Studies Leading to Potent, Dual Inhibitors of Bcl-2 and Bcl-xL

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Overexpression of the antiapototic proteins Bcl-2 and Bcl-xL provides a common mechanism through which cancer cells gain a survival advantage and become resistant to conventional chemotherapy. Inhibition of these prosurvival proteins is an attractive strategy for cancer therapy. We recently described the discovery of a selective Bcl-xL antagonist that potentiates the antitumor activity of chemotherapy and radiation. Here we describe the use of structure-guided design to exploit a deep hydrophobic binding pocket on the surface of these proteins to develop the first dual, subnanomolar inhibitors of Bcl-xL and Bcl-2. This study culminated in the identification of **2**, which exhibited EC_{50} values of 8 nM and 30 nM in Bcl-2 and Bcl-xL dependent cells, respectively. Compound **2** demonstrated single agent efficacy against human follicular lymphoma cell lines that overexpress Bcl-2, and efficacy in a murine xenograft model of lymphoma when given both as a single agent and in combination with etoposide.

Introduction

Normal tissue homeostasis requires the proper balance between cellular proliferation and attrition. The genetic instability inherent to cancer gives rise to defects in both cell growth and cell death pathways that tip the scales to allow tumor initiation and progression. Apoptosis, or programmed cell death, is an evolutionarily conserved and highly regulated process that is the primary mechanism for the removal of aged, damaged, and unnecessary cells.[1] One of the fundamental hallmarks of cancer is the ability to evade or ignore physiologic cues that would initiate this form of cellular suicide in normal cells.[2] This is often accomplished through dysregulation of apoptotic signaling pathways.

The B-cell lymphoma 2 (Bcl-2)[a] family of proteins is composed of both proapoptotic (prodeath) and antiapoptotic (prosurvival) members that cooperate through a complex series of protein−protein interactions to mediate the intrinsic or mitochondrial apoptotic pathway.[3−5] The prodeath proteins can be subcategorized into two groups; those that contain three Bcl homology (BH) domains (BH1−BH3) (Bax, Bak) and those that contain a single BH3 domain (BH3-only) (Bad, Bik, Bid, Bim, Hrk, Bmf, Noxa and Puma). These proteins propagate the death signal by inducing permeabilization of the mitochondrial membrane, release of cytochrome C, and the activation of a group of intracellular cysteine proteases called caspases. The resulting proteolytic cascade gives rise to the targeted degradation of both cytoplasmic and nuclear structures and the formation of apoptotic bodies that are rapidly engulfed and cleared by phagocytic scavenger cells.[6,7] Prosurvival Bcl-2 family members contain four BH domains (BH1−BH4) and include Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and Bcl2-A1. These proteins exert their protective effects by directly binding to and sequestering their prodeath counterparts. Cancer cells frequently overexpress the prosurvival Bcl-2 family members to suppress the apoptotic signal in order to promote survival or confer resistance to chemotherapy.[8,9] Inhibition of these antiapoptotic Bcl-2 family members should specifically target the abnormal cell death pathway found in these cancer cells and offers an attractive target for therapeutic intervention.

Three-dimensional structural studies of antiapoptotic Bcl-2 family proteins have provided invaluable insights into how these proteins interact with their prodeath counterparts.[10−13] These globular proteins consist of a bundle of eight to nine α-helices in which two mostly hydrophobic α-helices form a structural backbone that is surrounded by six to seven amphipathic α-helices. An elongated hydrophobic groove thus formed along the protein surface that spans approximately 20 Å serves as the binding site for the amphipathic α-helical BH3 domain of their proapoptotic partners. An improved understanding of these protein−protein interactions[14,15] has enabled strategies for inhibition and potential therapeutic intervention that include modified peptides, natural products, and small synthetic organic molecules.[16,17]

We have recently described the discovery of a class of potent biarylacylsulfonamide antagonists of the antiapoptotic protein Bcl-xL.[18] These studies led to the identification of **1a** (Figure 2) which bound Bcl-xL with a K_i of 0.8 nM. **1a** effectively negated the survival advantage provided by Bcl-xL overexpression against cytokine withdrawal in FL5.12 cells and enhanced the cytotoxic activity of multiple cytotoxic agents and UV irradiation in human tumor cell lines.[19] However, **1a** showed little or no single agent efficacy across a diverse panel of human tumor cell lines. Because this compound was developed by structure-based design targeting Bcl-xL, it is not surprising that

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[a] Abbreviations: Bcl-2, B-cell lymphoma 2; Bax, Bcl-2 related protein X; Bak, Bcl-2 antagonist/killer; Bad, Bcl-2 antagonist of cell death; Bik, bcl-2 interacting killer; Bid, BH3 interacting death domain; Bim, Bcl-2 interacting mediator; Hrk, Harakiri; Bmf, Bcl-2 modifying factor; Bcl-xL, B-cell lymphoma x long; Mcl-1, myeloid cell leukemia 1; Bcl2-A1, B-cell lymphoma 2 related protein A1; BH, Bcl homology; IL-3, interleukin 3; FPA, fluorescence polarization assay.

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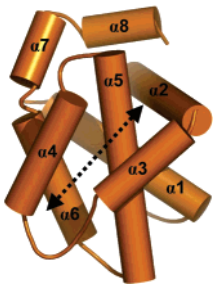


Figure 1. Generic, cylinder depiction of the three-dimensional structures of Bcl-xL and Bcl-2 proteins with the helices labeled. The dotted line is drawn along the axis of the hydrophobic binding groove formed largely by the α3, α4, and α5 helices.

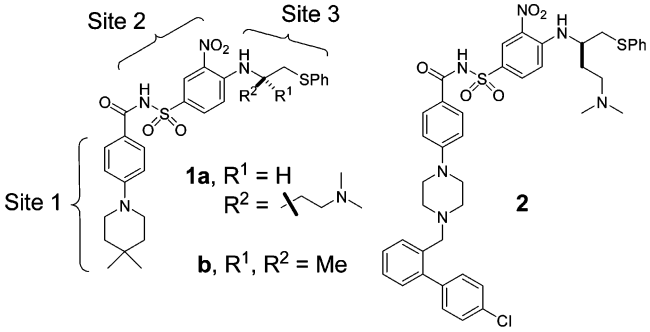


Figure 2. Structures of and substructure nomenclature for biarylacylsulfonamide Bcl-2 family protein inhibitors.

it exhibited considerably lower affinity for Bcl-2. Given the widespread overexpression of Bcl-2 in human cancers, the identification of the t(14;18) chromosomal translocation involving Bcl-2 overexpression as the initiating genetic lesion in non-Hodgkin's lymphoma and the potential for redundant antiapoptotic function of Bcl-2 and Bcl-xL, we sought to broaden the binding profile of compounds in this series to include high affinity for Bcl-2.

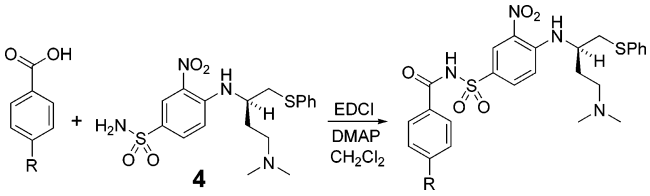
Although the overall sequence identity between Bcl-xL and Bcl-2 is only 49%, their three-dimensional architecture is quite similar.[20] In fact, if one omits the large unstructured loop between α1 and α2, the global root-mean-square deviation (rmsd) of their backbones is only ~1.85 Å. The binding groove is composed largely of a cleft between the α3 and α4 helices that has a floor made up of the central α5 and α6 helices (Figure 1). The largest difference between the two proteins in their unbound state is a slightly different helical fold of their α3 helices. Since the α3 helix borders one side of the hydrophobic binding groove, this results in a distinctly wider groove for Bcl-2 compared to Bcl-xL. Within the groove itself, there exist only three differences in primary sequence located at positions 104 (Ala in Bcl-xL, Asp in Bcl-2), 108 (Leu in Bcl-xL, Met in Bcl-2), and 122 (Ser in Bcl-xL, Arg in Bcl-2). Most notable is the potential for the flexible side chain of M108 in the center of the α3 helix of Bcl-2 to allow penetration into a deep hydrophobic pocket within the groove compared to the more rigid L108 found in Bcl-xL. Given this difference along with the inherently wider Bcl-2 groove, we postulated that accessing this deep hydrophobic pocket in the floor of the groove might significantly enhance the Bcl-2 affinity of our inhibitors. Herein we describe the molecular design considerations and structure−activity relationships that began with **1a** and culminated in the discovery of the potent, dual Bcl-2/Bcl-xL inhibitor **2** (ABT-737), for which the biological activity has recently been described[21] (Figure 2).

Synthesis

The convergent synthesis of the site-1 piperidine-containing acylsulfonamide inhibitors described in this study employed an EDCI coupling of an appropriately substituted benzoic acid and the previously described benzenesulfonamide **4**[18] as the final step (Chart 1). 4-Alkyl-4-methoxypiperidine analogues were synthesized as shown in Scheme 1. Addition of alkyl or benzyl Grignard reagents directly to ethyl 4-(4-oxopiperidin-1-yl)-benzoate[22] **5** yielded the tertiary alcohols **6a**−**n** in moderate yield. Methylation of the tertiary alcohols and saponification of the ethyl esters **7a**−**n** yielded the necessary benzoic acids that were condensed with **4** to yield acylsulfonamides **8a**−**n**.

4-Benzylidenepiperidine analogues were prepared according to Scheme 2. Wittig olefination of **5** allowed the generation of

Chart 1. General Coupling Procedure



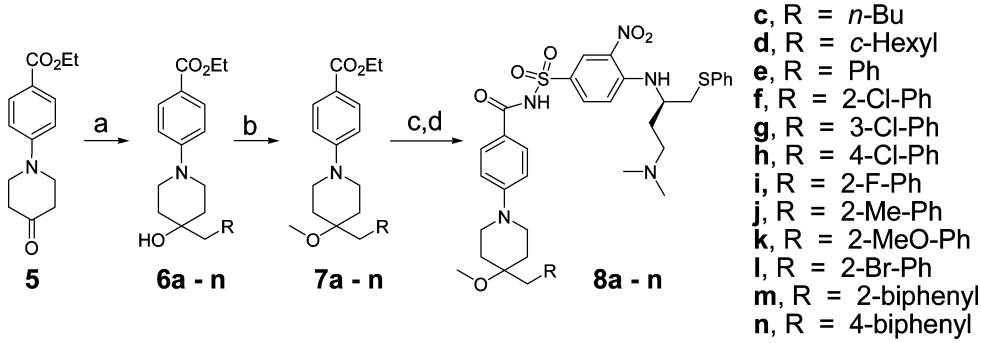
a variety of benzylidene analogues that were saponified to provide benzoic acids **9a**−**k**. Condensation with benzenesulfonamide **4** yielded the desired acylsulfonamides **10a**−**k**.

Synthesis of isoxazolines began with Wittig olefination of **5** followed by ester hydrolysis and EDCI coupling of the resulting carboxylic acid with sulfonamide **4** to provide the exomethylene **13**. Slow addition of a *N*-hydroxybenzimidoyl chloride[23] or phenylacetohydroximoyl chloride[24] to a warm chloroform solution of **13** in the presence of triethylamine yielded the cycloaddition adducts **14a** and **14b**, respectively. Treatment of ketone **5** with (±)-lactamide in the presence of acid yielded the racemic oxazolidin-4-one **15** directly. N-Benzylation followed by ester hydrolysis and coupling of the resulting carboxylic acid **4** furnished the oxazolidinone derivative **18** (Scheme 3).

The synthesis of piperazine containing analogues **20**−**22** and **23a**−**h** was achieved through functionalization of phenyl piperazine **19b**, which was obtained by coupling of 4-(4-(tert-butoxycarbonyl)piperazin-1-yl)benzoic acid[18] to **4** followed by deprotection of the amine (Scheme 4). Compounds **20** and **21** were easily prepared via reaction of **19b** with benzoyl chloride and tosyl chloride, respectively. Substituted phenyl urea **22** was formed by reaction of **19b** with phenyl isocyanate. Substituted benzyl piperazines **23a**−**h** were prepared from **19b** in a single step by either reductive alkylation with benzaldehydes or alkylation with substituted benzyl bromides. In the case of **23f**, 2-cyclohexylaminobenzaldehyde **26** was prepared by microwave-assisted aromatic nucleophilic substitution of 2-fluorobenzonitrile **24** with cyclohexylamine followed by DIBAL-H reduction of the nitrile (Scheme 5).

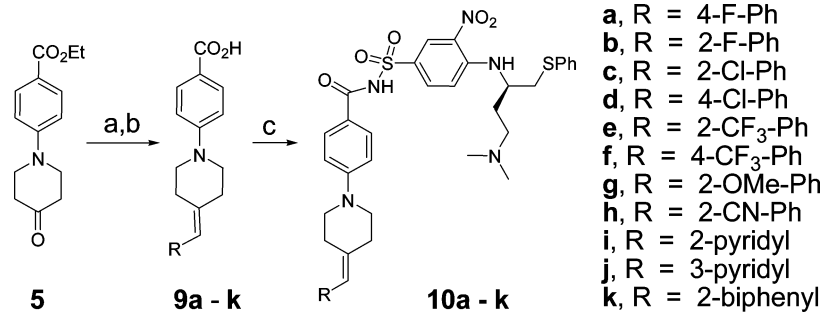
Alkylation of ethyl 4-piperazinylbenzoate **27**[25] with 2-bromomethylbiphenyl or 2-trifluoromethylbenzyl bromide gave **28** and **29**, respectively. Reductive alkylation of **27** with 2-bromobenzylaldehyde provided *o*-bromo analogue **30** that served as a useful intermediate for further functionalization. Microwave-assisted Suzuki coupling employing commercially available aryl boronic acids in the presence of dichlorobistriphenylphosphinepalladium yielded substituted 2-phenylbenzyl analogues **31**−**40** in good yields. Hydrolysis of ethyl esters **28**, **29**, and **31**−**40** followed by coupling of the resulting acids to **4** yielded **23i**−**s** and **2** (Scheme 6).

Scheme 1. Synthesis of 4-Alkyl-4-methoxypiperidine Analogues[a]



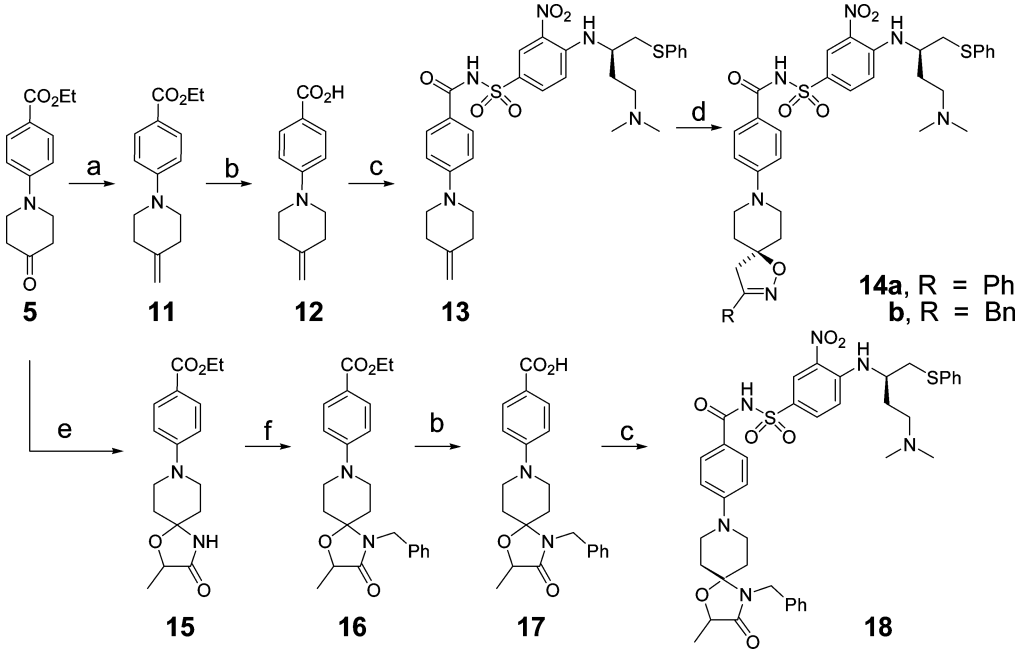
[a] Reagents and conditions: (a) RMgX, Et_2O/THF; (b) NaH, MeI, THF/HMPA, 50 °C; (c) LiOH, H_2O, THF, MeOH, 50 °C; (d) **4**, EDCI, DMAP, CH_2Cl_2.

Scheme 2. Synthesis of 4-Benzylidenepiperidine Analogues[a]



[a] Reagents and conditions: (a) $ArCH_2PPh_3Br$, NaH, DMSO, 80 °C; (b) 1 N aq NaOH/dioxane, 90 °C; (c) **4**, EDCI, DMAP, CH_2Cl_2.

Scheme 3. Synthesis of Spirocyclic Isoxazoline and Oxazolidin-4-one Analogues[a]



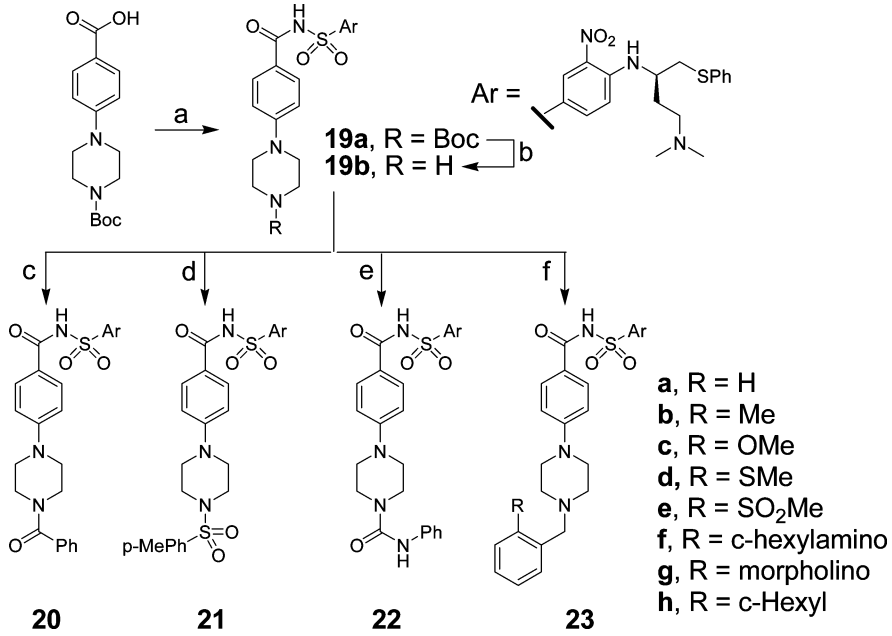
[a] Reagents and conditions: (a) $PPh_3CH_3{}^+I^-$, BuLi, THF; (b) LiOH/THF/EtOH/water; (c) **4**, EDCI, DMAP, CH_2Cl_2; (d) *N*-hydroxybenzimidoyl chloride or phenylacetohydroximoyl chloride, Et_3N; (e) (±)-lactamide, p-TSA; (f) NaH, benzyl bromide.

Compounds containing the 1,1-dimethyl-2-phenylthioethylamine side chain used in structural studies were prepared by the two-step process outlined in Scheme 7. Nucleophilic aromatic substitution of 4-fluoro-3-nitrobenzenesulfonamide with 2-amino-2,2-dimethylethyl phenyl thioether **41**[26] yielded **42** which was subsequently coupled to the indicated benzoic acids to provide **1b**, **43a**, and **43b**.

Results and Discussion

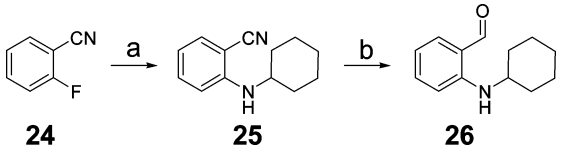
Rationale. The ability to access an additional Bcl-2 hydrophobic binding pocket was confirmed when an NMR-derived structure of the benzothiazole analogue **3** (Figure 3a) bound to Bcl-2 revealed deep penetration of its phenethyl side chain into the Bcl-2 groove[21] (Figures 3c). Interestingly, the NMR-derived structure of the analogous Bcl-xL complex (Figures 3b) shows

Scheme 4. Synthesis of Various 4-Substituted-*N*-phenylpiperazine Analogues[a]



[a] Reagents and conditions: (a) **4**, EDCI, DMAP, CH_2Cl_2; (b) 4 M HCl, dioxane; (c) BzCl, Et_3N, DMA; (d) TosCl, Et_3N, DMA; (e) PhNCO, Et_3N, DMA; (f) ArCHO, RBH_3CN, MeOH, CH_2Cl_2.

Scheme 5. Synthesis of 2-(Cyclohexylamino)benzaldehyde, **26**[a]



[a] Reagents and conditions: (a) c-hexylamine, DMSO, microwave, 180 °C, 15 min; (b) DIBAL, toluene.

3 to adopt an extended conformation lying along the hydrophobic surface of the protein. This suggested that substitution of our acylsulfonamide inhibitors at the site-1 terminus had the potential to enhance binding affinity to Bcl-2 while not adversely affecting affinity to Bcl-xL.

Although this served as a proof of principle, benzothiazole analogues akin to **3** lacked sufficient biological activity to pursue as a viable lead series. To explore the possibility of utilizing the structural scaffold found in **1a**, the most advanced lead identified in our previous study, an NMR-derived structure of **1b** bound to Bcl-2 was determined. The average-minimized structure is shown in Figure 3d. Compound **1b** (Figure 2) is a close structural analogue of **1a** in which the basic side chain has been removed and the 1,1-dimethyl-2-phenylthioethanamine moiety was incorporated at site-3. These modifications typically rendered this class of compounds more amenable to structural study. As shown in Figure 3d, the positioning of the terminal piperidine ring of **1b** within the groove is quite similar to that of the benzothiazole ring of **3**. We surmised that the 4-position of the piperidine ring of **1a** might offer a surrogate platform from which to further probe Bcl-2 binding with minimal change to the overall structure of **1a**.

