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Structure-Based Discovery of BM-957 as a Potent Small-Molecule Inhibitor of Bcl-2 and Bcl-xL Capable of Achieving Complete Tumor Regression

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Abstract

Bcl-2 and Bcl-xL anti-apoptotic proteins are attractive cancer therapeutic targets. We have previously reported the design of 4,5-diphenyl-1*H*-pyrrole-3-carboxylic acids as a class of potent Bcl-2/Bcl-xL inhibitors. In the present study, we report our structure-based optimization for this class of compounds based upon the crystal structure of Bcl-xL complexed with a potent lead compound. Our efforts accumulated into the design of compound **30** (BM-957), which binds to Bcl-2 and Bcl-xL with $K_i < 1$ nM and has low nanomolar IC_{50} values in cell growth inhibition in cancer cell lines. Significantly, compound **30** achieves rapid, complete and durable tumor regression in the H146 small-cell lung cancer xenograft model at a well-tolerated dose-schedule.

Introduction

Apoptosis is a tightly regulated cellular process to eliminate damaged and unwanted cells and plays a critical role in normal development and homeostasis of multicellular organisms. Evasion of apoptosis is a hallmark of human cancer^{1–3} and targeting key apoptotic regulators with the goal of restoring apoptosis in tumor cells is a promising cancer therapeutic strategy.^{2, 4}

The B-cell lymphoma 2 (Bcl-2) family proteins are key regulators of apoptosis and consist of both pro-apoptotic and anti-apoptotic members.^{5–7} Pro-apoptotic Bcl-2 proteins are structurally subdivided into two groups: those like Bax, Bak and Bok, which contain three Bcl-2 homology (BH) domains (BH1-BH3) and Bad, Bid, Bim, Bik, Puma, Noxa, and others that contain only the BH3 domain.⁸ Although the precise mechanism of the Bcl-2 and Bcl-xL pro-survival function is not completely understood, it is clear that these proteins inhibit apoptosis by directly binding to and sequestering their pro-death counterparts such as Bim, Bid and Bad.⁹ Bcl-2 and Bcl-xL are frequently overexpressed in cancer cell lines and human cancer tissues. Such overexpression helps cancer cells suppress a variety of apoptotic stimuli including those associated with cancer chemotherapeutic agents and confers on tumor cells resistance to current therapeutic agents.^{10, 11} Thus, inhibition of these anti-

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apoptotic Bcl-2 family members offers an attractive approach for the development of new cancer therapeutics.

Experimentally determined three-dimensional structures of Bcl-2 and its closely related homologous protein Bcl-xL showed that the BH1, BH2 and BH3 domains in these proteins form a well-defined hydrophobic surface binding groove (the BH3 binding groove), which interacts with BH3-only pro-apoptotic proteins such as Bad, Bid, and Bim.^{12–14} It has been proposed that small-molecule inhibitors designed to bind to the BH3 surface binding groove in Bcl-2 and Bcl-xL will inhibit their anti-apoptotic function and promote apoptosis in tumor cells with high expression of these proteins. In the past ten years, a number of classes of non-peptide, small-molecule inhibitors have been designed to target the BH3 binding grooves in Bcl-2 and Bcl-xL proteins.^{15–36} ABT-737 (**1**) and its analogue ABT-263 (**2**) (Figure 1), from Abbott Laboratories, represent two of the most potent small molecule inhibitors of Bcl-2 and Bcl-xL proteins reported to date. Both compounds bind to Bcl-2 and Bcl-xL with very high affinities ($K_i < 1$ nM) and efficiently induce tumor regression in multiple xenograft tumor models.^{24–32} In Phase I/II clinical trials, ABT-263 shows evidence of promising antitumor activity in patients in chronic lymphocytic leukemia³⁷ but has a very limited single-agent activity in patients with small cell lung cancer³⁸.

