Discovery of an Orally Bioavailable Small Molecule Inhibitor of Prosurvival B-Cell Lymphoma 2 Proteins

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Overexpression of prosurvival proteins such as Bcl-2 and Bcl-X_L has been correlated with tumorigenesis and resistance to chemotherapy, and thus, the development of antagonists of these proteins may provide a novel means for the treatment of cancer. We recently described the discovery of **1** (ABT-737), which binds Bcl-2, Bcl-X_L, and Bcl-w with high affinity, shows robust antitumor activity in murine tumor xenograft models, but is not orally bioavailable. Herein, we report that targeted modifications at three key positions of **1** resulted in a 20-fold improvement in the pharmacokinetic/pharmacodynamic relationship (PK/PD) between oral exposure (AUC) and in vitro efficacy in human tumor cell lines (EC_{50}). The resulting compound, **2** (ABT-263), is orally efficacious in an established xenograft model of human small cell lung cancer, inducing complete tumor regressions in all animals. Compound **2** is currently in multiple phase 1 clinical trials in patients with small cell lung cancer and hematological malignancies.

Introduction

Apoptosis or programmed cell death is an evolutionarily conserved process that regulates normal development and tissue homeostasis by eliminating unwanted and damaged cells. Alterations in this highly regulated process are implicated in a number of diseases including cancer.[1] Inability to respond to apoptotic stimuli leads to the accumulation of defective cells and tumorigenesis. Furthermore, this cellular defect confers resistance to cytotoxic anticancer agents that typically exert their effects indirectly through the apoptotic pathway.[2]

Bcl-2[a] family proteins are the key regulators of apoptosis in the mitochondria-mediated pathway and comprise both pro- and antiapoptotic proteins.[3–5] This large family of proteins can be structurally subdivided into three distinct groups based on the presence of Bcl-2 homology (BH) domains. Direct binding interactions between these three groups of proteins dictates the apoptotic fate of cells. The most structurally disparate of the Bcl-2 family members are those that contain a single BH domain. These proapoptotic BH3-only proteins (e.g., Bad, Bim, Noxa) act as molecular sentinels and are activated to propagate death signaling in response to cellular damage. The multidomain proapoptotic members, Bax and Bak, contain three BH domains (BH1−BH3) and respond to these signals by forming membrane bound oligomers on the mitochondrial surface that results in loss of membrane integrity, the release of cytochrome C, and downstream caspase activation.[6] The antiapoptotic members (e.g., Bcl-2, Bcl-X_L, Mcl-1) contain four BH domains and serve to protect cells by directly binding to and sequestering their proapoptotic counterparts. The overexpression of antiapoptotic proteins in cancer cells leads to a shift in the balance between anti- and proapoptotic proteins, resulting in the inhibition of the normal apoptotic pathway. Thus, a small molecule antagonist of the antiapoptotic proteins may restore normal apoptotic signaling in tumor cells. Such a molecule not only should be directly cytotoxic to those cancer cells reliant upon antiapoptotic proteins for survival but should also enhance the cytotoxicity of chemotherapeutic agents, such as DNA damaging agents or taxanes, that exert their effects in part by the up-regulation of proapoptotic factors.[7–9]

We have recently described our own efforts to discover potent inhibitors of Bcl-2 family proteins that culminated in the identification of **1** (ABT-737), which inhibits Bcl-2, Bcl-X_L, and Bcl-w with subnanomolar affinity.[10–14] This compound exhibits robust single agent activity, eliciting complete tumor regressions in established tumor xenograft models, and potentiates the effects of cytotoxic agents in a variety of settings.[10,15–20] However, this compound is not orally bioavailable and its low aqueous solubility makes formulation for intravenous delivery extremely difficult. Moreover, oral delivery should allow greater flexibility with regard to dosing schedules as a single agent or in combination regimens with parenteral chemotherapeutic agents in a clinical setting. Herein, we describe our efforts to impart oral bioavailability to **1** that resulted in the discovery of **2** (ABT-263) (Figure 1).[21]

Chemistry

Our strategy for the construction of the acylsulfonamides is outlined in the retrosynthetic analysis shown in Figure 2. Carbodiimide-mediated coupling was employed for the formation of the acylsulfonamides between carboxylic acids and sulfonamides.[22] Introduction of the amino side chains was achieved either by nucleophilic aromatic substitution prior to the formation of acylsulfonamides or by palladium-mediated coupling after acylsulfonamide formation to avoid catalyst poisoning by the free sulfonamides.

3-Substituted-4-fluoroarylsulfonamides were either obtained from commercial sources or prepared via chlorosulfonylation

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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 × 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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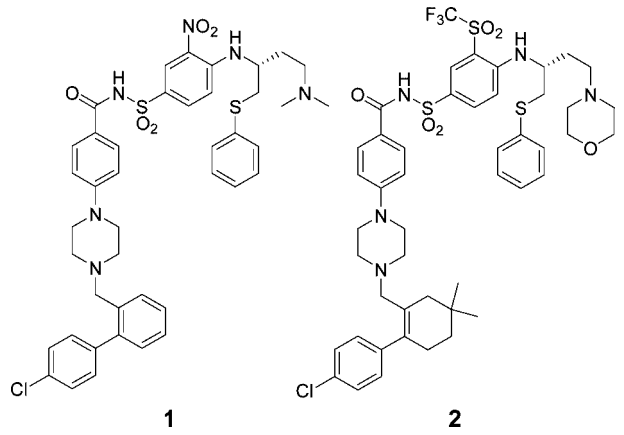


Figure 1. Structures of acylsulfonamide Bcl-2 family protein inhibitors.

and subsequent amination (Scheme 1, eq 1). Elaborated sulfonamides **10a**−**d** were prepared from the corresponding arylhalides **8a**−**d** by nucleophilic aromatic substitution with amine **9**[12] (eq 2). It is of note that the highly electrophilic trifluoroacetyl group prefers either a keto or a hydrated form depending on the electronic nature of its ortho substituent. Thus, while the trifluoroacetyl group in **8a** exists as a stable hydrate, it reverts to the keto form in **10a**, which contains an electron donating ortho amino substituent. This is readily apparent by the nuclear magnetic resonance signal of the carbonyl carbon. For the sulfonamide lacking an electron withdrawing group in the 3-position, palladium mediated coupling[23] of the bis-4-methoxybenzyl protected 4-bromosulfonamide with amine **9** followed by deprotection under acidic conditions smoothly furnished the desired product **14** (eq 3).

Scheme 2 illustrates the synthesis of trifluoromethylsulfone analogues **20a** and **20b**. Alkylation of 2-fluorobenzenethiol **15** with trifluoromethyl iodide under radical conditions[24] afforded the corresponding sulfide.[25] Ruthenium catalyzed oxidation of this highly deactivated sulfide yielded sulfone **17**.[26] Finally, sulfonamides **20a** and **20b** were prepared via nucleophilic aromatic substitution of aryl fluoride **18** by the chiral amines **9** and **19**, respectively. Acylsulfonamide formation between carboxylic acid **21** and sulfonamides **10a**−**d**, **14**, and **20a**,**b** was achieved in the presence of EDC and DMAP as shown in Scheme 3.

Compounds **25a** and **25b** were prepared according to Scheme 4. Sulfonamide **23d** was prepared from 4-bromo-3-trifluoromethoxyaniline in two steps via diazotization.[27] Coupling of **23d** or the commercially available 4-bromo-3-methylbenzenesulfonamide **23a** to carboxylic acid **21** yielded the acylsulfonamides **24a** and **24b**. Palladium mediated coupling of arylbromides **24a**,**b** and amine **9** gave access to the fully elaborated acylsulfonamide (**25a**, **25b**).

The synthesis of the carboxylic acids (**31a**−**f**) containing cycloalkenyl groups of various ring sizes is outlined in Scheme 5. The intermediates **29a**−**f** were obtained via reductive amination of piperazine **28** and 2-bromocycloalkene-1-carboxaldehydes (**27a**−**f**) that were readily accessible from the corresponding cyclic ketones (**26a**−**f**) by treatment with PBr_3 and DMF.[28]

Suzuki cross-coupling of bromides **29a**−**f** with 4-chlorophenylboronic acid and subsequent saponification of the ethyl esters (**30a**−**f**) afforded carboxylic acids **31a**−**f**. Carbodiimide-mediated acylsulfonamide formation of the carboxylic acids and sulfonamide **32**[12] yielded the desired compounds **33a**−**f**. Likewise, compounds **34** and **2** were prepared by condensation of **20b/31b** and **20b/31e**, respectively (Scheme 6).

Results and Discussion

Target affinities (K_i) were determined using fluorescence polarization assays (FPA), which are competition binding assays employing a Bad- or Bax-derived BH3 peptide fluorescent probe.[29] Our choice in structural modifications was directed to those areas that were not likely to disrupt the overall core binding motif and significantly impact the high binding affinity displayed by **1**. This turned out to be the case, as the vast majority of compounds presented here exhibited binding affinities at or below the detection limit of the Bcl-X_L and Bcl-2 FPA ($K_i \approx 1$ nM, Supporting Information Table S1). Therefore, the SAR was driven largely by cellular efficacy. To demonstrate mechanism-based cellular activity, compounds were first evaluated for their ability to reverse the protection afforded by the overexpression of human Bcl-2 or Bcl-X_L in IL-3 dependent murine tumor pro-B cell FL5.12 lines in the absence of added serum proteins. To evaluate their potential relevance to human cancer, compounds were then evaluated in the human tumor cell line H146 (small cell lung cancer) in the presence of 10% human serum. This cell line is known to be dependent upon Bcl-2 and/or Bcl-xL[30] and responsive to **1**.[10] Those compounds with promising activity profiles were evaluated for pharmacokinetic properties in rats, while their pharmacokinetic properties in higher species, combined with efficacy in tumor xenograft models, were used as the final arbiters for compound selection.

Small molecule drugs that have high molecular weight (>500), high number of hydrogen bond donors (>5) or acceptors (>10), and high lipophilicity (clogP) are predicted to have poor oral absorption and permeability by Lipinski's rule of five.[31] These predictions hold true for **1**. However, many of the structural features in **1** that impart undesirable drug properties are important for pharmacological activity. They are the result of design elements essential for inhibition of a large surface area, hydrophobic protein−protein interaction, and the reduction of serum albumin binding.[11–13] Thus, to improve the oral efficacy of this relatively large (MW > 800 Da) molecule, a careful balance had to be achieved wherein improved oral pharmacokinetic properties were not deleterious to target binding affinity or cellular efficacy.[32]

The low oral bioavailability of **1** was thought to be the combined result of low aqueous solubility, poor absorption (permeability) and metabolism. Given the large molecular size and hydrophobicity of compounds within this series, it seemed unlikely that significant improvements in aqueous solubility would be easily obtained by utilizing traditional strategies such as reducing nonpolar surface area. However, early in these studies, we uncovered strong evidence that relatively small modifications that were designed to alter the ionization state by modulating pK_a values of the acylsulfonamide or amine could significantly enhance oral absorption (Table 1). For example, simple replacement of the arylnitro group with the less electron withdrawing trifluoromethyl group (**3**) imparted a 16-fold increase in systemic exposure (pharmacokinetic area under the plasma concentration curve, AUC) after oral dosing in Sprague−Dawley rats with 24% oral bioavailability. However, this change was also accompanied by a large decrease in cellular efficacy. Since N-demethylation was identified as a significant route of metabolism for **1** and we sought to decrease overall molecular charge, the highly basic (p$K_a \approx 10$) dimethylamino group was also an early focus of our efforts. Removal of the dimethylaminoethyl side chain (**4**) altogether resulted in a 37-