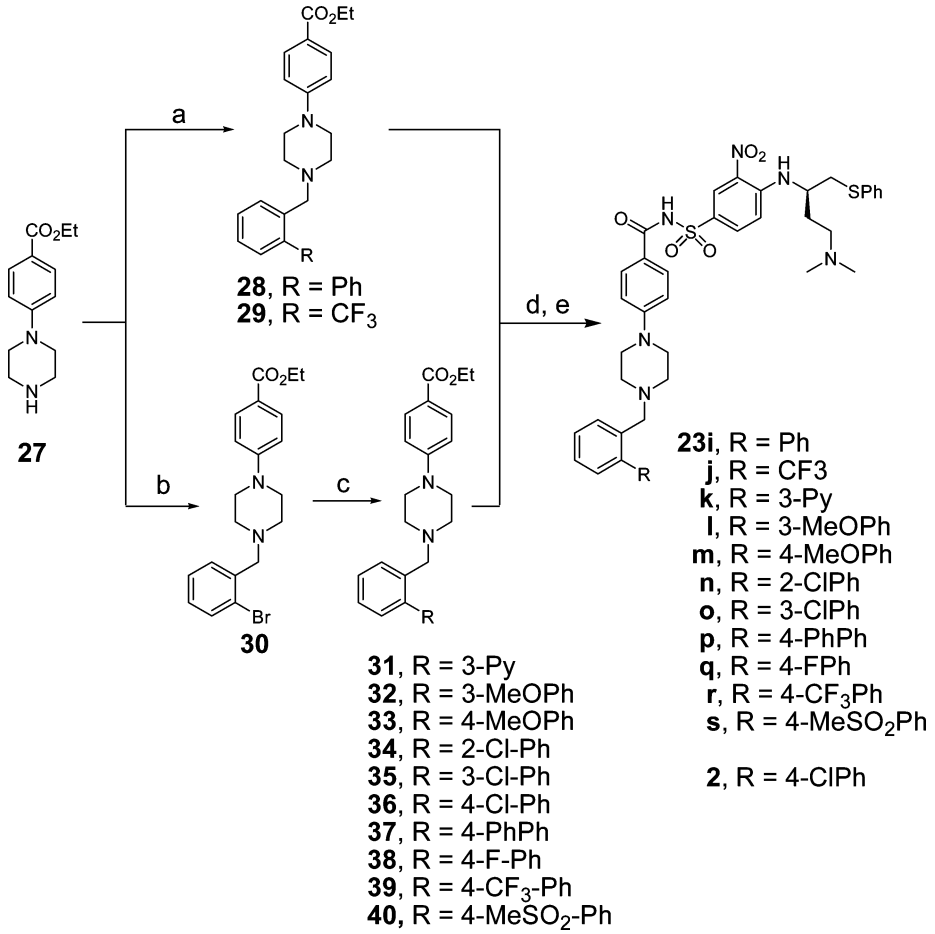
Structure−Activity Relationships. The binding affinities (K_i) of compounds were determined using fluorescence polarization assays (FPA) that measure their ability to competitively displace a Bad-derived peptide from Bcl-xL, and a Bax-derived peptide from Bcl-2 as described in the Experimental Section. Our previous studies identified the tendency for biarylacylsulfonamide inhibitors to bind serum albumin. Therefore, to assess the potential of serum components to attenuate compound activity, binding affinities for Bcl-xL were also obtained in the presence of 10% human serum. Compound efficacy in a cellular context was evaluated by testing their ability to reverse the protection from cytokine withdrawal afforded by overexpression of Bcl-xL and Bcl-2 in the IL-3 dependent murine pro-B cell line FL5.12. To examine the serum effect in this context, cellular assays were conducted both in the presence and absence of 3% fetal bovine serum for the most potent compounds.

We initially sought to survey a variety of piperidine and piperazine structural motifs bearing simple hydrophobic substituents at their 4-positions in order to identify those with appropriate trajectory and/or rigidity to enhance Bcl-2 affinity while maintaining Bcl-xL affinity. For the most direct compound comparison, all analogues prepared contained the site-2 - site-3 (*R*)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide found in **1a**. Table 1 summarizes the activities of four piperidine and four piperazine scaffolds that were initially sampled.

4-Substituted piperidines provided an attractive template because their C-2 symmetry allows the potential for two different substitution vectors without introduction of an additional chiral center. The solvent-exposed nature of the binding groove should allow one substituent access to the protein surface while the other projects toward solvent. Of the piperidines described in Table 1, 4-benzyl-4-methoxy analogue **8e** is the most structurally analogous to **1a.** Replacement of the terminal methyl groups of **1a** with benzyl and methoxy groups to give **8e** resulted in an increase in both Bcl-2 binding affinity and efficacy in Bcl-2 overexpressing FL5.12 cells. Bcl-xL activity of **8e** was similar to that of **1a**.

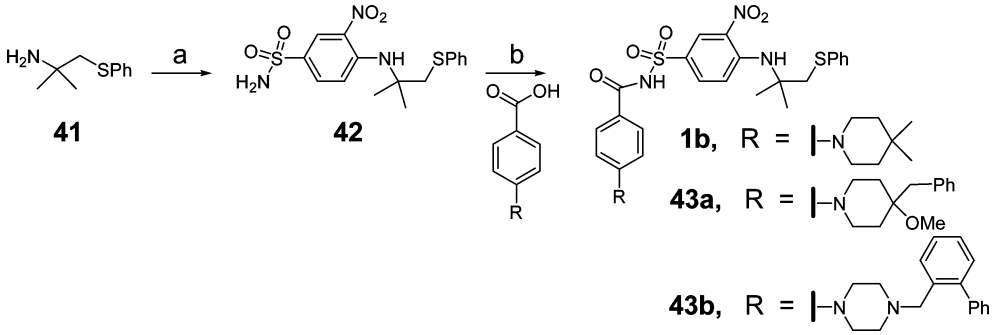
To confirm our working hypothesis of the binding orientation of these compounds, NMR-derived structures for the site-3 *gem*-dimethyl analogue **43a** bound to both Bcl-2 and Bcl-xL were determined. The average-minimized structures are depicted in Figures 4a and 4b. When bound to Bcl-2, the piperidine ring of **43a** adopts a chair conformation similar to that observed for **1b** when bound to Bcl-2. It projects a pseudoequatorial benzyl group that makes extensive contact with hydrophobic residues deep in the binding groove (Figure 4a). This binding orientation

Scheme 6. Synthesis of Substituted 2-Aryl-benzylpiperazine Analogues[a]



[a] Reagents and conditions: (a) $ArCH_2Br$, Et_3N, dioxane; (b) ArCHO, $NaBH(OAc)_3$, 1,2-dichloroethane; (c) $ArB(OH)_2$, Na_2CO_3, $PdCl_2(PPh_3)_2$, microwave, 150 °C; (d) LiOH, H_2O, MeOH, THF; (e) **4**, EDCI, DMAP, CH_2Cl_2.

Scheme 7. Synthesis of Compounds Containing the 1,1-Dimethyl-2-phenylthio Ethylamine Site-3 Used in Structural Studies[a]



[a] Reagents and conditions: (a) 4-fluoro-3-nitrobenzenesulfonamide, DMSO, DIEA, rt; (b) EDCI, DMAP, CH_2Cl_2.

is similar to that observed for benzothiazole **3** when bound to Bcl-2. When bound to Bcl-xL, the piperidine ring of **43a** exists in a chair conformation with a pseudoaxial benzyl group (Figure 4b) that permeates the hydrophobic surface to a much greater extent than the phenethyl group of benzothiazole **3**. Although not as dramatic as for Bcl-2, this suggests at least some capacity for the Bcl-xL groove to accommodate deep-binding hydrophobic substitution. The methoxy group of **43a** is solvent-exposed when bound to both proteins and presumably contributes little to binding energy.

In this initial study, we also explored the effect of scaffold rigidification by introduction of an sp^2 benzylidene linkage or by incorporation of spirocyclic isoxazoline or oxazolidin-4-one rings. Benzylidene **10a** displayed a 20-fold increase in Bcl-2 binding affinity relative to **1a** while maintaining subnanomolar affinity for Bcl-xL. This also translated into improved cellular efficacy. While the substituted spirocyclic analogues **14a**, **14b**, and **18** either maintained or increased Bcl-2 binding affinity, all but **14b** lost measurable cellular activity (Table 1).

Replacement of the piperidine ring of **1a** with piperazine was examined as a simple isosteric replacement that offered a more synthetically tractable core for SAR development. Our initial goal was to examine the effect of aryl substitution tethered to the piperazine nitrogen via a variety of linkers. Benzyl (**23a**), benzoyl (**20**), and tosyl (**21**) substitution yielded compounds with Bcl-2 affinities similar to or better than that of **1a**. *N*-Phenylurea **22** was significantly less active (K_i = 300 nM). However, when tested in FL5.12 cells only the benzyl derivative **23a** maintained cellular efficacy, while the three analogues with heteroatom-containing linkers exhibited little to no cellular

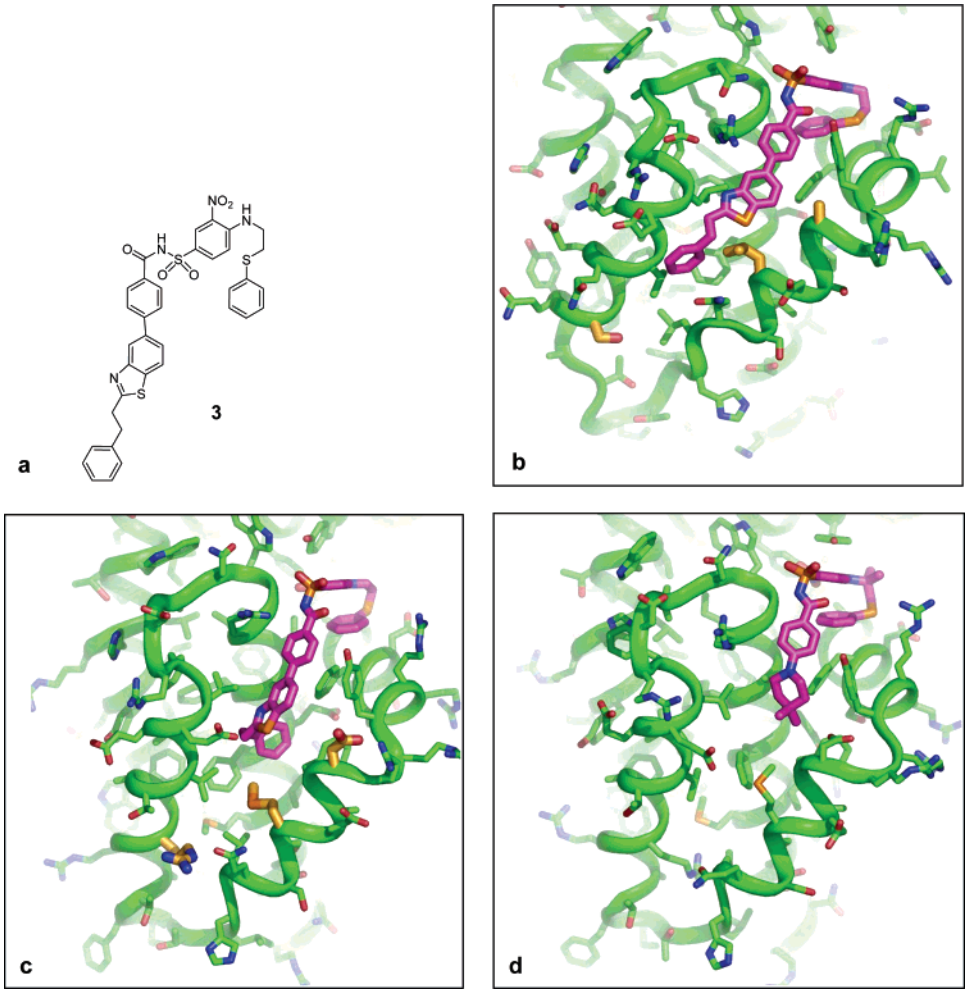


Figure 3. (a) Structure of benzothiazole inhibitor **3**. (b) NMR-derived structure of **3** bound to Bcl-xL. Ala104, Leu108, and Ser122 are highlighted in yellow (PDB code 2O1Y). (c) NMR-derived structure of **3** bound to Bcl-2. Asp104, Met108, and Arg122 are highlighted in yellow (PDB code 2O21). (d) NMR-derived structure of **1b** bound to Bcl-2 (PDB code 2O22). For all structures, protein backbone and residue sidechains are depicted in green with the α3 and α4 helices emphasized.

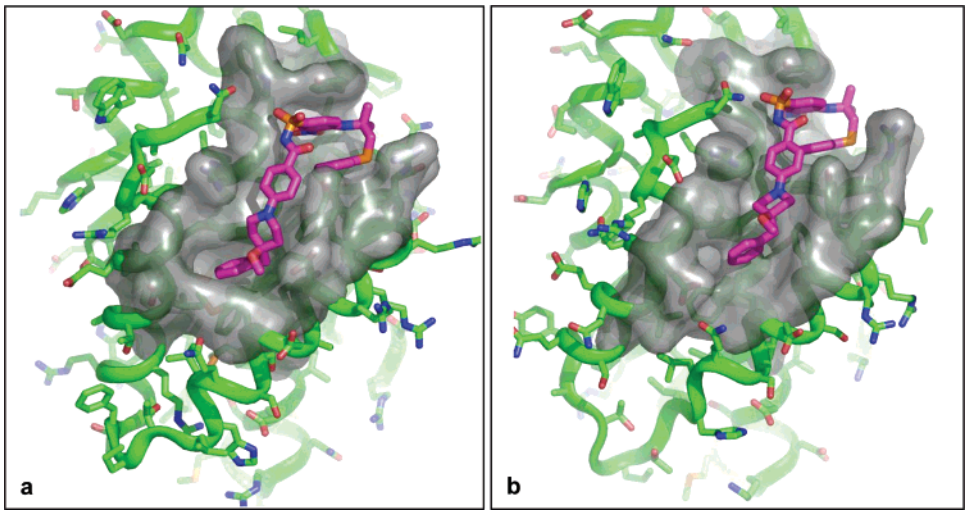
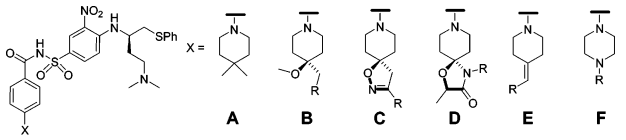


Figure 4. (a) NMR-derived structure of **43a** bound to Bcl-2 (PDB code 2O2F). (b) NMR-derived structure of **43a** bound to Bcl-xL (PDB code 2O2M). Protein backbone and residue sidechains are depicted in green with the α3 and α4 helices emphasized. The solvent exposed surface of residue sidechains making up the binding groove surrounding the ligand are highlighted in gray.

activity. On the basis of these data, we identified 4-methoxy-4-benzylpiperidines, 4-benzylidenepiperidines, and benzylpiperazines as the most promising series to explore in more depth.

Given the close structural similarity between the three chemical series, we focused first on development of the 4-methoxy-4-alkylpiperidine SAR with the hope of extending our findings to the other scaffolds. A detailed SAR of the 4-disubstituted piperidines is outlined in Table 2. A progressive increase in steric bulk of the terminal substituent (**1a**, **8a**−**e**) resulted in a corresponding increase in Bcl-2 affinity. A survey

Table 1. SAR of Various 4-Substituted Piperidine and Piperazine Structural Scaffolds



			FPA		FL5.12 cells	
			K_i (nM)[a]		EC_{50}, [uM][a]	
	X	R	Bcl-2	Bcl-xL	Bcl-2	Bcl- xL
1a	A	---	67 ± 6	0.8 ± 0.2	2.2 ± 0.15	0.47 ± 0.05
8e	B		8.1	1.8 ± 0.3*	0.93 ± 0.37*	0.68 ± 0.10*
14a	C		35.2 ± 4.3*	4.4 ± 0.3*	>50*	>50*
14b	C		6.5 ± 0.2*	1.7 ± 0.4*	2.93 ± 0.02*	2.34 ± 0.08*
18	D		9.6 ± 1.0*	3.7 ± 1.1*	>50	>20
10a	E		3.4 ± 1.4*	< 0.5	0.60	0.38 ± 0.13*
23a	F		39.8	2.6	1.7 ± 0.1	1.1 ± 0
20	F		61.9 ± 3.9*	<1	> 50*	14.6 ± 2.5*
21	F		6.1	4.0	> 20	> 20
22	F		300 ± 27*	2.5 ± 0.3*	> 50*	> 50*

[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate.

of chlorophenyl positional isomers (**8f**−**h**) revealed a preference for ortho-substitution (**8f**) that gave subnanomolar affinities for both Bcl-2 and Bcl-xL and significantly enhanced affinity in the presence of human serum. The very potent and balanced binding affinity of **8f** also translated into balanced cellular potency with EC_{50} values of 0.23 μM and 0.18 μM in Bcl-xL and Bcl-2 transfected cell lines, respectively. Evaluation of a series of ortho-substituted and ring fused analogues identified a phenyl group as the optimum ortho-substitutent. 4-(Biphenyl-2-ylmethyl)-4-methoxypiperidine analog, **8m**, maintains high target affinities that is below the detection limit of the FPA both in the absence and presence of added serum. In addition, **8m** possesses cellular EC_{50} values (0.035 μM and 0.020 μM in Bcl-xL and Bcl-2 transfected cell lines, respectively) that are approximately 10-fold and 100-fold more potent than the parent phenylpiperidine, **1a**.

Incorporation of a benzylidene at the piperidine 4-position imparts rigidity to the system, and we speculated that 1,3-allylic strain inherent to this structure would force the aromatic ring out of plane and adopt a similar binding orientation to that of the 4-benzyl-4-methoxypiperidines. Table 3 shows the SAR of subsitituted 4-benzylidenepiperidine analogues. Again, there is a significant preference for ortho- over para-substitution (**10c** vs **10d**, **10e** vs **10f**) in cellular assays. Interestingly, neither 2-pyridyl (**10i**) nor 3-pyridyl (**10j**) substitution was well tolerated. As in the 4-benzyl-4-methoxy series, 2-aryl substitution was optimal to give balanced and potent binding affinity that was superior in the presence of added human serum. Although **10k** possesses balanced cellular activity with EC_{50} values of 0.18 μM and 0.16 μM for Bcl-2 and Bcl-xL, respectively, this is 5-fold to 10-fold less potent than that of the corresponding 4-methoxy-4-benzyl analogue **8m**.

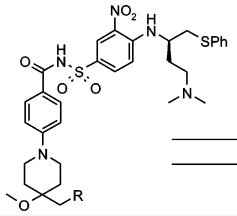
The ability of an *N*-phenylpiperazine group to adopt a similar conformation to *N*-phenylpiperidine led us to employ a scaffold-hopping strategy. This allowed us to focus on the SAR of ortho-substituted *N*-benzylpiperazine derivatives. Table 4 summarizes the activity of a variety of 1-(2-substituted benzyl)-4-phenylpiperazine derivatives. With the exception of the methylsulfone **23e**, all 2-substituted benzyl analogues exhibited significantly improved Bcl-2 affinity and enhanced cellular efficacy in Bcl-2 overexpressing cells compared to the unsubstituted parent compound, **23a**. Both nonpolar (alkyl, aryl) and polar (cyclohexylamine, morpholine, pyridyl) substituents were well tolerated with **23d**, **23g** and **23i** showing affinities below limits of detection in all binding assays. The biphenyl-2yl-methylpiperazine analogue **23i**, however, exhibited significantly enhanced cellular potency relative to other analogues in Table 4 that is 50-fold (Bcl-xL) to 100-fold (Bcl-2) greater than that of the *N*-benzyl analogue **23a**.

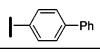
Further exploration of the SAR associated with terminal biphenyl ring substitution (Table 5) reveals that nonpolar substitution is well tolerated in the para-position. With the exception of the methylsulfone **23s**, all the para-substituted analogues exhibited cellular activities in the absence of serum similar to that of the unsubstituted biphenyl **23i**. However, when tested in the presence of added serum the *p*-chloro analogue **2** clearly shows superior cellular efficacy with EC_{50} values of 0.05 μM and 0.22 μM for Bcl-2 and Bcl-xL overexpressing cells, respectively, corresponding to a 5-fold to 25-fold greater potency than the unsubstituted biphenyl **23i**. This improved cellular activity may well be the result of decreased interaction with serum components as opposed to increased affinity of **2** for Bcl-2 family proteins. Nonetheless, compound **2** exhibits a >20-fold (Bcl-xL) and >250-fold (Bcl-2) improvement in cellular efficacy compared to our starting compound **1a**.

To better understand the origin of the improved activities imparted by biphenyl substitution, we determined the NMR-derived structure of site-3 *gem*-dimethyl analogue **43b** bound to Bcl-xL. The average minimized structure is shown in Figure 5b where **43b** (magenta) is bound to the hydrophobic groove of Bcl-xL depicted as an electrostatic potential surface. The piperazine ring adopts a chairlike conformation with its hydrophobic substituent in a pseudoaxial orientation similar to that of the piperidine of **43a** bound to Bcl-xL. However, the 2-substituted biphenyl group forces the biphenyl A-ring (Figure 5a) to stack under the piperazine making extensive hydrophobic contacts deep within the binding groove. This allows the biphenyl B-ring to more effectively occupy the pocket that had been only partially occupied by the benzyl group of **43a**. Although we were unable to generate sufficient data to determine the structure of the analogous Bcl-2 complex, we believe a similar binding arrangement is in operation when **43b** binds Bcl-2.

One of the striking features of this ligand-bound complex is the extent to which **43b** binds deeply in the groove and is enveloped by the protein. When compared to the unbound protein structure,[10] this represents a sizable conformational change of the protein upon ligand binding. In fact, the BH3 binding groove of Bcl-xL is not readily apparent on its surface when in an unbound state. In addition, the bound protein conformation of Bcl-xL depicted in Figure 5 is quite different than that when bound to a peptide derived from an endogenous BH3 only binding partner, Bad. In this instance, the protein binding groove is held in a much more open conformation while the peptide spans nearly the entire surface of the protein.[15] These observations dramatically highlight the importance of the protein dynamics in these binding interactions. Moreover, they illustrate the need for consideration of protein dynamics in inhibitor design as well as potential pitfalls of design against a static target.

