the ER, viral infection, heme deficiency, and amino acid starvation. The four ISR sensors (PERK, PKR, HRI, and GCN2) are eIF2α kinases that become activated in response to cellular stress and result in phosphorylation of eIF2α. This phosphorylated form of eIF2α (eIF2α-P) inhibits the eIF2B-catalyzed guanine nucleotide exchange reaction of eIF2 and results in both the attenuation of global protein synthesis and de-repression of ATF4 mRNA translation.

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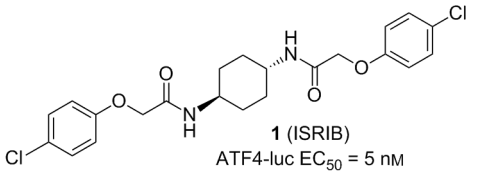


Figure 2. Structure of the prototypical ISR inhibitor **1**.[7]

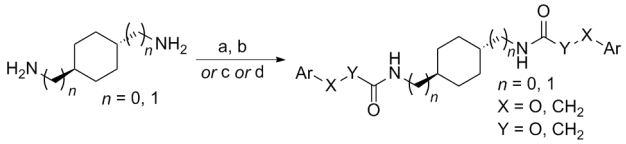
Using genetic, biochemical, and biophysical approaches, two groups[9,10] independently identified the molecular target of ISRIB as eIF2B—a multimeric protein complex that serves as the guanine nucleotide exchange factor (GEF) for eIF2 and which is inhibited by phosphorylated eIF2α. ISRIB analogues bind to and enhance the GEF activity of eIF2B, allowing protein synthesis to proceed with residual unphosphorylated eIF2α.[10] Although the precise binding site remains unknown, the weight of current evidence suggests that ISRIB analogues stabilize the dimeric form[11,12] of the eIF2B pentamer by binding across a protein–protein interface formed between eIF2Bδ subunits or between eIF2Bβ and eIF2Bδ regulatory subunits of the decamer (dimer of pentamers). This putative mechanism of action—the stabilization of an existing protein–protein interaction that may be important for GEF activity—also provides a satisfying explanation for the exceptional cellular potency and ligand efficiency of **1** and closely related analogues.

The notion of an ISRIB binding site that spans a symmetrical protein–protein interface is consistent with the results of extensive structure–activity studies performed on the ISRIB series to date, as initially described previously[7,10] and more fully detailed here. Specifically, we find that both halves of the symmetrical structure are required for ISR antagonism and that the spatial orientation and distance between the distal aryl ether groups is strongly correlated with biological activity. Furthermore, the exquisite sensitivity of the aryl moieties to substituent effects indicates that these groups mediate key binding interactions with the target and are important drivers of binding affinity. By subtle optimization of ring substitution and electronics we identified new ISRIB analogues with potencies in the picomolar range in a cell-based ATF4 translational reporter assay.

Results and Discussion

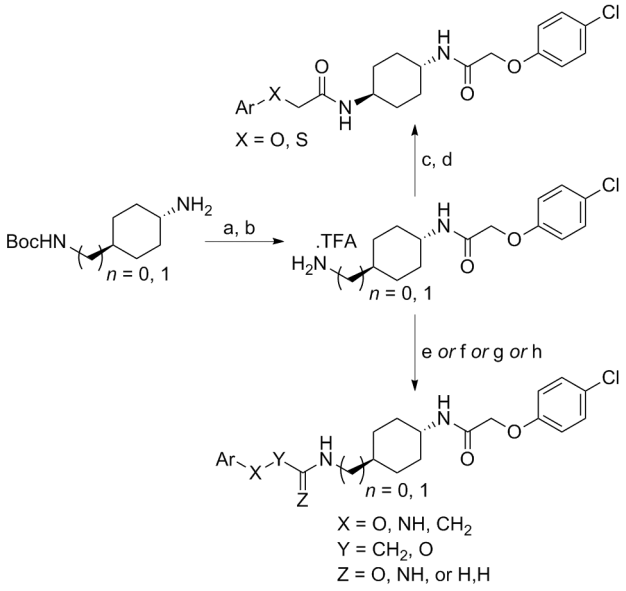
All structure–activity studies were guided by the cell-based luciferase reporter assay described previously.[7] Briefly, this assay employs stably transfected HEK293T cells harboring a retroviral vector containing the ORF of firefly luciferase fused to the 5′UTR of ATF4 mRNA. Induction of ER stress with tunicamycin in these cells leads to eIF2α phosphorylation, de-repression of ATF4 translation and the induction of luciferase activity. The EC_{50} values reported herein reflect the compound concentration that produces a half-maximal response in the luminescence signal following induction of ER stress.

ISRIB analogues **1**–**6**, **15**, **30**–**35**, **44**, and **45** were prepared as previously described.[7,10] Detailed experimental procedures for all new compounds described herein are provided in the experimental section. For symmetric ISRIB analogues, a central diamine core was elaborated in one or two steps to the desired compounds, as illustrated in Scheme 1.



Scheme 1. Synthetic approaches to symmetric ISRIB analogues. *Reagents and conditions*: a) $ClCH_2C(O)Cl$, DIEA, CH_2Cl_2; b) ArOH, K_2CO_3, acetone; c) $ArOCH_2COOH$, EDC, HOBt, DIEA, DMF or $ArCH_2OCOOH$, HATU, DIEA, DMF; d) triphosgene, ArOH, DIEA, CH_2Cl_2.

Pseudo-symmetric ISRIB analogues were prepared from partially Boc-protected diamines as illustrated in Scheme 2. Acylation of the free amino group to install a single *O*-arylglycolamide side chain was followed by removal of the Boc group to afford a key amine intermediate as a TFA salt. Various reactions of this key intermediate were then used to introduce the second *O*-arylglycolamide or related side chain. For example, *O*-arylglycolamide or *N*-arylglycinamide side chains could be introduced in one step by coupling the key intermediate to the corresponding acids or acid chlorides (Scheme 2, step e). An *S*-arylthioacetamide side chain was introduced in two steps via acylation with chloroacetyl chloride followed by reaction of the resulting chloroacetamide intermediate with an arylthiol (steps c and d). Carbamate-linked side chains were prepared by reacting the key amine intermediate (where $n=1$) with triphosgene and a substituted phenol (step f). A saturated amino

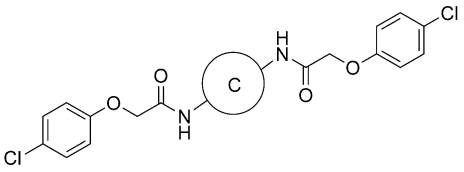


Scheme 2. Synthetic approach to pseudo-symmetric ISRIB analogues. *Reagents and conditions*: a) 4-$ClPhCH_2C(O)Cl$, DIEA, THF, 82%; b) TFA, Et_3SiH, CH_2Cl_2, H_2O; c) $ClCH_2C(O)Cl$, DIEA, CH_2Cl_2; d) ArOH, K_2CO_3, acetone or ArSH, DIEA, CH_2Cl_2; e) $ArXCH_2COOH$, EDC, HOBt, DIEA, DMF (X = O or NH) or $ArOCH_2C(O)Cl$, DIEA, THF; f) triphosgene, ArOH, DIEA, CH_2Cl_2; g) $ArOCH_2CHO$, $NaBH(OAc)_3$, AcOH, CH_2Cl_2; h) $ArOCH_2C(NH)OEt$, DIEA, EtOH.

side chain could be prepared from the key amine intermediate via reductive amination with the appropriate aldehyde building blocks (step g). Finally, an amidine-linked side chain was introduced by reaction with the appropriate carboximidate reagent (step h).

Our SAR studies began with a focus on the central ring that joins the *O*-arylglycolamide side chains. The original screening hit possessed a symmetric a 1,4-cyclohexane ring in this position, but with undefined stereochemistry. The two possible diastereomers were thus synthesized and evaluated in the reporter assay, where the *trans* diastereomer **1** was found to be at least 100-fold more potent than the *cis* diastereomer **2** (Table 1). Next, we explored ISRIB analogues bearing a smaller

Table 1. SAR of the central core (C) of ISRIB analogues.



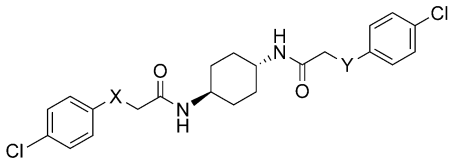
Compd	C	ATF4-luc EC_{50} [nM][a]
1	*trans*-1,4-cyclohexyl	5
2	*cis*-1,4-cyclohexyl	600
3	*trans*-1,3-cyclobutyl	142
4	*cis*-1,3-cyclobutyl	1000
5	1,4-*n*-butyl	306
6	1,3-*n*-propyl	>10000
7	1,4-but-2-ynyl	>10000
8	1,4-(*Z*)-but-2-enyl	200
9	1,4-phenyl	53

[a] ATF4-luciferase reporter assay EC_{50} (single determinations); EC_{50} data for **1**–**7** and **9** were reported previously.[10]

cyclobutane core (**3** and **4**) as well as those with acyclic linkers of four (**5**) or three carbons (**6**). All of these analogues were significantly less potent than **1**, but the new SAR revealed a preference for a cyclic central core over acyclic, and for four carbon over three carbon spacing (cf. **1** vs. **3** and **5** vs. **6**). This implied that the central ring/spacer plays a crucial role in properly orienting the two side chains in space. Consistent with this, we found that forcing the side chains further apart with an alkynyl spacer as in analogue **7** led to a complete loss of measureable activity. Reducing the alkyne to a (*Z*)-alkene as in **8** restored activity that was similar to or somewhat higher than the saturated, unconstrained comparator **5**. A simple aryl core as in **9** turned out to be the best tolerated replacement for *trans*-1,4-cyclohexane, but still tenfold less potent than **1**. Thus, the spatial orientation and distance separating the *O*-arylglycolamide side chains is strongly correlated with the activity of ISRIB analogues. Moreover, this preliminary SAR indicated a preference for an extended binding conformation in which both side chains project equatorially, or nearly so, from the plane of the central ring.