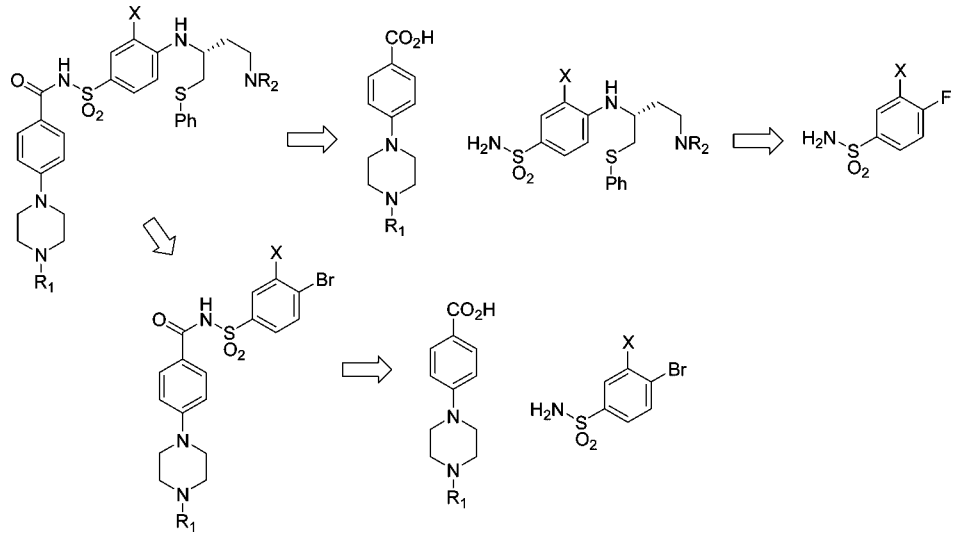
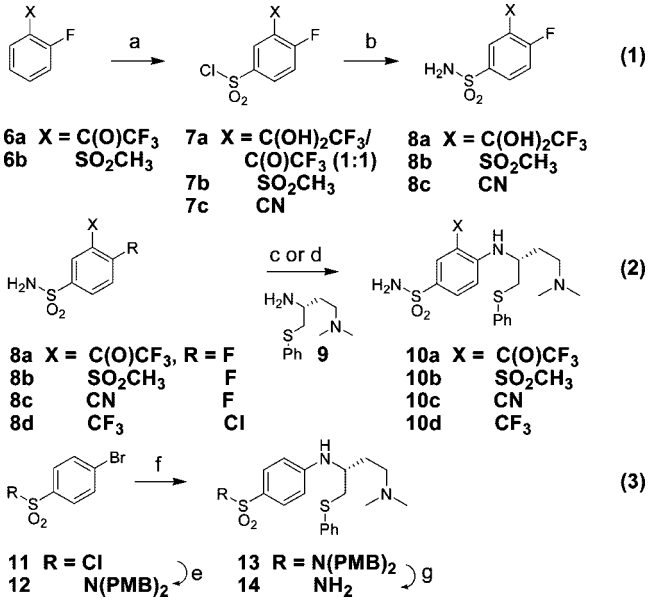


Figure 2. Retrosynthetic analysis of acylsulfonamides.

Scheme 1. Synthesis of Sulfonamides **10a**−**d** and **14**[a]



[a] Reagents and conditions: (a) $ClSO_3H$, 80 °C, 15 h; (b) NH_4OH, THF−MeOH (10:1), −50 to 0 °C, 3 h; (c) **9**, DIPEA, THF, 50 °C, 24 h for **10a**−**c**; (d) **9**, DIPEA, DMSO, 140 °C, 15 h for **10d**; (e) $(PMB)_2NH$, TEA, DMAP, CH_2Cl_2, room temp, 15 h; (f) **9**, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, toluene, reflux, 15 h; (g) TFA, CH_2Cl_2, room temp, 15 h.

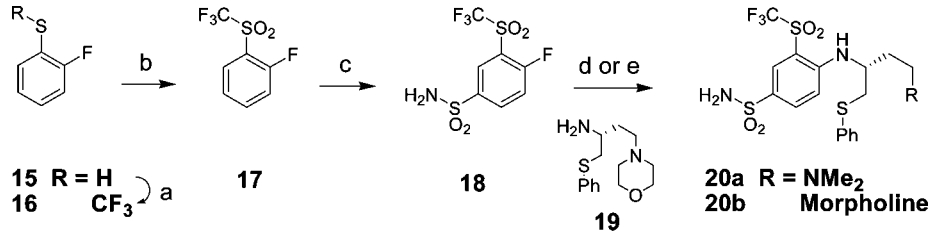
fold increase in oral exposure and 28% oral bioavailability. Again, this was accompanied by a drastic reduction in cellular efficacy that is likely driven by high serum binding, since this substituent was designed specifically to reduce binding to human serum albumin domain III.[12] Replacement of the dimethylamine with a less basic morpholino group ($pK_a \approx 7.5$) provided a more modest 4-fold enhancement in oral exposure with 16% oral bioavailability; however, the compound retained submicromolar cellular activity (**5**). This suggested that the morpholino group might be a suitable replacement if combined with other changes that similarly increased oral exposure and/or cellular activity.

Since in vivo antitumor efficacy is ultimately a function of the ability of a drug to kill tumor cells and to attain adequate concentrations in the body, these two parameters were quantified by examining the ratio of the pharmacokinetic AUC to cellular EC_{50} (AUC/EC_{50}) as a crude pharmacokinetic/pharmacodynamic (PK/PD) measure. The AUC after oral dosing in rats provides a measure of whether a given compound can achieve and maintain circulating concentrations. The EC_{50} in the H146 small cell lung cancer human tumor cell line obtained in the presence of 10% human serum provides a measure of tumor cell killing in the presence of human serum. Even though **3**, **4**, and **5** possess significantly improved oral pharmacokinetic properties, when compared to that of **1**, their AUC/EC_{50} ratios reveal a less than optimal tradeoff between cellular potency and exposure. Nonetheless, though these early modifications were not effective in improving the AUC/EC_{50} ratio, they offered evidence that the oral pharmacokinetic properties of **1** could be meaningfully improved.

Encouraged by these findings, key positions along the backbone of **1** were targeted where changes may impact molecular charge and/or metabolism. Given the likely effect of the nitro group on the acylsulfonamide pK_a, the known propensity for arylnitro groups to produce potentially toxic metabolites, and the dramatic effects of nitro group replacement outlined above, this position was an obvious choice. Because oxidative metabolism of the 4-chlorobiphenyl moiety was another metabolic pathway and little SAR of the internal aromatic ring had been explored, this area was also targeted. Each of these areas was initially optimized independently with the goal of later combining the most desired structural attributes with the morpholino substituent.

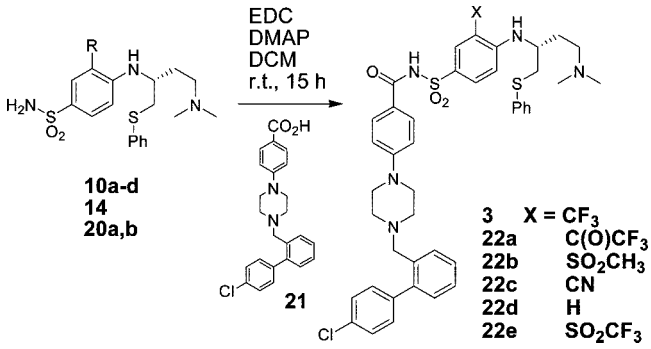
Table 2 summarizes the effects of nitro group replacement. Target affinities of these analogues remained below the FPA detection limit for both Bcl-2 and Bcl- X_L with the exception of **22d** (R = H), which showed a moderate loss against Bcl-X_L (K_i = 14 nM). However, modification of the nitroarene had a dramatic impact on activity against both FL5.12 and H146 cell lines, where compounds with weaker electron withdrawing groups (**3**, **25b**, **22c**, **22a**, and **22b**) exhibited a significant loss of potency. In fact, within this small group of compounds, there is a good correlation between the aromatic substituent Hammett σ_m value,[33,34] and thus the acylsulfonamide pK_a, and EC_{50} in H146 cells. It is not clear if the decreased cellular activity observed with less acidic cores is driven by reduced cellular penetration, increased sequestration by the added serum proteins, or other cellular compartments. The oral pharmacokinetics of compounds that retained H146 efficacy at or below $EC_{50} \approx 2$ μM were evaluated in Sprague−Dawley rats. Not surprisingly, oral exposure correlated roughly with the calculated polar

Scheme 2. Synthesis of Sulfonamides **20a**,**b**[a]



[a] Reagents and conditions: (a) CF_3I, TEA, methyl viologen dichloride, DMF, room temp, 15 h; (b) $RuCl_3$, $NaIO_4$, CCl_4−acetonitrile−H_2O (1:1:2), room temp, 15 h; (c) (i) $ClSO_3H$, 80−120 °C, 15 h; (ii) NH_4OH, ^{i}PrOH, −78 °C, 1 h; (d) **9**, DIPEA, THF, 50 °C, 15 h for **20a**; (e) **19**, DIPEA, THF, 50 °C, 15 h for **20b**.

Scheme 3. Synthesis of Target Compounds **3** and **22a**−**e**



surface area (PSA) with compounds with lower PSA exhibiting higher AUC values. The trifluoromethanesulfonyl group (triflone), which has slightly greater electron withdrawing capacity than the nitro group, gave a correspondingly small increase in cellular efficacy (**22e**). However, the lower PSA imparted by the triflone compared to the nitro group also corresponded to a modest increase in drug plasma concentrations. The net effect was a 7-fold increase in AUC/EC_{50} ratio.

We next investigated replacements for the 4-chlorobiphenyl moiety of **1** with the goal of enhancing metabolic stability and maintaining or improving cellular efficacy. We chose to examine cycloalkenyl groups of varied ring size as surrogates for the internal aromatic ring in order to maintain the rigid molecular geometry essential for the high affinity binding mode of **1**.[34] These modifications were well-tolerated, as all of the analogues fell at or below the limits of detection in the FPA assay. Cellular efficacy better differentiated the compounds, as the six-membered ring (**33b**) was more efficacious in the H146 cellular assay than its five-, seven-, or eight-membered ring counterparts (**33a**, **33c**, or **33d**). Investigation of a variety of six-membered ring substitutions or replacements identified the *gem*-dimethylcyclohexene **33e** (Table 3). Although this compound exhibited slightly lower H146 efficacy compared to the parent cyclohexenyl, it displayed subnanomolar EC_{50} values against both the Bcl-2 and Bcl-X_L dependent FL5.12 cells. The cyclohexene and *gem*-dimethylcyclohexene were chosen for further study.

The cellular efficacy and oral exposure of compounds resulting from the combination of modifications identified in the independent optimization processes are outlined in Table 4. First, incorporation of the cyclohexene alone (**33b**) resulted in both improved cellular efficacy and a modest 2-fold increase in oral exposure relative to **1**. The effect of combining the morpholine and the triflone with either cyclohexene (**34**) or *gem*-dimethylcyclohexene (**2**) on oral PK was nearly additive, resulting in a significant enhancement of oral exposure (14- to 22-fold relative to **1**) while maintaining cellular efficacy. The net result is approximately 20-fold increase in AUC/EC_{50} for **34** and **2** relative to **1**.

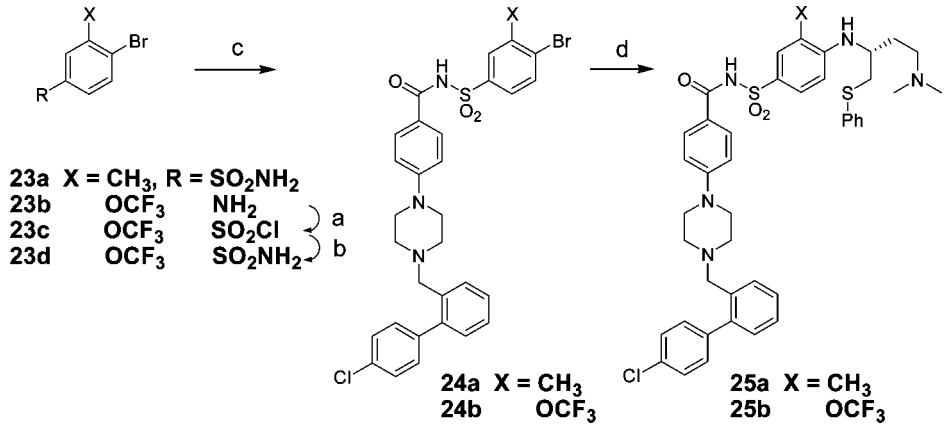
With these two promising candidates in hand, we opted to further differentiate them by characterization of their pharmacokinetic profiles in additional species. As shown in Table 5, **2** displays oral exposure superior to that of **34** in all species tested (rat, mouse, dog, and monkey). The biological properties and pharmacokinetic parameters for **2** have been recently reported.[35] This compound exhibits low volumes of distribution and plasma clearance values with an oral elimination half-life from 4.0 to 6.4 h and absolute oral bioavailability of approximately 20% in all four species above. Figure 3A shows a representative plasma concentration curve after intravenous or oral dosing in dog. When examined in a 3-day steady-state pharmacokinetics study in non-tumor-bearing scid-bg mice, both peak plasma concentration (C_{max}) and AUC increased in a dose proportional fashion.[35] In scid-bg mice bearing H146 flank tumor xenografts, **2** exhibits good tissue distribution after oral dosing with a plasma to tumor concentration ratio of near unity (Figure 3B).