Our laboratory has recently reported the design of compound **3** as a potent Bcl-2 and Bcl-xL inhibitor.³⁹ Compound **3** binds to Bcl-2 and Bcl-xL with K_i values of <1 nM and potently inhibits cancer cell growth with IC_{50} values of approximately 10 nM in multiple cancer cell lines. Compound **3** is also capable of achieving a strong *in vivo* antitumor activity in the H146 xenograft model in mice at a well-tolerated dose-schedule. Although compound **3** shows strong *in vivo* antitumor activity and in fact induces tumor regression during the treatment in the H146 xenograft tumor model, it fails to achieve durable tumor regression. After the treatment was stopped, tumors quickly regrew, suggesting that further optimization is needed toward our goal of obtaining a highly optimized Bcl-2/Bcl-xL inhibitor for clinical development. In the present study, we have performed further structure-based optimization for this class of compounds based upon a co-crystal structure of Bcl-xL complexed with compound **4** (BM-903), an analogue of compound **3** (Figure 2). Our efforts accumulated into the design of a new, highly potent Bcl-2/Bcl-xL inhibitor, which is capable of achieving complete and durable tumor regression in the H146 xenograft tumor model.

Results and Discussion

Analysis of the co-crystal structure of compound **4** complexed with Bcl-xL showed that the nitro group in **4** binds to a hydrophobic pocket, which can accommodate a larger group than the nitro group (Figure 2B). We have therefore modified the nitro group to determine structure-activity relationship at this site. Since **4** binds to Bcl-2 and Bcl-xL with very high affinities (K_i value <1 nM to both Bcl-2 and Bcl-xL), exceeding the lower limits of our binding assays for these two proteins, we designed and synthesized compound **5** (BM-916, Table 1) as a less potent but more soluble compound and used it as the template for further modifications of the nitro group. Compound **5** has a K_i value of 31.3 nM to Bcl-2 and 37.7 nM to Bcl-xL, respectively (Table 1).

To determine the contribution of the nitro group, we first synthesized compound **6**, in which the nitro group is replaced with a hydrogen atom. Compound **6** is 18- and 30-times less potent than **5** in its binding affinities to Bcl-2 and Bcl-xL, respectively, confirming the importance of the nitro group.

Since the nitro group inserts into a small hydrophobic pocket in Bcl-xL in the crystal structure of **4** complexed with Bcl-xL (Figure 2), we designed and synthesized a series of

analogues of **5** by replacing the nitro group with a small hydrophobic group. These include **7-9**, in which the nitro group is replaced with a halogen atom (F, Cl and Br), **10-13**, in which the nitro group is replaced with a small alkyl group, **14-17** with a small alkyloxyl group, and **18-21** with an alkylsulfonyl group. The binding data (Table 1) show that with the exception of compounds **9** and **18**, in which the nitro group is replaced by Br and trifluoromethylsulfonyl, respectively, these analogues bind to Bcl-2 and Bcl-xL 5-20 times less potently than **5**. While **9** is still slightly less potent than **5**, **18** is as potent as **5** in binding to both Bcl-2 and Bcl-xL.

Having identified trifluoromethylsulfonyl as a suitable replacement group for the nitro group for effective interaction with both Bcl-2 and Bcl-xL, we next synthesized **22**, in which the nitro group in **4** is replaced with trifluoromethylsulfonyl. Compound **22** binds to Bcl-2 and Bcl-xL with high affinities ($K_i = 2.1$ nM for Bcl-2 and < 1 nM for Bcl-xL). Similar to **3** and **4**, compound **22** has no appreciable binding to Mcl-1 at concentrations as high as 5 μ M, indicating that it is a potent and specific Bcl-2/Bcl-xL inhibitor.

We next evaluated **22** for its activity in inhibition of cell growth in the H1417 and H146 small-cell lung cancer cell lines, which are sensitive to potent and specific Bcl-2/Bcl-xL inhibitors such as compounds **1-4**.^{24, 27, 40} Compound **22** potently inhibits cell growth in the H1417 and H146 cancer cell lines, with IC_{50} values of 151 nM and 98 nM, respectively. The binding and cellular data thus indicated that **22** is a promising lead compound for further modifications.