Next, we altered the nature of the glycolamide linkage between the central cyclohexane and distal aryl rings of ISRIB analogues. Remarkably, we found that replacing just one of the aryl ether bonds with analogous nitrogen, sulfur, or carbon linkages resulted in a near or complete loss of effect in the reporter assay (Table 2). Of the analogues prepared, only the glycinamide **10** retained measurable activity, albeit ~100-fold less

Table 2. SAR of the glycolamide linker in ISRIB analogues.



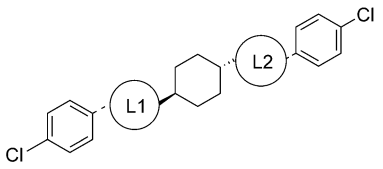
Compd	X	Y	ATF4-luc EC_{50} [nM][a]
1	O	O	5
10	NH	O	700
11	S	O	>10000
12	S=O	O	>10000
13	SO_2	O	>10000
14	CH_2	O	>10000
15	CH_2	CH_2	>10000

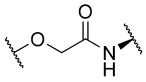
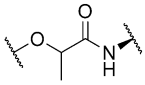
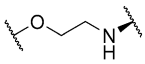
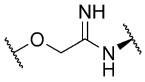
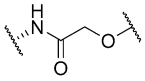
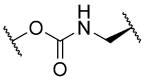
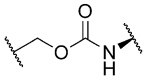
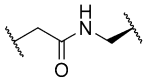
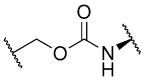
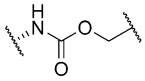
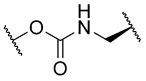
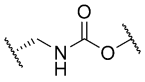
[a] ATF4-luciferase reporter assay EC_{50} (single determinations).

potent than **1**. Changes in bond lengths and/or angles in these analogues seemed insufficient to explain the dramatic effects and so we considered two alternate explanations for the loss of activity in analogues **10**–**15**. The first possibility was that binding of ISRIB analogues to their target is exquisitely dependent on the electronic nature of the distal aryl moieties and that a 4-chlorophenyl ether as in **1** is optimal or nearly so. A second possibility was that ISRIB analogues act by covalent modification of the target, which could occur via reaction at the α-carbon of the glycolamide, with 4-chlorophenoxide serving as the leaving group. Arguing against this possibility was the lack of activity observed for analogue **11**, in which putative S_N2 reaction should be *more* favorable given the presence of a superior leaving group. To more conclusively distinguish between these possibilities, a more extensive SAR survey of the glycolamide linker and aryl ring substitution was undertaken.

A series of ISRIB analogues bearing four-atom linkages of various chemotypes were prepared and evaluated (Table 3). The addition of a methyl group at the α-position of the glycolamide linker (analogue **16**) led to a significant loss of potency (EC_{50} = 210 nM vs. 5 nM for **1**). Replacement of the carbonyl function with methylene (compound **17**) produced a similar decrease in potency. Amidine **18** and carbamate **19** were the most potent of the new analogues, with EC_{50} values in the low-nanomolar range, but still less potent than **1**. Carbamate **20** and amide **21**, though less potent, demonstrated that at least one carbon-linked aryl side chain can be tolerated in ISRIB analogues. Replacing both *O*-arylglycolamide side chains with the new (and inferior) side chains (analogues **22** and **23**) produced the expected additional loss of potency, consistent with the putative interaction of these compounds with analogous binding sites on either side of a protein–protein interface.

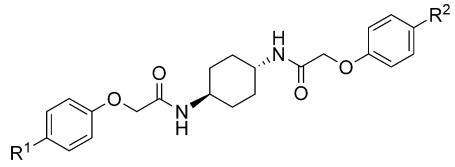
Table 3. SAR of the glycolamide linker (L) in ISRIB analogues.



Compd	L1	L2	ATF4-luc EC_{50} [nM][a]
1			5
16			210
17			170
18			60
19			30
20			122
21			150
22			>10000
23			>10000

[a] ATF4-luciferase reporter assay EC_{50} (single determinations).

Table 4. SAR of aryl substitution in ISRIB analogues.



Compd	R^1	R^2	ATF4-luc EC_{50} [nM][a]
1	Cl	Cl	5
24	I	Cl	5
25	I	I	51
26	CF_3	Cl	10
27	CF_3	CF_3	13
28	CCH	Cl	14
29	CCH	CCH	158
30	F	Cl	48
31	F	F	270
32	CH_3	Cl	95
33	CH_3	CH_3	327
34	CN	Cl	263
35	CN	CN	>10000
36	OMe	Cl	250
37	OMe	OMe	>10000
38	S(O)Me	Cl	>10000
39	C(O)Me	Cl	>10000

[a] ATF4-luciferase reporter assay EC_{50} (single determinations); EC_{50} data for **1** and **30**–**34** were reported previously.[10]

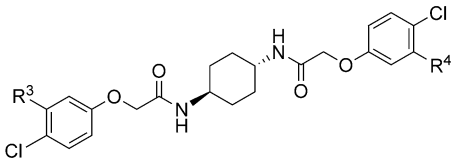
The reasonable (mid-nanomolar) activity observed for analogues such as **17**, **20**, and **21** argued against the possibility that ISRIB analogues act as covalent cross-linkers, as the modified (L1) side chains in these analogues cannot serve as viable electrophiles in biological settings. Rather, the superior potency of analogues **20** and **21** over that of **14** is most consistent with the notion that subtle electronic effects of the distal aryl rings drive binding and potency in ISRIB analogues. Because none of the modified linkers examined in these studies proved superior to the glycolamide linker, further optimization of the ISRIB scaffold was carried out in the context of the bis-*O*-aryl-glycolamide pharmacophore exemplified by **1**.

The electronics of the aryl moiety were explored systematically by replacement of one or both chlorine atoms in **1** (Table 4). Among pseudo-symmetric ISRIB analogues in which one of the two aryl rings was altered, the best tolerated substituents were iodo (**24**; EC_{50} = 5 nM), trifluoromethyl (**26**; 10 nM), alkynyl (**28**; 14 nM), and fluoro (**30**; 48 nM). Less-well-tolerated substituents included methyl (**32**; 95 nM), cyano (**34**; 263 nM), and methoxy (**36**; 250 nM), while the introduction of branched substituents such as methyl sulfoxide (**38**) or methyl ketone (**39**) was not tolerated. These data suggested a general preference for spheroid hydrophobes with moderate or strongly electron-withdrawing character. Interestingly, the least-well-tolerated substituents examined were those possessing a significant dipole moment (i.e., OMe, CN, C(O)Me, and S(O)Me). The polarizability of the *para* substituent, by contrast, appears less important, with both highly polarizable groups (iodo and alkynyl) and non-polarizable (fluoro and trifluoromethyl) groups tolerated.

The trends noted above were largely recapitulated in the case of symmetric ISRIB analogues in which both side chains were modified. Thus, the trend in potency for symmetrical *para*-substituted analogues was in the order Cl (**1**; EC_{50} = 5 nM) > CF_3 (**27**; 14 nM) > I (**25**; 51 nM) > CCH (**29**; 158 nM) > F (**31**; 270 nM) ~ CH_3 (**33**; 327 nM) ≫ CN (**35**; >10000 nM) ~ OMe (**37**; >10000 nM). In each case, modification of both side chains produced an additive effect when compared with modification of a single side chain. While the magnitude of the additive effect differed by substituent, the overall trend was consistent and in line with the idea that each half of the molecule engages in a similar binding interaction.

Next we explored the introduction of multiple substituents on the aryl ring, focusing on the groups CF_3, I, F, Cl, CCH, and CH_3 that had proven reasonably well tolerated at the *para* position. Initial SAR studies involving multiple chloro substituents indicated that *meta*/*para* substitution was strongly preferred over *ortho*/*para* substitution and so further SAR was focused on the *meta*/*para* substitution pattern (Table 5). Introduction of a *meta* substituent in the context of *para*-chloro substitution provided pseudo-symmetric ISRIB analogues with potencies measurably superior to **1**, including analogues **44** (R^3 = Cl;

Table 5. SAR of aryl substitution in ISRIB analogues.



Compd	R^3	R^4	ATF4-luc EC_{50} [nM][a]
1	H	H	5
40	CF_3	H	11
41	I	H	5.2
42	CH_3	H	2.4
43	CCH	H	12
44	Cl	H	1.0
45	F	H	1.9
46	CH_3	CH_3	3.0
47	Cl	Cl	0.8
48	F	F	0.6

[a] ATF4-luciferase reporter assay EC_{50} (single determinations); EC_{50} data for **1**, **45**, **47**, and **48** were reported previously.[10]

EC_{50} = 1.0 nM), **45** (R^3 = F; 1.9 nM), and **42** (R^3 = Me; 2.4 nM). Only slightly less potent were analogues **40**, **41**, and **43**, bearing respectively CF_3, I, and CCH substituents at the *meta* position (Table 5). As expected, introducing these more optimized aryl rings in both side chains produced further enhancements in potency, culminating in the symmetrical analogues **47** and **48** respectively, which remarkably exhibited cellular EC_{50} values in the high picomolar range (EC_{50} = 0.8 and 0.6 nM).