Table 2. SAR of 4-Alkyl-4-methoxypiperidine Derivatives



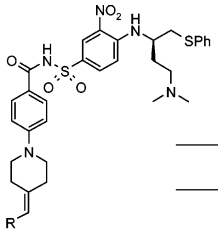
		FPA			FL5.12 cells	
		K_i (nM)[a]		10% HS IC_{50}, (nM)	EC_{50}, [uM][a]	
	R	Bcl-2	Bcl-xL	Bcl-xL	Bcl-2	Bcl-xL
1a	NA	67 ± 6	0.8 ± 0.2	360 ± 67	2.2 ± 0.15	0.47 ± 0.05
8a		56 ± 4*	6.1 ± 1.2*	360	0.7	0.58 ± 0.18*
8b		16	4.6	440	1.0	0.75 ± 0.15
8c		20 ± 2*	1.3	312	3.0	8.0
8d		6.6	0.8 ± 0.2	300	0.2 ± 0	0.32 ± 0.07*
8e		8.1	1.8 ± 0.3	150	0.93 ± 0.37*	0.68 ± 0.10*
8f		< 1	< 0.5	62	0.18 ± 0.05*	0.23 ± 0.09*
8g		1.8 ± 0.4	< 0.5	170	0.3 ± 0.0	0.25 ± 0.05
8h		2.7 ± 0.2	0.9	200	0.5 ± 0.1	0.4 ± 0.0
8i		3.1 ± 0.8*	0.9 ± 0.1*	79	0.10 ± 0.0	0.18 ± 0.04*
8j		1.4 ± 0.3*	<0.5	71	0.2	0.15 ± 0.05
8k		1.6 ± 0.6	1.1	75	0.25 ± 0.05	0.15 ± 0.05
8l		1.5 ± 0.5	< 0.5	< 60	0.2 ± 0.0	0.1 ± 0.0
8m		< 1	< 0.5	< 60	0.02 ± 0.00	0.035 ± 0.005
8n		< 1	0.9	160	1.1 ± 0.2	0.6 ± 0.1

[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate.

Activity in Human Tumor Cell Lines. The goal of this study was specifically to enhance the activity of our biarylacylsulfonamide inhibitors against Bcl-2. Indeed, the best compound identified (**2**) shows several orders of magnitude greater efficacy than our starting prototype compound (**1a**) against a murine pro-B cell line engineered to overexpress Bcl-2. To better examine the potential relevance to human disease, we examined the activity of compounds against human tumor cell lines derived from patients with follicular lymphoma. Bcl-2 is known to be highly overexpressed in follicular lymphoma due to the presence of a t(14;18) chromosomal translocation that is thought to be the initiating genetic lesion in these tumors.[27] This translocation places the bcl-2 gene of the 18q21 chromosomal region under the transcriptional control of the immunoglobulin heavy chain gene (IgH) region. Linkage to the expression of such a ubiquitously expressed protein induces a massive overexpression of the Bcl-2 protein. We chose to examine the activity of **2** compared to **1a** in three human tumor cell lines, DoHH2, SUDHL-4, and RS11380 that are known to harbor the t(14;18) translocation, express high levels of Bcl-2 and low levels of Bcl-xL by western blot analysis (data not shown). As shown in Table 6, in 10% human serum **1a** shows no cellular activity up to 30 μM whereas **2** possess EC_{50} values <1 μM against all three cell lines. When examined under less stringent conditions employing 3% fetal bovine serum (FBS), **1a** does exhibit measurable efficacy with EC_{50} values in the low micromolar range. Under these conditions, **2** is significantly more efficacious exhibiting low nanomolar EC_{50} values against all cell lines.

In Vivo Evaluation. We next evaluated **2** for efficacy in a murine established tumor xenograft model. As the most rigorous test of the compound, we chose to evaluate SUDHL-4, the least sensitive follicular lymphoma cell line described above. For comparison we used the topoisomerase II inhibitor etoposide. This agent has demonstrated clinical efficacy in non-Hodgkin's lymphoma as both a single agent and as part of combination regimens.[28−30] A quantity of 3×10^6 SUDHL-4 cells was inoculated subcutaneously in the flank of male *scid* mice, and the tumors were allowed to grow to an average size of 225 mm^3 prior to initiation of therapy. Twice daily treatment with 50 mg/kg of **2** for 21 consecutive days was well tolerated and achieved 60−65% tumor growth inhibition during therapy prior to tumor rebound (Figure 6). This effect is similar to that observed for the maximum tolerated dose (MTD) and schedule of etoposide.

Table 3. SAR of 4-Piperidinebenzylidene Derivatives



		FPA			FL5.12 cells	
		K_i (nM)[a]		10% HS IC_{50} (nM)[a]	EC_{50}, [uM][a]	
	R	Bcl-2	Bcl-xL	Bcl-xL	Bcl-2	Bcl-xL
1a	NA	67 ± 6	0.8 ± 0.2	360 ± 67	2.2 ± 0.15	0.47 ± 0.05
10a		3.4 ± 1.4*	< 0.5	520	0.60	0.38 ± 0.13*
10b		2.5	< 0.5	750	0.35 ± 0.05	0.25 ± 0.05
10c		0.7	< 0.5	700	0.45 ± 0.05	0.35 ± 0.05
10d		2.1	< 0.5	510	1.18 ± 0.08	0.62 ± 0.09
10e		2.0	< 0.5	300	0.21 ± 0.06*	0.15 ± 0.03*
10f		9.4	1.4	1300	3.6 ± 0.6	2.6 ± 0.6
10g		2.0	0.6	760	0.25 ± 0.06	0.30 ± 0.06
10h		13	< 0.5	520	1.6 ± 0.4	1.10 ± 0.05
10i		46	3.6	700	1.38 ± 0.08	0.55 ± 0.05
10j		68	1.3	2800	5.3 ± 1.8	1.9 ± 0.8
10k		< 1	< 0.5	83	0.18 ± 0.03	0.16 ± 0.05

[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate.

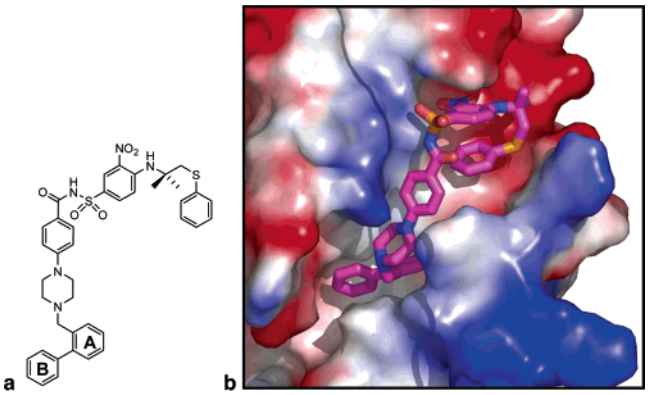


Figure 5. (a) Chemical structure of **43b** showing biphenyl nomenclature. (b) NMR-derived structure of **43b** bound to Bcl-xL (PDB code 2O2N). The solvent accessible surface of the protein is shown with coloration according to electrostatic potential.

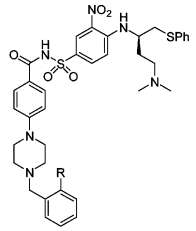
Combination of etoposide and **2** produced additive efficacy with up to 90% tumor growth inhibition during therapy and increased tumor growth delay compared to either agent alone.

Conclusions

We describe here the use of structure-guided design to develop the first potent, dual inhibitors of Bcl-xL and Bcl-2 with subnanomolar target affinities. Three distinct structural series were identified that utilized 4-substituted *N*-phenylpiperidine or *N*-phenylpiperazine templates to access a previously under utilized hydrophobic binding pocket deep in the Bcl-2 binding groove. This study culminated in the identification of **2**, which exhibited >250-fold and >20-fold greater efficacy in cells reliant on Bcl-2 and Bcl-xL, respectively, for survival compared to previously disclosed biarylacylsulfonamide inhibitors. We show that this improved activity also translates to enhanced efficacy against human follicular lymphoma cell lines that massively upregulate Bcl-2, and to efficacy in a murine xenograft model of lymphoma when given as both a single agent and in combination with etoposide. We have also previously reported the activity of **2** in primary patient-derived samples of follicular lymphoma and chronic lymphocytic leukemia (CLL),[21] both of which are characterized by high Bcl-2 expression. Although **2** is a balanced inhibitor of both Bcl-2 and Bcl-xL compared to the relatively selective Bcl-xL inhibitor **1a**, as outlined above **2** is also significantly more potent against each. Therefore, it is not known whether the increased efficacy of **2** compared to **1a** is due to its improved binding profile, or simply improved potency. True delineation of the contribution to efficacy by inhibition of each of these targets will ultimately require the development of potent and selective inhibitors of each antiapoptotic Bcl-2 family protein.

While our working hypothesis for accessing a deep hydrophobic binding pocket in the Bcl-2 groove was confirmed

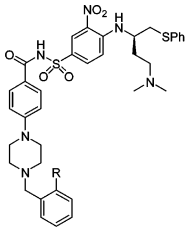
Table 4. SAR of 2-Substituted *N*-Benylpiperazine Derivatives

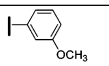
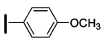
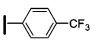
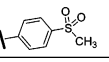


		FPA			FL5.12 cells	
		K_i (nM)[a]		10%HS IC_{50}, (nM)	EC_{50}, [uM][a]	
	R	Bcl-2	Bcl-xL	Bcl-xL	Bcl-2	Bcl-xL
1b	NA	67 ± 6	0.8 ± 0.2	360 ± 67	2.2 ± 0.15	0.47 ± 0.05
23a	-H	39.8	2.6	350 ± 180	1.7 ± 0.1	1.1 ± 0
23b	$-CH_3$	4.5	3.0	140	0.94 ± 0.37	0.41 ± 0.11
23j	$-CF_3$	< 1	< 0.5	85	0.13	0.39 ± 0.25*
23c	$-OCH_3$	3.2	< 0.5	80	0.95	0.39 ± 0.14
23d	$-SCH_3$	< 1	< 0.5	< 60	0.13 ± 0.025	0.095 ± 0.045
23e	$-SO_2CH_3$	4.6	0.9	170	6.8 ± 2.9	5.5 ± 2
23f		3.5	0.6	370	0.34 ± 0.09*	0.66 ± 0.015
23g		< 1	< 0.5	< 60	0.46 ± 0.17	0.43 ± 0.1
23h		< 1	< 0.5	61	0.12 ± 0.022	0.34 ± 0.11
23i		< 1	< 0.5	< 60	0.016 ± 0.004	0.018 ± 0.004
23k		1.0	< 0.5	81	0.82 ± 0.025	0.8 ± 0.21

[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate.

Table 5. SAR of 2-Aryl-substituted *N*-Benylpiperazine Derivatives



		FPA			FL5.12, EC_{50}, [uM][a]			
		K_i (nM)[a]		10%HS IC_{50}, (nM)	Bcl-2		Bcl-xL	
	R	Bcl-2	Bcl-xL	Bcl-xL	gel	3% FBS	gel	3% FBS
1a	NA	67 ± 6	0.8 ± 0.2	360 ± 67	2.2 ± 0.15	13 ± 0.79	0.47 ± 0.05	5.1 ± 0.53
23i		< 1	< 0.5	< 60	0.016 ± 0.004	1.3 ± 0.37	0.018 ± 0.004	1.2 ± 0.52
23l		< 1	< 0.5	178	0.46 ± 0.14	3.3	0.38 ± 0.01	4.5
23m		< 1	< 0.5	< 60	0.025 ± 0.012	0.39 ± 0.15*	0.03 ± 0.013*	0.7 ± 0.17*
23n		< 1	< 0.5	< 60	0.027 ± 0.0001*	1.9 ± 0.63*	0.056 ± 0.009*	2.5 ± 0.57*
23o		16.9	< 0.5	140	0.033	1.1	0.18 ± 0.02	2.8
2		< 1	< 0.5	< 60*	0.008 ± 0.002*	0.050 ± 0.015*	0.030 ± 0.0088*	0.22 ± 0.05*
23p		< 1	1	100	0.068 ± 0.031*	0.36 ± 0.16	0.022	0.12 ± 0.068
23q		< 1	< 0.5	< 60	0.027	0.66 ± 0.34	0.13 ± 0.055	0.71
23r		< 1	< 0.5	< 60	0.013	0.17 ± 0.025	0.04 ± 0.016*	0.12
23s		< 1	9.1[b]	< 60	0.53 ± 0.04	>20	0.46 ± 0.02	6.7

[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate. [b] IC_{50} value.

Table 6. Efficacy of **1a** and **2** in Follicular Lymphoma Cell Lines That Highly Overexpress Bcl-2

		EC_{50}, [μM][a]	
cell lines	conditions	**1a**	**2**
DoHH2	3% FBS[b]	7.25 ± 0.18	0.0083 ± 0.0003
	10% HS[c]	>30	0.13 ± 0.01
RS11380	3% FBS	10.85 ± 1.55*	0.014 ± 0.004
	10% HS	>30	0.15 ± 0.003
SUDHL-4	3% FBS	5.25 ± 1.94*	0.22 ± 0.09
	10% HS	>30	0.85 ± 0.14

[a] Mean values ± sem for three or more experiments run in duplicate; *mean values ± standard deviation for two experiments run in duplicate. [b] FBS = fetal bovine serum. [c] HS = human serum.

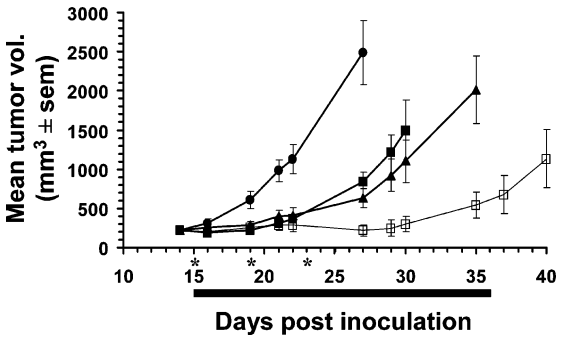


Figure 6. Effect of **2**, etoposide, or the combination of **2** and etoposide in SUDHL-4 xenograft models of lymphoma. Compound **2** (■)was administered ip twice daily at 50 mg/kg on days 15−36 (black bar). Etoposide (▲) was administered at 20 mg/ kg ip on days 15, 19, and 23 (asterisks). (□) The combination of **2** and etoposide. (●) Represents mice treated with combination vehicles. Compound **2** and etoposide monotherapies showed significant inhibition of tumor growth relative to vehicle controls from day 19 onward ($P < 0.02$, Wilcoxon Rank Sum test). The combination therapy showed significant tumor growth inhibition relative to monotherapy treatment from day 27 onward ($P < 0.01$).

throughout this study, we were surprised to discover that a similar binding mode was in operation for analogues bound to Bcl-xL. We have observed that the conformation of the Bcl-xL protein is quite different in its bound and unbound state. Moreover, the conformation of Bcl-xL bound to an endogenous BH3 peptide is distinctly different than its conformation when bound to **43b**, yet both ligands are functionally similar. It is reasonable to speculate that future, structurally novel inhibitors of Bcl-2 family proteins will utilize previously unobserved protein conformations. Whether this is due to an 'induced fit'[31] or 'conformational ensemble'[32] binding phenomena, this study provides another striking example of the importance of protein conformational flexibility in ligand binding.[33] However, traditional molecular modeling, virtual screening, and 'rational' drug design approaches typically treat proteins as static models, utilizing structures of either native unbound protein or high affinity ligand/protein complexes. The future success in targeting such conformationally mobile protein targets should be significantly enhanced by the development of computational approaches that take into account the protein's conformational flexibility and allow iterative design against a dynamic target.

Experimental Section

General Methods. All reactions were carried out under inert atmosphere (N_2) and at room temperature unless otherwise noted. Solvents and reagents were obtained commercially and were used without further purification. All reported yields are of isolated products and are not optimized. ^{1}H NMR spectra were obtained on a Varian UNITY or Inova (500 MHz), Varian UNITY (400 MHz), or Varian UNITY plus or Mercury (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the usual manner. Mass spectra determinations were performed by the Analytical Research Department, Abbott Laboratories; DCI indicates chemical ionization in the presence of ammonia, ESI indicates electron spray ionization, and APCI indicates atmospheric pressure chemical ionization with ammonia. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Column chromatography was carried out in flash mode on silica gel (Merck Kieselgel 60, 230−400 mesh). Unless otherwise noted, preparative HPLC samples were purified on a Zorbax Stable Bond C18 column (21.4 × 250 mm, 7 μm particle size) using a gradient of 20−100% CH_3CN/water/0.1% TFA over 45 min at a flow rate of 15 mL/min. HPLC purifications were performed in high-throughput format and the isolated products concentrated in parallel under unoptimized conditions using a Savant Speed Vac concentrator to provide the final products as hydrated trifluoroacetic acid salts.

Protein Preparation. A previously described loop-deleted version of Bcl-xL which lacked the putative transmembrane helix was employed for NMR studies and biological assays. The Bcl-2 protein used was a chimera based on isoform 2 (A96T and G110R) in which residues 35−91 were replaced with residues 35−50 from Bcl-xL, and the C-terminal end (residues 208−219) was excised. For both Bcl-xL and Bcl-2, uniformly ^{15}N-labeled and ^{15}N-,^{13}C-labeled protein was expressed in *E. coli* containing the appropriate plasmid, on minimal media containing ^{15}N-labeled ammonium chloride as the sole nitrogen source with or without ^{13}C-labeled glucose as the sole carbon source. Proteins were purified by affinity chromatography on a Nickel-ProBond column (Invitrogen), concentrated, and exchanged into 40 mM disodium phosphate buffer, pH 7.0, containing either 10% or 100% D_2O plus 5 mM deuterated dithiothreitol. Protein samples for NMR were 0.5−1.0 mM in microcells. Ligands were added to the protein from concentrated (100 mM) stock solutions prepared in DMSO-d_6.

NMR-Based Structural Studies. NMR spectra for structural studies were recorded on Bruker DRX600 and DRX800 spectrometers at 303 K for Bcl-xL and 298 K for Bcl-2. Protein solutions were prepared at pH 7.0 in 10 %D_2O. Ligands were added as concentrated stock solutions in deuterated DMSO to achieve a one to one ratio of ligand to protein. Although these ligands display poor aqueous solubility, they were highly soluble in these studies in the presence of protein. Resonance assignments for ligand-bound Bcl-xL and Bcl-2 were extrapolated from those of the respective apo proteins by comparing two-dimensional ^{13}C- and ^{15}N-HSQC spectra and three-dimensional ^{13}C-edited and ^{15}N-edited NOESY spectra of the liganded to the unliganded protein. An example of the HSQC spectra for Bcl-xL in the presence and absence of compound **2** is provided in Figure S1. Protein−ligand NOEs were extracted from three-dimensional ^{13}C-edited, ^{12}C-filtered NOESY spectra recorded with mixing times ranging from 150 to 250 ms.

Structure Calculations. For both Bcl-xL and Bcl-2, the program CNX was used for all structure calculations.[34] Ligands were first positioned randomly near the binding groove of the protein, and the observed intermolecular NOEs were used to dock the ligands into the groove. This docking was followed by energy minimization and a standard simulated annealing protocol.[35] During the simulated annealing, coordinates of the protein were held fixed with the exception of those residues that line the binding groove (residues 96−112, 127−142, 191−194). This protocol was based on our structural studies of Bak peptide binding to Bcl-xL for which we observed structural rearrangements only for residues lining the hydrophobic groove upon peptide binding.[14] For compound **3** bound to Bcl-xL, a total of 66 intermolecular NOEs were used to dock the ligand to the protein, whereas for Bcl-2, a total of 86 NOEs were employed. Docking of compound **1b** to Bcl-2 involved 72 intermolecular NOEs. For compound **43a**, 94 NOEs were used in docking to Bcl-xL and 68 in docking to Bcl-2. Finally, for compound **43b** bound to Bcl-xL, a total of 61 intermolecular NOEs were employed.

Fluorescence Polarization Assay. K_i and IC_{50} values were determined using a competitive fluorescence polarization assay. Compounds were serially diluted and added to each well of a 96-well microtiter plate. To determine Bcl-xL K_i values, a mixture totaling 125 μL per well of assay buffer (20 mM phosphate buffer, pH 7.4), 1 mM EDTA, 50 mM NaCl, 0.05% PF-68), 6 nM Bcl-xL protein[14] 1 nM fluorescein-labeled BAD peptide (NLWAAQRYG-RELRRMSDK(FITC)FVD, prepared in-house), and the DMSO solution of compound was shaken for 2 min and then placed in a LJL Analyst (LJL Bio Systems, CA). A negative control (DMSO, 1 nM BAD peptide, assay buffer) and a positive control (DMSO, 1 nM BAD peptide, 6 nM Bcl-xL, assay buffer) were used to determine the range of the assay. The effects of 10% human serum were detected as described above using 30 nM f-Bad peptide and 60 nM Bcl-xL. To determine Bcl-2 K_i values, a mixture totaling 125 μL per well of assay buffer (20 mM phosphate buffer, pH 7.4), 1 mM EDTA, 50 mM NaCl, 0.05% PF-68), 10 nM Bcl-2 protein,[11] 1 nM fluorescein-labeled BAX peptide (FITC-QDASTKKLSE-CLKRIGDELDS, prepared in-house), and the DMSO solution of the test compound was shaken for 2 min and placed in the LJL Analyst. Polarization was measured at 25 °C using a continuous fluorescein lamp (excitation 485 nm; emission 530 nm). Percentage of inhibition was determined by (1-((mP value of well-negative control)/range)) × 100%. K_i, and IC_{50} values were calculated using Microsoft Excel.

FL5.12 Cellular Assay. Mouse FL5.12 cells transfected with Bcl-xL were cultured under standard conditions in RPMI with 2 mM glutamine, 1% 100 mM sodium pyruvate, 2% 1 M HEPES, 4 μL/L of β-mercaptoethanol, 1% penicillin−streptomycin, 10% FBS, and 10% WEHI-3B conditioned media (for IL-3). For assaying the compound activity, the cells were exchanged into an IL-3-depleted deprivation media, which was identical to the growth media except for the absence of FBS and WEHI-3B conditional media, for 2 days. Then the cells were exchanged to either gelatin assay media (RPMI with 2 mM glutamine, 2% 1 M HEPES, 3.4 mg/mL bovine gelatin (Sigma)) or 3% FBS assay media (RPMI with 2 mM glutamine, 1% 100 mM sodium pyruvate, 2% 1 M HEPES, 4 μL/L of β-mercaptoethanol, 1% penicillin−streptomycin, 3% FBS). Compounds in series dilutions were added, and the cells were cultured for 24 h. Cell viability was assayed using the colorimetric 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium MTS assay or the CellTiter-Glo assay (Promega Corp., Madison, WI) according to the manufacturer instructions. Individual determinations were the result of duplicate values.