The co-crystal structure (Figure 2C) also showed that the dimethylamino group of compound **4** forms hydrogen bonds with side chains of E96 and Y195 residues of Bcl-xL. We next modified the dimethylamino group in **22** using different sized nitrogen containing rings with or without a hydroxyl group. Compound **23** with morpholinyl shows weaker binding affinity than **22** to Bcl-2, but all other compounds have similar potencies to both Bcl-2 and Bcl-xL, as compared to **22**. Consistent with its weaker binding affinity to Bcl-2, compound **23** is 3–5 times less potent than **22** in inhibition of cell growth in both H146 and H1417 cancer cell lines. All other compounds show similar potencies with IC_{50} values of 100–200 nM in inhibition of cell growth in the H1417 and H146 cell lines.

In our subsequent *in vivo* evaluation, we found that compound **28** is effective in inhibition of tumor growth in the H146 tumor xenograft model at a well-tolerated dose-schedule (see below). Therefore, we focused subsequent modifications on **28**.

The co-crystal structure (Figure 2D) shows that the *N*-methyl group in the core structure of compound **4** inserts into the well-defined hydrophobic pocket formed by L112, V126 and F146 in Bcl-xL and there is more space available in this pocket to accommodate a larger hydrophobic group than methyl. We therefore probed this hydrophobic binding pocket by replacing the methyl in compound **28** with ethyl, propyl, isopropyl and butyl groups (Table 3). Compound **30** with ethyl and compound **31** with isopropyl bind to both Bcl-2 and Bcl-xL with very high affinities. While **30** and **31** bind to Bcl-2 with IC_{50} values of 5.4 and 4.0 nM, respectively (K_i values = 1.2 and 0.8 nM, respectively), they bind to Bcl-xL with IC_{50} values of 6.0 and 3.9 nM, respectively (K_i values < 1 nM). However, compound **32** with propyl and compound **33** with butyl have lower affinities to Bcl-2 than **30** and **31**. Interestingly, these four compounds have similar high binding affinities to Bcl-xL. Similar to **22**, compounds **28-33** have no appreciable binding to Mcl-1 at concentrations as high as 5 μ M.

Testing of compounds **30-33** in the H1417 and H146 cell lines showed that these compounds are very potent in inhibition of cell growth and are 5–10 times more potent than

28. While **30** has IC₅₀ values of 21 nM and 22 nM, respectively, in these two cancer cell lines, compound **31** has IC₅₀ values of 9 and 13 nM, respectively. However, compounds **32** and **33** are >10-times less potent than **30** and **31**.

Further *in vitro* evaluations of potent Bcl-2 and Bcl-xL inhibitors

We next tested compounds **28**, **30** and **31** for their ability to induce cell death in the H146 cancer cell line, in direct comparison to compounds **1** and **2**. The results are shown in Figure 3.

All these compounds induce cell death in a dose-dependent manner but have different potencies. While **28** is somewhat less potent than **1** and **2**, **30** and **31** are several times more potent than **1** and **2**. For example, **30** and **31** at 10 nM with 24 hr-treatment induces >50% of the H146 cells to undergo cell death, whereas **1** and **2** at 30–100 nM have a similar effect.

We further tested compounds **2**, **28** and **30** in the H146 cell line for their ability to induce cleavage of poly(ADP-ribose) polymerase (PARP) and caspase-3, two key biochemical markers of apoptosis. The results are shown in Figure 4. Compound **30** at 10 nM, **28** at 100 nM and **2** at 30 nM all induce clear cleavage of PARP and activation of caspase-3 and have similar effects. Hence, the potencies for these three compounds in induction of cleavage of PARP and activation of caspase-3 in the H146 cells are consistent with their potencies in induction of cell death.