Conclusions

Targeting protein–protein interactions (PPI) with small molecules remains a frontier of drug discovery,[13] despite the fact that many examples of druggable PPI are now known.[14] Molecules that bind at PPI interfaces often stretch the boundaries of what might be considered "drug-like" or "beautiful"[15] molecules. This is particularly true for PPI inhibitors, which typically must bind a significant portion of the buried surface area comprising the targeted PPI interface, resulting in ungainly molecules that bind with relatively modest ligand efficiency. In contrast, the *stabilization* of a pharmacologically relevant PPI might well be achieved with smaller molecules binding at higher ligand efficiencies. It would appear based on current data that the compounds described herein fall into this latter category. At the very least, the exceptional cellular potency of **1** and analogues like **44**–**48** is more easily reconciled with a mechanism of PPI stabilization/activation than with PPI disruption/inhibition. Additional studies will be required however to structurally define the eIF2B binding site of ISRIB analogues and to better understand the effects of dimerization on the GEF activity of eIF2B.

Experimental Section

ATF4-luc reporter assay

HEK293T cells containing an ATF4 luciferase reporter as previously described[7,10] were plated on polylysine-coated 96-well plates (Greiner Bio-One, Monroe, NC, USA) at 30000 cells per well in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, and antibiotics (penicillin and streptomycin). Cells were then treated the following day with tunicamycin at 1 μg mL^{-1} along with various concentrations of each compound for 7 h. Luminescence was measured using One Glo (Promega, Madison, WI, USA) as specified by the manufacturer. EC_{50} values were calculated by plotting $\log_{10}$ [μM] for each compound as a function of the relative luminescence intensity or response.

Chemistry

Synthesis. Unless otherwise noted, all reagents and solvents used were commercially available. Compounds **1** and **2** were prepared as described previously.[7] Compounds **3**–**6**, **15**, **30**–**35**, **44**, and **45** were prepared as described previously.[10] Compound **9** was purchased from Specs (The Netherlands). Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere in oven-dried glassware using anhydrous solvents from commercial suppliers. Air- and/or moisture-sensitive reagents were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa. Solvent removal was carried out with a rotary evaporator at ~10–50 Torr. ^{1}H NMR spectra were recorded on a Varian INOVA-400 400 MHz spectrometer and a Bruker AvanceIII HD 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm. NMR spectra were referenced relative to residual NMR solvent peaks. Coupling constants (J) are reported in hertz (Hz). Microwave reactions were carried out in a CEM Discover microwave reactor. Column chromatography was carried out using Biotage SP1 and Isolera Four flash chromatography system and Silia*Sep* silica gel cartridges from Silicycle. Reversed-phase chromatography was carried out on a Waters 2535 Separation Module with a Waters 2998 Photodiode Array Detector. Separations were carried out on XBridge Preparative C_{18} 19×50 mm columns at ambient temperature using a mobile phase of water/methanol containing a constant amount (0.05%) of trifluoroacetic acid. LC–MS data were acquired on a Waters Micromass ZQ mass spectrometer equipped with a Waters 2795 Separation Module, a Waters 2424 Evaporative Light-Scattering Detector, and a Waters 2996 Photodiode Array Detector. Separations were carried out with an XTerra MS C_{18} column (5 μm, 4.6×50 mm) at ambient temperature (unregulated) using a mobile phase of water/methanol containing a constant amount (0.1%) of formic acid.

General Procedure A for amide coupling. To a solution of the carboxylic acid (2 equiv) in *N*,*N*-dimethylformamide were sequentially added 1-hydroxybenzotriazole hydrate (2 equiv), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2 equiv), the diamine (1.0 equiv), and *N*,*N*-diisopropylethylamine (6 equiv). The reaction mixture was stirred at room temperature until determined complete by LC–MS and then diluted with water. The precipitate formed was washed with water and 10% diethyl ether in dichloromethane. The precipitate was dried in vacuo to obtain the product.

General Procedure B for amide coupling. To a solution of the carboxylic acid (1 equiv) in *N*,*N*-dimethylformamide, were sequentially added 1-hydroxybenzotriazole hydrate (1.2 equiv), 1-(3-dimethyla-

minopropyl)-3-ethylcarbodiimide hydrochloride (1.2 equiv), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (1.0 equiv), and *N*,*N*-diisopropylethylamine (2.0 equiv). The reaction mixture was stirred at room temperature until determined complete by LC–MS and then diluted with water. The mixture was vigorously vortexed, centrifuged, and the water layer was decanted. This washing protocol was repeated with water and then with diethyl ether. The wet solid was dissolved in dichloromethane and dried over anhydrous magnesium sulfate. The solids were removed by filtration, and the filtrate was concentrated by rotary evaporation to obtain the product.

2-(4-Chlorophenoxy)-*N*-{4-[2-(4-chlorophenoxy)acetamido]but-2-yn-1-yl}acetamide (7). To a solution of but-2-yne-1,4-diamine dihydrochloride (0.05 g, 0.31 mmol) in a 1:1.5 mixture of tetrahydrofuran/water (2.5 mL) were sequentially added potassium carbonate (0.27 g, 1.86 mmol) and 2-(4-chlorophenoxy)acetyl chloride (0.094 mL, 0.6 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS. The reaction mixture was then diluted with ethyl acetate, washed with 5% potassium bisulfate, saturated sodium bicarbonate solution and brine. The organic layer was dried over magnesium sulfate and concentrated down to obtain a white solid. The white solid was then triturated with diethyl ether twice to obtain 51 mg (39%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.54 (br s, 2H), 7.32 (d, J = 8 Hz, 4H), 6.95 (d, J = 8 Hz, 4H), 4.47 (s, 4H), 3.91 ppm (d, J = 8 Hz, 4H); LC–MS: m/z = 421 [M + H, ^{35}Cl]$^+$, 423 [M + H, ^{37}Cl]$^+$.

2-(4-Chlorophenoxy)-*N*-[(2*Z*)-4-[2-(4-chlorophenoxy)acetamido]but-2-en-1-yl]acetamide (8). To a solution of **7** (0.016 g, 0.037 mmol) in a 10:1 mixture of ethyl acetate/methanol (1.5 mL) were added pyridine (0.15 mL) and Lindlar's catalyst (0.016 mg). The suspension was stirred under hydrogen at atmospheric pressure and room temperature for 30 min. The reaction mixture was filtered through Celite, concentrated and isolated by flash column chromatography (5–50% acetone/dichloromethane) to obtain 5.8 mg (37%) of the title compound. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.32 (br s, 2H), 7.26 (d, J = 8 Hz, 4H), 6.85 (d, J = 8 Hz, 4H), 5.60 (d, J = 4.9 Hz, 2H), 4.44 (s, 4H), 4.04 ppm (t, J = 5.6 Hz, 4H); LC–MS: m/z = 423 [M + H, ^{35}Cl]$^+$, 425 [M + H, ^{37}Cl]$^+$.

2-[(4-Chlorophenyl)amino]-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (10). To a mixture of *tert*-butyl *N*-[(1*R*,4*R*)-4-aminocyclohexyl]carbamate (750 mg, 3.5 mmol) in THF (20 mL) were sequentially added *N*,*N*-diisopropylethylamine (0.914 mL, 5.25 mmol) and 4-chlorophenoxyacetyl chloride (0.573 mL, 3.78 mmol). The reaction mixture was vigorously stirred at ambient temperature for 3 h then diluted with water (100 mL). The precipitate was filtered, washed with water (2×20 mL), washed with diethyl ether (3×20 mL) and dried under vacuum to afford 1.1 g (82%) of *tert*-butyl *N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]carbamate as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.88 (d, J = 7.87 Hz, 1H), 7.25–7.37 (m, 2H), 6.93 (d, J = 8.97 Hz, 2H), 6.68 (d, J = 7.69 Hz, 1H), 4.41 (s, 2H), 3.51 (m, 1H), 3.13 (br s, 1H), 1.72 (t, J = 13.19 Hz, 4H), 1.34 (s, 9H), 1.09–1.30 ppm (m, 4H); LC–MS: m/z = 405 [M + Na, ^{35}Cl]$^+$, 407 [M + Na, ^{37}Cl]$^+$.

To a suspension of *tert*-butyl *N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]carbamate (500 mg, 1.31 mmol) in dichloromethane (9 mL) were sequentially added triethylsilane (0.300 mL, 1.88 mmol), water (0.200 mL, 11.1 mmol) and trifluoroacetic acid (3.00 mL, 39.2 mmol). The reaction mixture was vigorously stirred at ambient temperature for 30 min then the solvent was removed by rotary evaporation. The resulting colorless oil was triturated with diethyl ether (2×15 mL). After decanting the ether washes, residual solvent was removed under vacuum to afford 499 mg (96%) of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.95 (d, J = 7.8 Hz, 1H), 7.77 (br s, 3H), 7.31 (d, J = 9.0 Hz, 2H), 6.93 (d, J = 9.0 Hz, 2H), 4.43 (s, 2H), 3.54 (m, 1H), 2.93 (br s, 1H), 1.90 (d, J = 9.2 Hz, 2H), 1.77 (d, J = 9.3 Hz, 2H), 1.31 ppm (sxt, J = 11.5 Hz, 4H); LC–MS: m/z = 283 [M + H, ^{35}Cl]$^+$, 285 [M + H, ^{37}Cl]$^+$.