Human Tumor Cell Line Viability Assay. DoHH-2 human B-cell lymphoma cells was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). SUDHL-4 human tumor cell line was obtained from American Type Culture Collection (Manassas, VA). RS11380 human follicular lymphoma cell line was a generous gift from Dr. John Reed (the Burnham Institute, San Diego, CA). Cells were cultured in RPMI 1640 medium supplemented with 1% penicillin−streptomycin and 10% fetal bovine serum. To determine the effect of Bcl-2 inhibitors, cells were plated in 96 well plates at 50 000 cells per well and treated with compounds in RPMI 1640 medium supplemented with either 3% fetal bovine serum or 10% human serum for 48 h. Cell viability was analyzed using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium MTS assay (Promega Corp., Madison, WI) according to the manufactures instructions.

In Vivo Xenograft Modeling. All animal studies were conducted in accordance with the guidelines established by the internal Institutional Animal Care and Use Committee. C.B.-17 *scid* (*scid*) mice (Charles River Laboratories, Wilmington, MA) were implanted with 3×10^6 SUDHL-4 cells subcutaneously into the right flank. Inoculation volume was 0.2 mL consisting of 50% matrigel (BD Biosciences, Bedford, MA). When tumors reached the appropriate tumor volume, mice were size-matched (day 14) into treatment and control groups with treatment commencing the following day. Each animal was ear-tagged and followed individually throughout the experiment. Tumor volume was estimated by twice weekly measurements of the length and width of the tumor by electronic calipers and applying the following equation: $V = L \times W^2/2$. Compound **2** was formulated in <1% DMSO, 5% Tween 80, 30% propylene glycol, and ∼65% sterile 5% dextrose (pH ∼ 3−4). Etoposide (Bedford Laboratories, Bedford, OH) was administered ip and formulated according to the manufacturer's recommendations.

4-(4-Isobutyl-4-hydroxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (**6a**). A solution of *i*-butylmagnesium bromide (3.6 mL, 2.0 M solution in diethyl ether, 7.2 mmol) in diethyl ether (30 mL) was cooled to 0 °C and treated dropwise with a solution of 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester[22] (1.48 g, 6.0 mmol) in a 1:1 mixture of diethyl ether : THF (10 mL). The reaction mixture was allowed to come to rt, stirred overnight, and diluted with saturated aqueous NH_4Cl (30 mL). The resulting mixture was diluted with EtOAc, and the organic phase washed with water and brine and dried over $MgSO_4$. The volatiles were removed in vacuo, and the remaining residue was purified by silica gel chromatography eluting with 20% EtOAc in hexanes to yield 370 mg (20%) of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 9.2 Hz, 2H), 6.94 (d, J = 9.2 Hz, 2H), 4.22 (q, J = 7.1 Hz, 2H), 4.15 (s, 1H), 3.58 (d, J = 12.9 Hz, 2H), 3.13−3.27 (m, 2H), 1.73−1.88 (m, 1H), 1.40−1.60 (m, 4H), 1.21−1.35 (m, 5H), 0.91 (d, J = 6.8 Hz, 6H). MS (DCI), m/z 306 $[M + H]^+$.

4-[4-(2,2-Dimethyl-propyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6b). 6b was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and neopentylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 9.2 Hz, 2H), 4.22 (q, J = 7.1 Hz, 2H), 4.06 (s, 1H), 3.60 (d, J = 13.2 Hz, 2H), 3.07−3.25 (m, 2H), 1.45−1.74 (m, 4H), 1.39 (s, 2H), 1.28 (t, J = 7.1 Hz, 3H), 1.00 (s, 9H). MS (CI), m/z 320 $[M + H]^+$.

4-(4-*n*-Butyl-4-hydroxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (6c). 6c was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and n-butylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 8.9 Hz, 2H), 6.94 (d, J = 9.2 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 3.59 (m, 2H), 3.20 (m, 2H), 1.46−1.56 (m, 4H), 1.23−1.40 (m, 9H), 0.87 (t, J = 7.0 Hz, 3H). MS (ESI) m/z 306 $[M + H]^+$.

4-(4-Cyclohexylmethyl-4-hydroxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (6d). **6d** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and cyclohexylmethylmagnesium bromide using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 9.2 Hz, 2H), 4.17−4.27 (m, 2H), 4.14 (s, 1H), 3.49−3.66 (m, 2H), 3.12−3.26 (m, 2H), 1.74 (d, J = 12.2 Hz, 2H), 1.41−1.68 (m, 6H), 1.04−1.36 (m, 6H), 0.83−1.00 (m, 2H). MS (DCI), m/z 346 $[M + H]^+$.

4-(4-Benzyl-4-hydroxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (6e). **6e** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and benzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, J = 8.1 Hz, 2H), 7.10−7.34 (m, 5H), 6.93 (d, J = 8.5 Hz, 2H), 4.42 (s, 1H), 4.22 (q, J = 7.1 Hz, 2H), 3.62 (d, J = 12.9 Hz, 2H), 3.05−3.26 (m, 2H), 2.70 (s, 2H), 1.37−1.64 (m, 4H), 1.18−1.34 (t, J = 7.1 Hz, 3H). MS (CI), m/z 340 $[M + H]^+$.

4-[4-(2-Chlorobenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6f). **6f** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 2-chlorobenzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, J = 9.2 Hz, 2H), 7.31−7.50 (m, 2H), 7.18−7.31 (m, 2H), 6.94 (d, J = 9.2 Hz, 2H), 4.58 (s, 1H), 4.14−4.30 (m, 2H), 3.66 (d, J = 12.9 Hz, 2H), 3.05−3.20 (m, 2H), 2.89 (s, 2H), 1.44−1.69 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H). MS (DCI), m/z 374 $[M + H]^+$.

4-[4-(3-Chlorobenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6g). **6g** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 3-chlorobenzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 8.8 Hz, 2H), 7.25−7.32 (m, 2H), 7.19 (d, J = 6.8 Hz, 1H), 7.12 (d, J = 5.1 Hz, 1H), 6.95 (d, J = 9.2 Hz,

2H), 4.51 (s, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.63 (d, $J = 12.9$ Hz, 2H), 3.05−3.24 (m, 2H), 2.71 (s, 2H), 1.48−1.62 (m, 2H), 1.37−1.48 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 374 $[M + H]^+$.

4-[4-(4-Chlorobenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6h). **6h** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 4-chlorobenzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 9.2$ Hz, 2H), 7.29−7.33 (m, 2H), 7.21−7.27 (m, 2H), 6.94 (d, $J = 9.2$ Hz, 2H), 4.46 (s, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.56−3.67 (m, 2H), 3.10−3.23 (m, 2H), 2.69 (s, 2H), 1.47−1.60 (m, 2H), 1.37−1.47 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 374 $[M + H]^+$.

4-[4-(2-Fluorobenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6i). **6i** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 2-fluorobenzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 9.2$ Hz, 2H), 7.29−7.37 (m, 1H), 7.20−7.27 (m, 1H), 7.07−7.16 (m, 2H), 6.94 (d, $J = 9.2$ Hz, 2H), 4.53 (s, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.57−3.72 (m, 2H), 3.09−3.23 (m, 2H), 2.74 (s, 2H), 1.53−1.62 (m, 2H), 1.43−1.54 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 358 $[M + H]^+$.

4-[4-(2-Methylbenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6j). **6j** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 2-methylbenzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (d, $J = 9.2$ Hz, 2H), 7.15−7.23 (m, 1H), 7.02−7.15 (m, 3H), 6.93 (d, $J = 9.2$ Hz, 2H), 4.42 (s, 1H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.65 (d, $J = 13.2$ Hz, 2H), 3.04−3.20 (m, 2H), 2.74 (s, 2H), 2.31 (s, 3H), 1.44−1.65 (m, 4H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 354 $[M + H]^+$.

4-[4-(2-Methoxybenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6k). **6k** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 2-methoxybenzylmagnesium chloride using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (d, $J = 8.8$ Hz, 2H), 7.13−7.22 (m, 2H), 6.89−6.96 (m, 3H), 6.82−6.88 (m, 1H), 4.35 (s, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.74 (s, 3H), 3.55−3.66 (m, 2H), 3.08−3.21 (m, 2H), 2.74 (s, 2H), 1.50−1.59 (m, 2H), 1.48 (d, $J = 3.4$ Hz, 2H), 1.27 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 370 $[M + H]^+$.

4-[4-(2-Bromobenzyl)-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6l). **6l** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 2-bromobenzylmagnesium bromide using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 9.2$ Hz, 2H), 7.57 (dd, $J = 8.1$, 1.4 Hz, 1H), 7.46 (dd, $J = 7.8$, 1.7 Hz, 1H), 7.26−7.34 (m, 1H), 7.10−7.18 (m, 1H), 6.94 (d, $J = 9.2$ Hz, 2H), 4.60 (s, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.67 (d, $J = 13.2$ Hz, 2H), 3.06−3.20 (m, 2H), 2.92 (s, 2H), 1.56−1.68 (m, 2H), 1.46−1.56 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 420 $[M + H]^+$.

4-(4-Biphenyl-2ylmethyl-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6m). **6m** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 2-phenylbenzylmagnesium bromide using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81 (d, $J = 9.2$ Hz, 1H), 7.69 (d, $J = 9.2$ Hz, 2H), 7.40 (d, $J = 7.8$ Hz, 2H), 7.25−7.33 (m, 5H), 7.03 (d, $J = 9.2$ Hz, 1H), 6.82 (d, $J = 9.2$ Hz, 2H), 4.38 (s, 1H), 4.17−4.25 (m, 2H), 3.71−3.78 (m, 2H), 2.93−3.07 (m, 2H), 2.84 (s, 2H), 2.45 (t, $J = 6.1$ Hz, 2H), 1.23−1.30 (m, 3H). MS (DCI), *m*/*z* 416 $[M + H]^+$.

4-(4-Biphenyl-4ylmethyl-4-hydroxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (6n). **6n** was prepared from 4-(4-oxo-piperidin-1-yl)-benzoic acid ethyl ester and 4-phenylbenzylmagnesium bromide using the procedure for the preparation of **6a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 8.8$ Hz, 2H), 7.61−7.67 (m, 2H), 7.56 (d, $J = 8.1$ Hz, 2H), 7.41−7.49 (m, 2H), 7.28−7.36 (m, 3H), 6.95 (d, $J = 9.2$ Hz, 2H), 4.47 (s, 1H), 4.17−4.26 (m, 3H), 3.74 (t, $J = 6.1$ Hz, 1H), 3.64 (d, $J = 12.9$ Hz, 2H), 3.12−3.24 (m, 2H), 2.75 (s, 2H), 1.53−1.66 (m, 2H), 1.44−1.52 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI), *m*/*z* 416 $[M + H]^+$.

4-(4-Isobutyl-4-methoxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (**7a**). A solution of the **6a** (370 mg, 1.2 mmol) in THF (5 mL) was treated with NaH (96 mg, 2.4 mmol, 60% dispersion in mineral oil), heated to 50 °C for 2 h, and treated with HMPA (1 mL) followed by MeI (1 mL). The reaction mixture was refluxed overnight, cooled to 0 °C, and diluted with saturated aqueous $NaHSO_4$ solution (10 mL). The resulting two-phase mixture was separated, the aqueous phase was extracted twice with ether, and the combined organic layers washed with water and brine. After drying over $MgSO_4$, the mixture was concentrated in vacuo and purified by silica gel chromatography eluting with 15% EtOAc in hexanes to yield 190 mg (49%) of **7a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 9.0$ Hz, 2H), 6.94 (d, $J = 9.0$ Hz, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.58 (m, 2H), 3.09 (s, 3H), 3.05 (m, 2H), 1.70−1.85 (m, 3H), 1.47 (m, 2H), 1.36 (d, $J = 5.8$ Hz, 2H), 1.28 (t, $J = 7.0$ Hz, 3H), 0.92 (d, $J = 6.8$ Hz, 6H). MS (ESI) *m*/*z* 320 $[M + H]^+$.

4-[4-(2,2-Dimethyl-propyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7b). 7b was prepared from **6b** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, $J = 9.2$ Hz, 2H), 6.95 (d, $J = 9.2$ Hz, 2H), 4.21 (q, $J = 7.1$ Hz, 2H), 3.61 (m, 2H), 3.10 (s, 3H), 3.00 (m, 2H), 1.89 (m, 2H), 1.54 (m, 2H), 1.43 (s, 2H), 1.28 (t, $J = 7.1$ Hz, 3H), 0.98 (s, 9H). MS (DCI) *m*/*z* 334 $[M + H]^+$.

4-(4-*n*-Butyl-4-methoxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (7c). 7c was prepared from **6c** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, $J = 9.2$ Hz, 2H), 6.95 (d, $J = 9.1$ Hz, 2H), 4.23 (q, $J = 7.0$ Hz, 2H), 3.58 (m, 2H), 3.09 (s, 3H), 3.06 (m, 2H), 1.77 (m, 2H), 1.46 (m, 4H), 1.20−1.33 (m, 7H), 0.89 (t, $J = 7.0$ Hz, 3H). MS (ESI) *m*/*z* 320 $[M + H]^+$.

4-(4-Cyclohexylmethyl-4-methoxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (7d). **7d** was prepared from **6d** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, $J = 9.1$ Hz, 2H), 6.95 (d, $J = 9.1$ Hz, 2H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.58 (m, 2H), 3.08 (s, 3H), 3.05 (m, 2H), 1.77 (m, 2H), 0.89−1.68 (m, 18H). MS (ESI) *m*/*z* 360 $[M + H]^+$.

4-(4-Benzyl-4-methoxy-piperidin-1-yl)-benzoic Acid Ethyl Ester (7e). **7e** was prepared from **6e** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 8.8$ Hz, 2H), 7.27 (m, 2H), 7.19 (m, 3H), 6.94 (d, $J = 9.2$ Hz, 2H), 4.22 (q, $J = 7.0$ Hz, 2H), 3.62 (m, 2H), 3.24 (s, 3H), 3.02 (m, 2H), 2.79 (s, 2H), 1.69 (m, 2H), 1.51 (m, 2H), 1.27 (t, $J = 7.1$ Hz, 3H). MS (ESI) *m*/*z* 354 $[M + H]^+$.

4-[4-(2-Chlorobenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7f). **7f** was prepared from **6f** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (d, $J = 9.1$ Hz, 2H), 7.42 (dd, $J = 7.4$ Hz, 1.7 Hz, 1H), 7.28 (m, 3H), 6.93 (d, $J = 9.1$ Hz, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.65 (m, 2H), 3.31 (s, 3H), 2.97 (m, 4H), 1.77 (m, 2H), 1.54 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI) *m*/*z* 388 $[M + H]^+$.

4-[4-(3-Chlorobenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7g). **7g** was prepared from **6g** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, $J = 8.8$ Hz, 2H), 7.27 (m, 3H), 7.15 (m, 1H), 6.95 (d, $J = 8.8$ Hz, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.63 (m, 2H), 3.29 (s, 3H), 3.03 (t, $J = 10.8$ Hz, 2H), 2.81 (s, 2H), 1.67 (m, 2H), 1.51 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI) *m*/*z* 388 $[M + H]^+$.

4-[4-(4-Chlorobenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7h). **7h** was prepared from **6h** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 8.8$ Hz, 2H), 7.33 (d, $J = 8.5$ Hz, 2H), 7.20 (d, $J = 8.5$ Hz, 2H), 6.94 (d, $J = 8.8$ Hz, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.61 (m, 2H), 3.28 (s, 3H), 3.02 (m, 2H), 2.79 (s, 2H), 1.67 (m, 2H), 1.50 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (DCI) *m*/*z* 388 $[M + H]^+$.

4-[4-(2-Fluorobenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7i). **7i** was prepared from **6i** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, $J = 9.0$ Hz, 2H), 7.26 (m, 2H), 7.13 (m, 2H), 6.94 (d, $J = 9.0$ Hz, 2H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.64 (m, 2H), 3.29 (s, 3H), 3.00 (m, 2H), 2.83 (s, 2H), 1.73 (m, 2H), 1.52 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H). MS (ESI) *m*/*z* 372 $[M + H]^+$.

4-[4-(2-Methylbenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7j). **7j** was prepared from **6j** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (d, J = 9.2 Hz, 2H), 7.16−7.21 (m, 1H), 7.05−7.15 (m, 3H), 6.93 (d, J = 9.2 Hz, 2H), 4.42 (s, 1H), 4.22 (q, J = 7.1 Hz, 2H), 3.65 (d, J = 13.2 Hz, 2H), 3.05−3.20 (m, 2H), 2.74 (s, 2H), 2.31 (s, 3H), 1.56−1.66 (m, 2H), 1.48−1.57 (m, 2H), 1.28 (t, J = 7.1 Hz, 3H). MS (DCI), m/z 368 $[M + H]^+$.

4-[4-(2-Methoxybenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7k). **7k** was prepared from **6k** using the procedure described for the preparation of **7a.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73 (d, J = 9.1 Hz, 2H), 7.18 (m, 1H), 7.12 (m, 1H), 6.93 (m, 3H), 6.86 (m, 1H), 4.22 (q, J = 7.1 Hz, 2H), 3.75 (s, 3H), 3.61 (m, 2H), 3.28 (s, 3H), 2.98 (m, 2H), 2.80 (s, 2H), 1.73 (m, 2H), 1.49 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 384 $[M + H]^+$.

4-[4-(2-Bromobenzyl)-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7l). **7l** was prepared from **6l** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, J = 9.1 Hz, 2H), 7.60 (m, 1H), 7.35 (m, 2H), 7.17 (m, 1H), 6.95 (d, J = 9.1 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 3.67 (m, 2H), 3.34 (s, 3H), 3.02 (s, 2H), 2.99 (m, 2H), 1.79 (m, 2H), 1.57 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 432 $[M + H]^+$.

4-(4-Biphenyl-2ylmethyl-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7m). **7m** was prepared from **6m** using the procedure described for the preparation of **7a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.69 (d, J = 9.2 Hz, 2H), 7.25−7.45 (m, 8H), 7.15 (m, 1H), 6.83 (d, J = 9.1 Hz, 2H), 4.21 (q, J = 7.1 Hz, 2H), 3.38 (m, 2H), 3.04 (s, 3H), 2.90 (s, 2H), 2.85 (m, 2H), 1.48 (m, 2H), 1.27 (t, J = 7.0 Hz, 3H), 1.20 (m, 2H). MS (ESI) m/z 430 $[M + H]^+$.

4-(4-Biphenyl-4ylmethyl-4-methoxy-piperidin-1-yl]-benzoic Acid Ethyl Ester (7n). **7n** was prepared from **6n** using the procedure described for the preparation of **7a.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73 (d, J = 9.1 Hz, 2H), 7.63 (m, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.43 (m, 2H), 7.33 (m, 1H), 7.26 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 4.22 (q, J = 7.0 Hz, 2H), 3.63 (m, 2H), 3.03 (m, 2H), 2.83 (s, 2H), 1.73 (m, 2H), 1.55 (m, 2H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 430 $[M + H]^+$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-[4-(4-isobutyl-4-methoxy-piperidin-1-yl)-benzoyl]-3-nitrobenzenesulfonamide, Trifluoroacetate Salt, (**8a**). A solution of **7a** (190 mg, 0.59 mmol) and LiOH (43 mg, 1.8 mmol) in 1:1:1 H_2O/MeOH/THF (15 mL) was heated at 50 °C overnight. After removal of the volatiles in vacuo, the residue was acidified with 1 M $NaHSO_4$ solution (5 mL) and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to yield 170 mg (98%) of the intermediate acid that was used without further purification.

A solution of the above intermediate acid (47 mg, 0.16 mmol), EDCI (70 mg, 0.36 mmol), DMAP (44 mg, 0.36 mmol), and **4** (74 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) was stirred for 12 h and the mixture partitioned between water and CH_2Cl_2. The organic phase was washed twice with saturated aqueous NH_4Cl and dried over $MgSO_4$. After concentration, the residue was purified by reversed phase HPLC to yield 64 mg (56%) of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.40 (br s, 1H), 8.54 (d, J = 2.3 Hz, 1H), 8.27 (d, J = 9.3 Hz, 1H), 7.87 (dd, J = 9.4, 2.0 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.23 (m, 2H), 7.09−7.19 (m, 4H), 6.92 (d, J = 9.1 Hz, 2H), 4.19 (m, 1H), 3.60 (m, 2H), 3.38 (m, 2H), 3.02−3.18 (m, 7H), 2.75 (s, 6H), 2.14 (m, 2H), 1.78 (m, 2H), 1.73 (m, 1H), 1.45 (m, 2H), 1.34 (d, J = 6.0 Hz, 2H), 0.91 (d, J = 6.6 Hz, 6H). MS (ESI) m/z 696 $[M - H]^-$. Anal. ($C_{35}H_{47}N_5O_6S_2 \cdot 1.5CF_3CO_2H \cdot H_2O$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2,2-dimethyl-propyl)-4-methoxy-piperidin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, Trifluoroacetate Salt, (**8b**). **8b** was prepared from **7b** using the procedure described for the preparation of **8a.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.95 (br s, 1H), 9.48 (br s, 1H), 8.54 (d, J = 2.2 Hz, 1H), 8.28 (d, J = 9.4 Hz, 1H), 7.86 (dd, J = 9.2, 2.0 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.23 (m, 2H), 7.09−7.19 (m, 4H), 6.92 (d, J = 9.1 Hz, 2H), 4.19 (m, 1H), 3.62 (m, 2H), 3.39 (d, J = 6.2 Hz, 2H), 3.15 (m, 2H), 3.10 (s, 3H), 3.02 (m, 2H), 2.75 (s, 6H), 2.14 (m, 2H), 1.88 (m, 2H), 1.51 (m, 2H), 1.41 (s, 2H), 0.98 (s, 9H). MS (ESI) m/z 710 $(M - H)^-$. Anal. ($C_{36}H_{49}N_5O_6S_2 \cdot 1.5\ CF_3CO_2H \cdot H_2O$) C, H, N.