In vivo evaluation of compounds **28**, **30** and **31**

We determined the maximum tolerated dose (MTD) for compounds **28**, **30** and **31** in severe combined immunodeficiency (SCID) mice. It was found that **28** at 50 mg/kg, **30** at 25 mg/kg and **31** at 10 mg/kg, daily, intravenous dosing, 5 days a week for 2 weeks were well tolerated in SCID mice and the animals had less than 10% of weight loss. Higher doses of these compounds (75 mg/kg for **28**, 50 mg/kg for **30** and 25 mg/kg for **31**) caused more than 10% of weight loss. These experiments established the MTDs for these three compounds in SCID mice for our subsequent pharmacodynamics (PD) and efficacy experiments. Based upon the toxicity data, we decided to further evaluate compounds **28** and **30** for their *in vivo* antitumor activity.

We tested compounds **28** and **30** for their ability to induce cleavage of PARP and activation of caspase-3 in the H146 xenograft tumor tissue in mice at their respective MTD in a pharmacodynamics experiment. Mice bearing H146 tumors were given a single *i.v.* dose of **28** at 50 mg/kg or **30** at 25 mg/kg. The mice were then sacrificed at 3-, 6- and, 24-h time points and tumors were harvested for analysis. Western blotting analysis showed that both **28** and **30** effectively induce robust cleavage of PARP and caspase-3 in H146 tumor tissues at 3- and 6-h time-points, indicative of strong apoptosis induction by both compounds in tumor tissues (Figure 5).

Based upon the encouraging *in vivo* pharmacodynamic data, we next evaluated **28** and **30** for their antitumor efficacy in the H146 xenograft tumor model (Figure 6). Our data showed that although compound **28** at 50 mg/kg effectively inhibits tumor growth, it fails to induce tumor regression. In contrast, compound **30** at 25 mg/kg is capable of achieving complete tumor regression. Of 7 mice treated with **30**, all mice were tumor-free at day 47 (15 days post-treatment) and five (71%) remained tumor-free on day 58 (28 days post-treatment). Similar to the data obtained from our MTD experiment, both compounds **28** and **30** are well tolerated in tumor-bearing animals. All treated animals experienced less than 10% weight loss compared to the vehicle control and all regained their weight quickly after the treatments were finished. This *in vivo* experiment thus established that **30** achieves complete

and durable tumor regression in the H146 xenograft tumor model and is more efficacious than **28**.

Synthesis of Designed Bcl-2 family protein inhibitors

The general synthetic route to compounds in Table 1 and Table 2 is shown in Scheme 1. Compound **34**, which was prepared according to a previously reported method³⁹, was coupled with 3-(4-Methyl-piperazin-1-yl)-propylamine to give phenylnitro **35**. Reduction of the phenylnitro under hydrogen atmosphere in the presence of Pd/C yielded the corresponding aniline, which was subjected to different *m*-substituted benzenesulfonyl chloride in pyridine to generate compounds in Table 1.

Using a converged synthetic strategy, compounds in Table 2 were prepared by coupling amine **37a-f** with highly activated fluoro containing intermediate **38**. Amine **37a-f** were obtained by treatment of **36** with different amines, followed by removal of the Fmoc group with Et₂NH and reduction with borane. Phenylnitro **34** was reduced by hydrogenation and then treated with commercially available 4-fluoro-3-(trifluoromethylsulfonyl)benzenesulfonyl chloride to yield **38**.

The general synthetic route for compounds in Table 3 is shown in Scheme 2. Briefly, condensation of **39** with primary amines resulted in pyrrole **40a-b**. Phenylnitro **41a-b** were prepared by Ullman coupling of 1-(*p*-nitrophenyl)piperazine with **40a-b**, followed by hydrolysis of these ethyl esters, which yielded free carboxylic acid **42a-b**. Reduction of the nitro group of **42a-b** and **42c-d**³⁹, followed by coupling of 4-fluoro-3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride, yielded sulfonanilide **43a-d**, treatment of which with **37e** in the presence of DIPEA in DMF, gave compounds in Table 3.