To a solution of 2-[(4-chlorophenyl)amino]acetic acid (9.3 mg, 0.05 mmol) in *N*,*N*-dimethylformamide (0.6 mL), were sequentially added 1-hydroxybenzotriazole hydrate (9.2 mg, 0.06 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (11.5 mg, 0.06 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (40 mg, 0.1 mmol) and *N*,*N*-diisopropylethylamine (0.035 mL, 0.2 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS. The reaction mixture was then diluted with ethyl acetate and was washed with 5% potassium hydrogen sulfate, water, saturated sodium bicarbonate solution and brine. The organic layer was dried over anhydrous magnesium sulfate, concentrated by rotary evaporation and triturated with water and ether to obtain 8.4 mg (37%) of the title compound. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.89 (d, J = 8.1 Hz, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 8.6 Hz, 2H), 7.06 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.50 (d, J = 8.8 Hz, 2H), 6.03 (t, J = 5.8 Hz, 1H), 4.41 (s, 2H), 3.54–3.56 (m, 4H), 1.71 (d, J = 11.4 Hz, 3H), 1.13–1.34 ppm (m, 5H); LC–MS: m/z = 450 [M + H, ^{35}Cl]$^+$, 452 [M + H, ^{37}Cl]$^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-{2-[(4-chlorophenyl)sulfanyl]acetamido}cyclohexyl]acetamide (11). To a cooled (0 °C) solution of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (0.127 g, 0.32 mmol) in dichloromethane (3 mL) were added sequentially *N*,*N*-diisopropylethylamine (0.174 mL, 1.0 mmol) and chloroacetyl chloride (0.04 mL, 0.5 mmol). The reaction mixture was warmed up to room temperature and stirred for four days. The reaction mixture was concentrated down to obtain 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(2-chloroacetamido)cyclohexyl]acetamide, which was used without further purification.

To a solution of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(2-chloroacetamido)cyclohexyl]acetamide (0.118 g, 0.32 mmol) in dichloromethane (6.0 mL) were sequentially added *N*,*N*-diisopropylethylamine (0.078 mL, 0.45 mmol) and 4-chlorothiophenol (0.043 g, 0.3 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was then concentrated down, triturated with diethyl ether followed by 1:10 mixture of methanol/water and then dried in vacuo to obtain 96 mg (64%) of the title compound as a light-brown solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.98 (d, J = 7.7 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.29–7.32 (m, 6H), 6.93 (d, J = 9.0 Hz, 2H), 4.41 (s, 2H), 3.57 (s, 2H), 3.32 (br s, 2H), 1.70–1.72 (m, 3H), 1.16–1.32 ppm (m, 5H); LC–MS: m/z = 467 [M + H, ^{35}Cl]$^+$, 469 [M + H, ^{37}Cl]$^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorobenzenesulfinyl)acetamido]cyclohexyl]acetamide (12). To a solution of **11** (10 mg, 0.021 mmol) in dichloromethane (0.5 mL) was added 3-chloroperoxybenzoic acid (4.7 mg, 0.021 mmol). The reaction mixture was stirred at room temperature for 1 h and then partitioned between dichloromethane and saturated sodium bicarbonate solution. The organic layer was then washed with brine, filtered and concentrated to obtain 4 mg (38%) of the title compound as a white solid. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.52 (s, 2H), 7.25 (s, 2H), 6.84 (d, J = 9.0 Hz, 2H), 6.65 (d, J = 7.9 Hz, 1H), 6.29 (d, J = 8.2 Hz, 1H), 4.42 (s, 2H), 3.82–3.84 (m, 1H), 3.65–3.73 (m, 2H), 3.38 (d, J = 15 Hz, 1H),

2.02–2.06 (m, 3H), 1.82 (brs, 1H), 1.22–1.36 ppm (m, 4H); LC–MS: m/z=483 $[M+H, {}^{35}Cl]^+$, 485 $[M+H, {}^{37}Cl]^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorobenzenesulfonyl)-acetamido]cyclohexyl]acetamide (13). To a solution of **11** (20 mg, 0.043 mmol) in dichloromethane (1.0 mL) was added 3-chloroperoxybenzoic acid (19.8 mg, 0.086 mmol). The reaction mixture was stirred at room temperature overnight and then partitioned between dichloromethane and 10% aqueous sodium thiosulfate. The organic layer was then washed with saturated sodium bicarbonate solution, brine and concentrated to obtain 8 mg (37%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.10 (d, J=8 Hz, 1H), 7.89 (d, J=8 Hz, 1H), 7.82 (d, J=8.6 Hz, 2H), 7.70 (d, J=8.4 Hz, 2H), 7.30 (d, J=8.8 Hz, 2H), 6.93 (d, J=8.8 Hz, 2H), 4.41 (s, 2H), 4.22 (s, 2H), 1.68–1.71 ppm (m, 2H); LC–MS: m/z=499 $[M+H, {}^{35}Cl]^+$, 501 $[M+H, {}^{37}Cl]^+$.

3-(4-Chlorophenyl)-*N*-[(1*R*,4*R*)-4-[3-(4-chlorophenyl)propanamido]cyclohexyl]propanamide (14). To a solution of the 3-(4-chlorophenyl)propionic acid (0.018 g, 0.101 mmol) in *N*,*N*-dimethylformamide (0.17 mL) were sequentially added 1-hydroxybenzotriazole hydrate (0.094 g, 0.70 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.014 g, 0.101 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (0.04 g, 0.101 mmol) and *N*,*N*-diisopropylethylamine (0.027 mL, 0.15 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS and subjected to conditions described in procedure A to obtain 9 mg (20%) of the title compound. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.90 (d, J=7.7 Hz, 1H), 7.67 (d, J=7.5 Hz, 1H), 7.29 (d, J=9.0 Hz, 4H), 7.18 (d, J=7.7 Hz, 2H), 6.93 (d, J=8.2 Hz, 2H), 4.41 (s, 2H), 3.42–3.54 (m, 2H), 2.75 (t, J=7.1 Hz, 2H), 2.29 (t, J=7.1 Hz, 2H), 1.70 (d, J=10.4 Hz, 4H), 1.11–1.32 ppm (m, 4H); LC–MS: m/z=447 $[M+H, {}^{35}Cl]^+$, 449 $[M+H, {}^{37}Cl]^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]propanamide (16). To a solution of 2-(4-chlorophenoxy)propionic acid (10 mg, 0.05 mmol) in *N*,*N*-dimethylformamide (1.0 mL), were sequentially added 1-hydroxybenzotriazole hydrate (9 mg, 0.06 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (11 mg, 0.06 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (20 mg, 0.05 mmol) and *N*,*N*-diisopropylethylamine (0.013 mL, 0.075 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS and subjected to conditions described in procedure B to obtain 17 mg (73%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.93 (d, J=8.1 Hz, 1H), 7.90 (d, J=8.2 Hz, 1H), 7.28–7.32 (m, 4H), 6.86–6.94 (m, 4H), 4.60 (q, J=6.4 Hz, 1H), 4.41 (s, 2H), 3.53 (brs, 2H), 1.62–1.72 (m, 4H), 1.37 (d, J=6.4 Hz, 3H), 1.20–1.31 ppm (m, 4H); LC–MS: m/z=465 $[M+H, {}^{35}Cl]^+$, 467 $[M+H, {}^{37}Cl]^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-{[2-(4-chlorophenoxy)ethyl]-amino}cyclohexyl]acetamide (17). To a suspension of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (0.119 g, 0.3 mmol) in dichloromethane (2.0 mL) was added *N*,*N*-diisopropylethylamine (0.07 mL, 0.4 mmol). After stirring at room temperature for 10 min, 2-(4-chlorophenoxy)acetaldehyde (0.034 g, 0.2 mmol) and acetic acid (0.4 mL) were sequentially added, followed by addition of sodium triacetoxyborohydride (0.042 g, 0.2 mmol) after 1 h. The reaction mixture was stirred at room temperature overnight, diluted with 10:1 mixture of dichloromethane/methanol, washed with dilute ammonium hydroxide, dilute potassium hydrogen sulfate solution, sodium bicarbonate solution and brine. The organic layer was concentrated and isolated by reversed-phase column chromatography to obtain 2 mg (2%) of the title compound as a trifluoroacetate salt. LC–MS: m/z=437 $[M+H, {}^{35}Cl]^+$, 439 $[M+H, {}^{37}Cl]^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)ethanimidamido]cyclohexyl]acetamide (18). To a solution of 4-chlorophenoxyacetonitrile (0.42 g, 2.49 mmol) in diethyl ether (2.0 mL) were sequentially added 4 M solution of hydrochloric acid in dioxane (0.59 mL, 2.36 mmol) and ethanol (0.145 mL, 2.49 mmol). The reaction mixture was stirred at room temperature overnight and concentrated to obtain 0.49 g of crude ethyl 2-(4-chlorophenoxy)-ethanecarboximidate hydrochloride as white solid that was used without further purification.

To a solution of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]-acetamide (0.100 g, 0.3 mmol) in ethanol (1.0 mL) were added *N*,*N*-diisopropylethylamine (0.063 mL, 0.4 mmol) and ethyl 2-(4-chlorophenoxy)ethanecarboximidate (0.082 g, 0.4 mmol). The mixture was stirred at room temperature for 24 h, concentrated and suspended in diethyl ether (10.0 mL). The suspension was vortexed, and the ether layer was decanted. The ether washes were repeated twice to obtain a white gel that was suspended in methanol (0.15 mL) and dichloromethane (5.0 mL). The suspension was gently heated and cooled to room temperature. The suspension was vortexed, the organic solvents were decanted and the solids were dissolved in methanol (5.0 mL). To this methanolic mixture was added Silicycle Si-TMA acetate resin (0.9 g) and stirred at room temperature overnight. The mixture was filtered, filtrate concentrated and triturated vigorously with hot diethyl ether (10 mL) twice. The resulting white solids were filtered and dried to obtain 79 mg (61%) of the product as an acetate salt. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.93 (d, J=8.1 Hz, 1H), 7.32 (dd, J=8.3, 6.6 Hz, 4H), 6.94 (dd, J=8.8, 6.6 Hz, 4H), 4.41 (s, 2H), 4.42 (s, 2H), 3.58 (brs, 2H), 1.72–1.75 (m, 3H), 1.17–1.36 ppm (m, 5H); LC–MS: m/z=450 $[M+H, {}^{35}Cl]^+$, 452 $[M+H, {}^{37}Cl]^+$.