We next evaluated **2** for oral efficacy in an established murine tumor xenograft model (Figure 4). In order to directly compare the efficacy of **2** with that of **1**, we chose the NCI-H1963 SCLC cell line. Compound **1** has been reported to produce complete tumor regression in this model when delivered once daily by ip injection for 21 days.[10] A quantity of 5×10^6 NCI-H1963 cells was inoculated subcutaneously in the flank of scid-bg mice. The tumors were allowed to grow to an average size of 235 mm^3, and mice were size-matched (day 0) into treatment and control groups. Once daily treatment with 100 mg/kg given orally for 21 days was well tolerated (<5% weight loss) and achieved 100% tumor growth inhibition with complete regression of all tumors ($N = 10$) at the end of the dosing period. These results demonstrate that when given at the same dose and schedule, oral administration of **2** achieves similar efficacy to that of **1** when dosed via ip injection.

Conclusions

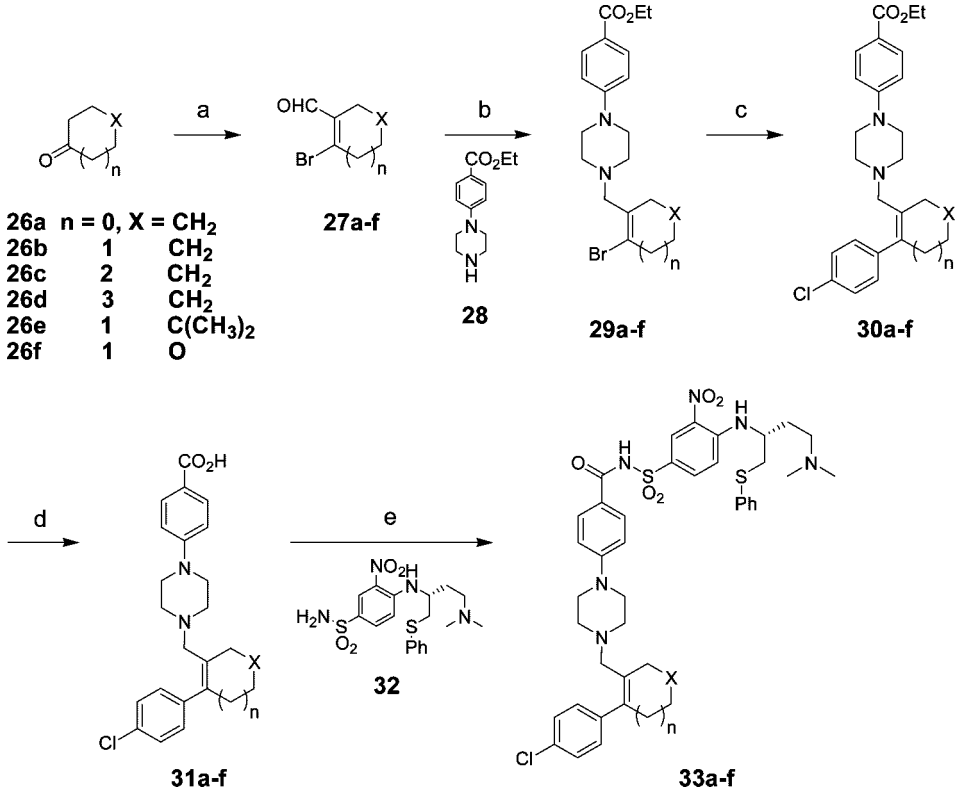
Discovery of small molecule inhibitors of protein−protein interactions has traditionally been a challenge because of the requirement to disrupt large, often hydrophobic and seemingly featureless surface interactions. BH3-mimetics are particularly interesting from a compound design standpoint. The native α-helical BH3-domain of proapoptotic proteins derive their high binding energy through the summation of multiple interactions with distinct, discontinuous hydrophobic pockets or "hotspots" (P1−P4) along the surface groove of their binding partners, such as Bcl-2 and Bcl-xL. Our initial efforts to mimic this interaction generated compound **1**, which efficiently binds two nonadjacent hot spots of Bcl-xL (P2 and P4)[35] and is exquisitely effective in this regard. Perhaps not surprisingly though, these efforts

Scheme 4. Synthesis of Target Compounds **25a**,**b**[a]



[a] Reagents and conditions: (a) $NaNO_2$, HCl−AcOH, −10 °C, 45 min; SO_2, CuCl, AcOH, room temp, 10 min; (b) NH_4OH, ^{i}PrOH, 0 °C to room temp, 15 h; (c) **21**, EDC, DMAP, CH_2Cl_2, room temp, 15 h; (d) **9**, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, toluene, reflux, 15 h.

Scheme 5. Synthesis of Target Compounds **33a**−**f**[a]

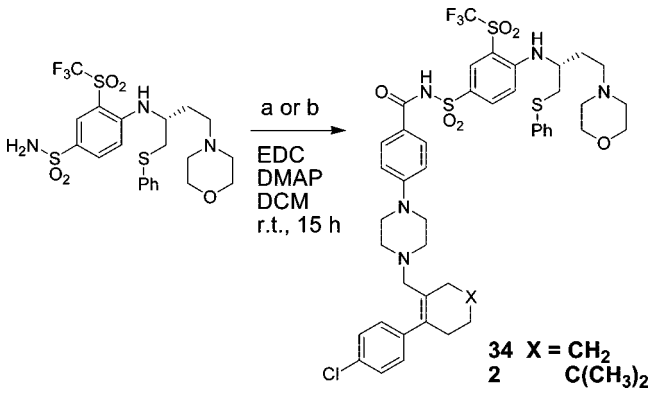


[a] Reagents and conditions: (a) DMF, PBr_3, CH_2Cl_2, room temp, 20 min; (b) $Na(CN)BH_3$, AcOH, EtOH, 15 h; (c) 4-chlorophenylboronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, DME−H_2O−EtOH (7:3:2), 85 °C, 3 h; (d) LiOH, dioxane−H_2O (5:1), 100 °C, 24 h; (e) **32**, EDC, DMAP, CH_2Cl_2, room temp, 15 h.

resulted in a compound with a high molecular weight (813 Da) and hydrophobicity that has poor solubility and oral absorption.

Three key sites were identified along the backbone of **1** that were targeted in order to decrease molecular charge and metabolism while simultaneously minimizing the disruption of the overall molecular scaffold in order to maintain high target affinity. Thus, most of the compounds prepared exhibited high target binding affinity (<1 nM), and the work here was driven entirely by cell-based activity and pharmacokinetic behavior. However, relatively minor changes in these large molecules had a significant impact on oral absorption. For example, replacement of the arylnitro group with trifluoromethyl (**3**) or removal of the basic amine (**4**) yielded a 16- and 37-fold increase in oral exposure, respectively. As is so often the case when attempting to optimize multiple variables simultaneously, we also found these improvements in PK parameters often came at the expense of other desired properties such as cellular efficacy. In order to account for cellular efficacy, protein binding, and oral absorption in a single, semiquantitative measure, we utilized the PK/PD relationship for AUC/EC_{50}, which has commonly been employed in other settings to predict in vivo efficacy.[36] Independent optimization of multiple sites led to modifications at each locale that gave incremental improvements in the AUC/EC_{50} ratio. When combined, these effects were additive and resulted in compound **2**, which has roughly 20-fold improved oral absorption compared to **1**. This compound is orally bioavailable in multiple species, demonstrates good tissue distribution, and is orally efficacious in tumor xenograft

Scheme 6. Synthesis of Target Compounds **34** and **2**[a]



[a] Reagents and conditions: (a) **31b** for **34**; (b) **31e** for **2**.

models. More recently, **2** was shown to display dose proportional oral pharmacokinetics in humans.[37]

It is interesting to note that the ultimate compound identified in these studies is far from what is traditionally thought of as "druglike". The molecular weight of **2** (974 Da) is actually greater than that of the prototype compound **1** and violates three of the four rules of five. To a large degree, the size and hydrophobicity of this chemical series are due to the nature of the drug target itself and the requirements for efficiently mimicking the native BH3 peptide interaction. However, it became apparent throughout this process that simple changes that impact its underlying physiochemical properties (pK_a of the charged functionalities or the polar surface area) or its metabolic potential had a meaningful impact on oral absorption and exposure, even within this large molecular framework. Although this is but a single example, it represents progress toward targeting protein−protein interactions that have typically been thought of as "undruggable".

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 NH_3, and ESI indicates electron spray ionization. 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 mm × 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.

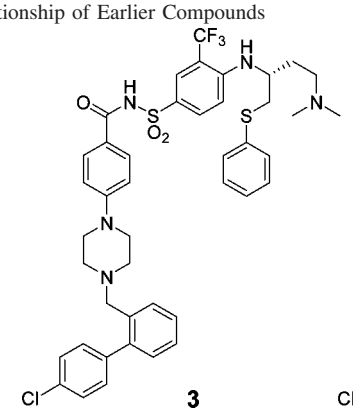
Fluorescence Polarization Assay. K_i and IC_{50} values were determined using a competitive fluorescence polarization assay. Compounds were serially diluted and added to each well of a 96-well microtiter plate. To determine Bcl-X_L K_i values, a mixture totaling 125 μL per well of assay buffer (20 mM phosphate buffer, pH 7.4, 1 mM EDTA, 50 mM NaCl, 0.05% PF-68), 6 nM Bcl-X_L protein,[38] 1 nM fluorescein-labeled BAD peptide (NLWAAQRYGRELRRMSDK(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-X_L, 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-X_L. 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,[39] 1 nM fluorescein-labeled Bax peptide (FITC-QDASTKKLSECLKRIGDELDS, 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-X_L 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.

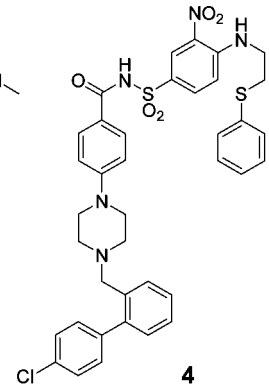
Human Tumor Cell Line Viability Assay. The SCLC cell line NCI-H146 was purchased from American Type Culture Collection, ATCC, Manassas, VA. Cells were cultured in RPMI 1640 (Invitrogen Corp., Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 1% sodium pyruvate, 25 mM HEPES, 4.5 g/L glucose, and 1% penicillin−streptomycin (Sigma) and maintained in a humidified chamber at 37 °C containing 5% CO_2. Cells (50 000 per cell) were treated for 48 h in 96-well tissue culture plates in a total volume of 100 μL of tissue culture medium supplemented with 10% human serum (Invitrogen). Cell viability was determined using the CellTiter-Glo luminescent cell viability assay (Promega Corp., Madison, WI).

Pharmacokinetics. The single dose pharmacokinetics of compounds **1**−**5**, **22a**, **22c**, **22e**, **33b**, and **34** were evaluated in Sprague−Dawley rats (Charles River) after a 5 mg/kg oral dose (n = 3) (10% DMSO in PEG-400) administered by gavage. The single dose pharmacokinetics of compounds **34** and **2** were evaluated in beagle dogs after a 2.5 mg/kg intravenous (n = 3) or oral (n = 3) dose in 10% DMSO in PEG-400. The iv dose was administered as a slow bolus in a cephalic vein, and oral doses were administered by gavage followed by 10 mL of water. The single dose pharmacokinetics of compounds **34** and **2** were evaluated in cynomolgus monkeys after a 2.5 mg/kg oral (n = 3) dose in 10% DMSO in PEG-400 administered by gastric intubation followed by 5 mL of water. Heparinized blood samples were obtained from each animal prior to dosing and at selected time points after compound administration. The single dose pharmacokinetics of compounds **34** and **2** were evaluated in CD-1 mice after a 50 mg/kg oral dose (n = 27) (2% DMSO/5% Tween-80/20% propylene glycol/73% D5W) administered by gavage. The plasma and tumor concentration profile of **2** (50 mg/kg) in tumor bearing female scid-bg mice was examined on the third day of 3 consecutive days of dosing by oral gavage in 10% EtOH, 30% PEG 400, and 60% Phosal 50 PG (n =

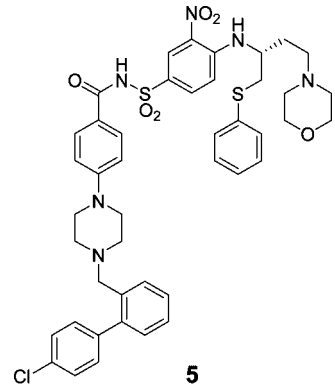
Table 1. PK/PD Relationship of Earlier Compounds



3



4

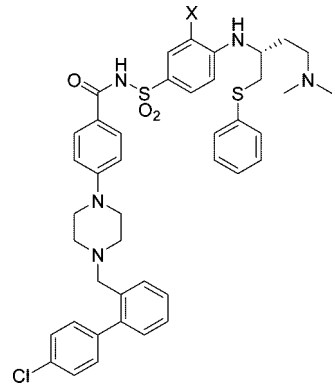


5

	C_{max}[a] (μM)	AUC[a] (μM·h)	F[a] (%)	H146/10% HS EC_{50} (μM)	AUC/EC_{50}
1	0.039	0.28	6	0.087	3.2
3	0.37	4.41	24	2.46	1.8
4	1.15	10.4	28	33.0	0.32
5	0.20	1.16	16	0.61	1.9

[a] Dosed orally at 5 mg/kg.