Summary

We have performed further structure-based optimization for a new class of Bcl-2/Bcl-xL inhibitors containing 4,5-diphenyl-1*H*-pyrrole-3-carboxylic acid as the core structure. Our efforts accumulated into the design of compound **30** (BM-957), which binds to Bcl-2 and Bcl-xL with the K_i values <1 nM and shows potent activity in cell growth inhibition in cancer cell lines, with IC₅₀ values of ~20 nM against the H1147 and H146 small-cell lung cancer cell lines. Compound **30** induces robust cleavage of PARP and caspase-3 in 24 h at concentrations as low 10 nM in the H146 cell line. *In vivo*, compound **30** achieves complete and durable tumor regression in H146 xenograft tumors and is thus more efficacious than compound **3** in the same tumor model. The efficacy data thus suggest that compound **30** has a superior pharmacokinetic property to compound **3**. Taken together, compound **30** represents a promising Bcl-2/Bcl-xL inhibitor for extensive evaluations as a new anticancer agent.

Experimental Section

Chemistry

General Methods—Unless otherwise noted, all reactions were performed under nitrogen atmosphere in dry solvents under anhydrous conditions and reagents were used as supplied without further purification. NMR spectra were acquired at a proton frequency of 300 MHz and chemical shifts are reported in parts per million (ppm) relative to an internal standard. The final products were purified by a C18 reverse phase semi-preparative HPLC column with solvent A (0.1% of TFA in H₂O) and solvent B (0.1% of TFA in CH₃CN) as eluents. All the target compounds have purities of >95% based upon UPLC analysis. Compounds **1** (ABT-737) and **2** (ABT-263) were purchased from SellechBio.com and their purity was confirmed to be >95% based upon UPLC analysis.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (35): A solution of **34** (1.06 g, 2.0 mmol), 3-(4-methylpiperazin-1-yl)propan-1-amine (472 mg, 3.0 mmol), EDCI (768 mg, 4.0 mmol), and DMAP (244 mg, 2.0 mmol) in CH₂Cl₂ (10 mL) was stirred for 3 h until no **34** was observed by TLC. The mixture was diluted with EtOAc (200 mL), washed sequentially with 1 M HCl (50 mL), H₂O (50 mL), and brine (20 mL), dried (Na₂SO₄), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 5% MeOH/CH₂Cl₂ to provide 1.15 g (86%) of **35**. ¹H NMR (300 MHz, CDCl₃): δ 8.14 (d, *J* = 10.8 Hz, 2H), 7.27-7.05 (m, 5H), 6.89-6.68 (m, 5H), 3.53-3.47 (m, 4H), 3.42 (s, 3H), 3.26-3.19 (m, 6H), 2.61 (s, 3H), 2.45-2.09 (m, 8H), 2.24 (s, 3H), 2.11 (t, *J* = 6.4, 2H), 1.50-1.41 (m, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 165.99, 154.57, 150.26, 138.54, 135.84, 133.68, 133.31, 132.28, 130.58, 129.41, 129.17, 128.42, 125.88, 122.37, 121.04, 118.58, 114.83, 114.33, 112.67, 55.90, 55.07, 53.48, 53.03, 48.39, 46.72, 46.00, 37.63, 31.58, 26.47, 11.40. MS (ESI): *m/z* 670.92 (M + H)⁺.