4-Chlorophenyl *N*-{[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]methyl}carbamate (19). To a suspension of *tert*-butyl *N*-{[(1*R*,4*R*)-4-aminocyclohexyl]methyl}carbamate (0.068 g, 0.3 mmol) was in tetrahydrofuran (2.0 mL) were added *N*,*N*-diisopropylethylamine (0.058 g, 0.4 mmol) and 4-chlorophenoxyacetyl chloride (0.046 mL, 0.3 mmol) and stirred at room temperature overnight. The reaction mixture was partitioned in 1:1 mixture of ethyl acetate/5% aqueous potassium hydrogen sulfate The organic layer was washed with brine, dried over magnesium sulfate, concentrated, triturated with 2:1 mixture of hexanes/diethyl ether (2×10 mL) and dried to obtain 80 mg (68%) of *tert*-butyl *N*-{[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]methyl}carbamate as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.84 (d, J=8.2 Hz, 1H), 7.31 (d, J=9 Hz, 2H), 6.93 (d, J=9 Hz, 2H), 6.77 (brs, 1H), 4.40 (s, 2H), 3.51–3.53 (brs, 2H), 2.73 (t, J=6.3 Hz, 2H), 1.63–1.73 (m, 4H), 1.34 (s, 9H), 1.13–1.22 (m, 2H), 0.83–0.92 ppm (m, 2H); LC–MS: m/z=397 $[M+H, {}^{35}Cl]^+$, 399 $[M+H, {}^{37}Cl]^+$.

To a solution of *tert*-butyl *N*-{[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]methyl}carbamate (0.079 g, 0.2 mmol) in dichloromethane (4.0 mL) were sequentially added triethylsilane (0.1 mL, 0.6 mmol), water (0.1 mL) and trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 30 min, concentrated, triturated with diethyl ether (10.0 mL) and dried to obtain 76 mg (93%) of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(aminomethyl)cyclohexyl]acetamide as trifluoroacetic acid salt. LC–MS: m/z=297 $[M+H, {}^{35}Cl]^+$, 299 $[M+H, {}^{37}Cl]^+$.

To a solution of 4-chlorophenyl chloroformate (0.009 mL, 0.1 mmol) in tetrahydrofuran (1.0 mL) were added dropwise a mixture of 2-(4-

chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(aminomethyl)cyclohexyl]acetamide trifluoroacetic acid (0.025 g, 0.1 mmol) and *N*,*N*-diisopropylethylamine (0.026 mL, 0.2 mmol) in tetrahydrofuran (0.5 mL). The mixture was stirred at room temperature for 2 h and then partitioned between a mixture of dichloromethane/5% aqueous potassium hydrogen sulfate. The organic layer was dried, concentrated and purified by flash column chromatography (0–50% acetone/dichloromethane) to obtain 13 mg (47%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.87 (d, J=8.1 Hz, 1H), 7.80 (t, J=5.8 Hz, 1H), 7.39 (d, J=8.8 Hz, 2H), 7.31 (d, J=8.8 Hz, 2H), 7.11 (d, J=8.8 Hz, 2H), 6.94 (d, J=9 Hz, 2H), 4.41 (s, 2H), 3.53 (br s, 1H), 2.89 (t, J=6.1 Hz, 2H), 1.73 (t, J=10.4 Hz, 4H), 1.17–1.26 (m, 2H), 0.91–1.00 ppm (m, 2H); LC–MS: m/z=451 [M+H, ^{35}Cl]$^+$, 453 [M+H, ^{37}Cl]$^+$.

(4-Chlorophenyl)methyl *N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]carbamate (20). To a cooled solution (0 °C) of 4-chlorobenzyl alcohol (0.022 g, 0.2 mmol) and triphosgene (0.015 g, 0.1 mmol) in dichloromethane (1.0 mL), was added dropwise *N*,*N*-diisopropylethylamine (0.026 mL, 0.15 mmol). After warming up to room temperature and stirring for 1 h, the reaction mixture was added dropwise to a solution of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide (0.040 g, 0.1 mmol) and *N*,*N*-diisopropylethylamine (0.052 mL, 0.3 mmol) in dichloromethane (1.0 mL). After stirring the mixture at room temperature for 2 h, the reaction was quenched with methanol (0.5 mL) and partitioned between 1:1 dichloromethane/5% aqueous potassium hydrogen sulfate. The organic layer was concentrated and the resulting solid was washed with diethyl ether thrice to obtain 29 mg (64%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.89 (d, J=7.9 Hz, 1H), 7.40 (d, J=8 Hz, 1H), 7.33 (d, J=8.6 Hz, 1H), 7.31 (d, J=9.0 Hz, 2H), 7.20 (d, J=7.7 Hz, 1H), 6.93 (d, J=9.0 Hz, 2H), 4.96 (s, 2H), 4.41 (s, 2H), 3.51–3.53 (m, 1H), 3.20–3.23 (m, 1H), 1.70–1.79 (m, 4H), 1.18–1.33 ppm (m, 4H); LC–MS: m/z=451 [M+H, ^{35}Cl]$^+$, 453 [M+H, ^{37}Cl]$^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-{[2-(4-chlorophenyl)acetamido]methyl}cyclohexyl]acetamide (21). To a solution of 4-chlorophenylacetic acid (0.010 g, 0.1 mmol) in *N*,*N*-dimethylformamide (0.5 mL) were added sequentially 1-hydroxybenzotriazole hydrate (0.011 g, 0.1 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.014 g, 0.1 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(aminomethyl)cyclohexyl]acetamide (0.025 g, 0.1 mmol), and *N*,*N*-diisopropylethylamine (0.026 mL, 0.2 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS. The reaction mixture was then diluted with ethyl acetate and was washed with 5% potassium hydrogen sulfate, water, saturated sodium bicarbonate solution and brine. The organic layer was dried over anhydrous magnesium sulfate, concentrated by rotary evaporation and triturated with water and ether to obtain 9.3 mg (33%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.99 (br s, 1H), 7.85 (d, J=8.1 Hz, 1H), 7.31 (dd, J=8.4, 4.2 Hz, 4H), 7.24 (d, J=8.0 Hz, 2H), 6.94 (d, J=8.8 Hz, 2H), 4.41 (s, 2H), 3.53 (br s, 2H), 3.37 (s, 2H), 2.86 (t, J=6.0 Hz, 2H), 1.64–1.73 (m, 4H), 1.13–1.19 (m, 2H), 0.89–0.95 ppm (m, 2H); LC–MS: m/z=449 [M+H]$^+$.

(4-Chlorophenyl)methyl *N*-[(1*R*,4*R*)-4-({[(4-chlorophenyl)methoxy]carbonyl}amino) cyclohexyl]carbamate (22). To a solution of 4-chlorobenzyl alcohol (0.052 g, 0.4 mmol) in dichloromethane (3.0 mL) was added triphosgene (0.036 g, 0.1 mmol) and cooled to 0 °C. To this mixture was added dropwise a solution of *N*,*N*-diisopropylethylamine (0.064 mL, 0.37 mmol) in dichloromethane (1.0 mL) and stirred at room temperature for 2 h. To a mixture of (1*R*,4*R*)-cyclohexane-1,4-diamine (0.020 g, 0.2 mmol) and *N*,*N*-diisopropylethylamine (0.064 mL, 0.37 mmol) in dichloromethane (1.0 mL) was added dropwise the pre-formed chloroformate solution and was stirred at room temperature overnight. To the reaction mixture was added methanol (0.3 mL) to quench unreacted triphosgene and concentrated to dryness. The residue was washed with water (2×10 mL), diethyl ether (3×10 mL) and dried to obtain 25 mg (32%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.32–7.41 (m, 8H), 7.19 (d, J=7.5 Hz, 2H), 4.95 (s, 4H), 3.18 (br s, 2H), 1.74–1.76 (m, 3H), 1.18–1.23 ppm (m, 5H); LC–MS: m/z=449 [M+H]$^+$.

4-Chlorophenyl *N*-{[(1*R*,4*R*)-4-{[(4-chlorophenoxycarbonyl)amino]methyl}cyclohexyl]methyl}carbamate (23). To a solution of 4-chlorophenyl *N*-{[(1*R*,4*R*)-4-(aminomethyl)cyclohexyl]methyl}carbamate (0.03 g, 0.1 mmol) and *N*,*N*-diisopropylethylamine (0.032 mL, 0.2 mmol) in tetrahydrofuran (0.5 mL) was added dropwise 4-chlorophenyl chloroformate (0.01 mL, 0.1 mmol). After stirring at room temperature for 20 min, water (3 mL) was added to the reaction mixture. The precipitate was washed with water (3×2 mL), diethyl ether (4×2 mL) and dried to obtain 23 mg (70%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.79 (t, J=5.7 Hz, 2H), 7.39 (d, J=8.8 Hz, 4H), 7.11 (d, J=8.8 Hz, 4H), 3.29 (br s, 2H), 2.89 (t, J=6.2 Hz, 4H), 1.73 (d, J=7 Hz, 4H), 0.85–0.89 ppm (m, 4H); LC–MS: m/z=451 [M+H]$^+$.