Table 2. SAR of Nitro Group Replacements



			EC_{50} (nM)[a]					
			serum free		10% HS			
	X	σ_m	FL5.12/Bcl-2	FL5.12/Bcl-X_L	H146	PSA[b] (Å^2)	AUC[c] (μM·h)	AUC/EC_{50}
1	NO_2	0.71	7.7 ± 2.0*	30 ± 8.8*	87 ± 3.5*	135	0.28	3.2
3	CF_3	0.43	42 ± 5.0	122 ± 38*	2460 ± 558*	88	4.41	1.8
25a	CH_3	−0.07	673 ± 278*	ND[c]	28800 ± 3480*	ND[d]	ND[d]	ND[d]
22d	H	0	2700 ± 566	15000 ± 4200	78700 ± 18700*	ND[d]	ND[d]	ND[d]
25b	OCF_3	0.38	230	805	5080 ± 624*	ND[d]	ND[d]	ND[d]
22c	CN	0.56	49 ± 13	225 ± 7.1	1930 ± 51*	112	2.19	1.1
22a	$C(O)CF_3$	0.63	55 ± 1.2*	329 ± 29.0*	2010 ± 300	108	1.25	0.62
22b	SO_2Me	0.60	170 ± 5.8*	415 ± 20.2*	3490 ± 92.7*	ND[d]	ND[d]	ND[d]
22e	SO_2CF_3	0.79	4.7 ± 0.9*	12 ± 1.6*	39.4 ± 10.9	121	0.83	21

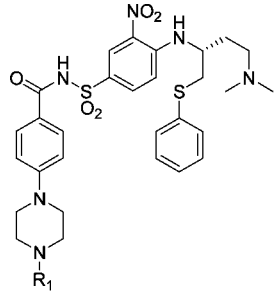
[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate. [b] Polar surface areas were calculated using Savol software (Tripos) from 3D coordinates generated using Concord (Tripos). [c] Dosed orally at 5 mg/kg in rats. [d] ND = not determined.

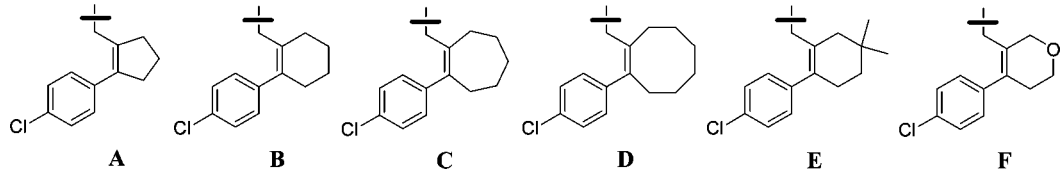
18). At selected time points, groups of three animals were euthanized with carbon dioxide, exsanguinated by cardiac puncture, and heparinized blood and/or tumor samples obtained. Plasma from all samples was separated by centrifugation, and compounds were separated using protein precipitation with acetonitrile.

Compound and the internal standard were separated from each other and coextracted contaminants on a 50 mm × 3 mm Keystone Betasil CN 5 μm column with an acetonitrile/0.1% trifluoroacetic acid mobile phase (50:50, by volume) at a flow rate of 0.7 mL/min. Analysis was performed on a Sciex API3000 biomolecular mass analyzer with a heated nebulizer interface. Compound and internal standard peak areas were determined using Sciex MacQuan software. The plasma drug concentration of each sample was calculated by least-squares linear regression analysis (nonweighted) of the peak area ratio (parent/internal standard) of the spiked plasma standards versus concentration. The plasma concentration data were submitted to multiexponential curve fitting using WinNonlin.3. The area under the plasma concentration−time curve was calculated using the linear trapezoidal rule for the plasma concentration−time profiles.

In Vivo Xenograft Modeling. Animal studies were conducted in accordance with the guidelines established by the internal Institutional Animal Care and Use Committee. C.B.-17 scid-bg mice (Charles River Laboratories, Wilmington, MA) were implanted with 5×10^6 NCI-H1963 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 0) into treatment and control groups. 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: $V =$

Table 3. SAR of 4-Chlorobiphenyl Group Replacements



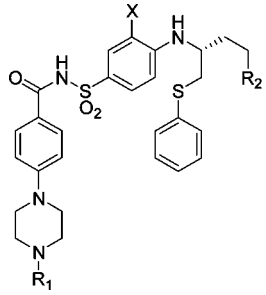


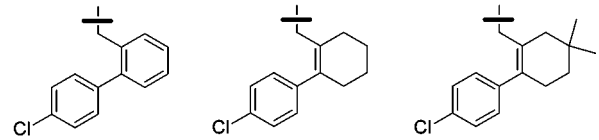
A **B** **C** **D** **E** **F**

		EC$_{50}$ (nM)[a]		
		serum free		
	R$_1$	FL5.12/Bcl-2	FL5.12/Bcl-X$_L$	10% HS H146
33a	A	51 ± 6.6*	143 ± 16.5*	691
33b	B	11 ± 3.3*	29 ± 3.0*	25.7 ± 6.9
33c	C	<1	5.0	51.9
33d	D	39 ± 8.8*	21 ± 1.4	200
33e	E	0.6	0.9	98
33f	F	33 ± 9.9	55 ± 17*	266

[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 4. PK/PD Relationship of Selected Compounds





A **B** **C**

				EC$_{50}$ (nM)[a]				
				serum free				
	R$_1$	X	R$_2$	FL5.12/Bcl-2	FL5.12/Bcl-X$_L$	10% HS H146[c]	AUC[b] (μM·h)	AUC/EC$_{50}$ (h)
1	A	NO_2	NMe_2	7.7 ± 2.0*	30 ± 8.8*	87 ± 3.5*	0.28	3.2
33b	B	NO_2	NMe_2	11 ± 3.3*	29 ± 3.0*	25.7 ± 6.9	0.65	25.3
34	B	SO_2CF_3	morpholine	1.1 ± 0.3	0.7 ± 0.1	58.9 ± 5.3*	3.87	65.7
2	C	SO_2CF_3	morpholine	5.9 ± 0.7	4.2 ± 1.3	86.7 ± 8.3*	6.26	72.2

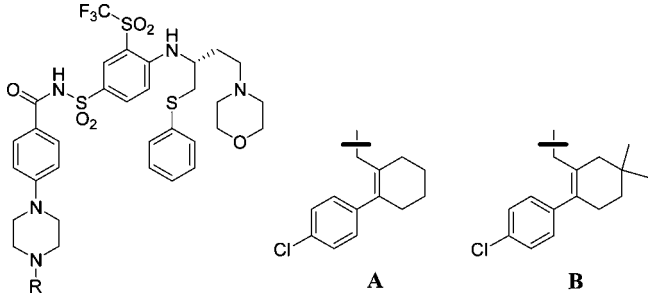
[a] Values are mean ± standard deviation for two experiments run in duplicate. Asterisk indicates mean ± standard error for three or more experiments run in duplicate. Values without error are single experiments run in duplicate. [b] Dosed orally at 5 mg/kg in rats. [c] H146 in the presence of 10% HS.

$L(W^2/2)$. Tumor growth inhibition (%TGI) was calculated on the basis of the difference in mean tumor volumes for the treated group relative to the appropriate vehicle control. Complete response (%CR) was defined as regression of established tumor beyond the level of detection by palpation. **2** was formulated in 10% EtOH, 30% PEG 400, and 60% Phosal 50 PG.

General Procedure I. 4-Fluoro-3-(methylsulfonyl)benzenesulfonamide (8b). A solution of 1-fluoro-2-(methylsulfonyl)benzene[40] (1.0 g, 5.7 mmol) in chlorosulfonic acid (4 mL) was heated at 80 °C overnight and poured onto crushed ice. The mixture was diluted with ethyl acetate, and the layers were separated. The aqueous layer was extracted with ethyl acetate (×2), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give a crude product (1.4 g, 93%), which was used in the next step without further purification. To a solution of the sulfonyl chloride (1.4 g, 5.3 mmol) in THF−MeOH (10 mL-10 mL) cooled to −50 °C was added ammonium hydroxide (28% aqueous solution, 1.8 mL, 26.5 mmol). The reaction mixture was allowed to warm to 0 °C, and the solvent was removed in vacuo. The residue was purified by flash chromatography (SiO_2, 10−100% ethyl acetate in hexanes) to give **8b** (945 mg, 65% over two steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (dd, J = 2.2, 6.2 Hz, 1H), 8.20 (ddd, J = 2.5, 4.4, 8.4 Hz, 1H), 7.76 (dd, J = 8.7, 9.7 Hz, 1H), 7.64 (s, 2H), 3.41 (s, 3H); MS (DCI) m/z 271.0 $(M + NH_4)^+$.

4-Fluoro-3-(2,2,2-trifluoro-1,1-dihydroxyethyl)benzenesulfonamide (8a). 8a was prepared from 2,2,2-trifluoro-1-(2-fluorophenyl)ethanone according to general procedure I. **7a** (hydrated form): ^{1}H NMR (500 MHz, C_6D_6) 8.54 (dd, J = 2.6, 6.6 Hz, 1H), 7.51 (ddd, J = 2.6, 4.0, 8.8 Hz, 1H), 6.52 (dd, J = 9.0, 10.4 Hz, 1H); ^{13}C NMR (125 MHz, C_6D_6) 164.5 (d, J = 266.0 Hz, CF), 131.6,

Table 5. Comparison of **34** and **2** Pharmacokinetics in Multiple Species



		AUC (μM·h)			
	R	rat, 5 mg/kg	mouse, 50 mg/kg	dog, 2.5 mg/kg	monkey, 2.5 mg/kg
34	A	3.87	25.2	3.8	1.90
2	B	6.26	56.7	27.8	6.61

140.3, 130.5, 126.1, 123.0 (q, J = 287.7 Hz, CF_3), 118.9, 93.0 (q, J = 34.4 Hz, $C(OH)_2$). **7a** (keto form): ^{1}H NMR (500 MHz, C_6D_6) 8.19 (dd, J = 2.6, 6.2 Hz, 1H), 7.44 (ddd, J = 2.9, 4.4, 9.2 Hz, 1H), 6.30 (dd, J = 9.2, 9.2 Hz, 1H); ^{13}C NMR (125 MHz, C_6D_6) 177.1 (C(O)), 164.6 (d, J = 273.3 Hz, CF), 140.9, 135.2, 131.4, 120.5, 119.3, 116.1 (q, J = 289.6 Hz, CF_3). **8a**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (dd, J = 2.5, 7.2 Hz, 1H), 7.95 (s, 2H), 7.91 (ddd, J = 2.8, 4.4, 8.7 Hz, 1H), 7.46 (s, 2H), 7.41 (dd, J = 8.6, 11.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.73 (d, J = 259.0 Hz, CF), 139.82, 129.50 (d, J = 10.1 Hz), 128.52, 125.90 (d, J = 12.2 Hz), 123.08 (q, J = 287.7 Hz, CF_3), 117.45 (d, J = 25.0 Hz), 91.70 (dq, J = 3.0, 33.3 Hz, $C(OH)_2$); MS (DCI) m/z 289.0 $(M + NH_4)^+$.

3-Cyano-4-fluorobenzenesulfonamide (8c). 8c was prepared from 3-cyano-4-fluorobenzene-1-sulfonyl chloride according to general procedure I. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.32 (dd, J = 2.2, 5.9 Hz, 1H), 8.18 (ddd, J = 2.4, 5.1, 8.8 Hz, 1H), 7.76 (dd, J = 9.0, 9.0 Hz, 1H), 7.60 (s, 2H); MS (DCI) m/z 219.7 $(M + NH_4)^+$.

General Procedure II. (*R*)-3-Cyano-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)benzenesulfonamide (10c). To a solution of **8c** (170 mg, 0.849 mmol) and **9**[12] (191 mg, 0.849 mmol) in THF (10 mL) was added DIPEA (297 μL, 1.698 mmol). The solution was stirred at 50 °C overnight and concentrated. The residue was purified by flash chromatography (SiO_2, 0−10% 7 N NH_3−MeOH in CH_2Cl_2) to give **10c** (160 mg, 47%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, J = 2.1 Hz, 1H), 7.63 (dd, J = 9.2, 2.2 Hz, 1H), 7.38−7.29 (m, 5H), 7.24 (m, 1H), 7.14 (br s, 2H), 6.66 (d, J = 9.2, 1H), 3.90 (s, 1H), 3.26 (m, 2H), 2.44−2.20 (m, 2H), 2.14 (s, 6H), 1.91−1.79 (m, 2H); MS (ESI) m/z 405 $(M + H)^+$.