***N*-[4-(4-Butyl-4-methoxy-piperidin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**8c**). **8c** was prepared from **7c** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.40 (br s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 7.86 (dd, J = 9.1, 2.4 Hz, 1H), 7.73 (d, J = 9.2 Hz, 2H), 7.10−7.25 (m, 6H), 6.93 (d, J = 9.2 Hz, 2H), 4.17 (m, 1H), 3.60 (m, 2H), 3.39 (m, 2H), 3.10 (m, 2H), 3.07 (s, 3H), 3.02 (m, 2H), 2.75 (m, 6H), 2.15 (m, 2H), 1.75 (m, 2H), 1.43 (m, 4H), 1.24 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H). MS (ESI) m/z 696 $(M - H)^-$. Anal. ($C_{35}H_{47}N_5O_6S_2 \cdot 1.5\ CF_3CO_2H$) C, H, N.

***N*-[4-(4-Cyclohexylmethyl-4-methoxy-piperidin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt,** (**8d**). **8d** was prepared from **7d** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.41 (br s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.28 (d, J = 9.3 Hz, 1H), 7.87 (dd, J = 9.0, 2.1 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.23 (m, 2H), 7.08−7.18 (m, 4H), 6.92 (d, J = 9.5 Hz, 2H), 4.19 (m, 1H), 3.59 (m, 2H), 3.39 (m, 2H), 3.13 (m, 2H), 3.07 (s, 3H), 3.03 (m, 2H), 2.75 (s, 6H), 2.15 (m, 2H), 1.78 (m, 2H), 1.72 (m, 2H), 1.53−1.65 (m, 3H), 1.44 (m, 3H), 1.32 (d, J = 5.6 Hz, 2H), 1.20 (m, 2H), 1.10 (m, 1H), 0.95 (m, 2H). MS (ESI) m/z 736 $(M - H)^-$.

***N*-[4-(4-Benzyl-4-methoxy-piperidin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt,** (**8e**). **8e** was prepared from **7e** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.97 (br s, 1H), 9.43 (br s, 1H), 8.55 (d, J = 2.2 Hz, 1H), 8.29 (d, J = 9.4 Hz, 1H), 7.87 (dd, J = 9.0, 2.2 Hz, 1H), 7.72 (d, J = 9.0 Hz, 2H), 7.21−7.29 (m, 4H), 7.09−7.21 (m, 7H), 6.91 (d, J = 9.0 Hz, 2H), 4.19 (m, 1H), 3.63 (m, 2H), 3.39 (d, J = 6.2 Hz, 2H), 3.28 (s, 3H), 3.06−3.20 (m, 2H), 2.97−3.06 (m, 2H), 2.78 (s, 2H), 2.74 (s, 6H), 2.08−2.19 (m, 2H), 1.68 (m, 2H), 1.41−1.53 (m, 2H). MS (ESI) m/z 730 $(M - H)^-$. Anal. ($C_{38}H_{45}N_5O_6S_2 \cdot 1.4\ CF_3CO_2H$) C, H, N.

***N*-{4-[4-(2-Chloro-benzyl)-4-methoxy-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt,** (**8f**). **8f** was prepared from **7f** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.40 (br s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 7.86 (dd, J = 9.5, 2.1 Hz, 1H), 7.72 (d, J = 9.1 Hz, 2H), 7.40 (m, 1H), 7.08−7.34 (m, 9H), 6.91 (d, J = 9.1 Hz, 2H), 4.18 (m, 1H), 3.67 (m, 2H), 3.39 (m, 2H), 3.09−3.19 (m, 5H), 2.91−3.03 (m, 4H), 2.74 (m, 6H), 2.14 (m, 2H), 1.75 (m, 2H), 1.50 (m, 2H). MS (ESI) m/z 764 $(M - H)^-$. Anal. ($C_{38}H_{44}ClN_5O_6S_2 \cdot 2.0\ CF_3CO_2H$) C, H, N.

***N*-{4-[4-(3-Chloro-benzyl)-4-methoxy-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**8g**). **8g** was prepared from **7g** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.33 (br s, 1H), 8.53 (d, J = 2.2 Hz, 1H), 8.29 (d, J = 9.5 Hz, 1H), 7.86 (dd, J = 9.2, 2.3 Hz, 1H), 7.72 (d, J = 9.2 Hz, 2H), 7.09−7.32 (m, 10H), 6.91 (d, J = 9.2 Hz, 2H), 4.18 (m, 1H), 3.64 (m, 2H), 3.39 (m, 2H), 3.07−3.20 (m, 5H), 3.01 (m, 4H), 2.79 (s, 2H), 2.74 (s, 6H), 2.13 (m, 2H), 1.65 (m, 2H), 1.47 (m, 2H). MS (ESI) m/z 764 $(M - H)^-$. Anal. ($C_{38}H_{44}ClN_5O_6S_2 \cdot 1.5\ CF_3CO_2H$) C, H, N.

***N*-{4-[4-(4-Chloro-benzyl)-4-methoxy-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonami de, Trifluoroacetate Salt**, (**8h**). **8h** was prepared from **7h** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.35 (br s, 1H), 8.54 (d, J = 2.0 Hz, 1H), 8.29 (d, J = 9.5 Hz, 1H), 7.87 (dd, J = 9.2, 2.4 Hz, 1H), 7.73 (d, J = 9.1 Hz, 2H), 7.32 (m, 2H),

7.09−7.26 (m, 8H), 6.92 (d, J = 9.1 Hz, 2H), 4.19 (m, 1H), 3.63 (m, 2H), 3.39 (m, 2H), 3.08−3.20 (m, 5H), 3.01 (m, 2H), 2.78 (s, 2H), 2.74 (s, 6H), 2.14 (m, 2H), 1.65 (m, 2H), 1.46 (m, 2H). MS (ESI) *m*/*z* 766 [M − H]$^-$. Anal. ($C_{38}H_{44}ClN_5O_6S_2 \cdot CF_3CO_2H \cdot 1.5\ H_2O$) C, H, N.

***N*-{4-[4-(2-Fluoro-benzyl)-4-methoxy-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Hydrochloride Salt**, (**8i**). **8i** was prepared from **7i** using the procedure described for the preparation of **8a**. The resulting product was dissolved in 4N HCl in dioxane and concentrated to provide the HCl salt. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 9.36 (br s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 9.6 Hz, 1H), 7.87 (dd, J = 9.1, 2.3 Hz, 1H), 7.72 (d, J = 9.1 Hz, 2H), 7.08−7.30 (m, 10H), 6.92 (d, J = 9.1 Hz, 2H), 4.18 (m, 1H), 3.67 (m, 2H), 3.39 (m, 2H), 3.09−3.18 (m, 5H), 2.99 (m, 2H), 2.82 (s, 2H), 2.74 (m, 6H), 2.15 (m, 2H), 1.71 (m, 2H), 1.49 (m, 2H). MS (ESI) *m*/*z* 748 (M − H)$^-$. Anal. ($C_{38}H_{44}FN_5O_6S_2 \cdot 2\ HCl \cdot 2\ H_2O$) C, H, N.

***N*-{4-[4-Methoxy-4-(2-methylbenzyl)-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**8j**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.36 (s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 9.5 Hz, 1H), 7.82−7.89 (m, 1H), 7.71 (d, J = 9.2 Hz, 2H), 7.20−7.27 (m, 2H), 7.03−7.19 (m, 7H), 6.90 (d, J = 9.2 Hz, 2H), 4.08−4.26 (m, 1H), 3.66 (d, J = 12.5 Hz, 2H), 3.34−3.42 (m, 2H), 3.28 (s, 3H), 3.12 (d, J = 11.2 Hz, 2H), 2.90−3.04 (m, 2H), 2.80 (s, 2H), 2.74 (s, 6H), 2.27 (s, 3H), 2.08−2.19 (m, 2H), 1.75 (d, J = 13.2 Hz, 2H), 1.39−1.56 (m, 2H). MS (ESI), *m*/*z* 744 [M − H]$^-$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-methoxy-4-(2-methoxy-benzyl)-piperidin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, Trifluoroacetate Salt, (**8k**). **8k** was prepared from **7k** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.40 (br s, 1H), 8.54 (d, J = 2.1 Hz, 1H), 8.30 (d, J = 9.6 Hz, 1H), 7.86 (dd, J = 9.2, 2.3 Hz, 1H), 7.71 (d, J = 9.0 Hz, 2H), 7.09−7.27 (m, 8H), 6.82−6.97 (m, 4H), 4.18 (m, 1H), 3.74 (s, 3H), 3.63 (m, 2H), 3.39 (m, 2H), 3.27 (s, 3H), 3.14 (m, 2H), 2.98 (m, 2H), 2.79 (s, 2H), 2.74 (s, 6H), 2.15 (m, 2H), 1.71 (m, 2H), 1.45 (m, 2H). MS (ESI) *m*/*z* 760 (M − H)$^-$. Anal. ($C_{39}H_{47}N_5O_7S_2 \cdot 1.5\ CF_3CO_2H$) C, H, N.

***N*-{4-[4-(2-Bromo-benzyl)-4-methoxy-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**8l**). **8l** was prepared from **7l** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 9.32 (br s, 1H), 8.54 (d, J = 2.3 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 7.86 (dd, J = 9.0, 2.3 Hz, 1H), 7.72 (d, J = 9.2 Hz, 2H), 7.58 (d, J = 7.7 Hz, 1H), 7.32 (m, 2H), 7.24 (m, 2H), 7.09−7.20 (m, 5H), 6.91 (d, J = 9.2 Hz, 2H), 4.18 (m, 1H), 3.68 (m, 2H), 3.39 (m, 2H), 3.31 (s, 3H), 3.12 (m, 2H), 2.92−3.03 (m, 4H), 2.74 (s, 6H), 2.14 (m, 2H), 1.75 (m, 2H), 1.51 (m, 2H). MS (ESI) *m*/*z* 810 (M − H)$^-$. Anal. ($C_{38}H_{44}BrN_5O_7S_2 \cdot 1.5\ CF_3CO_2H \cdot H_2O$) C, H, N.

***N*-[4-(4-Biphenyl-2-ylmethyl-4-methoxy-piperidin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**8m**). **8m** was prepared from **7m** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.96 (br s, 1H), 9.29 (br s, 1H), 8.53 (d, J = 2.0 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.85 (dd, J = 9.1, 2.2 Hz, 1H), 7.68 (d, J = 9.2 Hz, 2H), 7.09−7.44 (m, 15H), 6.81 (d, J = 9.2 Hz, 2H), 4.18 (m, 1H), 3.39 (m, 4H), 3.13 (m, 2H), 3.02 (s, 3H), 2.89 (s, 2H), 2.83 (m, 2H), 2.73 (m, 6H), 2.14 (m, 2H), 1.46 (m, 2H), 1.16 (m, 2H). MS (ESI) *m*/*z* 806 (M − H)$^-$. Anal. ($C_{44}H_{49}N_5O_6S_2 \cdot 1.5\ CF_3CO_2H$) C, H, N.

***N*-[4-(4-Biphenyl-4-ylmethyl-4-methoxy-piperidin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**8n**). **8n** was prepared from **7n** using the procedure described for the preparation of **8a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.32 (br s, 1H), 8.53 (d, J = 2.4 Hz, 1H), 8.28 (d, J = 9.4 Hz, 1H), 7.86 (dd, J = 9.1, 2.2 Hz, 1H), 7.72 (d, J = 9.7 Hz, 2H), 7.63 (m, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.44 (m, 2H), 7.33 (m, 1H), 7.24 (m, 4H), 7.14 (m, 4H), 6.92 (d, J = 9.1 Hz, 2H), 4.18 (m, 1H), 3.65 (m, 2H), 3.39 (m, 2H), 3.31 (s, 3H), 2.96−3.20 (m, 4H), 2.83 (s, 2H), 2.74 (s, 6H), 2.13 (m, 2H), 1.72 (m, 2H), 1.52 (m, 2H). MS (ESI) *m*/*z* 806 (M − H)$^-$. Anal. ($C_{44}H_{49}N_5O_6S_2 \cdot 1.25\ CF_3CO_2H$) C, H, N.

4-[4-(4-Fluoro-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9a**). A solution of triphenylphosphine (5.24 g, 20 mmol) and 4-fluorobenzyl bromide (2.5 mL, 20 mmol) was refluxed overnight in toluene (50 mL) and allowed to cool to room temperature, and the resulting precipitate was collected by filtration, washed with Et_2O, and dried in vacuo to yield 8.42 g (93%) (4-fluorobenzyl)-triphenylphosphonium bromide as a white solid.

A suspension of sodium hydride (220 mg, 5.5 mmol, 60% dispersion in mineral oil) in DMSO (20 mL) was heated for 1 h at 80 °C, cooled to 5 °C, and treated with (4-fluorobenzyl)-triphenylphosphonium bromide (2.24 g, 5.5 mmol). The resulting red solution was stirred for 10 min and treated with **5** (1.36 g, 5.5 mmol) and the reaction mixture heated for 3 h at 80 °C. After standing overnight at room temperature, the reaction mixture was poured into aqueous $NaHSO_4$ solution and extracted twice with Et_2O. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography eluting with a gradient from 10 to 20% EtOAc in hexanes yielded 1.74 g (93%) of the intermediate ethyl ester as a white crystalline solid.

A solution of the intermediate ethyl ester (1.74 g, 5.1 mmol) in 1 N aqueous NaOH (10 mL) and dioxane (10 mL) was heated at 90 °C for 5 h. After concentration in vacuo, the residue was diluted in 1 M HCl and extracted with EtOAc. After drying the organic phase over $MgSO_4$, concentration yielded 1.37 g (86%) of **9a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (br s, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.28 (m, 2H), 7.15 (m, 2H), 6.96 (d, J = 8.8 Hz, 2H), 6.38 (s, 1H), 3.50 (m, 2H), 3.42 (m, 2H), 2.53 (m, 2H), 2.43 (m, 2H). MS (ESI) *m*/*z* 312 [M + H]$^+$.

4-[4-(2-Fluoro-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9b**). **9b** was prepared starting from 2-fluorobenzyl bromide using the procedure described for the preparation of **9a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.21 (br s, 1H), 7.75 (d, J = 9.1 Hz, 2H), 7.05−7.35 (m, 4H), 6.97 (d, J = 9.1 Hz, 2H), 6.32 (s, 1H), 3.52 (m, 2H), 3.43 (m, 2H), 2.47 (m, 2H), 2.39 (m, 2H). MS (ESI) *m*/*z* 312 [M + H]$^+$.

4-[4-(2-Chloro-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9c**). **9c** was prepared starting from 2-chlorobenzyl bromide using the procedure described for the preparation of **9a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.79 (br s, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.46 (d, J = 7.1 Hz, 1H), 7.38 (d, J = 8.1 Hz, 2H), 7.23−7.35 (m, 3H), 6.29 (s, 1H), 2.81−2.96 (m, 1H), 2.65 (d, J = 13.6 Hz, 1H), 2.33−2.47 (m, 1H), 1.96−2.15 (m, 2H), 1.84−1.95 (m, 1H), 1.44−1.69 (m, 2H). MS (ESI), *m*/*z* 326 [M − H]$^-$.

4-[4-(4-Chloro-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9d**). **9d** was prepared starting from 4-chlorobenzyl bromide using the procedure described for the preparation of **9a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 7.72 (d, J = 9.1 Hz, 2H), 7.36 (m, 2H), 7.23 (m, 2H), 6.93 (d, J = 9.1 Hz, 2H), 6.35 (s, 1H), 3.48 (m, 2H), 3.40 (m, 2H), 2.52 (m, 2H), 2.41 (m, 2H). MS (ESI) *m*/*z* 328 [M + H]$^+$.

4-[4-(2-Trifluoromethyl-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9e**). **9e** was prepared starting from 2-trifluoromethylbenzyl bromide using the procedure described for the preparation of **9a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.23 (br s, 1H), 7.77 (d, J = 9.2 Hz, 2H), 7.73 (d, J = 7.5 Hz, 1H), 7.65 (dd, J = 7.8, 7.4 Hz, 1H), 7.48 (dd, J = 7.8, 7.5 Hz, 1H), 7.38 (d, J = 7.1 Hz, 1H), 6.98 (d, J = 9.1 Hz, 2H), 6.51 (s, 1H), 3.51 (dd, J = 5.8, 5.4 Hz, 2H), 3.38 (dd, J = 6.1, 5.4 Hz, 2H), 2.44 (dd, J = 5.7, 5.1 Hz, 2H), 2.27 (dd, J = 5.8, 5.0 Hz, 2H). MS (ESI) *m*/*z* 362 [M + H]$^+$.

4-[4-(4-Trifluoromethyl-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9f**). **9f** was prepared starting from 4-trifluoromethylbenzyl bromide using the procedure described for the preparation of **9a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (br s, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H),

6.97 (d, $J = 9.1$ Hz, 2H), 6.48 (s, 1H), 3.52 (m, 2H), 3.43 (m, 2H), 2.56 (m, 2H), 2.46 (m, 2H). MS (ESI) *m/z* 362 $[M + H]^+$.

4-[4-(2-Methoxy-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9g**). **9g** was prepared starting from 2-methoxybenzyl bromide using the procedure described for the preparation of **9a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.19 (br s, 1H), 7.76 (d, $J = 8.9$ Hz, 2H), 7.23 (m, 1H), 7.14 (dd, $J = 7.4$, 1.7 Hz, 1H), 6.94−7.00 (m, 3H), 6.90 (t, $J = 7.3$ Hz, 1H), 6.33 (s, 1H), 3.77 (s, 3H), 3.49 (m, 2H), 3.40 (m, 2H), 2.42 (m, 4H). MS (ESI) *m/z* 324 $[M + H]^+$.

4-[4-(2-Cyano-benzylidene)-piperidin-1-yl]-benzoic Acid, (**9h**). **9h** was prepared starting from 2-cyanobenzyl bromide using the procedure described for the preparation of **9a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (br s, 1H), 7.82 (d, $J = 7.7$ Hz, 1H), 7.75 (d, $J = 9.0$ Hz, 2H), 7.67 (td, $J = 7.6$, 1.4 Hz, 1H), 7.45 (m, 2H), 6.97 (d, $J = 9.1$ Hz, 2H), 6.52 (s, 1H), 3.53 (m, 2H), 3.44 (m, 2H), 2.47 (m, 2H), 2.39 (m, 2H). MS (ESI) *m/z* 319 $[M + H]^+$.

4-(4-Pyridin-2-ylmethylene-piperidin-1-yl)-benzoic Acid, (**9i**). **9i** was prepared starting from 2-(bromomethyl)pyridine using the procedure described for the preparation of **9a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.19 (br s, 1H), 8.53 (m, 1H), 7.76 (d, $J = 9.0$ Hz, 2H), 7.71 (dd, $J = 8.5$, 2.0 Hz, 1H), 7.26 (m, 1H), 7.18 (m, 1H), 6.97 (d, $J = 8.8$ Hz, 2H), 6.39 (s, 1H), 3.52 (m, 2H), 3.46 (m, 2H), 3.05 (m, 2H), 2.46 (m, 2H). MS (ESI) *m/z* 295 $[M + H]^+$.

4-(4-Pyridin-3-ylmethylene-piperidin-1-yl)-benzoic Acid, (**9j**). **9j** was prepared starting from 4-(bromomethyl)pyridine using the procedure described for the preparation of **9a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.15 (br s, 1H), 8.43 (m, 1H), 8.38 (dd, $J = 4.7$, 1.8 Hz, 1H), 7.73 (d, $J = 9.2$, 2H), 7.63 (m, 1H), 7.33 (m, 1H), 6.94 (d, $J = 9.1$ Hz, 2H), 6.36 (s, 1H), 3.50 (m, 2H), 3.43 (m, 2H), 2.51 (m, 2H), 2.45 (m, 2H). MS (ESI) *m/z* 295 $[M + H]^+$.

4-(4-Biphenyl-2-ylmethylene-piperidin-1-yl)-benzoic Acid, (**9k**). **9k** was prepared starting from 2-(bromomethyl)biphenyl using the procedure described for the preparation of **9a.** ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.22 (br s, 1H), 7.73 (m, $J = 8.8$ Hz, 2H), 7.39 (m, 4H), 7.34 (m, 4H), 7.27 (m, 1H), 6.94 (d, $J = 9.1$ Hz, 2H), 6.17 (s, 1H), 3.40 (dd, $J = 6.1$, 5.0 Hz, 2H), 3.22 (dd, $J = 6.2$, 5.0 Hz, 2H), 2.28 (m, 4H). MS (ESI) *m/z* 370 $[M + H]^+$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(4-fluoro-benzylidene)-piperidin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, **Hydrochloride Salt** (**10a**). A solution of **9a** (0.81 g, 2.6 mmol), **4** (1.0 g, 2.4 mmol), EDCI (1.10 g, 5.7 mmol), and DMAP (0.70 g, 5.7 mmol) in CH_2Cl_2 (25 mL) was stirred overnight and partitioned between CH_2Cl_2 and saturated aqueous ammonium chloride. The organic phase was dried over $MgSO_4$ and concentrated and the resulting residue purified by silica gel chromatography eluting with a gradient from 0−12% 7N NH_3 in MeOH solution in CH_2Cl_2 to give 1.21 g (72%) **10a** as a yellow foam. The hydrochloride salt was prepared by lyophilization of a frozen solution of the product in 2 N aqueous HCl (20 mL) and 1:1 CH_3CN/H_2O (50 mL). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 10.05 (br s, 1H), 8.54 (d, $J = 2.3$ Hz, 1H), 8.28 (d, $J = 9.4$ Hz, 1H), 7.85 (dd, $J = 9.3$, 2.2 Hz, 1H), 7.76 (d, $J = 8.7$ Hz, 2H), 7.10−7.30 (m, 10H), 6.95 (d, $J = 9.1$ Hz, 2H), 6.37 (s, 1H), 4.22 (m, 1H), 3.44 (m, 2H), 3.39 (m, 2H), 3.12 (m, 2H), 2.72 (m, 6H), 2.41 (m, 2H), 2.16 (m, 2H). MS (ESI) *m/z* 764 $(M - H)^-$. MS (ESI) *m/z* 716 $(M - H)^-$. Anal. ($C_{37}H_{40}FN_5O_5S_2 \cdot 2$ HCl $\cdot 2$ H_2O) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-fluoro-benzylidene)-piperidin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, Trifluoroacetate Salt, (**10b**). A solution of **9b** (69 mg, 0.22 mmol), **4** (85 mg, 0.20 mmol), EDCI (84 mg, 0.44 mmol), and DMAP (55 mg, 0.44 mmol) in CH_2Cl_2 (5 mL) was stirred overnight and partitioned between CH_2Cl_2 and saturated aqueous ammonium chloride. The organic phase was dried over $MgSO_4$ and concentrated and the resulting residue purified by reverse phase HPLC to yield 119 mg (72%) of **10b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.40 (br s, 1H), 8.55 (d, $J = 2.2$ Hz, 1H), 8.28 (d, $J = 9.0$ Hz, 1H), 7.87 (dd, $J = 9.5$, 2.1 Hz, 1H), 7.77 (d, $J = 9.0$ Hz, 2H), 7.10−7.32 (m, 10H), 6.96 (d, $J = 9.5$ Hz, 2H), 6.31 (s, 1H), 4.18 (m, 1H), 3.54 (m, 2H), 3.44 (m, 2H), 3.38 (m, 2H), 3.15 (m, 2H), 2.74 (s, 6H), 2.44 (t, $J = 5.4$ Hz, 2H), 2.38 (t, $J = 5.4$ Hz, 2H), 2.13 (m, 2H). MS (ESI) *m/z* 716 $(M - H)^-$. Anal. ($C_{37}H_{40}FN_5O_5S_2 \cdot 1.5$ $CF_3CO_2H \cdot 0.5$ H_2O) C, H, N.