2-(4-Iodophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]-cyclohexyl]acetamide (24). To a suspension of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(2-chloroacetamido)cyclohexyl]acetamide (0.085 g, 0.2 mmol,) in acetone (5.0 mL) were added potassium carbonate (0.070 g, 0.4 mmol) and 4-iodophenol (0.092 g, 0.4 mmol) and heated at 120 °C for 1 h in the microwave reactor. The reaction mixture was diluted with water (6 mL) and resulting solids were washed with water (5 mL) and diethyl ether (5 mL). The solids were suspended in dichloromethane and concentrated by rotary evaporation to obtain 9.5 mg (52%) of the title compound as a brown solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.91 (d, J=7.9 Hz, 2H), 7.57 (d, J=9.0 Hz, 2H), 7.31 (d, J=9.0 Hz, 2H), 6.94 (d, J=9.0 Hz, 2H), 6.76 (d, J=9.0 Hz, 2H), 4.40–4.42 (m, 4H), 3.55 (br s, 2H), 1.74 (d, J=5.9 Hz, 4H), 1.25–1.35 ppm (m, 4H); LC–MS: m/z=543 [M+H]$^+$.

2-(4-Iodophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-iodophenoxy)acetamido]cyclohexyl]acetamide (25). To a suspension of 2-chloro-*N*-[(1*R*,4*R*)-4-(2-chloroacetamido)cyclohexyl]acetamide (0.2 g, 0.7 mmol) and potassium carbonate (0.517 g, 3.7 mmol) in *N*,*N*-dimethylformamide (0.058 mL), were added 4-iodophenol (0.659 g, 3.0 mmol) and sodium iodide (0.006 g, 0.037 mmol), and the mixture was heated in the microwave at 100 °C for 30 min. The reaction mixture was diluted with water (20 mL). The mixture was centrifuged and the water layer was decanted. This washing protocol was repeated thrice and the resulting wet solid was concentrated down with toluene (10 mL) in a rotary evaporator. The residual product was washed with diethyl ether (10 mL) and concentrated using rotary evaporation to afford 0.28 g (59%) of the title compound as a brown solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.90 (d, J=8.1 Hz, 2H), 7.57 (d, J=8.8 Hz, 4H), 6.76 (d, J=8.8 Hz, 4H), 4.40 (s, 4H), 3.54 (br s, 2H), 1.72 (d, J=5.9 Hz, 4H), 1.27–1.32 ppm (m, 4H); LC–MS: m/z=634 [M+H]$^+$.

***N*-[(1*R*,4*R*)-4-[2-(4-Chlorophenoxy)acetamido]cyclohexyl]-2-[4-(trifluoromethyl)phenoxy]acetamide (26)**. To a solution of 2-[4-(trifluoromethyl)phenoxy]acetic acid (0.011 g, 0.052 mmol) in *N*,*N*-dimethylformamide (0.3 mL), were sequentially added 1-hydroxybenzotriazole hydrate (0.008 g, 0.055 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.011 g, 0.055 mmol), 2-(4-

chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (0.020 g, 0.05 mmol) and *N*,*N*-diisopropylethylamine (0.021 mL, 0.12 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS and then subjected to conditions described in procedure B to obtain 17.7 mg (69%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.98 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 9.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.0 Hz, 2H), 4.53 (s, 2H), 4.42 (s, 2H), 3.56 (br s, 2H), 1.74 (d, J = 7.9 Hz, 4H), 1.28–1.33 ppm (m, 4H); LC–MS: m/z = 485 [M + H, ^{35}Cl]$^+$, 487 [M + H, ^{37}Cl]$^+$.

***N*-[(1*R*,4*R*)-4-{2-[4-(Trifluoromethyl)phenoxy]acetamido}cyclohexyl]-2-[4-(trifluoromethyl)phenoxy]acetamide (27)**. To a solution of 4-(trifluoromethy)phenoxyacetic acid (0.19 g, 1.76 mmol) in *N*,*N*-dimethylformamide (1.75 mL) were sequentially added 1-hydroxybenzotriazole hydrate (0.12 g, 1.76 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.175 g, 1.76 mmol), (1*R*,4*R*)-cyclohexane-1,4-diamine (0.05 g, 0.87 mmol) and *N*,*N*-diisopropylethylamine (0.47 mL, 5.62 mmol). The reaction mixture was subjected to conditions described in procedure A to obtain 0.15 g (66%) of the title compound. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.98 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 8.2 Hz, 4H), 7.09 (d, J = 8.1 Hz, 4H), 4.53 (s, 4H), 3.57 (br s, 2H), 1.75–1.76 (m, 4H), 1.29–1.31 ppm (m, 4H); LC–MS: m/z = 519 [M + H]$^+$.

2-(4-Ethynylphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (28). To a solution of 2-(4-iodophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido] cyclohexyl]acetamide (**24**) (0.02 g, 0.036 mmol), dichlorobis(triphenylphosphine)palladium (0.003 g, 0.0036 mmol) and copper(I) Iodide (0.003 g, 0.016 mmol) in a degassed mixture of triethylamine/*N*,*N*-dimethylformamide (1:1, 0.4 mL), was added a solution of ethynyltrimethylsilane (0.009 mL, 0.1 mmol) in triethylamine/*N*,*N*-dimethylformamide (1:1, 0.4 mL) as a single portion and the mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with ethyl acetate (25 mL) and washed with 5% aqueous potassium hydrogen sulfate solution (20 mL) and brine (20 mL). The organic phase was dried over magnesium sulfate, filtered and concentrated to obtain a brown colored oil. Purification by flash column chromatography (20–100% ethyl acetate/hexanes) afforded 20 mg (105%) of *N*-{4-[2-(4-chlorophenoxy)acetamido]cyclohexyl}-2-{4-[2-(trimethylsilyl)ethynyl]phenoxy}acetamide (contaminated with triphenylphosphine oxide) as a white solid. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.44 (d, J = 8.6 Hz, 2H), 7.27–7.30 (m, 2H), 6.84–6.88 (m, 2H), 6.34 (d, J = 7.9 Hz, 2H), 4.46 (s, 2H), 4.44 (s, 2H), 3.86 (br s, 2H), 2.05 (d, J = 5.5 Hz, 4H), 1.32–1.34 (m, 4H), 0.25 ppm (s, 9H); LC–MS: m/z = 513 [M + H]$^+$.

To a solution of *N*-{4-[2-(4-chlorophenoxy)acetamido]cyclohexyl}-2-{4-[2-(trimethylsilyl)ethynyl]phenoxy}acetamide (0.015 g, 0.03 mmol) in a 1:1 mixture of methanol/tetrahydrofuran (2.0 mL), was added potassium carbonate (0.006 g, 0.04 mmol). The reaction mixture was stirred at ambient temperature for 30 min and then concentrated down to dryness. The residue was triturated thrice with water (5.0 mL) and the resulting wet solid was suspended in toluene (5.0 mL) and concentrated down to dryness to obtain 10 mg (78%) of the title compound as a white solid. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.45 (d, J = 8.6 Hz, 2H), 7.25–7.28 (m, 2H), 6.85 (dd, J = 8.6, 5.1 Hz, 4H), 6.32 (d, J = 7.9 Hz, 2H), 4.45 (s, 2H), 4.42 (s, 2H), 3.85 (br s, 2H), 3.01 (s, 2H), 2.04 (d, J = 6 Hz, 4H), 1.24–1.38 ppm (m, 4H); LC–MS: m/z = 441 [M + H]$^+$.

2-(4-Ethynylphenoxy)-*N*-{4-[2-(4-ethynylphenoxy)acetamido]cyclohexyl}acetamide (29). To a solution of 2-(4-iodophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-iodophenoxy)acetamido]cyclohexyl]acetamide (**25**) (0.2 g, 0.3 mmol), dichlorobis(triphenylphosphine)palladium (0.023 g, 0.03 mmol) and copper(I) Iodide (0.03 mg, 0.015 mmol) in a degassed mixture of triethylamine/*N*,*N*-dimethylformamide (1:1, 2 mL), was added a solution of ethynyltrimethylsilane (0.178 mL, 1.5 mmol) in triethylamine/*N*,*N*-dimethylformamide (1:1, 2 mL) as a single portion and the mixture was stirred at room temperature for 4 h. The reaction mixture was then diluted with ethyl acetate (25 mL) and washed with 5% aqueous potassium hydrogen sulfate solution (20 mL). The emulsion formed was filtered through a Celite plug and washed with dichloromethane (150 mL). The organic layers were dried over magnesium sulfate, filtered and concentrated down to a brown solid. Purification by flash column chromatography (0–80% acetone/dichloromethane) afforded 58 mg (32%) of 2-{4-[2-(trimethylsilyl)ethynyl]phenoxy}-*N*-[4-(2-{4-[2-(trimethylsilyl)ethynyl]phenoxy}acetamido)cyclohexyl]acetamide as a brown solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.92 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 8.8 Hz, 4H), 6.89 (d, J = 8.8 Hz, 4H), 4.44 (s, 4H), 1.73 (d, J = 5.9 Hz, 4H), 1.29–1.32 (m, 4H), 0.18 ppm (s, 18H); LC–MS: m/z = 575 [M + H]$^+$.