(*R*)-4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(2,2,2-trifluoroacetyl)benzenesulfonamide (10a). 10a was prepared from **8a** and **9** according to general procedure II. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (d, J = 9.0 Hz, 1H), 8.04 (s, 1H), 7.79 (dd, J = 1.9, 9.4 Hz, 1H), 7.29 (m, 4H), 7.23 (t, J = 7.6 Hz, 2H), 7.16 (t, J = 7.3 Hz, 1H), 7.07 (d, J = 9.4 Hz, 1H), 4.16 (m, 1H), 3.40 (dd, J = 5.0, 14.0 Hz, 2H), 2.35 (m, 1H), 2.24 (m, 1H), 2.12 (s, 6H), 1.94 (m, 1H), 1.78 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.60 (q, J = 33.3 Hz, C=O), 154.09, 135.40, 133.86, 129.97, 129.45, 128.94, 128.86, 126.07, 116.64 (q, J = 291.7 Hz, CF_3), 114.01, 107.62, 55.06, 50.64, 45.02, 37.20, 31.22; MS (ESI) m/z 476.2 $(M + H)^+$.

(*R*)-4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(methylsulfonyl)benzenesulfonamide (10b). 10b was prepared from **8b** and **9** according to general procedure II. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (d, J = 2.2 Hz, 1H), 7.73 (dd, J = 9.0, 2.5 Hz, 1H), 7.38−7.29 (m, 4H), 7.22 (m, 1H), 7.18 (br s, 2H), 6.85 (d, J = 9.0, 1H), 6.78 (d, J = 8.4, 1H), 3.94 (s, 1H), 3.34 (m, 2H), 3.17 (s, 3H), 2.37 (m, 1H), 2.23 (m, 1H), 2.11 (s, 6H), 1.93 (m, 1H), 1.75 (m, 1H); MS (ESI) m/z 458 $(M + H)^+$.

(*R*)-4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethyl)benzenesulfonamide (10d). 10d was prepared from **8d** and **9** according to general procedure II with modification (140 °C, 24 h). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (d, J = 2.0 Hz, 1H), 7.64 (dd, J = 9.0, 2.0 Hz, 1H), 7.42−7.25 (m, 5H), 7.13 (s, 2H), 6.84 (d, J = 7.5 Hz, 1H), 6.65 (d, J = 8.8 Hz, 1H), 3.87 (m, 1H), 3.22 (d, J = 6.5 Hz, 2H), 2.20 (m, 2H), 2.11 (s, 6H), 1.89 (m, 1H), 1.81 (m, 1H); MS (ESI) m/z 448 $(M + H)^+$.

4-Bromo-*N*,*N*-bis(2,4-dimethoxybenzyl)benzenesulfonamide (12). To a solution of **11** (1.3 g, 5.0 mmol) and bis(*p*-methoxybenzyl)ammonium tosylate (2.2 g, 5.0 mmol) in CH_2Cl_2 (20 mL) was added triethylamine (5 mL) and DMAP (61 mg, 0.050 mmol). The mixture was stirred at room temperature overnight and diluted with ethyl acetate. The resulting solution was washed with 5% HCl, water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 20% ethyl acetate in hexanes) to give **12** (2.1 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (dd, J = 9.1, 9.1 Hz, 4H), 6.98 (d, J = 9.0 Hz, 4H), 6.76 (d, J = 9.0 Hz, 4H), 4.25 (s, 4H), 3.79 (s, 6H); MS (ESI) m/z 475.9 $(M + H)^+$.

General Procedure III. (*R*)-*N*,*N*-Bis(2,4-dimethoxybenzyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)benzenesulfonamide (13). To a solution of **12** (475 mg, 1.00 mmol) and **9** (280 mg, 1.25 mmol) in toluene (20 mL) was added $Pd_2(dba)_3$ (60 mg, 0.085 mmol), BINAP (120 mg, 0.20 mmol), and Cs_2CO_3 (1.0 g, 3.0 mmol). The mixture was heated under reflux in a nitrogen atmosphere overnight and diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (×2), and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 5−10% 7 N NH_3−MeOH in CH_2Cl_2) to give **13** (400 mg, 60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.55 (m, 2H), 7.40 (m, 2H), 7.40−7.24 (m, 9H), 6.98 (d, J = 8.3 Hz, 4H), 6.77 (d, J = 8.3 Hz, 4H), 6.39 (d, J = 8.7 Hz, 2H), 4.18 (s, 4H), 3.77 (s, 6H), 3.18 (m, 1H), 3.03 (m, 1H), 2.45 (m, 1H), 2.35 (m, 1H), 2.23 (s, 6H), 2.00 (m, 1H), 1.89 (m, 1H); MS (ESI) m/z 620.3 $(M + H)^+$.

(*R*)-4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)benzenesulfonamide (14). To a solution of **13** (310 mg, 0.50 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL). The solution was stirred at room temperature overnight and concentrated. The residue was diluted with CH_2Cl_2 and washed with saturated sodium bicarbonate, brine, dried over Na_2SO_4 and filtered. The solution was concentrated and the residue was purified by flash chromatography (SiO_2, 5−10% 7N NH_3-MeOH in CH_2Cl_2) to give **14** (176 mg, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.60 (d, J = 8.9 Hz, 2H), 7.40−7.24 (m, 5H), 6.38 (d, J = 8.9 Hz, 2H), 5.60 (m, 1H), 4.60 (m, 2H), 3.70 (m, 1H), 3.15 (m, 1H), 2.99 (m, 1H), 2.45 (m, 1H), 2.35 (m, 1H), 2.23 (s, 6H), 2.00 (m, 1H), 1.76 (m, 1H); MS (ESI) m/z 380.0 $(M + H)^+$.

(2-Fluorophenyl)(trifluoromethyl)sulfane (16). To a suspension of methyl viologen dichloride (2.65 g, 10.3 mmol) in DMF (180 mL) saturated with trifluoromethyl iodide (81 g, 412 mmol) in a 3-neck flask equipped with a coldfinger was added **15** (22 mL, 206 mmol). The reaction mixture was treated with triethylamine (40 mL, 290 mmol) dropwise, and stirred at room temperature overnight. The solution was diluted with water (480 mL), and the layers were separated. The aqueous layer was extracted with ether (×2), and the combined organic layers were washed with 1 N aqueous NaOH, saturated NH_4Cl, and brine, dried over $MgSO_4$, and filtered. Fractional distillation of the filtrate provided **16** (32.8 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 1H), 7.51 (m, 1H), 7.20 (m, 2H); MS (EI) m/z 196.1 $(M)^+$.

1-Fluoro-2-(trifluoromethylsulfonyl)benzene (17). To a solution of **16** (32.76 g, 167.2 mmol) in CCl_4−acetonitrile−H_2O (100 mL−100 mL−200 mL) cooled with a water bath was added sodium periodate (107.3 g, 501.5 mmol) and ruthenium(III) chloride hydrate (346 mg, 1.672 mmol). The reaction mixture was stirred at room temperature overnight and diluted with CH_2Cl_2 (200 mL). The mixture was filtered through a pad of Celite, and the filtrate was diluted with aqueous sodium bicarbonate. The aqueous layer was

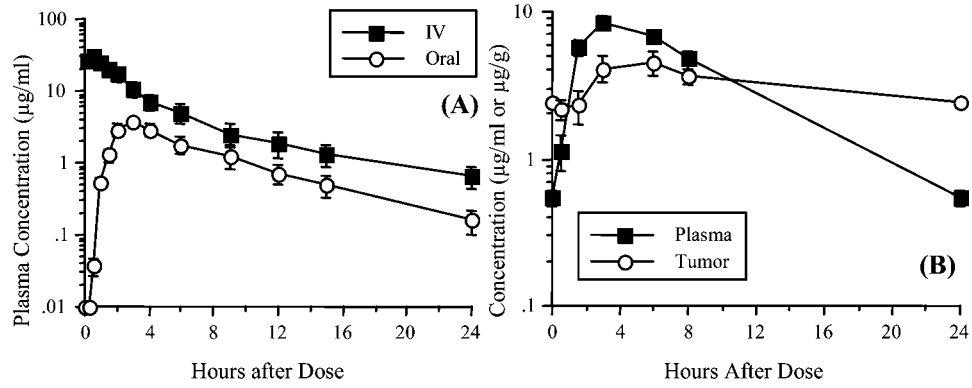


Figure 3. (A) Mean (±SEM, $n = 6$) plasma concentrations of **2** after a 2.5 mg/kg iv or oral dose in dog. (B) Plasma and tumor concentrations of **2** following 50 mg/kg once-daily oral dosing in female scid-bg mice (mean ± SEM, $n = 3$ mice per time point).

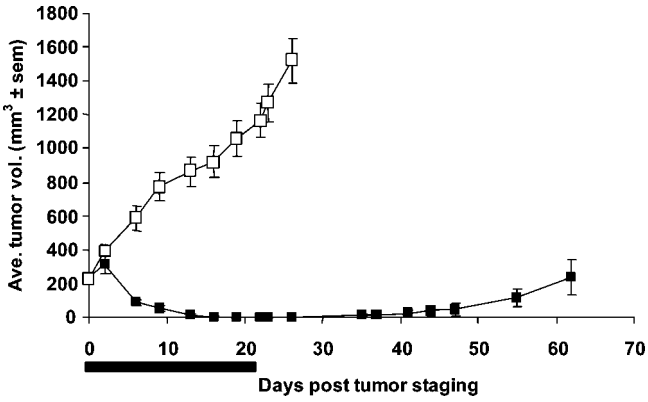


Figure 4. Effect of **2** in the xenograft model of H1963 SCLC. Compound **2** (■) was administered orally once daily at 100 mg/kg for 21 days (black bar). Open squares (□) represent the vehicle. 100% inhibition of tumor growth was observed with treatment of **2** with complete regression of all tumors ($N = 10$) by the end of the therapy period.

extracted with CH_2Cl_2 (×2), and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was filtered through a pad of silica gel to give **17** (37.3 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (ddd, $J = 1.7$, 6.7, 8.2 Hz, 1H), 7.85 (dddd, $J = 1.8$, 4.9, 7.5, 12.4 Hz, 1H), 7.45 (ddd, $J = 1.1$, 7.7, 7.7 Hz, 1H), 7.35 (ddd, $J = 1.0$, 8.4, 9.6 Hz, 1H); MS (EI) m/z 228.1 $(M)^+$.

4-Fluoro-3-(trifluoromethylsulfonyl)benzenesulfonamide (18). **17** (37.3 g, 163.6 mmol) was dissolved in chlorosulfonic acid (32.8 mL, 490.8 mmol) and heated at 120 °C overnight. The solution was slowly poured onto crushed ice and extracted with ethyl acetate. The combined organic layers were washed with water (until the pH of the aqueous layer increased to 3−4) and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give the sulfonyl chloride (46 g, 86%).

To a solution of the crude sulfonyl chloride (23 g, 70.6 mmol) in isopropanol (706 mL) cooled to −78 °C in a three-neck flask equipped with a dropping funnel and mechanical stirrer was added ammonium hydroxide (98 mL, 706 mmol) dropwise. The reaction mixture was stirred at −78 °C for an hour and slowly quenched with 6 N HCl. The solution was allowed to warm to room temperature and concentrated. The residue was diluted with ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate (×2). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was recrystallized from hot ethyl acetate and hexanes to give **18** (17.5 g, 81%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (ddd, $J =$ 2.5, 4.4, 8.7 Hz, 1H), 8.43 (dd, $J = 2.5$, 6.2 Hz, 1H), 7.97 (dd, J = 8.7, 10.0 Hz, 1H), 7.78 (s, 2H); MS (EI) m/z 307.1 $(M)^+$.

(*R*)-4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)benzenesulfonamide (20a). 20awas prepared from **18** and **9** according to general procedure II. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (d, $J = 2.4$ Hz, 1H), 7.84 (dd, $J =$ 9.2, 2.4 Hz, 1H), 7.43−7.29 (m, 7H), 7.22 (m, 1H), 6.99 (d, $J =$ 9.2, 1H), 4.05 (s, 1H), 3.25 (m, 2H), 2.37−2.17 (m, 2H), 2.09 (s, 6H), 1.92−1.74 (m, 2H); MS (ESI) m/z 510 $(M - H)^-$.