***N*-{4-[4-(2-Chloro-benzylidene)-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethyl amino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**10c**). **10c** was prepared from **9c** using the procedure described for the preparation of **10b.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.35 (br s, 1H), 8.55 (d, $J = 2.5$ Hz, 1H), 8.27 (d, $J = 9.3$ Hz, 1H), 7.87 (dd, $J = 9.2$, 2.2 Hz, 1H), 7.76 (d, $J = 9.0$ Hz, 2H), 7.46 (d, $J = 7.6$ Hz, 1H), 7.10−7.32 (m, 9H), 6.96 (d, $J = 9.0$ Hz, 2H), 6.37 (s, 1H), 4.18 (m, 1H), 3.54 (m, 2H), 3.44 (m, 2H), 3.39 (m, 2H), 3.15 (m, 2H), 2.74 (s, 6H), 2.44 (m, 2H), 2.35 (m, 2H) 2.14 (m, 2H). MS (ESI) *m/z* 732 $(M - H)^-$. Anal. ($C_{37}H_{40}ClN_5O_5S_2 \cdot 1.5$ CF_3CO_2H) C, H, N.

***N*-{4-[4-(4-Chloro-benzylidene)-piperidin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt**, (**10d**). **10d** was prepared starting from **9d** using the procedure described for the preparation of **10b.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.44 (br s, 1H), 8.55 (d, $J = 2.4$ Hz, 1H), 8.28 (d, $J = 9.1$ Hz, 1H), 7.87 (dd, $J = 9.2$, 2.2 Hz, 1H), 7.76 (d, $J = 9.1$ Hz, 2H), 7.38 (m, 2H), 7.25 (m, 4H), 7.10−7.20 (m, 4H), 6.95 (d, $J = 9.1$ Hz, 2H), 6.37 (s, 1H), 4.18 (m, 1H), 3.51 (m, 2H), 3.45 (m, 2H), 3.39 (m, 2H), 3.13 (m, 2H), 2.74 (s, 6H), 2.50 (m, 2H), 2.42 (m, 2H), 2.14 (m, 2H). MS (ESI) *m/z* 732 $(M - H)^-$. Anal. ($C_{37}H_{40}ClN_5O_5S_2 \cdot 1.5$ CF_3CO_2H) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(2-trifluoromethyl-benzylidene)-piperidin-1-yl]-benzoyl}-benzenesulfonamide, Trifluoroacetate Salt, (**10e**). **10e** was prepared starting from **9e** using the procedure described for the preparation of **10b.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.50 (br s, 1H), 8.55 (d, $J = 2.5$ Hz, 1H), 8.29 (d, $J = 9.4$ Hz, 1H), 7.87 (dd, $J = 9.3$, 2.2 Hz, 1H), 7.76 (d, $J = 9.0$ Hz, 2H), 7.72 (d, $J = 7.8$ Hz, 1H), 7.64 (t, $J = 7.4$ Hz, 1H), 7.47 (t, $J = 7.8$ Hz, 1H), 7.35 (d, $J = 7.6$ Hz, 1H), 7.24 (m, 2H), 7.10−7.20 (m, 4H), 6.96 (d, $J = 9.2$ Hz, 2H), 6.50 (s, 1H), 4.19 (m, 1H), 3.52 (m, 2H), 3.40 (m, 4H), 3.14 (m, 2H), 2.75 (s, 6H), 2.42 (m, 2H), 2.24 (m, 2H), 2.15 (m, 2H). MS (ESI) *m/z* 766 $(M - H)^-$. Anal. ($C_{38}H_{40}F_3N_5O_5S_2 \cdot 2.0$ CF_3CO_2H) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(4-trifluoromethyl-benzylidene)-piperidin-1-yl]-benzoyl}-benzenesulfonamide, Trifluoroacetate Salt, (**10f**). **10f** was prepared starting from **9f** using the procedure described for the preparation of **10b.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.50 (br s, 1H), 8.56 (d, $J = 2.3$ Hz, 1H), 8.29 (d, $J = 9.3$ Hz, 1H), 7.87 (dd, $J = 9.2$, 2.2 Hz, 1H), 7.77 (d, $J = 9.0$ Hz, 2H), 7.68 (d, $J = 8.0$ Hz, 2H), 7.46 (d, $J = 8.0$ Hz, 2H), 7.24 (m, 2H), 7.10−7.20 (m, 4H), 6.96 (d, $J = 9.0$ Hz, 2H), 6.48 (s, 1H), 4.19 (m, 1H), 3.54 (m, 2H), 3.46 (m, 2H), 3.39 (m, 2H), 3.14 (m, 2H), 2.75 (s, 6H), 2.54 (m, 2H), 2.46 (m, 2H), 2.14 (m, 2H). MS (ESI) *m/z* 766 $(M - H)^-$. MS (ESI) *m/z* 766 $(M - H)^-$. Anal. ($C_{38}H_{40}F_3N_5O_5S_2 \cdot 1.5$ CF_3CO_2H) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(2-methoxy-benzylidene)-piperidin-1-yl]-benzoyl}-benzenesulfonamide, Trifluoroacetate Salt, (**10g**). **10g** was prepared starting from **9g** using the procedure described for the preparation of **10b.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.40 (br s, 1H), 8.55 (d, $J = 2.3$ Hz, 1H), 8.28 (d, $J = 9.3$ Hz, 2H), 7.87 (dd, $J = 9.4$, 2.1 Hz, 1H), 7.75 (d, $J = 9.0$ Hz, 2H), 7.09−7.26 (m, 8H), 6.97 (m, 3H), 6.90 (t, $J = 7.4$ Hz, 1H), 6.33 (s, 1H), 4.19 (m, 1H), 3.78 (s, 3H), 3.52 (m, 2H), 3.46 (m, 2H), 3.39 (m, 2H), 3.13 (m, 2H), 2.74 (m, 6H), 2.40 (m, 4H), 2.14 (m, 2H). MS (ESI) *m/z* 728 $(M - H)^-$. Anal. ($C_{38}H_{43}N_5O_5S_2 \cdot 2.5$ CF_3CO_2H) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(2-cyano-benzylidene)-piperidin-1-yl]-benzoyl}-benzenesulfonamide, Trifluoroacetate Salt, (**10h**). **10h** was prepared starting from **9h** using the procedure described for the preparation of **10b.** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.43 (br s, 1H), 8.55 (d, $J = 2.4$ Hz, 1H), 8.29 (d, $J = 9.1$ Hz, 1H), 7.87 (dd, $J = 9.1$, 2.0 Hz, 1H), 7.83 (d, $J = 7.5$ Hz, 1H), 7.76

(d, J = 9.0 Hz, 2H), 7.68 (td, J = 7.6, 1.5 Hz, 1H), 7.45 (m, 2H), 7.24 (m, 2H), 7.09−7.18 (m, 4H), 6.98 (d, J = 9.1 Hz, 2H), 6.52 (s, 1H), 4.18 (m, 1H), 3.55 (m, 2H), 3.47 (m, 2H), 3.38 (m, 2H), 3.13 (m, 2H), 2.74 (s, 6H), 2.46 (m, 2H), 2.38 (m, 2H), 2.15 (m, 2H). MS (ESI) m/z 723 $(M - H)^-$. Anal. ($C_{38}H_{40}N_6O_5S_2 \cdot 1.5\ CF_3CO_2H$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-pyridin-2-ylmethylene-piperidin-1-yl]-benzoyl}-benzenesulfonamide, (10i). **10i** was prepared starting from **9i** using the procedure described for the preparation of **10a.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.05 (br s, 1H), 9.53 (br s, 1H), 8.57 (m, 1H), 8.29 (d, J = 9.3 Hz, 1H), 7.85−7.95 (m, 2H), 7.77 (d, J = 9.1 Hz, 2H), 7.44 (d, J = 7.8 Hz, 1H), 7.34 (m, 1H), 7.09−7.26 (m, 6H), 6.97 (d, J = 9.0 Hz, 2H), 6.44 (s, 1H), 4.18 (m, 1H), 3.55 (m, 2H), 3.49 (m, 2H), 3.39 (m, 2H), 3.13 (m, 2H), 2.94 (m, 2H), 2.74 (s, 6H), 2.47 (m, 2H), 2.14 (m, 2H). MS (ESI) m/z 699 $(M - H)^-$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-pyridin-3-ylmethylene-piperidin-1-yl]-benzoyl}-benzenesulfonamide, (10j). **10j** was prepared starting from **9j** using the procedure described for the preparation of **10a.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 9.60 (br s, 1H), 8.60 (br s, 1H), 8.55 (m, 2H), 8.29 (d, J = 9.3 Hz, 1H), 7.94 (m, 1H), 7.87 (dd, J = 9.5, 2.1 Hz, 1H), 7.60 (m, 1H), 7.09−7.26 (m, 7H), 6.97 (d, J = 9.1 Hz, 2H), 6.44 (s, 1H), 4.19 (m, 1H), 3.55 (m, 2H), 3.48 (m, 2H), 3.39 (m, 2H), 3.15 (m, 2H), 2.74 (m, 6H), 2.44−2.55 (m, 4H), 2.15 (m, 2H). MS (ESI) m/z 699 $[M - H]^-$.

***N*-[4-(4-Biphenyl-2-ylmethylene-piperidin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, Trifluoroacetate Salt, (10k).** **10k** was prepared starting from **9k** using the procedure described for the preparation of **10b.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.38 (br s, 1H), 8.55 (d, J = 2.5 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H), 7.87 (dd, J = 9.1, 2.4 Hz, 1H), 7.74 (d, J = 8.9 Hz, 2H), 7.10−7.43 (m, 15H), 6.92 (d, J = 9.1 Hz, 2H), 6.17 (s, 1H), 4.18 (m, 1H), 3.40 (m, 4H), 3.24 (m, 2H), 3.14 (m, 2H), 2.75 (s, 6H), 2.25 (m, 4H), 2.14 (m, 2H). MS (ESI) m/z 774 $[M - H]^-$.

Ethyl 4-(4-Methylenepiperidin-1-yl)benzoate, (11). To a suspension of CH_3PPh_3I salt (1.972 g, 4.858 mmol) in THF (20 mL) at 0 °C was added nBuLi (1.9 mL, 4.858 mmol) and stirred at 0 °C for 30 min. A solution of ketone **5** (1.0 g, 4.049 mmol) in THF (5 mL) was added dropwise, and the resulting solution was allowed to warm to r.t. over 2 h. The reaction mixture was quenched with saturated NH_4Cl solution, diluted with ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate (100 mL × 2). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography (0−80% ethyl acetate−hexane) to give the desired product **11** (784 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.77 (d, J = 9.2 Hz, 2H), 6.99 (d, J = 9.2 Hz, 2H), 4.78 (s, 2H), 4.23 (q, J = 7.1 Hz, 2H), 3.43 (t, J = 5.8 Hz, 4H), 2.24 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H). MS (DCI) m/z 246.1 $[M + H]^+$.

4-(4-Methylenepiperidin-1-yl)benzoic Acid, (12). To a solution of ester **11** (480 mg, 1.959 mmol) in THF−EtOH−H_2O (8 mL-2 mL-2 mL) was added LiOH−H_2O (165 mg, 3.918 mmol) and heated under reflux for 6 h. The solution was partitioned between ethyl acetate and saturated NH_4Cl solution, and the layers were separated. The aqueous layer was extracted with ethyl acetate (100 mL × 2), and the combined organic layers were dried over $MgSO_4$. The solution was concentrated to give the desired product **12** (376 mg, 88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 9.2 Hz, 2H), 6.95 (d, J = 9.2 Hz, 2H), 4.77 (s, 2H), 3.40 (m, 4H), 2.24 (m, 4H). MS (DCI) m/z 218.0 $[M + H]^+$.

(*R*)-*N*-(4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)-4-(4-methylenepiperidin-1-yl)-benzamide, (13). To a solution of carboxylic acid **12** (300 mg, 1.382 mmol) and sulfonamide **4** (586 mg, 1.382 mmol) in dichloromethane (7 mL) were added EDCI (531 mg, 2.764 mmol) and DMAP (169 mg, 1.382 mmol) and stirred at ambient temperature overnight. The solution was diluted with dichloromethane and washed with saturated NH_4Cl. The aqueous layer was extracted with dichloromethane (50 mL × 3), and the combined organic layers were dried over $MgSO_4$. The solution was concentrated, and the residue was purified by flash chromatography (0−10% 7N NH_3 in MeOH−DCM) to give the desired product **13** (590 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (d, J = 2.2 Hz, 1H), 8.26 (d, J = 9.2 Hz, 1H), 7.80 (dd, J = 8.9, 1.8 Hz, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.32 (m, 2H), 7.25 (t, J = 7.5 Hz, 2H), 7.17 (t, J = 7.2 Hz, 1H), 6.89 (d, J = 9.2 Hz, 1H), 6.84 (d, J = 9.2 Hz, 2H), 4.74 (s, 2H), 4.05 (m, 1H), 3.35 (br, 6H), 2.81 (m, 1H), 2.70 (m, 1H), 2.45 (s, 6H), 2.23 (m, 4H), 2.06 (m, 1H), 1.97 (m, 1H). MS (DCI) m/z 624.3 $[M + H]^+$.

(*R*)-*N*-(4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)-4-(3-phenyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-8-yl)benzamide, (14a). To a solution of alkene **13** (112 mg, 0.180 mmol) in $CHCl_3$ (2 mL) at 50 °C were simultaneously added a solution of *N*-hydroxybenzimidoyl chloride (84 mg, 0.540 mmol) in $CHCl_3$ (1 mL) and a solution of triethylamine (75 μL, 0.540 mmol) in $CHCl_3$ (1 mL) via syringe pump over 5 h. The solution was concentrated and the residue was purified by flash chromatography (0−10% 7 N NH_3 in MeOH−CH_2Cl_2) followed by HPLC (C8 reverse phase column, 20−80% acetonitrile-0.1% TFA in water) to give the desired product **14a** as TFA salt (75 mg, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (br s, 1H), 9.51 (br s, 1H), 8.56 (d, J = 2.2 Hz, 1H), 8.30 (d, J = 9.2 Hz, 1H), 7.88 (dd, J = 9.2, 2.2 Hz, 1H), 7.77 (d, J = 8.9 Hz, 2H), 7.66 (m, 2H), 7.45 (m, 3H), 7.25 (m, 2H), 7.14 (m, 4H), 7.01 (d, J = 8.9 Hz, 2H), 4.18 (m, 1H), 3.79 (br, 2H), 3.53 (m, 4H), 3.40 (d, J = 5.8 Hz, 2H), 3.15 (m, 2H), 2.75 (s, 6H), 1.82 (t, J = 5.5 Hz, 4H). MS (ESI) m/z 743.4 $[M + H]^+$. Anal. ($C_{38}H_{42}N_6O_6S_2 \cdot 2CF_3CO_2H \cdot 0.5H_2O$) C, H, N.

(*R*)-4-(3-Benzyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-8-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide, (14b). **14b** was prepared from **13** and phenylacetohydroximoyl chloride according to the procedure for the preparation of **14a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.49 (br s, 1H), 8.55 (d, J = 2.2 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.87 (dd, J = 9.2, 2.2 Hz, 1H), 7.76 (m, 3H), 7.34 (t, J = 7.2 Hz, 2H), 7.25 (m, 4H), 7.14 (m, 4H), 6.95 (d, J = 9.2 Hz, 2H), 4.18 (m, 1H), 3.64 (s, 2H), 3.39 (br, 6H), 3.14 (m, 2H), 2.75 (s, 6H), 2.66 (s, 2H), 2.14 (m, 2H), 1.66 (m, 4H). MS (ESI) m/z 757.4 $[M + H]^+$. Anal. ($C_{39}H_{44}N_6O_6S_2 \cdot 1.5CF_3CO_2H \cdot 0.5\ H_2O$) C, H, N.

Ethyl 4-(2-Methyl-3-oxo-1-oxa-4,8-diazaspiro[4.5]decan-8-yl)-benzoate, (15). A solution of ketone **5** (500 mg, 2.024 mmol) and lactamide (198 mg, 2.227 mmol) in benzene (5 mL) was treated with *p*-toluenesulfonic acid (19 mg, 0.101 mmol) and heated under reflux with azeotropic removal of water for 36 h. The reaction mixture was quenched with a few drops of triethylamine and concentrated. The residue was purified by flash chromatography (0−80% acetonitrile−CH_2Cl_2) to give the desired product **15** (415 mg, 64%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.02 (s, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 4.33 (q, J = 6.7 Hz, 1H), 4.23 (q, J = 7.1 Hz, 2H), 3.65 (m, 2H), 3.38 (m, 2H), 1.74 (m, 4H), 1.27 (m, 6H). MS (ESI) m/z 319 $[M + H]^+$.

Ethyl 4-(4-Benzyl-2-methyl-3-oxo-1-oxa-4,8-diazaspiro[4.5]-decan-8-yl)benzoate, (16). To a solution of oxazolidinone **15** (30 mg, 0.0943 mmol) in THF (1 mL) was added NaH (5.7 mg, 0.142 mmol) and stirred at r.t. for 20 min. Benzyl bromide (17 μL, 0.141 mmol) was added, and the solution was heated at 60 °C overnight. The mixture was quenched with saturated NH_4Cl solution and partitioned between ethyl acetate and saturated NH_4Cl solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate (20 mL × 3). The combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (0−80% acetonitrile−CH_2Cl_2) to give the desired product **16** (31 mg, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 9.2 Hz, 2H), 7.28 (m, 5H), 6.96 (d, J = 9.2 Hz, 2H), 4.48 (m, 3H), 4.23 (q, J = 7.0 Hz, 2H), 3.87 (dd, J = 13.1, 2.2 Hz, 2H), 3.04 (t, J = 12.6 Hz, 2H), 1.96 (m, 1H), 1.80 (m, 1H), 1.63 (m, 1H), 1.42 (m, 1H), 1.37 (d, J = 6.4 Hz, 3H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 409.1 $[M + H]^+$.

4-(4-Benzyl-2-methyl-3-oxo-1-oxa-4,8-diazaspiro[4.5]decan-8-yl)benzoic Acid, (17). 17 was prepared from **16** according to the procedure for the preparation of **12**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (br, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.27 (m, 5H), 6.94 (d, J = 8.8 Hz, 2H), 4.48 (m, 3H), 3.85 (m, 2H), 3.03 (t, J = 12.5 Hz, 2H), 1.96 (m, 1H), 1.79 (m, 1H), 1.62 (dd, J = 13.4, 2.2 Hz, 1H), 1.41 (m, 1H), 1.37 (d, J = 6.8 Hz, 3H). MS (ESI) m/z 381.1 $[M + H]^+$.

(*R*)-4-(4-Benzyl-2-methyl-3-oxo-1-oxa-4,8-diazaspiro[4.5]decan-8-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide, (18). 18 was prepared from **17** according to the procedure for the preparation of **13**. ^{1}H NMR (500 MHz, CD_3OD) δ 8.68 (d, J = 2.5 Hz, 1H), 7.95 (dd, J = 9.4, 2.2 Hz, 1H), 7.72 (d, J = 9.1 Hz, 2H), 7.23 (m, 7H), 7.06 (m, 3H), 7.01 (d, J = 9.1 Hz, 1H), 6.90 (d, J = 9.4 Hz, 2H), 4.47 (m, 3H), 4.17 (m, 1H), 3.84 (m, 2H), 3.41 (dd, J = 14.7, 5.0 Hz, 1H), 3.21 (m, 5H), 2.88 (s, 6H), 2.24 (m, 2H), 1.99 (m, 1H), 1.79 (m, 1H), 1.61 (dd, J = 13.3, 2.3 Hz, 1H), 1.48 (m, 1H), 1.45 (d, 3H). MS (ESI) m/z 787.4 $[M + H]^+$. Anal. ($C_{40}H_{46}N_6O_7S_2 \cdot 1.5\ CF_3CO_2H \cdot 0.5\ H_2O$) C, H, N.

(*R*)-*tert*-Butyl 4-(4-(4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonylcarbamoyl)phenyl)-piperazine-1-carboxylate, (19b). Compound **19a** was prepared from 4-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)benzoic acid according to the procedure described for the preparation of **8a**. A solution of compound **19a** in dioxane was treated with an excess of 4 M HCl in dioxane and stirred overnight. The reaction mixture was concentrated to near dryness and diluted with Et_2O and the resulting precipitate (**19b**) collected by filtration, dried in vacuo, and carried on to the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (br s, 1H), 10.32 (br s, 1H), 9.09−9.28 (m, 2H), 8.54 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 9.5 Hz, 1H), 7.86 (dd, J = 9.2, 2.4 Hz, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.20−7.28 (m, 2H), 7.08−7.15 (m, 3H), 7.02 (d, J = 9.2 Hz, 2H), 4.17−4.35 (m, 1H), 3.55 (m, 4H), 3.35−3.41 (m, 2H), 3.14−3.23 (m, 4H), 3.06−3.15 (m, 2H), 2.66−2.75 (m, 6H), 2.11−2.24 (m, 2H). MS (ESI), m/z 611 $[M - H]^-$.