To a solution of (trimethylsilyl)ethynyl]phenoxy}-*N*-[4-(2-{4-[2-(trimethylsilyl)ethynyl]phenoxy}acetamido)cyclohexyl]acetamide (0.020 g, 0.035 mmol) in 1:1 mixture of methanol/tetrahydrofuran (2.0 mL), was added potassium carbonate (0.014 g, 0.1 mmol). The reaction mixture was stirred at ambient temperature for 20 min and then concentrated down to dryness. The residue was triturated thrice with water (5 mL) and the resulting wet solid was suspended in toluene (5 mL) and concentrated down to dryness to obtain 12 mg (80%) of the title compound as a tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.92 (d, J = 7.9 Hz, 2H), 7.38 (d, J = 8.8 Hz, 4H), 6.91 (d, J = 8.6 Hz, 4H), 4.45 (s, 4H), 4.00 (s, 2H), 1.74 (d, J = 5.9 Hz, 4H), 1.28–1.33 ppm (m, 4H); LC–MS: m/z = 431 [M + H]$^+$.

2-(4-Methoxyphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-methylphenoxy)acetamido]cyclohexyl]acetamide (36). To a solution of 4-(methoxy)phenoxyacetic acid (0.014 g, 0.076 mmol) in *N*,*N*-dimethylformamide (0.3 mL) were sequentially added 1-hydroxybenzotriazole hydrate (0.01 g, 0.076 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.015 g, 0.076 mmol), (1*R*,4*R*)-cyclohexane-1,4-diamine (0.03 g, 0.076 mmol) and *N*,*N*-diisopropylethylamine (0.04 mL, 0.23 mmol). The reaction mixture was then diluted with 5% methanol/dichloromethane and was washed with 5% potassium hydrogen sulfate, water, saturated sodium bicarbonate solution and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered through a silica plug and concentrated by rotary evaporation to obtain 34 mg (58%) of the title compound. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.23–7.27 (m, 2H), 6.78–6.83 (m, 6H), 6.42 (d, J = 8.2 Hz, 1H), 6.35 (d, J = 8.1 Hz, 1H), 4.38–4.40 (m, 4H), 3.84 (br s, 2H), 2.02 (d, J = 5.5 Hz, 4H), 1.27–1.38 ppm (m, 4H); LC–MS: m/z = 447 [M + H]$^+$.

2-(4-Methoxyphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-methoxyphenoxy)acetamido]cyclohexyl]acetamide (37). To a solution of the 2-(4-methoxyphenoxy)acetic acid (0.32 g, 1.75 mmol) in *N*,*N*-dimethylformamide (1 mL) were sequentially added 1-hydroxybenzotriazole hydrate (0.236 g, 1.75 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.345 g, 1.75 mmol), *trans*-1,4-diaminocyclohexane (0.1 g, 0.87 mmol) and *N*,*N*-diisopropylethylamine (1.0 mL, 5.2 mmol). The reaction mixture was stirred at 52 °C until determined complete by LC–MS and then subjected to conditions described in procedure A to obtain 150 mg (62%) of the title compound. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.83 (s, 8H), 6.41 (d, J = 8.1 Hz, 2H), 4.39 (m, 4H), 3.83 (br s, 2H), 2.03 (d, J = 6.4 Hz, 4H),

1.28–1.38 ppm (m, 4H); LC–MS: m/z=443 [M+H, ^{35}Cl]$^+$, 445 [M+H, ^{37}Cl]$^+$.

2-(4-Methanesulfinylphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)-acetamido]cyclohexyl]acetamide (38). To a solution of 2-(4-methanesulfinylphenoxy)acetic acid (0.011 g, 0.052 mmol) in *N*,*N*-dimethylformamide (0.3 mL), were sequentially added 1-hydroxybenzotriazole hydrate (0.008 g, 0.055 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.011 mg, 0.055 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (20 mg, 0.05 mmol) and *N*,*N*-diisopropylethylamine (0.021 mL, 0.12 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS and then subjected to conditions described in procedure B to obtain 16 mg (66%) of the title compound as an off-white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.96 (d, J=8.2 Hz, 1H), 7.91 (d, J=8.2 Hz, 1H), 7.60 (d, J=8.8 Hz, 2H), 7.31 (d, J=9.0 Hz, 2H), 7.10 (d, J=8.6 Hz, 2H), 6.94 (d, J=9.0 Hz, 2H), 4.50 (s, 2H), 4.42 (s, 2H), 3.57 (brs, 2H), 2.67 (s, 3H), 1.75 (d, J=8.1 Hz, 4H), 1.21–1.34 ppm (m, 4H); LC–MS: m/z=479 [M+H, ^{35}Cl]$^+$, 481 [M+H, ^{37}Cl]$^+$.

2-(4-Acetylphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (39). To a solution of 4-acetylphenoxyacetic acid (0.012 g, 0.063 mmol) in *N*,*N*-dimethylformamide (0.3 mL), were sequentially added 1-hydroxybenzotriazole hydrate (0.012 g, 0.076 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.014 g, 0.076 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (0.049 g, 0.1 mmol) and *N*,*N*-diisopropylethylamine (0.022 mL, 0.1 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS and then subjected to conditions described in procedure B to obtain 21.5 mg (39%) of the title compound as a tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.97 (d, J=8.1 Hz, 2H), 7.88–7.92 (m, 2H), 7.31 (d, J=8.0 Hz, 2H), 6.93–7.02 (m, 4H), 4.52 (s, 2H), 4.42 (s, 2H), 3.55 (brs, 2H), 3.28 (s, 3H), 1.74 (d, J=9.2 Hz, 4H), 1.28–1.33 ppm (m, 4H); LC–MS: m/z=459 [M+H, ^{35}Cl]$^+$, 461 [M+H, ^{37}Cl]$^+$.

2-[4-Chloro-3-(trifluoromethyl)phenoxy]-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (40). To a suspension of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(2-chloroacetamido)cyclohexyl]acetamide (0.036 g, 0.1 mmol) in acetone (1.0 mL) were added potassium carbonate (0.021 g, 0.2 mmol) and 2-chloro-5-hydroxybenzotrifluoride (0.027 g, 0.13 mmol) and heated at 120 °C for 20 min in the microwave reactor. The reaction mixture was diluted with water (6 mL) and resulting solids were washed with water (5 mL) and diethyl ether (5 mL). The solids were suspended in dichloromethane and concentrated by rotary evaporation to obtain 28 mg (54%) of the title compound as a brown solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.99 (d, J=8.1 Hz, 1H), 7.91 (d, J=8.1 Hz, 1H), 7.62 (d, J=8.8 Hz, 1H), 7.37 (d, J=2.9 Hz, 1H), 7.31 (d, J=8.0 Hz, 2H), 7.23 (dd, J=8.9, 3 Hz, 1H), 6.94 (d, J=8.0 Hz, 2H), 4.55 (s, 2H), 4.42 (s, 2H), 3.56 (brs, 2H), 3.28 (s, 3H), 1.74 (d, J=5.9 Hz, 4H), 1.28–1.36 ppm (m, 4H); LC–MS: m/z=519 [M+H]$^+$.

2-(4-Chloro-3-iodophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)-acetamido]cyclohexyl]acetamide (41). To a suspension of 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-(2-chloroacetamido)cyclohexyl]acetamide (0.071 g, 0.2 mmol) in acetone (1.5 mL) were added potassium carbonate (0.041 g, 0.3 mmol) and 4-chloro-3-iodophenol (0.050 g, 0.2 mmol) and heated at 120 °C for 30 min in the microwave reactor. The reaction mixture was diluted with water (10 mL) and resulting solids were washed with water (10 mL) and diethyl ether (10 mL). The solids were suspended in dichloromethane and concentrated by rotary evaporation to obtain 57 mg (50%) of the title compound as a tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.93 (t, J=8.7 Hz, 2H), 7.43–7.48 (m, 2H), 7.31 (d, J=8.8 Hz, 2H), 6.93–6.99 (m, 3H), 4.40–4.43 (m, 4H), 3.55 (brs, 2H), 3.28 (s, 3H), 1.74 (d, J=5.5 Hz, 4H), 1.31 ppm (brs, 4H); LC–MS: m/z=577 [M+H, ^{35}Cl]$^+$, 579 [M+H, ^{37}Cl]$^+$.

2-(4-Chloro-3-methylphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (42). To a solution of 4-chloro-3-methylphenoxyacetic acid (0.010 g, 0.05 mmol) in *N*,*N*-dimethylformamide (1.0 mL), were sequentially added 1-hydroxybenzotriazole hydrate (0.009 g, 0.06 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.012 g, 0.06 mmol), 2-(4-chlorophenoxy)-*N*-[(1*R*,4*R*)-4-aminocyclohexyl]acetamide trifluoroacetic acid (0.020 g, 0.05 mmol) and *N*,*N*-diisopropylethylamine (0.013 mL, 0.075 mmol). The reaction mixture was stirred at room temperature until determined complete by LC–MS and then subjected to conditions described in procedure B to obtain 20 mg (85%) of the title compound as a white solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.88–7.92 (m, 2H), 7.26–7.33 (m, 3H), 6.93–6.99 (m, 2H), 6.77 (dd, J=8.7, 3 Hz, 2H), 4.40–4.42 (m, 4H), 3.56 (brs, 2H), 2.25 (s, 3H), 1.73 (d, J=6.0 Hz, 4H), 1.28–1.36 ppm (m, 4H); LC–MS: m/z=465 [M+H, ^{35}Cl]$^+$, 467 [M+H, ^{37}Cl]$^+$.