(*R*)-4-(4-Morpholino-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)benzenesulfonamide (20b). 20bwas prepared from **18** and **19**[12] according to general procedure II. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.98 (d, $J = 1.8$ Hz, 1H), 7.85 (dd, $J = 1.8$, 9.2 Hz, 1H), 7.38 (s, 2H), 7.35 (d, $J = 7.3$ Hz, 2H), 7.31 (t, $J = 7.6$ Hz, 2H), 7.22 (t, $J = 7.2$ Hz, 2H), 7.06 (d, $J = 9.5$ Hz, 1H), 6.91 (d, $J =$ 9.2 Hz, 1H), 4.10 (m, 1H), 3.50 (br, 4H), 3.37 (dd, $J = 5.2$, 14.0 Hz, 1H), 3.29 (dd, $J = 7.3$, 14.0 Hz, 1H), 2.29 (br, 4H), 2.18 (br, 2H), 1.95 (m, 1H), 1.74 (m, 1H); MS (ESI) m/z 554.0 $(M + H)^+$.

General Procedure IV. (*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(2,2,2-trifluoroacetyl)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (22a). To a solution of **21** (140 mg, 0.345 mmol) and **10a** (149 mg, 0.314 mmol) in CH_2Cl_2 (1.6 mL) were added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (121 mg, 0.628 mmol) and DMAP (38 mg, 0.314 mmol). The solution was stirred at room temperature for 15 h and diluted with saturated NH_4Cl. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under vacuo. The residue was purified by reverse phase HPLC to give **22a** (178 mg, 52%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.69 (br, 1H), 8.90 (d, J = 9.0 Hz, 1H), 8.24 (s, 1H), 7.92 (dd, $J = 1.9$, 9.4 Hz, 1H), 7.77 (m, 3H), 7.52 (m, 4H), 7.39 (d, $J = 8.4$ Hz, 2H), 7.34 (m, 1H), 7.21 (d, $J = 7.2$ Hz, 2H), 7.15 (d, $J = 9.4$ Hz, 1H), 7.11 (t, $J = 7.3$ Hz, 2H), 7.06 (t, $J = 7.3$ Hz, 1H), 6.93 (d, $J = 9.0$ Hz, 2H), 4.32 (br, 6H), 3.80 (m, 1H), 3.42 (dd, $J = 4.7$, 14.3 Hz, 1H), 3.34 (dd, $J = 6.6$, 14.3 Hz, 1H), 3.15 (br, 4H), 2.91 (br, 2H), 2.75 (s, 3H), 2.13 (m, 2H); MS (ESI) m/z 864.4 $(M + H)^+$. Anal. ($C_{44}H_{45}ClF_3N_5O_4S_2 \cdot 2.75CF_3CO_2H \cdot 0.15H_2O$) C, H, N.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(methylsulfonyl)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (22b). 22b was prepared from **10b** and **21** according to general procedure IV. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, $J = 2.4$ Hz, 1H), 7.89 (dd, $J = 2.1$, 9.2 Hz, 1H), 7.76 (d, $J = 8.9$ Hz, 3H), 7.53 (d, $J = 8.5$ Hz, 4H), 7.40 (d, $J = 8.5$ Hz, 2H), 7.34 (m, 3H), 7.28 (t, $J = 7.6$ Hz, 2H), 7.19 (t, $J = 7.2$ Hz, 1H), 6.96 (d, $J = 9.5$ Hz, 1H), 6.93 (d, $J = 9.2$ Hz, 2H), 6.67 (d, $J = 8.9$ Hz, 1H), 4.50−3.77 (br, 6H), 4.00 (m, 1H), 3.38 (dd, $J = 5.2$, 13.7 Hz, 1H), 3.33 (dd, $J = 7.3$, 13.7 Hz, 1H), 3.21 (s, 3H), 3.12 (m, 4H), 2.89 (br, 2H), 2.75 (s, 6H), 2.08 (m, 2H); MS (ESI) m/z 846 $(M + H)^+$.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(3-cyano-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (22c). 22c was prepared from **10c** and **21** according to general procedure IV. ^{1}H

NMR (400 MHz, DMSO-d_6) δ 7.93 (d, J = 2.1 Hz, 1H), 7.78 (m, 3H), 7.72 (br s, 1H), 7.52 (m, 4H), 7.40 (d, J = 8.6 Hz, 2H), 7.33 (m, 1H), 7.26 (m, 4H), 7.17 (m, 1H), 6.92 (d, J = 8.6 Hz, 2H), 6.81 (dd, J = 9.4, 2.6 Hz, 2H), 4.19−3.93 (m, 7H), 3.41 (m, 2H), 3.28 (m, 2H), 3.18−3.02 (m, 2H), 2.80 (m, 2H), 2.75 (d, 6H), 2.07 (m, 2H); MS (ESI) m/z 793 $(M + H)^+$. Anal. ($C_{43}H_{45}ClN_6O_3S_2 \cdot 2.3CF_3CO_2H \cdot 0.65H_2O$) C, H, N.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (22d). 22d was prepared from **14** and **21** according to general procedure IV. ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.27 (d, J = 9.2 Hz, 2H), 8.16 (d, J = 8.9 Hz, 2H), 7.51 (m, 4H), 7.43 (m, 4H), 7.35 (m, 2H), 7.26 (m, 2H), 7.18 (m, 1H), 6.91 (d, J = 9.2 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 3.83 (m, 1H), 3.39 (s, 2H), 3.29 (m, 2H), 3.16 (m, 4H), 3.14 (m, 2H), 2.74 (s, 6H), 2.50 (m, 1H), 2.38 (m, 4H), 2.20 (m, 1H); MS (ESI) m/z 768.1 $(M + H)^+$. Anal. ($C_{42}H_{46}ClN_5O_3S_2 \cdot 3.2CF_3CO_2H \cdot 0.3H_2O$) C, H, N.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (22e). 22e was prepared from **20a** and **21** according to general procedure IV. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 1.8 Hz, 1H), 7.95 (dd, J = 8.9, 1.8 Hz, 1H), 7.72 (d, J = 8.9 Hz, 2H), 7.51 (d, J = 7.3 Hz, 1H), 7.48 (s, 4H), 7.38 (m, 4H), 7.30 (t, J = 7.8 Hz, 2H), 7.24 (m, 1H), 7.21 (t, J = 7.3 Hz, 1H), 6.85−6.78 (m, 4H), 3.99 (m, 1H), 3.39−3.24 (m, 6H), 3.14 (m, 6H), 2.85−2.72 (m, 2H), 2.40 (d, 6H), 1.93 (m, 2H); MS (ESI) m/z 900 $(M + H)^+$.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethyl)phenylsulfonyl)benzamide (3). 3 was prepared from **10d** and **21** according to general procedure IV, and the product was purified by flash chromatography (0−10% MeOH in CH_2Cl_2 saturated with NH_3). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 (d, J = 2.0 Hz, 1H), 7.80 (dd, J = 9.0, 2.0 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.53 (d, J = 6.6 Hz, 1H), 7.47 (m, 4H), 7.41−7.26 (m, 7H), 7.19 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.1 Hz, 1H), 5.94 (s, 1H), 3.94 (m, 1H), 3.42 (m, 2H), 3.26 (m, 6H), 3.10 (m, 1H), 2.96 (m, 1H), 2.67 (s, 6H), 2.41 (m, 4H), 2.13 (m, 2H); MS (ESI) m/z 836 $(M + H)^+$.

4-Bromo-3-(trifluoromethoxy)aniline (23b). To a solution of 3-(trifluoromethoxy)aniline (3.5 g, 20 mmol) in CH_2Cl_2 (60 mL) was added pyridinium bromide perbromide (7.7 g, 24 mmol). After being stirred at 0 °C for 3 h, the mixture was diluted with ethyl acetate (400 mL), washed with aqueous $Na_2S_2O_3$, water, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (30% ethyl acetate in hexanes) to give **23b** (3.5 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.61 (m, 1H), 6.50 (m, 1H), 6.47 (m, 1H); MS (ESI) m/z 254 $(M - H)^-$.

4-Bromo-3-(trifluoromethoxy)benzenesulfonamide (23d). 23b (3.5 g, 13.7 mmol) was dissolved in concentrated HCl (10 mL) and AcOH (3 mL) and cooled to −15 °C with a dry ice ethanol bath. A solution of $NaNO_2$ (1.04 g, 15 mmol) in water (3 mL) was added slowly with the temperature not to exceed −5 °C and stirred at −10 °C for 45 min. In a separate flask, a saturated solution of SO_2 in AcOH (30 mL) was prepared by bubbling SO_2 for 45 min. To the resulting solution was added CuCl (1.1 g, 11 mmol) in one portion, and the solution was bubbled with SO_2 for an additional 15 min. The solution was cooled to 10 °C, and the solution of diazonium salt prepared above was added portionwise with the temperature not to exceed 30 °C. Upon completion of the addition, the mixture was poured onto crushed ice and stirred for 20 min. The mixture was extracted with ether (150 mL × 3), and the combined organic layers were washed with aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in ^{i}PrOH (100 mL) and cooled to 0 °C. A solution of ammonium hydroxide (50 mL) was added dropwise, and the resulting solution was allowed to warm to room temperature overnight. The solvent was removed in vacuo, and the residue was diluted with ethyl acetate (200 mL) and water (150 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (100 mL × 2). The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The crude material was used for the next step without further purification (2.5 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (m, 1H), 7.46 (m, 1H), 7.42 (m, 1H); MS (ESI) m/z 318 $(M - H)^-$.

***N*-(4-Bromo-3-methylphenylsulfonyl)-4-(4-((4′-chlorobiphenyl-2-yl)methyl)piperazin-1-yl)benzamide (24a). 24a** was prepared from **23a** and **21** according to general procedure IV, and the product was purified by flash chromatography (0−10% MeOH in CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (m, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.76 (m, 3H), 7.69 (dd, J = 8.5, 2.2 Hz, 1H), 7.52 (m, 4H), 7.40 (m, 2H), 7.33 (m, 1H), 6.92 (d, J = 9.1 Hz, 2H), 4.36 (br, 2H), 3.88 (br, 2H), 3.20 (br, 4H), 2.86 (m, 2H), 2.43 (s, 3H); MS (ESI) m/z 637.9 $(M + H)^+$.

***N*-(4-Bromo-3-(trifluoromethoxy)phenylsulfonyl)-4-(4-((4′-chlorobiphenyl-2-yl)methyl)piperazin-1-yl)benzamide (24b). 24b** was prepared from **23d** and **21** according to general procedure VI, and the product was purified by flash chromatography (0−10% MeOH in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (m, 2H), 7.65 (m, 5H), 7.25 (m, 5H), 6.69 (m, 3H), 3.25 (m, 5H), 2.57 (m, 5H); MS (ESI) m/z 710 $(M + H)^-$.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-methylphenylsulfonyl)benzamide (25a). 25a was prepared from **24a** and **9** according to general procedure III, and the product was purified by flash chromatography (0−10% MeOH in CH_2Cl_2 saturated with NH_3). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (d, J = 8.7 Hz, 2H), 7.49 (m, 6H), 7.37 (m, 4H), 7.30 (t, J = 7.6 Hz, 2H), 7.22 (m, 2H), 6.83 (d, J = 9.1 Hz, 2H), 6.34 (d, J = 9.1 Hz, 1H), 5.75 (s, 1H), 5.52 (d, J = 8.4 Hz, 1H), 3.72 (m, 1H), 3.38 (m, 2H), 3.19 (m, 5H), 3.12 (dd, J = 6.9, 13.4 Hz, 1H), 2.64 (m, 1H), 2.54 (m, 1H), 2.39 (m, 4H), 2.34 (s, 6H), 2.05 (s, 3H), 1.98 (m 1H), 1.87 (m, 1H); MS (ESI) m/z 782.2 $(M + H)^+$.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethoxy)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (25b). 25b was prepared from **24b** and **9** according to general procedure III. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.95 (m, 1H), 10.74 (m, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.50 (m, 5H), 7.27 (s, 10H), 6.92 (d, J = 9.2 Hz, 2H), 6.78 (d, J = 8.8 Hz, 1H), 6.55 (d, J = 9.2 Hz, 1H), 4.36 (m, 1H), 3.83 (m, 4H), 3.38 (m, 2H), 3.22 (m, 4H), 2.95 (m, 4H), 2.71 (s, 6H), 2.11 (m, 2H); MS (ESI) m/z 852 $(M + H)^+$. Anal. ($C_{43}H_{45}ClF_3N_5O_4S_2 \cdot 2.8CF_3CO_2H \cdot 0.05H_2O$) C, H, N.

General Procedure V. Ethyl 4-(4-((2-Bromocyclohex-1-enyl)-methyl)piperazin-1-yl)benzoate (29b). To a solution of DMF (20.0 g, 275 mmol) in CH_2Cl_2 (60 mL) cooled to 0 °C was added PBr_3 (23.5 mL, 249 mmol). The mixture was allowed to warm to room temperature and stirred for 20 min. A solution of **26b** in CH_2Cl_2 (10 mL) was added dropwise. After being stirred at room temperature for 20 min, the reaction mixture was poured onto crushed ice and neutralized with $NaHCO_3$. The mixture was diluted with ether, and the layers were separated. The aqueous layer was extracted with ether (×3), and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (5% ethyl acetate in hexanes) to give **27b** (7.99 g, 44%), which was used immediately for the next step because of instability.