***N*-[4-(4-Benzoyl-piperazin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide,** (**20**). To a suspension of HCl salt of amine **19b** (68.6 mg, 0.1 mmol) and triethylamine (40.4 mg, 56 μL, 0.4 mmol) in DMA (1.5 mL) was added benzoyl chloride (16.9 mg, 13.9 μL, 0.12 mmol). The reaction mixture was shaken for 16 h, then concentrated, redisolved in DMSO/MeOH (1 mL), and purified by reverse phase preparative HPLC, providing 42 mg (51%) of TFA salt of **20**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.09 (br s, 1H), 9.52 (br s, 1H), 8.55 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 9.5 Hz, 1H), 7.87 (dd, J = 9.2, 2.1 Hz, 1H), 7.78 (d, J = 9.2 Hz, 2H), 7.35−7.50 (m, 5H), 7.21−7.26 (m, 2H), 7.08−7.21 (m, 4H), 6.97 (d, J = 9.2 Hz, 2H), 4.13−4.25 (m, 1H), 3.73 (br s, 2H), 3.33−3.42 (m, 8H), 3.06−3.21 (m, 2H), 2.74 (s, 6H), 2.09−2.20 (m, 2H). MS (ESI), m/z 715 $[M - H]^-$. Anal. ($C_{36}H_{40}N_6O_6S_2 \cdot 1.5CF_3CO_2H \cdot 0.5\ H_2O$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(toluene-4-sulfonyl)-piperazin-1-yl]-benzoyl}-benzenesulfonamide, (21). To a suspension of HCl salt of amine **19b** (68.6 mg, 0.1 mmol) and triethylamine (40.4 mg, 56 μL, 0.4 mmol) in DMA (1.5 mL) was added tosyl chloride (22.9 mg, 0.12 mmol). The reaction mixture was shaken for 16 h, then concentrated, redisolved in DMSO/MeOH (1 mL), and purified by reverse phase preparative HPLC, providing 43 mg (49%) of TFA salt of **21**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.09 (br s, 1H), 9.56 (br s, 1H), 8.54 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 9.2 Hz, 1H), 7.84−7.88 (m, 1H), 7.74 (d, J = 9.2 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.21−7.25 (m, 2H), 7.07−7.20 (m, 4H), 6.92 (d, J = 9.2 Hz, 2H), 4.13−4.24 (m, 1H), 3.35−3.45 (m, 6H), 3.07−3.21 (m, 2H), 2.91−2.98 (m, 4H), 2.74 (s, 6H), 2.39 (s, 3H), 2.09−2.18 (m, 2H). MS (ESI), m/z 765 $[M - H]^-$. Anal. ($C_{36}H_{42}N_6O_7S_3 \cdot 1.5\ CF_3CO_2H$) C, H, N.

4-{4-[4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonylaminocarbonyl]-phenyl}-piperazine-1-carboxylic Acid Phenylamide, (22). To a suspension of HCl salt of amine **19b** (68.6 mg, 0.1 mmol) and triethylamine (40.4 mg, 56 μL, 0.4 mmol) in DMA (1.5 mL) was added phenyl isocyanate (14.3 mg, 13.1 μL, 0.12 mmol). The reaction mixture was shaken for 16 h, then concentrated, redisolved in DMSO/MeOH (1 mL), and purified by reverse phase preparative HPLC, providing 51 mg (60%) of TFA salt of **22**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (br s, 1H), 9.55 (br s, 1H), 8.61 (s, 1H), 8.56 (d, J = 2.1 Hz, 1H), 8.30 (d, J = 9.2 Hz, 1H), 7.85−7.91 (m, 1H), 7.79 (d, J = 9.2 Hz, 2H), 7.46 (d, J = 7.6 Hz, 2H), 7.08−7.27 (m, 8H), 7.00 (d, J = 9.2 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.15−4.26 (m, 1H), 3.55−3.63 (m, 4H), 3.36−3.42 (m, 6H), 3.07−3.22 (m, 2H), 2.75 (s, 6H), 2.09−2.21 (m, 2H). MS (ESI), m/z 730 $[M - H]^-$. Anal. ($C_{36}H_{41}N_7O_6S_2 \cdot 1.5\ CF_3CO_2H$) C, H, N.

***N*-[4-(4-Benzyl-piperazin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, (23a)**. To a suspension of **19b** (34.3 mg, 0.05 mmol) and benzaldehyde (10.6 mg, 0.1 mmol) in MeOH/dichloromethane (1/1, 2 mL) was added DIEA until pH was adjusted to 5 followed by the addition of MP−BH_3CN (2.42 mmol/g, 83 mg, 0.2 mmol). The reaction mixture was shaken for 16 h, the resin was removed by filtration and washed with MeOH/dichloromethane (3 × 5 mL), and the combined organic layers were concentrated under reduced presure. The residue was purified by reverse phase preparative HPLC providing 20 mg (43%) of **23a**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (s, 1H), 10.25 (s, 1H), 9.59 (s, 1H), 8.55 (d, J = 2.2 Hz, 1H), 8.29 (d, J = 9.4 Hz, 2H), 7.87 (dd, J = 9.0, 2.2 Hz, 2H), 7.81 (d, J = 9.0 Hz, 2H), 7.40−7.55 (m, 6H), 7.23 (d, J = 6.9 Hz, 2H), 7.08−7.20 (m, 4H), 7.01 (d, J = 9.0 Hz, 2H), 4.35 (s, 2H), 4.16−4.23 (m, 1H), 4.03 (s, 2H), 3.42−3.46 (m, 4H), 3.39 (d, J = 6.2 Hz, 2H), 3.03−3.28 (m, 6H), 2.75 (s, 6H), 2.10−2.19 (m, 2H). MS (ESI), m/z 701 $[M - H]^-$.

4-(3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-methyl-benzyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (**23b**). To a suspension of the piperazine **19b** (150 mg, 0.22 mmol), 2-methylbenzaldhehyde (32 mg, 0.26 mmol), and DIPEA resin (3.45 mmol/g, 127 mg, 0.44 mmol) in dichloromethane/methanol (1/1, 2 mL) was added sodium triacetoxyborohydride (97 mg, 0.46 mmol). The reaction mixture was shaken for 16 h, the resin was removed by filtration and washed with MeOH/dichloromethane (3 × 5 mL), and the combined organic layers were concentrated under reduced presure. The mixture was purified by silica gel chromatography eluting with 20% methanol in dichloromethane to yield **23b** in 29% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, J = 2.0 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.81 (dd, J = 9.2, 2.0 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.28−7.35 (m, 2H), 7.21−7.28 (m, 3H), 7.10−7.20 (m, 3H), 6.90 (d, J = 9.5 Hz, 1H), 6.81 (d, J = 8.8 Hz, 1H), 3.98−4.15 (m, J = 5.1 Hz, 1H), 3.47 (s, 2H), 3.33−3.38 (m, 3H), 3.10−3.23 (m, 4H), 2.81 (m, 3H), 2.55 (s, 5H), 2.33 (s, 3H), 1.94−2.16 (m, 2H), 1.91 (s, 3H). MS (ESI), m/z 807 $[M - H]^-$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-methoxy-benzyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (**23c**). **23c** was prepared from **19b** and 2-methoxybenzaldehyde using the procedure described for **23a**. (78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (br s, 1H), 9.82 (br s, 1H), 9.57 (s, 1H), 8.55 (d, J = 2.5 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.87 (dd, J = 9.2, 2.1 Hz, 1H), 7.81 (d, J = 8.9 Hz, 2H), 7.47 (d, J = 7.4 Hz, 2H), 7.21−7.26 (m, 2H), 7.09−7.20 (m, 5H), 7.03−7.07 (m, 1H), 7.01 (d, J = 9.2 Hz, 2H), 4.13−4.24 (m, 1H), 4.04 (br s, 2H), 3.86 (s, 3H), 3.45−3.71 (m, 4H), 3.39 (d, J = 6.1 Hz, 2H), 3.04−3.27 (m, 6H), 2.75 (s, 6H), 2.10−2.19 (m, 2H). MS (ESI), m/z 731 $[M - H]^-$. Anal. ($C_{37}H_{44}N_6O_6S_2 \cdot 2.25CF_3CO_2H$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-methylsulfanyl-benzyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (23d). **23d** was prepared from **19b** and 2-methanesulfanyl-benzaldehyde using the procedure described for the preparation of **23b**. (49% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (d, J = 2.0 Hz, 1H), 8.36 (d, J = 8.5 Hz, 1H), 7.78 (dd, J = 9.2, 2.0 Hz, 1H), 7.73 (d, J = 9.2 Hz, 2H), 7.21−7.38 (m,

7H), 7.10−7.21 (m, 2H), 6.86 (d, J = 9.5 Hz, 1H), 6.80 (d, J = 9.2 Hz, 2H), 3.99−4.12 (m, 1H), 3.52 (s, 2H), 3.22−3.30 (m, 6H), 3.13−3.21 (m, 4H), 2.51−2.62 (m, 2H), 2.43 (s, 3H), 2.27 (s, 6H), 1.81−2.07 (m, 2H). MS (ESI), *m*/*z* 747 [M − H]$^-$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-methanesulfonyl-benzyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide , (23e). **23e** was prepared from **19b** and 2-methylsulfonylbenzaldehyde[36] using the procedure described for the preparation of **23b**. (36% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, J = 2.0 Hz, 1H), 8.21 (d, J = 9.5 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.81 (dd, J = 9.2, 2.0 Hz, 1H), 7.67−7.76 (m, 3H), 7.60 (d, J = 7.5 Hz, 2H), 7.13−7.33 (m, 5H), 6.87−6.92 (m, 1H), 6.82 (d, J = 9.2 Hz, 2H), 4.01−4.12 (m, 1H), 3.92 (s, 2H), 3.44 (s, 3H), 3.33−3.40 (m, 6H), 3.14−3.24 (m, 4H), 2.82−3.00 (m, 2H), 2.56 (s, 6H), 1.98−2.13 (m, 2H). MS (ESI), *m*/*z* 779 [M − H]$^-$.

2-Cyclohexylamino-benzonitrile, (25). A solution of 2-fluorobenzonitrile **24** (500 mg, 4.2 mmol) and cyclohexylamine (1 mL) in DMSO (2 mL) was heated to 180 °C in a Personal Chemistry microwave reactor for 15 min. The reaction was cooled and poured into ether (50 mL), and the solution was washed with 1 M HCl (3 × 10 mL) and brine, dried over Na_2SO_4, filtered, and concentrated to yield 650 mg (79%) of **25**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.45 (m, 2H), 6.81 (d, J = 8.2 Hz, 1H), 6.62 (dd, J = 8.1, 6.8 Hz, 1H), 5.41 (d, J = 8.1 Hz, 1H), 3.40 (m, 1H), 1.91 (m, *2*H), 1.69 (m, 2H), 1.58 (m, 1H), 1.30 (m, 4H), 1.16 (m, 1H). MS (ESI), *m*/*z* 201 [M + H]$^+$.

2-Cyclohexylamino-benzaldehyde, (26). A solution of **25** (640 mg, 3.2 mmol) in toluene (5 mL) at 0 °C was treated with 1 M DIBAL in toluene (8 mL), and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched with methanol (5 mL) and taken up in 1 M HCl solution (20 mL). The solution was extracted with EtOAc (3 × 25 mL), and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by chromatography on silica gel using 10% EtOAc/hexanes to yield 80 mg (12%) of **26**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.30 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.41 (dd, J = 7.8, 8.5 Hz, 1H), 6.83 (d, J = 8.5 Hz, 1H), 6.66 (dd, J = 7.1, 6.8 Hz, 1H), 3.50 (m, 1H), 1.90 (m, *2*H), 1.66 (m, 2H), 1.39 (m, 2H), 1.28 (m, 2H). MS (ESI), *m*/*z* 204 [M + H]$^+$.

***N*-{4-[4-(2-Cyclohexylamino-benzyl)-piperazin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, (23f)**. **23f** was prepared from **19b** and **26** using the procedure described for the preparation of **23b**. (Yield 18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (d, J = 2.5 Hz, 1H), 8.22 (d, J = 8.9 Hz, 1H), 7.85 (dd, J = 9.2, 2.1 Hz, 1H), 7.72−7.79 (m, 2H), 7.26 (d, J = 7.1 Hz, 2H), 7.04−7.22 (m, 5H), 6.97 (d, J = 6.8 Hz, 1H), 6.91 (d, J = 8.9 Hz, 2H), 6.58 (d, J = 8.3 Hz, 1H), 6.48−6.52 (m, 1H), 4.10−4.24 (m, 1H), 3.45−3.56 (m, 2H), 3.37 (d, J = 5.8 Hz, 2H), 3.21−3.28 (m, 4H), 3.08−3.16 (m, 4H), 2.70 (s, 6H), 2.43−2.49 (m, 2H), 2.12−2.20 (m, 2H), 1.84−1.93 (m, 2H), 1.59−1.70 (m, 2H), 1.48−1.56 (m, 1H), 1.30−1.40 (m, 2H), 1.20−1.29 (m, 4H). MS (ESI), *m*/*z* 798 [M − H]$^-$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-morpholin-4-yl-benzyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (23g). **23g** was prepared from **19b** and 2-morpholin-4-yl-benzaldehyde using the procedure described for the preparation of **23b**. (Yield 23%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, J = 2.0 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.80 (dd, J = 8.8, 2.0 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.39 (dd, J = 7.5, 1.4 Hz, 1H), 7.21−7.35 (m, 5H), 7.02−7.20 (m, 3H), 6.89 (d, J = 9.5 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 4.06 (m, 1H), 3.68−3.79 (m, 4H), 3.57 (s, 2H), 3.34−3.41 (m, 4H), 3.25−3.28 (m, 2H), 3.13−3.21 (m, 4H), 2.89−2.98 (m, 4H), 2.77−2.87 (m, 2H), 2.52 (s, 6H), 1.92−2.14 (m, 2H). MS (ESI), *m*/*z* 786 [M − H]$^-$. Anal. ($C_{40}H_{49}N_7O_6S_2 \cdot 1.5H_2O$) C, H, N.

***N*-{4-[4-(2-Cyclohexyl-benzyl)-piperazin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, (23h)**. **23h** was prepared from **19b** and 2-cyclohexyl-benzaldehyde using the procedure described for the preparation of **23a**. (47% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (d, J = 2.0 Hz, 1H), 8.19 (d, J = 9.2 Hz, 1H), 7.82 (dd, J = 9.2, 1.7 Hz, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.27−7.34 (m, 2H), 7.06−7.28 (m, 7H), 6.92 (d, J = 9.2 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 3.98−4.15 (m, 1H), 3.51 (s, 2H), 3.32−3.39 (m, 4H), 3.25−3.29 (m, 2H), 3.10−3.20 (m, 4H), 2.85−3.05 (m, 3H), 2.62 (s, 6H), 2.00−2.17 (m, 2H), 1.72 (s, 4H), 1.24−1.50 (m, 6H). MS (ESI), *m*/*z* 783 [M − H]$^-$.

4-(4-Biphenyl-2-ylmethyl-piperazin-1-yl)-benzoic Acid Ethyl Ester, (**28**). 4-Piperazin-1-yl-benzoic acid ethyl ester (**27**) (0.20 g, 0.85 mmol) was dissolved in 1,4-dioxane (4 mL) and treated with 2-bromomethyl-biphenyl (0.23 g, 0.94 mmol) and *N*,*N*-diisopropylethylamine (0.17 g, 1.3 mmol). The solution was heated to 40 °C for 15 min, concentrated, and purified by silica gel chromatography eluting with 20% ethyl acetate in hexanes to yield 0.34 g (100%) of **28**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, J = 6.8 Hz, 2H), 7.55 (dd, J = 6.8, 0.9 Hz, 1 H), 7.46−7.32 (m, 7H), 7.24 (dd, J = 6.8 Hz, J = 1.2 Hz, 1H), 6.94 (d, J = 9.1 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 3.42 (s, 2H), 3.25 (t, J = 4.9 Hz, 4H), 2.39 (t, J = 4.9 Hz, 4H), 1.28 (t, J = 7.1 Hz, 3 H). MS (ESI) *m*/*z* 401 [M + H]$^+$.

4-[4-(2-Trifluoromethyl-benzyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (29). **29** was prepared from **27** and 2-trifluoromethylbenzyl bromide according to the procedure described for the preparation of **28**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.62−7.90 (m, 5H), 7.44−7.54 (m, 1H), 6.97 (d, J = 9.2 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.68 (s, 2H), 3.31−3.38 (m, 4H), 2.52−2.59 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI) *m*/*z* 393 [M + H]$^+$.

4-[4-(2-Bromo-benzyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**30**). A solution of **27** (1.200 g, 5.12 mmol) and 2-bromobenzaldehyde (1.04 g, 5.63 mmol) in 1,2-dichloroethane (25 mL) was treated with sodium triacetoxyborohydride (1.20 g, 5.63 mmol), and the reaction mixture was stirred for 75 min. The solution was filtered through silica gel, the eluent concentrated, and the crude material recrystallized from ethyl acetate to yield 1.84 g (89%) of **30**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 8.8 Hz, 2H), 7.62 (dd, J = 8.0, J = 1.2, 1H), 7.53 (dd, J = 7.8, J = 1.7, 1H), 7.40 (td, J = 7.5, J = 1.4, 1H), 7.22 (td, J = 7.5, J = 1.8, 1H), 6.98 (d, J = 9.2 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.61 (s, 2H), 3.32 (t, J = 4.9 Hz, 4H), 2.57 (t, J = 4.9 Hz, 4H), 1.28 (t, J = 7.0 Hz, 3H). MS (ESI) *m*/*z* 403/405 [M + H]$^+$.

4-[4-(2-Pyridin-3-yl-benzyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**31**). A suspension of **30** (0.20 g, 0.50 mmol), 3-pyridine boronic acid (0.074 g, 0.60 mmol), $PdCl_2(PPh_3)_2$ (cat.), and Na_2CO_3 (2 M, 0.60 mL) in DME/water/EtOH (7:3:2) (4 mL) was irradiated in a microwave reactor (Personal chemistry) at 150 °C for 3 min at normal absorbance solvent setting. The solvents were evaporated *in vacuo*, and the residue was partitioned between dichloromethane (5 mL) and water (1 mL). The mixture was loaded on the celite cartridge (5 g) and washed with dichloromethane (2 × 5 mL). The dichloromethane was evaporated and the residue redisolved in DMSO/MeOH (1:1, 2.5 mL) and purified by reverse phase HPLC to yield 0.10 g (50%) of **31**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.74 (br s, 1H), 8.71 (dd, J = 5.1, 1.7 Hz, 1H), 8.67 (s, 1H), 7.97 (d, J = 7.1 Hz, 1H), 7.79 (d, J = 9.2 Hz, 3H), 7.54−7.68 (m, 3H), 7.37−7.45 (m, 1H), 6.96 (d, J = 9.2 Hz, 2H), 4.38 (s, 2H), 4.23 (q, J = 7.1 Hz, 2H), 3.88 (br s, 2H), 3.27 (br s, 2H), 3.09 (br s, 2H), 2.93 (br s, 2H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI), *m*/*z* 402 [M + H]$^+$.

4-[4-(3′-Methoxy-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**32**). **32** was prepared from **30** and 3-methoxy-phenylboronic according to the procedure described for the preparation of **31**. (Yield 62%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.79 (d, J = 8.8 Hz, 3H), 7.52 (d, J = 4.1 Hz, 2H), 7.29−7.45 (m, 2H), 6.83−7.05 (m, 5H), 4.39 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.86 (s, 2H), 3.80 (s, 3H), 3.39 (br s, 2H), 3.12 (br s, 2H), 2.87 (br s, 2H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI), *m*/*z* 431 [M + H]$^+$.

4-[4-(4′-Methoxy-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**33**). **33** was prepared from **30** and 4-methoxy-phenylboronic acid according to the procedure described

for the preparation of **31**. (Yield 80%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.67−7.76 (m, 1H), 7.45−7.56 (m, 2H), 7.32−7.37 (m, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 9.2 Hz, 2H), 4.38 (s, 2H), 4.19−4.28 (m, 2H), 3.80 (s, 3H), 3.70−3.92 (m, 2H), 3.26 (s, 2H), 3.12 (s, 2H), 2.86 (s, 2H), 1.22−1.34 (m, 3H). MS (ESI), m/z 431 $[M + H]^+$.

4-[4-(2′-Chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**34**). **34** was prepared from **30** and 2-chlorophenylboronic acid according to the procedure described for the preparation of **31**. (Yield 54%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.02 (s, 1H), 7.79 (m, 3H), 7.34−7.67 (m, 6H), 7.21−7.35 (m, 1H), 6.97 (d, J = 9.2 Hz, 2H), 4.29−4.48 (m, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.61−4.16 (m, 4H), 2.86−3.44 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI), m/z 435 $[M + H]^+$.

4-[4-(3′-Chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**35**). **35** was prepared from **30** and 3-chlorophenylboronic acid according to the procedure described for the preparation of **31**. (Yield 73%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.11 (br s, 1H), 7.79 (m, 3H), 7.45−7.60 (m, 5H), 7.29−7.40 (m, 2H), 6.97 (d, J = 9.2 Hz, 2H), 4.33 (s, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.63−4.03 (m, 2H), 3.01−3.48 (m, 4H), 2.92 (br s, 2H), 1.28 (t, J = 7.1 Hz, 3H). MS (ESI), m/z 435 $[M + H]^+$.

4-[4-(4′-Chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**36**). A suspension of **30** (13.83 g, 34.3 mmol), 4-chlorophenylboronic acid (7.04 g, 45 mmol), $PdCl_2(PPh_3)_2$ (0.48 g, 0.686 mmol, 2 mol %), and 2 M aq Na_2CO_3 (22.5 mL) in DME/H_2O/EtOH (1:1:1, 200 mL) was heated at 90 °C for 4.5 h. The mixture was diluted with ethyl acetate (200 mL), the layers were separated, and the organic layer was dried ($MgSO_4$) and concentrated. The oily residue was purified by silica gel chromatography eluting with a gradient from 5% to 40% ethyl acetate in hexanes to yield 10.90 g (73%) of **36** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.36 (t, J = 7 Hz, 3H), 2.49 (m, 4H), 3.26 (m, 4H), 3.41 (s, 2H), 4.32 (q, J = 7 Hz, 2H), 6.83 (d, J = 9 Hz, 2H), 7.23−7.26 (m, 1H), 7.30−7.38 (m, 5H), 7.91 (d, J = 9 Hz, 2H). MS (ESI) m/z 435 $[M + H]^+$.