2-(4-Chlorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chloro-3-ethynylphenoxy)acetamido]cyclohexyl]acetamide (43). To a solution of 2-(4-chloro-3-iodophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (**41**) (0.03 g, 0.05 mmol), dichlorobis(triphenylphosphine)palladium (0.004 g, 0.005 mmol) and copper(I) Iodide (0.002 g, 0.01 mmol) in a degassed mixture of triethylamine/*N*,*N*-dimethylformamide (1:1, 0.4 mL), was added a solution of ethynyltrimethylsilane (0.014 mL, 0.1 mmol) in triethylamine/*N*,*N*-dimethylformamide (1:1, 0.4 mL) as a single portion and the mixture was stirred at room temperature for 20 min. The reaction mixture was then diluted with ethyl acetate and washed with 5% aqueous potassium hydrogen sulfate solution, saturated sodium thiosulfate solution and brine. The organic phase was dried over magnesium sulfate, filtered and concentrated to obtain the crude. Purification by flash column chromatography (0–40% acetone/dichloromethane) afforded 12 mg (42%) of 2-{4-chloro-3-[2-(trimethylsilyl)ethynyl]phenoxy}-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide as a light-brown solid. LC–MS: m/z=547 [M+H, ^{35}Cl]$^+$, 549 [M+H, ^{37}Cl]$^+$.

To a solution of 2-{4-chloro-3-[2-(trimethylsilyl)ethynyl]phenoxy}-*N*-[(1*R*,4*R*)-4-[2-(4-chlorophenoxy)acetamido]cyclohexyl]acetamide (0.01 g, 0.018 mmol) in a 1:1 mixture of methanol/tetrahydrofuran (0.9 mL), was added potassium carbonate (0.004 g, 0.027 mmol). The reaction mixture was stirred at ambient temperature for one hour and then concentrated down to dryness. The residue was triturated thrice with water (10 mL), diethyl ether (10 mL) and concentrated down to dryness to obtain 7 mg (81%) of the title compound as a tan solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=7.92 (t, J=7.5 Hz, 2H), 7.42 (d, J=9.0 Hz, 2H), 7.31 (d, J=8.0 Hz, 2H), 7.13 (d, J=2.9 Hz, 1H), 7.00 (dd, J=9.0, 3.1 Hz, 1H), 6.94 (d, J=8.0 Hz, 2H), 4.54 (s, 1H), 4.46 (s, 2H), 4.42 (s, 2H), 3.55 (brs, 2H), 1.74 (d, J=5.7 Hz, 4H), 1.28–1.33 ppm (m, 4H); LC–MS: m/z=475 [M+H, ^{35}Cl]$^+$, 477 [M+H, ^{37}Cl]$^+$.

2-(4-Chloro-3-methylphenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chloro-3-methylphenoxy)acetamido]cyclohexyl]acetamide (46). To a solution of (1*R*,4*R*)-cyclohexane-1,4-diamine (0.025 g, 0.2 mmol) in *N*,*N*-dimethylformamide (1 mL) were added 4-chloro-3-methylphenoxyacetic acid (0.088 g, 0.4 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (0.175 g, 0.5 mmol) and *N*,*N*-diisopropylethylamine (0.153 mL, 0.9 mmol).

The reaction mixture was vigorously stirred at room temperature until determined complete by LC–MS. The reaction mixture was diluted with water (10 mL) and resulting solids were washed thrice with water (10 mL). The wet solid was then concentrated down with toluene (10 mL) in a rotary evaporator. The residual product was washed with diethyl ether (10 mL) and dried to obtain 99 mg (94%) of the title compound as a cream colored solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.80$ (s, 2H), 6.68 (d, $J = 8.6$ Hz, 2H), 6.32 (d, $J = 8.1$ Hz, 2H), 4.41 (s, 4H), 3.84 (br s, 2H), 2.04 (d, $J = 6$ Hz, 4H), 1.33 ppm (t, $J = 9.8$ Hz, 4H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta =$ 166.9, 157.0, 136.8, 129.9, 125.5, 118.0, 114.3, 67.6, 47.3, 31.3, 20.2 ppm; LC–MS: $m/z = 479$ [$M+$H, ^{35}Cl]$^+$, 481 [$M+$H, ^{37}Cl]$^+$.

2-(3,4-Dichlorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(3,4-dichlorophenoxy)-acetamido]cyclohexyl]acetamide (47). To a solution of (1*R*,4*R*)-cyclohexane-1,4-diamine (0.025 g, 0.2 mmol) in *N*,*N*-dimethylformamide (1 mL) were added 3,4-dichlorophenoxyacetic acid (0.097 g, 0.4 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (0.175 g, 0.5 mmol) and *N*,*N*-diisopropylethylamine (0.153 mL, 0.9 mmol). The reaction mixture was vigorously stirred at ambient temperature for 2 h. The reaction mixture was diluted with water (10 mL) and resulting solids were washed thrice with water (10 mL). The wet solid was then concentrated down with toluene (10 mL) in a rotary evaporator. The residual product was washed with diethyl ether (10 mL) and dried under vacuum to obtain 107 mg (94%) of the title compound as a cream colored solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37$ (d, $J = 8.8$ Hz, 2H), 7.04 (s, 2H), 6.78 (d, $J = 8.8$ Hz, 4H), 6.26 (d, $J =$ 8.1 Hz, 2H), 4.42 (s, 4H), 3.85 (br s, 2H), 2.05 (d, $J = 6$ Hz, 4H), 1.31–1.39 ppm (m, 4H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 166.5$, 157.7, 131.9, 131.4, 123.4, 117.3, 116.1, 67.7, 47.4, 31.3 ppm; LC–MS: $m/z = 519$ [$M+$H, ^{35}Cl]$^+$, 521 [$M+$H, ^{37}Cl]$^+$.

2-(4-Chloro-3-fluorophenoxy)-*N*-[(1*R*,4*R*)-4-[2-(4-chloro-3-fluoro-phenoxy)acetamido]cyclohexyl]acetamide (48). To a solution 4-chloro-3-fluorophenol (0.100 g, 0.7 mmol) in *N*,*N*-dimethylformamide (2 mL), were added potassium carbonate (0.189 g, 1.4 mmol) and *tert*-butyl bromoacetate (0.111 mL, 0.8 mmol) and stirred at 65 °C for 2 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine. The organic layer was dried over magnesium sulfate and concentrated in a rotary evaporator to obtain 177 mg of tert-butyl 2-(4-chloro-3-fluorophenoxy)acetate as a colorless oil which was used without further purification.

To a solution of *tert*-butyl 2-(4-chloro-3-fluorophenoxy)acetate (177 mg, 0.7 mmol) in methanol/water (4.5 mL, 2:1) was added aqueous 5N NaOH solution (0.7 mL, 3.5 mmol) and stirred at ambient temperature for 1 h. The reaction mixture was concentrated in a rotary evaporator to remove methanol, diluted with water (5 mL) and extracted with ethyl acetate (5 mL). The aqueous layer was adjusted to about pH 2 with 1N HCl and extracted with ethyl acetate (3×5 mL). The organic extract was washed with brine (5 mL), dried over magnesium sulfate and concentrated to obtain 108 mg of 2-(4-chloro-3-fluorophenoxy)acetic acid as a white solid.

To a solution of (1*R*,4*R*)-cyclohexane-1,4-diamine (0.02 g, 0.2 mmol) in *N*,*N*-dimethylformamide (1 mL) were added 2-(4-chloro-3-fluorophenoxy)acetic acid (0.072 g, 0.4 mmol), 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (0.14 g, 0.4 mmol) and *N*,*N*-diisopropylethylamine (0.122 mL, 0.7 mmol). The reaction mixture was vigorously stirred at room temperature until determined complete by LC–MS. The reaction mixture was diluted with water (10 mL) and resulting solids were washed thrice with water (10 mL). The wet solid was then concentrated down with toluene (10 mL) in a rotary evaporator. The residual product was washed with diethyl ether (10 mL) and dried to obtain 85 mg (99%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$ with CD_3OD as co-solvent): $\delta =$ 7.23–7.28 (m, 4H), 6.61–6.73 (m, 4H), 4.36 (s, 4H), 3.56 (m, 2H), 1.95 (d, $J = 6.2$ Hz, 4H), 1.28–1.33 ppm (m, 4H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 166.5$, 159.1, 158.4, 158.3, 156.7, 131.1, 112.8,112.8, 111.6, 111.5, 104.6, 104.4, 67.8, 47.4, 31.3 ppm; LC–MS: $m/z = 487$ [$M+$H, ^{35}Cl]$^+$, 489 [$M+$H, ^{37}Cl]$^+$.

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Keywords: integrated stress response · ISRIB · protein–protein interactions · structure–activity relationships · unfolded protein response

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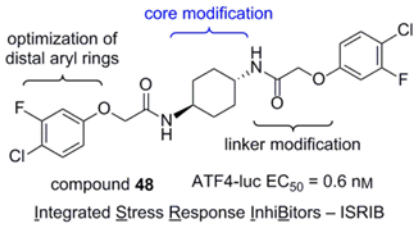
Structure–Activity Studies of Bis-*O*-Arylglycolamides: Inhibitors of the Integrated Stress Response



Stress management: Herein we describe structure–activity studies of bis-*O*-arylglycolamides, first-in-class Integrated Stress Response InhiBitors (ISRIB). ISRIB analogues make cells insensitive to the effects of eIF2α phosphorylation by activating eIF2B, the guanine exchange factor for eIF2. ISRIB analogues are thought to bind and stabilize a protein–protein interface in the dimeric form of the eIF2B heteropentameric protein complex.