To a solution of **27b** (7.99 g, 42.2 mmol) and **28** (10.0 g, 42.7 mmol) in EtOH (50 mL) was added sodium cyanoborohydride (2.68 g, 42.7 mmol) at room temperature. The pH of the solution was adjusted to 5 with acetic acid, and the suspension was stirred overnight. After filtration, the filter cake was washed with EtOH, and the combined filtrate was concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 10−20% ethyl acetate in hexanes) to give **29b** (6.59 g, 38%). **27b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (s, 1H), 2.79−2.71 (m, 2H), 2.32−2.24 (m, 2H), 1.80−1.63 (m, 4H). **29b**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (m, 2H), 6.98 (m, 2H), 4.26 (q, J = 7.17 Hz, 2H), 3.33−3.29 (m, 4H), 3.14 (s, 2H), 2.51−2.47 (m, 6H), 2.23 (m, 2H), 1.73−1.59 (m, 4H), 1.31 (t, J = 7.17 Hz, 3H); MS (ESI) m/z 407 $(M + H)^+$.

Ethyl 4-(4-((2-Bromocyclopent-1-enyl)methyl)piperazin-1-yl)-benzoate (29a). 29a was prepared from **26a** via **27a** according to general procedure V. **27a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.91 (s, 1H), 2.95−2.86 (m, 2H), 2.58−2.49 (m, 2H), 2.09−1.95 (m, 2H). **29a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97−7.87 (m, 2H), 6.90−6.80 (m, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.44−3.21 (m, 4H), 3.13 (s, 2H), 2.73−2.62 (m, 2H), 2.61−2.50 (m, 4H), 2.47−2.37 (m, 2H), 2.03−1.90 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H); MS (ESI) m/z 393 $(M + H)^+$.

(*Z*)-Ethyl 4-(4-((2-Bromocyclohept-1-enyl)methyl)piperazin-1-yl)benzoate (29c). 29c was prepared from **26c** via **27c** according to general procedure V. **27c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.93 (s, 1H), 3.05−2.96 (m, 2H), 2.53−2.45 (m, 2H), 1.85−1.76 (m, 2H), 1.71−1.61 (m, 2H), 1.51−1.40 (m, 2H). **29c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95−7.88 (m, 2H), 6.88−6.82 (m, 2H), 4.32 (q, J = 7.2 Hz, 2H), 3.33−3.28 (m, 4H), 3.14 (s, 2H), 2.82−2.74 (m, 2H), 2.63−2.56 (m, 4H), 2.40−2.33 (m, 2H), 1.81−1.69 (m, 2H), 1.63−1.44 (m, 4H), 1.36 (t, J = 7.2 Hz, 3H); MS (ESI) m/z 421 $(M + H)^+$.

(*Z*)-Ethyl 4-(4-((2-Bromocyclooct-1-enyl)methyl)piperazin-1-yl)benzoate (29d). 29d was prepared from **26d** via **27d** according to general procedure V. **29d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95−7.88 (m, 2H), 6.88−6.82 (m, 2H), 4.31 (q, J = 7.1 Hz, 2H), 3.34−3.27 (m, 4H), 3.16 (s, 2H), 2.77−2.66 (m, 4H), 2.63−2.56 (m, 4H), 2.45−2.36 (m, 4H), 1.72−1.56 (m, 4H), 1.36 (t, J = 7.1 Hz, 3H); MS (ESI) m/z 435 $(M + H)^+$.

Ethyl 4-(4-((2-Bromo-5,5-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)benzoate (29e). 29e was prepared from **26e** via **27e** according to general procedure V. **29e**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81−7.74 (m, 2H), 7.00−6.94 (m, 2H), 4.23 (q, J = 7.1 Hz, 2H), 3.09 (s, 2H), 2.48−2.40 (m, 4H), 2.06−1.95 (m, 4H), 1.51−1.35 (m, 4H), 1.28 (t, J = 6.8 Hz, 2H), 0.94−0.86 (m, 9H); MS (ESI), m/z 435 $(M + H)^+$.

Ethyl 4-(4-((4-Bromo-5,6-dihydro-2*H*-pyran-3-yl)methyl)piperazin-1-yl)benzoate (29f). 29f was prepared from **26f** via **27f** according to general procedure V. **27f**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.94 (s, 1H), 4.34 (t, J = 2.7 Hz, 2H), 3.84 (t, J = 5.8 Hz, 2H), 2.86−2.79 (m, 2H). **29f**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99−7.86 (m, 2H), 6.91−6.79 (m, 2H), 4.37−4.26 (m, 4H), 3.86−3.79 (m, 2H), 3.35−3.26 (m, 4H), 3.21−3.15 (m, 2H), 2.69−2.53 (m, 6H), 1.36 (t, J = 7.1 Hz, 3H); MS (ESI), m/z 409 $(M + H)^+$.

General Procedure VI. Ethyl 4-(4-((2-(4-Chlorophenyl)cyclohex-1-enyl)methyl)piperazin-1-yl)benzoate (30b). To a suspension of **29b** (1.86 g, 4.56 mmol), 4-chlorophenylboronic acid (0.80 g, 5.12 mmol), and aqueous sodium carbonate (2M, 2.5 mL, 5.0 mmol) in DME−H_2O−EtOH (14 mL−6 mL−4 mL) was added $PdCl_2(PPh_3)_2$ (124 mg, 0.177 mmol). The mixture was heated at 85 °C for 3 h under nitrogen atmosphere and filtered through a pad of Celite. The filter cake was washed with ethyl acetate, and the combined filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, 5−10% ethyl acetate in hexanes) to give **30b** (1.77 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.27 (m, 2H), 7.00 (m, 2H), 6.81 (m, 2H), 4.32 (q, J = 7.12 Hz, 2H), 3.30−3.22 (m, 4H), 2.81 (s, 2H), 2.40−2.33 (m, 4H), 2.28−2.17 (m, 4H), 1.78−1.67 (m, 4H), 1.36 (t, J = 7.12 Hz, 3H); MS (ESI) m/z 439 $(M + H)^+$.

Ethyl 4-(4-((2-(4-Chlorophenyl)cyclopent-1-enyl)methyl)piperazin-1-yl)benzoate (30a). 30a was prepared from **29a** according to general procedure VI. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97−7.91 (m, 2H), 7.41−7.34 (m, 2H), 7.10−7.05 (m, 2H), 6.86−6.78 (m, 2H), 4.33 (q, J = 7.2 Hz, 2H), 3.88 (s, 2H), 3.79−3.60 (m, 4H), 3.48−3.31 (m, 2H), 2.82−2.74 (m, 2H), 2.73−2.64 (m, 4H), 2.09−1.99 (m, 2H), 1.36 (t, J = 7.2 Hz, 3H); MS (ESI) m/z 425 $(M + H)^+$.

(*Z*)-Ethyl 4-(4-((2-(4-Chlorophenyl)cyclohept-1-enyl)methyl)piperazin-1-yl)benzoate (30c). 30c was prepared from **29c** according to general procedure VI. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93−7.85 (m, 2H), 7.28−7.22 (m, 2H), 7.02−6.95 (m, 2H), 6.83−6.78 (m, 2H), 4.31 (q, J = 7.2 Hz, 2H), 3.28−3.23 (m, 4H), 2.82 (s, 2H), 2.47−2.41 (m, 4H), 2.41−2.36 (m, 4H), 1.86−1.79 (m, 2H), 1.64−1.52 (m, 4H), 1.35 (t, J = 7.2 Hz, 3H); MS (ESI) m/z 453 $(M + H)^+$.

(*Z*)-Ethyl 4-(4-((2-(4-Chlorophenyl)cyclooct-1-enyl)methyl)piperazin-1-yl)benzoate (30d). 30d was prepared from **29d** according to general procedure VI. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98−7.89 (m, 2H), 7.41−7.33 (m, 2H), 7.03−6.95 (m, 2H), 6.85−6.76 (m, 2H), 4.33 (q, J = 7.1 Hz, 2H), 3.74−3.10 (m, 12H), 2.61−2.45 (m, 6H), 1.74−1.43 (m, 4H), 1.36 (t, J = 7.1 Hz, 3H); MS (ESI) m/z 467 $(M + H)^+$.

Ethyl 4-(4-((2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl)-methyl)piperazin-1-yl)benzoate (30e). 30e was prepared from **29e** according to general procedure VI. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94−7.86 (m, 2H), 7.31−7.24 (m, 2H), 7.04−6.96 (m, 2H), 6.85−6.78 (m, 2H), 4.31 (q, J = 7.12 Hz, 2H), 3.29−3.21 (m, 4H), 2.80 (s, 2H), 2.44−2.19 (m, 6H), 2.02 (s, 2H), 1.50−1.42 (m, 2H), 1.35 (t, J = 7.12 Hz, 3H), 0.99 (s, 6H); MS (ESI) m/z 467 $(M + H)^+$.

Ethyl 4-(4-((4-(4-Chlorophenyl)-5,6-dihydro-2*H*-pyran-3-yl)-methyl)piperazin-1-yl)benzoate (30f). 30f was prepared from **29f** according to general procedure VI. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.31 (m, 2H), 7.06 (m, 2H), 6.81 (m, 2H), 4.32 (m, 4H), 3.90 (t, J = 5.76 Hz, 2H), 3.25 (m, 4H), 2.91 (s, 2H), 2.38 (m, 6H,) 1.36 (t, J = 7.12 Hz, 3H); MS (ESI) m/z 441 $(M + H)^+$.

General Procedure VII. 4-(4-((2-(4-Chlorophenyl)cyclohex-1-enyl)methyl)piperazin-1-yl)benzoic Acid (31b). To a solution of **30a** (406 mg, 0.925 mmol) in dioxane (10 mL) was added aqueous LiOH (1 N, 2 mL, 3.00 mmol). The solution was heated at 100 °C for 24 h. The solution was concentrated, and the pH was adjusted to 6 with 2 N HCl. The precipitate was collected by vacuum filtration and dried in a vacuum oven to give **31b** (360 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (br s, 1H), 7.74 (m, 2H), 7.38 (m, 2H), 7.13 (m, 2H), 6.91 (m, 2H), 3.26−3.18 (m, 4H), 2.77 (s, 2H), 2.34−2.15 (m, 8H), 1.76−1.62 (m, 4H); MS (ESI) m/z 411 $(M + H)^+$.

4-(4-((2-(4-Chlorophenyl)cyclopent-1-enyl)methyl)piperazin-1-yl)benzoic Acid (31a). 31a was prepared from **30a** according to general procedure VII. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.23 (s, 1H), 7.77−7.72 (m, 2H), 7.44−7.28 (m, 4H), 6.96−6.88 (m, 2H), 3.38−3.18 (m, 4H), 3.09 (s, 2H), 2.76−2.69 (m, 2H), 2.61−2.54 (m, 2H), 2.43−2.34 (m, 4H), 1.94−1.84 (m, 2H); MS (ESI) m/z 397 $(M + H)^+$.

(*Z*)-4-(4-((2-(4-Chlorophenyl)cyclohept-1-enyl)methyl)piperazin-1-yl)benzoic Acid (31c). 31c was prepared from **30c** according to general procedure VII. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (br s, 1H), 7.76−7.71 (m, 2H), 7.41−7.34 (m, 2H), 7.13−7.06 (m, 2H), 6.94−6.87 (m, 2H), 3.26−3.19 (m, 4H), 2.78 (s, 2H), 2.45−2.36 (m, 4H), 2.35−2.27 (m, 4H), 1.87−1.70 (m, 2H), 1.64−1.44 (m, 4H); MS (ESI) m/z 425 $(M + H)^+$.

(*Z*)-4-(4-((2-(4-Chlorophenyl)cyclooct-1-enyl)methyl)piperazin-1-yl)benzoic Acid (31d). 31d was prepared from **30d** according to general procedure VII. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.27 (s, 1H), 7.81−7.76 (m, 2H), 7.46−7.41 (m, 2H), 7.15−7.10 (m, 2H), 6.97−6.92 (m, 2H), 3.29−3.25 (m, 4H), 2.81 (s, 2H), 2.58−2.45 (m, 4H), 2.36−2.29 (m, 4H), 1.71−1.56 (m, 6H), 1.53−1.46 (m, 2H); MS (ESI) m/z 439 $(M + H)^+$.