4-(4-[1,1′;4′,1″]Terphenyl-2-ylmethyl-piperazin-1-yl)-benzoic Acid Ethyl Ester, (**37**). **37** was prepared from **30** and 4-biphenylboronic acid according to the procedure described for the preparation of **31**. (Yield 38%) ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.74 (br s, 1H), 8.08 (s, 1H), 7.88 (d, J = 8.5 Hz, 2H), 7.60−7.83 (m, 7H), 7.35−7.60 (m, 7H), 6.95 (d, J = 8.8 Hz, 2H), 4.48 (s, 1H), 4.14−4.29 (m, 2H), 3.60−3.95 (m, 3H), 3.21−3.46 (m, 2H), 3.15 (m, 2H), 2.93 (br s, 2H), 1.22−1.31 (m, 3H). MS (ESI), m/z 477 $[M + H]^+$.

4-[4-(4′-Fluoro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**38**). **38** was prepared from **30** and 4-fluorophenylboronic acid according to the procedure described for the preparation of **31**. (Yield 66%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84−7.98 (m, 2H), 7.50 (d, J = 5.4 Hz, 1H), 7.29−7.45 (m, 4H), 7.23 − 7.26 (m, 1H), 7.02−7.13 (m, 2H), 6.82 (d, J = 9.2 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.41 (s, 2H), 3.14−3.34 (m, 4H), 2.40−2.60 (m, 4H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI), m/z 419 $[M + H]^+$.

4-[4-(4′-Trifluoromethyl-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**39**). **39** was prepared from **30** and 4-trifluoromethyl-phenylboronic acid according to the procedure described for the preparation of **31**. (Yield 68%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83−7.97 (m, 2H), 7.60−7.70 (m, 2H), 7.46−7.60 (m, 3H), 7.31−7.44 (m, 2H), 7.20−7.29 (m, 1H), 6.82 (d, J = 9.2 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.41 (s, 2H), 3.15−3.30 (m, 4H), 2.39−2.57 (m, 4H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI), m/z 469 $[M + H]^+$.

4-[4-(4′-methanesulfonyl-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoic Acid Ethyl Ester, (**40**). **40** was prepared from **30** and 4-methanesulfonyl-phenylboronic acid according to the procedure described for the preparation of **31**. (Yield 64%) ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 8.8 Hz, 2H), 7.68−7.74 (m, 2H), 7.51−7.58 (m, 1H), 7.36−7.45 (m, 2H), 7.27−7.31 (m, 1H), 6.89 (d, J = 9.2 Hz, 2H), 4.29 (q, J = 7.1 Hz, 2H), 3.48 (s, 2H), 3.21−3.25 (m, 4H), 3.17 (s, 3H), 2.43−2.47 (m, 4H), 1.34 (t, J = 7.1 Hz, 3H). MS (ESI), m/z 479 $[M + H]^+$.

***N*-[4-(4-Biphenyl-2-ylmethyl-piperazin-1-yl)-benzoyl]-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, (23i)**. A solution of **28** (340 mg, 0.85 mmol) in a 3:1:1 mixture of tetrahydrofuran/methanol/water (5 mL) was treated with lithium hydroxide monohydrate (143 mg, 3.40 mmol) and the solution stirred for 16 h. The reaction mixture was acidified with 4.0 M HCl (0.75 mL) and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, concentrated, and purified by silica gel chromatography eluting with 20% methanol in dichloromethane to yield 200 mg (63%) of the intermediate acid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, J = 9.0 Hz, 2H), 7.55 (dd, J = 7.5 Hz, 0.9 Hz, 1 H), 7.45−7.31 (m, 7H), 7.24 (dd, J = 7.5 Hz, 1.2 Hz, 1H), 6.91 (d, J = 9.0 Hz, 2H), 3.42 (s, 2H), 3.23 (t, J = 4.8 Hz, 4H), 2.39 (t, J = 4.8 Hz, 4H). MS (ESI) m/z 371 $[M - H]^-$.

A suspension of **4** (115 mg, 0.27 mmol), the above intermediate acid (112 mg, 0.30 mmol), EDCI (109 mg, 0.57 mmol), and DMAP (49 mg, 0.41 mmol) in dichloromethane (2.5 mL) was stirred for 16 h and the reaction mixture concentrated. The resulting residue was purified by silica gel chromatography eluting with 20% methanol in dichloromethane to yield 80 mg (38%) **23i**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (d, J = 2.0 Hz, 1H), 8.28 (d, J = 9.2 Hz, 1H), 7.76−7.82 (m, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.50−7.57 (m, 1H), 7.28−7.45 (m, 10H), 7.20−7.29 (m, 3H), 7.12−7.21 (m, 1H), 6.87 (d, J = 9.2 Hz, 1H), 6.78 (d, J = 9.2 Hz, 2H), 3.98−4.13 (m, 1H), 3.41 (s, 2H), 3.07−3.18 (m, 4H), 2.62−2.82 (m, 2H), 2.42 (s, 6H), 2.34−2.41 (m, 4H), 1.85−2.11 (m, 2H). MS (ESI), m/z 777 $[M - H]^-$.

4-(3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(2-trifluoromethyl-benzyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (23j). **23j** was prepared from **29** according to the procedure used for the preparation of **23i** and the product purified by reverse phase HPLC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.09 (s, 1H), 9.37 (s, 1H), 8.55 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 9.2 Hz, 1H), 7.83−7.92 (m, 3H), 7.79 (d, J = 8.8 Hz, 3H), 7.59 (s, 1H), 7.09−7.27 (m, 6H), 6.98 (d, J = 9.2 Hz, 2H), 4.12−4.26 (m, 1H), 3.78−4.08 (m, 2H), 3.25−3.48 (m, 6H), 3.13 (d, J = 13.9 Hz, 4H), 2.81−3.02 (m, 2H), 2.75 (d, J = 3.7 Hz, 6H), 2.07−2.21 (m, J = 7.1 Hz, 2H). MS (ESI), m/z 769 $[M - H]^-$. Anal. ($C_{37}H_{41}F_3N_6O_5S_2 \cdot 2.5\ CF_3CO_2H$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(2-pyridin-3-yl-benzyl)-piperazin-1-yl]-benzoyl}-benzenesulfonamide, (**23k**). A suspension of **31** (0.10 g, 0.25 mmol) in a 3:1 mixture of dioxane/water (4 mL) was treated with lithium hydroxide (0.75 mmol, 0.75 mL of a 1 M aqueous solution) and heated at 80 °C for 16 h. The solution was neutralized with 1 M HCl (0.75 mL) and the resulting precipitate collected by filtration, washed with water, and dried in vacuum oven for 24 h to yield 82 mg (80%) of the intermediate acid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 8.61 (d, J = 2.4 Hz, 1H), 8.57 (dd, J = 4.7, 1.7 Hz, 1H), 7.84−7.93 (m, 1H), 7.74 (d, J = 9.2 Hz, 2H), 7.49−7.56 (m, 1H), 7.36−7.49 (m, 3H), 7.26−7.32 (m, 1H), 6.91 (d, J = 9.2 Hz, 2H), 3.42 (s, 2H), 3.14−3.24 (m, 4H), 2.32−2.42 (m, 4H). MS (ESI), m/z 374 $[M + H]^+$.

23k was prepared from the above intermediate acid according to the procedure for the preparation of **10a** and the product purified by reverse phase HPLC. (Yield 44%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 9.66 (br s, 2H), 8.62−8.73 (m, 2H), 8.54 (d, J = 2.5 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.86 (dd, J = 9.2, 2.1 Hz, 1H), 7.77 (d, J = 8.9 Hz, 3H), 7.53−7.64 (m, 3H), 7.37−7.44 (m, 1H), 7.06−7.26 (m, 6H), 6.93 (d, J = 9.2 Hz, 2H), 4.30 (br s, 2H), 4.13−4.24 (m, 1H), 3.75 (br s, 2H), 3.39 (d, J = 6.1 Hz, 2H), 3.05−3.24 (m, 4H), 2.79−3.05 (m, 3H), 2.75 (s, 6H), 2.15 (q, J = 7.0 Hz, 2H). MS (ESI), m/z 778 $[M - H]^-$. Anal. ($C_{41}H_{45}N_7O_5S_2 \cdot 3.5\ CF_3CO_2H \cdot 0.5\ H_2O$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(3′-methoxy-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (**23l**). **23l** was prepared from

32 according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 15%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 9.58 (br s, 1H), 8.55 (d, J = 2.5 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.86 (dd, J = 2.3, 9.2 Hz, 1H), 7.77 (d, J = 8.9 Hz, 2H), 7.72 (m, 1H), 7.50 (m, 2H), 7.37 (m, 2H), 7.24 (m, 2H), 7.14 (m, 4H), 6.98 (m, 1H), 6.92 (m, 4H), 4.31 (br s, 2H), 4.18 (m, 1H), 3.79 (s, 3H), 3.39 (d, J = 6.1 Hz, 3H), 3.15 (m, 5H), 2.75 (s, 6H), 2.14 (q, J = 8 Hz, 2H). MS (ESI), m/z 807 [M − H]$^-$. Anal. ($C_{43}H_{48}N_6O_6S_2 \cdot 2.5\ CF_3CO_2H$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(4′-methoxy-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (**23m**). **23m** was prepared from **33** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 14%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 9.92 (br s, 1H), 9.57 (br s, 1H), 8.54 (d, J = 2.5 Hz, 1H), 8.29 (d, J = 9.5 Hz, 1H), 7.86 (dd, J = 9.2, 2.1 Hz, 1H), 7.77 (d, J = 8.9 Hz, 3H), 7.69−7.75 (m, 1H), 7.45−7.54 (m, 2H), 7.26−7.36 (m, 3H), 7.07−7.25 (m, 5H), 7.00−7.05 (m, 2H), 6.93 (d, J = 9.2 Hz, 2H), 4.36 (br s, 1H), 4.17 − 4.19 (m, 1H), 3.79 (s, 3H), 3.39 (d, J = 6.1 Hz, 2H), 2.97−3.31 (m, 6H), 2.77−2.95 (m, 2H), 2.75 (s, 3H), 2.74 (s, 3H), 2.15 (m, 2H). MS (ESI), m/z 807 [M − H]$^-$.

***N*-{4-[4-(2′-Chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide,** (**23n**). **23n** was prepared from **34** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 19%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (br s, 1H), 9.98 (br s, 1H), 9.59 (br s, 1H), 8.54 (d, J = 2.1 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.86 (dd, J = 9.2, 2.1 Hz, 1H), 7.77 (d, J = 9.2 Hz, 3H), 7.50−7.62 (m, 3H), 7.38−7.50 (m, 3H), 7.07−7.31 (m, 7H), 6.94 (d, J = 8.9 Hz, 2H), 4.31 (s, 2H), 4.12−4.25 (m, 1H), 3.92 (s, 3H), 3.39 (d, J = 6.1 Hz, 2H), 2.89−3.27 (m, 6H), 2.75 (s, 6H), 2.15 (q, J = 7 Hz, 2H). MS (ESI), m/z 811 [M − H]$^-$. Anal. ($C_{42}H_{45}ClN_6O_5S_2 \cdot 3\ CF_3CO_2H \cdot H_2O$) C, H, N.

***N*-{4-[4-(3′-Chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-4-((*R*)-3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide, (23o)**. **23o** was prepared from **35** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 19%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (br s, 1H), 9.88 (br s, 1H), 9.59 (br s, 1H), 8.54 (d, J = 2.1 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.86 (dd, J = 9.2, 2.1 Hz, 1H), 7.77 (d, J = 8.9 Hz, 2H), 7.70 (br s, 1H), 7.41−7.59 (m, 5H), 7.28−7.40 (m, 2H), 7.05−7.27 (m, 6H), 6.94 (d, J = 8.9 Hz, 2H), 4.11−4.22 (m, 1H), 3.90 (s, 5H), 3.39 (d, J = 6.1 Hz, 2H), 3.04−3.31 (m, 5H), 2.88 (s, 2H), 2.75 (s, 6H), 2.15 (m, 2H). MS (ESI), m/z 811 [M − H]$^-$. Anal. ($C_{42}H_{45}ClN_6O_5S_2 \cdot 2.5\ CF_3CO_2H$) C, H, N.

***N*-{4-[4-(4′-Chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-4-(3-dim ethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide**, (**2**). **2** was prepared from **36** according to the procedure described for the preparation of **23i** and the product purified by silica gel chromatography eluting with a step gradient of CH_2Cl_2 saturated with NH_3(g), 1% MeOH in CH_2Cl_2 saturated with NH_3(g), 2% MeOH/CH_2Cl_2 saturated with NH_3(g) by 5% MeOH/CH_2Cl_2 saturated with NH_3(g), 10% MeOH in CH_2Cl_2 saturated with NH_3(g), and 15% MeOH/CH_2Cl_2 saturated with NH_3(g) to yield 4.78 g (68%) of **2** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (d, J = 2 Hz, 1H), 8.24 (d, J = 9 Hz, 1H), 7.80 (dd, J = 2, 9 Hz, 1H), 7.72 (d, J = 9 Hz, 2H), 7.48−7.53 (m, 5H), 7.14−7.41 (m, 9H), 6.88 (d, J = 10 Hz, 2H), 6.79 (d, J = 9 Hz, 2H), 4.08 (m, 1H), 3.32−3.38 (m, 6H), 3.13 (m, 4H), 2.73−2.93 (m, 2H), 2.50 (br s, 6H), 1.92−2.13 (m, 2H). MS (ESI) m/z 813 [M + H]$^+$. Anal. ($C_{42}H_{45}N_6O_5S_2Cl$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-[4-(4-[1,1′;4′,1″]terphenyl-2-ylmethyl-piperazin-1-yl)-benzoyl]-benzenesulfonamide, (**23p**). **23p** was prepared from **37** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 20%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 9.96 (br s, 1H), 9.56 (s, 1H), 8.54 (d, J = 2.5 Hz, 1H), 8.28 (d, J = 9.2 Hz, 1H), 7.83−7.88 (m, 2H), 7.70−7.81 (m, 7H), 7.52−7.60 (m, 2H), 7.44−7.52 (m, 4H), 7.36−7.44 (m, 2H), 7.07−7.25 (m, 6H), 6.93 (d, J = 9.2 Hz, 2H), 4.42 (br s, 2H), 4.12−4.24 (m, 1H), 3.83 (br s, 2H), 3.39 (d, J = 6.1 Hz, 2H), 3.00−3.33 (m, 6H), 2.91 (br s, 2H), 2.74 (s, 3H), 2.74 (s, 3H), 2.09−2.20 (m, 2H). MS (ESI), m/z 853 [M − H]$^-$.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(4′-fluoro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (**23q**). **23q** was prepared from **38** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 52%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (br s, 1H), 9.65 (br s, 2H), 8.54 (d, J = 2.5 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.86 (dd, J = 9.2, 2.1 Hz, 1H), 7.69−7.80 (m, 3H), 7.46−7.56 (m, 2H), 7.37−7.44 (m, 2H), 7.25−7.36 (m, 3H), 7.06−7.25 (m, 6H), 6.93 (d, J = 8.9 Hz, 2H), 4.28 (s, 2H), 4.15−4.24 (m, 1H), 3.54−4.02 (m, 4H), 3.39 (d, J = 5.8 Hz, 2H), 3.02−3.28 (m, 4H), 2.92 (s, 2H), 2.74 (s, 6H), 2.15 (q, J = 8.0 Hz, 2H). MS (ESI), m/z 795 [M − H]$^-$. Anal. ($C_{42}H_{45}FN_6O_5S_2 \cdot 3C_2HF_3O_2$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-*N*-{4-[4-(4′-trifluoromethyl-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-benzenesulfonamide, (**23r**). **23r** was prepared from **39** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 45%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, 1H), 9.60 (s, 2H), 8.54 (d, J = 2.1 Hz, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.70−7.91 (m, 6H), 7.49−7.64 (m, 4H), 7.32−7.43 (m, 1H), 7.05−7.26 (m, 6H), 6.93 (d, J = 8.9 Hz, 2H), 4.12−4.41 (m, 5H), 3.39 (d, J = 5.8 Hz, 2H), 2.78−3.33 (m, 8H), 2.74 (s, 6H), 2.15 (q, J = 8.0 Hz, 2H). MS (ESI), m/z 845 [M − H]$^-$. Anal. ($C_{43}H_{45}F_3N_6O_5S_2 \cdot 3\ CF_3CO_2H$) C, H, N.

4-((*R*)-3-Dimethylamino-1-phenylsulfanylmethyl-propylamino)-*N*-{4-[4-(4′-methanesulfonyl-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-3-nitro-benzenesulfonamide, (**23s**). **23s** was prepared from **40** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. (Yield 76%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, J = 2.4 Hz, 1H), 8.57 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.46−7.52 (m, 1H), 7.34−7.41 (m, 2H), 7.14−7.31 (m, 7H), 6.65−6.78 (m, 3H), 4.04 (s, 1H), 3.39 (s, 2H), 3.17 (d, J = 4.7 Hz, 4H), 3.07−3.14 (m, 5H), 2.79−2.91 (m, 2H), 2.59 (s, 6H), 2.40−2.49 (m, 4H), 1.96−2.27 (m, 2H). MS (ESI), m/z 855 [M − H]$^-$.

4-(2-Methyl-1-(phenylthio)propan-2-ylamino)-3-nitrobenzenesulfonamide, (42). A suspension of 2-methyl-1-(phenylthio)propan-2-amine[24] (4.34 g, 20.0 mmol) and 4-fluoro-3-nitrobenzenesulfonamide (4.40 g, 20.0 mmol) in DMSO/Hunig's base (15 mL/10 mL) was stirred overnight. The upper layer (Hunig's base) was separated, and DMSO layer was poured to water (200 mL). The precipitated product was filtered off, washed with water, and dried in vacuum oven to yield 6.33 g (83%) of **42**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.38 (d, J = 2.2 Hz, 1H), 7.69−7.78 (m, 1H), 7.40 (d, J = 9.0 Hz, 1H), 7.26−7.36 (m, 4H), 7.03−7.20 (m, 3H), 3.56 (s, 2H), 1.57 (s, 6H). MS (ESI) m/z 380 [M − H]$^-$.

4-(4,4-Dimethylpiperidin-1-yl)-*N*-(4-(2-methyl-1-(phenylthio)-propan-2-ylamino)-3-nitrophenylsulfonyl)benzamide, (1b). 1b was prepared from **42** and 4-(4,4-dimethylpiperidin-1-yl)benzoic acid[18] according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H), 8.53 (s, 1H), 8.51 (d, J = 2.5 Hz, 1H), 7.82−7.87 (m, 1H), 7.76 (d, J = 9.2 Hz, 2H), 7.37 (d, J = 9.2 Hz, 1H), 7.24−7.28 (m, 2H), 6.98−7.04 (m, 2H), 6.91−6.97 (m, 3H), 3.54 (s, 2H), 3.29−3.40 (m, 4H), 1.57 (s, 6H), 1.34−1.42 (m, 4H), 0.95 (s, 6H). MS (ESI), m/z 595 [M − H]$^-$. Anal. ($C_{30}H_{36}N_4O_5S_2 \cdot 0.25\ CF_3CO_2H$) C, H, N.

4-(4-Benzyl-4-methoxypiperidin-1-yl)-*N*-(4-(2-methyl-1-(phenylthio)propan-2-ylamino)-3-nitrophenylsulfonyl)benzamide, (43a). 43a was prepared from **42** and the acid derived from **7e** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. ^{1}H NMR (400 MHz, DMSO-d_6)

δ 11.95 (s, 1H), 8.53 (s, 1H), 8.51 (d, $J = 2.5$ Hz, 1H), 7.81−7.87 (m, 1H), 7.75 (d, $J = 9.2$ Hz, 2H), 7.37 (d, $J = 9.5$ Hz, 1H), 7.22−7.29 (m, 4H), 7.14−7.21 (m, 3H), 6.99−7.05 (m, 2H), 6.89−6.96 (m, 3H), 3.58−3.69 (m, 2H), 3.54 (s, 2H), 3.28 (s, 3H), 2.96−3.08 (m, 2H), 2.78 (s, 2H), 1.68 (d, $J = 13.2$ Hz, 2H), 1.57 (s, 6H), 1.42−1.53 (m, 2H). MS (CI), *m*/*z* 689 $[M + H]^+$. Anal. ($C_{36}H_{40}N_4O_6S_2 \cdot 0.2\ CF_3CO_2H$) C, H, N.

4-(4-(Biphenyl-2-ylmethyl)piperazin-1-yl)-*N*-(4-(2-methyl-1-(phenylthio)propan-2-ylamino)-3-nitrophenylsulfonyl)benzamide, (43b). 43b was prepared from **42** and the acid derived from **28** according to the procedure described for the preparation of **23i** and the product purified by reverse phase HPLC. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.05 (br s, 1H), 9.78 (br s, 1H), 8.53 (s, 1H), 8.51 (d, $J = 2.2$ Hz, 1H), 7.83 (dd, $J = 9.4$, 2.5 Hz, 1H), 7.79 (d, $J = 8.7$ Hz, 2H), 7.72 (s, 1H), 7.50−7.58 (m, 2H), 7.44−7.50 (m, 2H), 7.39−7.44 (m, 1H), 7.31−7.39 (m, 4H), 7.26 (d, $J = 7.2$ Hz, 2H), 6.98−7.04 (m, 2H), 6.93 (d, $J = 8.4$ Hz, 2H), 6.90−6.93 (m, 1H), 4.29 (br s, 2H), 3.77 (br s, 2H), 3.41−3.53 (m, 4H), 3.15 (br s, 2H), 2.80 (br s, 2H), 1.56 (s, 6H). MS (ESI), *m*/*z* 734 $[M - H]^-$. Anal. ($C_{40}H_{41}N_5O_5S_2 \cdot C_2HF_3O_2$) C, H, N.

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Supporting Information Available: Elemental analysis and HPLC data for all compounds and HSQC spectra of Bcl-xL in the presence and absence of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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