4-(4-((2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)benzoic Acid (31e). 31e was prepared from **30e** according to general procedure VII. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40 (s, 1H), 7.83−7.73 (m, 2H), 7.46−7.38 (m, 2H), 7.22−7.15 (m, 2H), 7.00−6.92 (m, 2H), 3.92−3.79 (m, 2H), 3.64−3.56 (m, 2H), 3.43−3.19 (m, 4H), 2.90−2.71 (m, 2H), 2.35−2.20 (m, 2H), 2.13 (s, 2H), 1.53−1.43 (m, 2H), 1.00 (s, 6H); MS (ESI) m/z 439 $(M + H)^+$.

4-(4-((4-(4-Chlorophenyl)-5,6-dihydro-2*H*-pyran-3-yl)methyl)piperazin-1-yl)benzoic Acid (31f). 31f was prepared from **30f** according to general procedure VII. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.73 (d, J = 8.82 Hz, 2H), 7.41 (d, J = 8.48 Hz, 2H), 7.21 (d, J = 8.48 Hz, 2H), 6.90 (d, J = 8.82 Hz, 2H), 4.17 (s, 2H), 3.80 (t, J = 5.43 Hz, 2H), 3.23 (m, 4H), 2.89 (s, 2H), 2.32 (m, 6H); MS (ESI) m/z 413 $(M + H)^+$.

(*R*)-4-(4-((2-(4-Chlorophenyl)cyclopent-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (33a). 33a was prepared from **31a** and **32**[12] according to general procedure IV. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (br s, 1H), 8.30−8.28 (m, 1H), 8.28 (d, J = 9.2 Hz, 1H), 7.63−7.58 (m, 1H), 7.78 (d, J = 8.9 Hz, 2H), 7.32 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.6 Hz, 2H), 6.99−6.95 (m, 2H), 6.94−6.83 (m, 4H), 6.97 (d, J = 8.9 Hz, 2H), 3.98−3.88 (m, 1H), 3.71−3.60 (m, 4H), 3.31−3.27 (m, 4H), 3.15−3.12 (m, 2H), 2.95−2.84 (m, 4H), 2.53−2.46 (m, 8H), 2.43−2.33 (m, 2H), 1.93−1.85 (m, 2H), 1.76−1.66 (m, 2H); MS (ESI) m/z 803 (M + H)$^+$. Anal. ($C_{41}H_{47}ClN_6O_5S_2 \cdot 3.05CF_3CO_2H$) C, H, N.

(*R*)-4-(4-((2-(4-Chlorophenyl)cyclohex-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (33b). 33b was prepared from **31b** and **32** according to general procedure IV. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (br s, 1H), 8.29 (d, J = 2.5 Hz, 1H), 8.03 (d, J = 9.2 Hz, 1H), 7.63−7.59 (m, 1H), 7.53 (d, J = 8.9 Hz, 2H), 7.15 (d, J = 8.3 Hz, 2H), 6.99−6.95 (m, 2H), 6.95−6.83 (m, 6H), 6.70 (d, J = 9.2 Hz, 2H), 3.97−3.89 (m, 1H), 3.43−3.31 (m, 4H), 3.19−3.07 (m, 4H), 2.95−2.83 (m, 4H), 2.49 (s, 6H), 2.26−2.22 (m, 2H), 2.02−1.94 (m, 4H), 1.92−1.86 (m, 2H), 1.48−1.39 (m, 4H); MS (ESI) m/z 817 (M + H)$^+$. Anal. ($C_{42}H_{49}ClN_6O_5S_2 \cdot 3.05CF_3CO_2H \cdot 0.05H_2O$) C, H, N.

(*R,Z*)-4-(4-((2-(4-Chlorophenyl)cyclohept-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (33c). 33c was prepared from **31c** and **32** according to general procedure IV. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (br s, 1 H), 8.56−8.53 (m, 1H), 8.29 (d, J = 9.2 Hz, 1H), 7.88−7.84 (m, 1H), 7.78 (d, J = 8.9 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.26−7.06 (m, 8 H), 6.95 (d, J = 8.9 Hz, 2H), 4.23−4.15 (m, 1H), 3.93−3.82 (m, 2H), 3.66−3.56 (m, 2H), 3.44−3.33 (m, 4H), 3.21−3.09 (m, 4H), 2.80−2.70 (m, 8H), 2.49−2.42 (m, 4H), 2.18−2.10 (m, 2H), 1.85−1.76 (m, 2H), 1.60−1.52 (m, 4H); MS (ESI) m/z 831 (M + H)$^+$. Anal. ($C_{43}H_{51}ClN_6O_5S_2 \cdot 2.9CF_3CO_2H \cdot 0.05H_2O$) C, H, N.

(*R,Z*)-4-(4-((2-(4-Chlorophenyl)cyclooct-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (33d). 33d was prepared from **31d** and **32** according to general procedure IV. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.13 (s, 1H), 8.56−8.53 (m, 1H), 8.30 (d, J = 9.2 Hz, 1H), 7.90−7.83 (m, 1H), 7.78 (d, J = 9.2 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.24−7.09 (m, 8H), 6.95 (d, J = 9.2 Hz, 2H), 4.27−4.11 (m, 5H), 3.95−3.83 (m, 2H), 3.72−3.57 (m, 2H), 3.45−3.32 (m, 4H), 3.20−3.05 (m, 4H), 2.75 (s, 3H), 2.74 (s, 3H), 2.48−2.43 (m, 2H), 2.21−2.07 (m, 2H), 1.70−1.62 (m, 2H), 1.60−1.48 (m, 4H), 1.45−1.36 (m, 2H); MS (ESI) m/z 439 (M + H)$^+$. Anal. ($C_{44}H_{53}ClN_6O_5S_2 \cdot 3.45CF_3CO_2H$) C, H, N.

(*R*)-4-(4-((2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (33e). 33e was prepared from **31e** and **32** according to general procedure IV. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.10 (s, 1H), 8.54 (d, J = 2.5 Hz, 1H), 8.28 (d, J = 9.2 Hz, 1H), 7.89−7.84 (m, 1H), 7.79 (d, J = 8.9 Hz, 2H), 7.43−7.39 (m, 2H), 7.24−7.21 (m, 2H), 7.18−7.10 (m, 6H), 6.95 (d, J = 9.2 Hz, 2H), 4.23−4.16 (m, 1H), 3.95−3.85 (m, 1H), 3.39 (d, 2H), 3.15 (s, 6H), 2.76−2.71 (m, 8H), 2.54−2.53 (m, 3H), 2.31−2.24 (m, 2H), 2.18−2.10 (m, 2H), 2.03 (s, 2H), 1.51−1.43 (m, 2H), 0.98 (s, 6H); MS (ESI) m/z 845 (M + H)$^+$. Anal. ($C_{44}H_{53}ClN_6O_5S_2 \cdot 4.0CF_3CO_2H \cdot 0.7H_2O$) C, H, N.

(*R*)-4-(4-((4-(4-Chlorophenyl)-5,6-dihydro-2*H*-pyran-3-yl)methyl)piperazin-1-yl)-*N*-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (33f). 33f was prepared from **31f** and **32** according to general procedure IV. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.29 (d, J = 2.2 Hz, 1H), 8.03 (d, J = 9.4 Hz, 1H), 7.63−7.59 (m, 1H), 7.52 (d, J = 9.1 Hz, 2H), 7.20−7.17 (m, 2H), 7.00−6.96 (m, 4H), 6.93−6.84 (m, 4H), 6.70 (d, J = 9.1 Hz, 2H), 3.99 (s, 2H), 3.96−3.90 (m, 1H), 3.69 (s, 2H), 3.58 (t, J = 5.3 Hz, 2H), 3.13 (s, 8H), 2.93−2.84 (m, 4H), 2.49 (s, 6H), 2.15−2.09 (m, 2H), 1.93−1.85 (m, 2H); MS (ESI) m/z 819 (M + H)$^+$. Anal. ($C_{41}H_{47}ClN_6O_6S_2 \cdot 2.85CF_3CO_2H \cdot 0.65H_2O$) C, H, N.

(*R*)-4-(4-((2-(4-Chlorophenyl)cyclohex-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-morpholino-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonyl)benzamide Trifluoroacetic Acid Salt (34). 34 was prepared from **20b** and **31b** according to general procedure IV. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.10 (d, J = 2.03 Hz, 1H), 7.93 (dd, J = 9.15, 2.03 Hz, 1H), 7.71 (d, J = 8.81 Hz, 2H), 7.41−7.23 (m, 6H), 7.22−7.15 (m, 1H), 7.15−7.09 (m, 2H), 6.94 (d, J = 9.15 Hz, 1H), 6.83 (d, J = 8.81 Hz, 3H), 4.13−3.97 (m, 1H), 3.51 (br s, 4H), 3.39−3.26 (m, 2H), 3.23 (br s, 4H), 2.83 (br s, 2H), 2.48−2.24 (m, 10H), 2.18 (br s, 4H), 2.04−1.86 (m, 1H), 1.81−1.70 (m, 1H), 1.66 (br s, 4H); MS (ESI) m/z 946 (M + H)$^+$.

(*R*)-4-(4-((2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)-*N*-(4-(4-morpholino-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonyl)benzamide (2). 2 was prepared from **20b** and **31e** according to general procedure IV, and the product was purified by flash chromatography (0−10% MeOH in dichloromethane saturated with NH_3). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (d, J = 2.1 Hz, 1H), 7.92 (dd, J = 2.1, 9.2 Hz, 1H), 7.71 (d, J = 8.9 Hz, 2H), 7.36 (m, 4H), 7.28 (t, J = 7.8 Hz, 2H), 7.19 (t, J = 7.2 Hz, 1H), 7.12 (d, J = 8.2 Hz, 2H), 6.90 (d, J = 9.5 Hz, 1H), 6.81 (d, J = 8.9 Hz, 2H), 6.77 (d, J = 8.9 Hz, 1H), 4.03 (m, 1H), 3.51 (m, 4H), 3.33 (dd, J = 5.5, 13.7 Hz, 1H), 3.26 (dd, J = 6.7, 14.0 Hz, 1H), 3.17 (br, 4H), 2.78 (s, 2H), 2.32 (m, 12H), 1.99 (s, 2H), 1.95 (m, 1H), 1.73 (m, 1H), 1.43 (t, J = 6.4 Hz, 2H), 0.97 (s, 6H); MS (ESI) m/z 974.3 (M + H)$^+$. Anal. ($C_{47}H_{55}ClF_3N_5O_6S_3 \cdot 0.15H_2O$) C, H, N.

4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(3-nitro-4-(2-(phenylthio)ethylamino)phenylsulfonyl)benzamide (4). 4 was prepared from 3-nitro-4-(2-(phenylthio)ethylamino)benzenesulfonamide[12] and **21** according to general procedure IV, and the product was purified by flash chromatography (0−10% MeOH in dichloromethane). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.66 (br, 1H), 8.56 (d, J = 2.2 Hz, 1H), 7.89 (dd, J = 2.0, 9.2 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.51 (m, 1H), 7.47 (m, 4H), 7.37 (m, 4H), 7.28 (t, J = 7.6 Hz, 2H), 7.25 (m, 1H), 7.18 (t, J = 7.3 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 2H), 3.64 (q, J = 6.5 Hz, 2H), 3.40 (s, 2H), 3.27 (t, J = 6.9 Hz, 2H), 3.21 (br, 4H), 2.40 (br, 4H); MS (ESI) m/z 742.2 (M + H)$^+$. Anal. ($C_{38}H_{36}ClN_5O_5S_2 \cdot 0.15CH_2Cl_2$) C, H, N.

(*R*)-4-(4-((4′-Chlorobiphenyl-2-yl)methyl)piperazin-1-yl)-*N*-(4-(4-morpholino-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonyl)benzamide Trifluoroacetic Acid Salt (5). 5 was prepared from (*R*)-4-(4-morpholino-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide[12] and **21** according to general procedure IV. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (d, J = 2.5 Hz, 1H), 8.30 (d, J = 9.0 Hz, 1H), 7.87 (dd, J = 2.0, 9.2 Hz, 1H), 7.77 (d, J = 9.0 Hz, 2H), 7.73 (br, 1H), 7.52 (d, J = 8.1 Hz, 4H), 7.40 (d, J = 8.1 Hz, 2H), 7.34 (br, 1H), 7.23 (d, J = 7.2 Hz, 2H), 7.15 (m, 4H), 6.93 (d, J = 9.0 Hz, 2H), 4.20 (m, 1H), 3.95 (br, 4H), 3.63 (br, 4H), 3.40 (m, 4H), 3.18 (br, 4H), 3.02 (br, 4H), 2.18 (m, 2H); MS (ESI) m/z 855 (M + H)$^+$. Anal. ($C_{44}H_{47}ClN_6O_6S_2 \cdot 3CF_3CO_2H$) C, H, N.

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Supporting Information Available: Bcl-2 and Bcl-xL binding affinity data and elemental analysis and HPLC data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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