General Procedure I

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-(3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (5): To a solution of **35** (100 mg, 0.15 mmol) in 15 mL of methanol was added 10% wt. Pd/C (10 mg, 0.1 eq. m/m). The solution was stirred under hydrogen atmosphere for about 20 min at room temperature until no **35** was observed by TLC. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was used for next step directly without purification. To the solution of this aniline in pyridine, 3-nitrobenzene-1-sulfonyl chloride (40 mg, 0.18 mmol) was added at 0 °C. The mixture was stirred at 0 °C to room temperature for 1 hour until no aniline was observed by TLC. H₂O (10 mL) was added and the mixture was extracted with EtOAc (2×30 mL). The EtOAc solution was washed with brine (50 mL), dried over Na₂SO₄ and concentrated *in vacuo*. The concentrate was purified by HPLC to give the pure product **5** (as the trifluoroacetate salt, 89 mg), yield 72% over two steps. The gradient ran from 80% of solvent A and 20% of solvent B to 40% of solvent A and 60% of solvent B in 40 min. ¹H NMR (300 MHz, CD₃OD): δ 8.44-8.39 (m, 2H), 8.09-8.06 (m, 1H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.32-7.22 (m, 3H), 7.14-7.04 (m, 7H), 6.86 (t, *J* = 2.2 Hz, 2H), 3.51 (br, 4H), 3.42-3.34 (m, 17H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.92 (s, 3H), 2.45 (s, 3H), 1.87-1.83 (m, 2H). MS (ESI): *m/z* 826.00 (M + H)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-(phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (6): **6** was prepared from **35** and benzenesulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.76-7.74 (m, 2H), 7.60-7.45 (m, 3H), 7.34-7.23 (m, 3H), 7.16-7.04 (m, 7H), 6.90-6.86 (m, 2H), 3.52-3.9 (m, 21H), 3.00 (t, *J* = 7.4 Hz, 2H), 2.93 (s, 3H), 2.47 (s, 3H), 1.89-1.84 (m, 2H). MS (ESI): *m/z* 780.75 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-fluorophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (7): **7** was prepared from **35** and 3-fluorobenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.50-7.36 (m, 3H), 7.26-7.18 (m, 4H), 7.13-6.95 (m, 7H), 6.87-6.81 (m, 2H), 3.52-3.22 (m, 21H), 2.99 (t, *J* = 7.0 Hz, 2H), 2.88 (s, 3H), 2.37 (s, 3H), 1.82-1.75 (m, 2H). MS (ESI): *m/z* 799.00 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-chlorophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (8): **8** was prepared from **35** and 3-chlorobenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.67-7.40 (m, 4H),

7.30-7.20 (m, 3H), 7.11-7.00 (m, 7H), 6.87-6.84 (m, 2H), 3.52-3.26 (m, 21H), 2.98 (t, J = 7.4 Hz, 2H), 2.90 (s, 3H), 2.42 (s, 3H), 1.87-1.82 (m, 2H). MS (ESI): m/z 815.42 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-bromophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (9): **9** was prepared from **35** and 3-bromobenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.81-7.64 (m, 3H), 7.38-7.21 (m, 4H), 7.11-7.00 (m, 7H), 6.88-6.82 (m, 2H), 3.53-3.26 (m, 21H), 2.99 (t, J = 6.9 Hz, 2H), 2.91 (s, 3H), 2.42 (s, 3H), 1.87-1.82 (m, 2H). MS (ESI): m/z 859.92 (M + H)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-(3-(tri fluoromethyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (10): **10** was prepared from **35** and 3-trifluoromethylbenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.94-7.86 (m, 3H), 7.68 (t, J = 8.0 Hz, 1H), 7.32-7.01 (m, 10H), 6.88-6.86 (m, 2H), 3.52-3.28 (m, 21H), 2.98 (t, J = 7.5 Hz, 2H), 2.91 (s, 3H), 2.44 (s, 3H), 1.91-1.81 (m, 2H). MS (ESI): m/z 849.08 (M + H)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(4-(3-methylphenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (11): **11** was prepared from **35** and 3-methylbenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.56-7.51 (m, 2H), 7.38-7.21 (m, 5H), 7.13-7.00 (m, 7H), 6.87-6.84 (m, 2H), 3.52-3.28 (m, 21H), 2.98 (t, J = 7.1 Hz, 2H), 2.91 (s, 3H), 2.44 (s, 3H), 2.33 (s, 3H), 1.90-1.81 (m, 2H). MS (ESI): m/z 795.00 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-ethylphenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (12): **12** was prepared from **35** and 3-ethylbenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.58-7.34 (m, 6H), 7.24-7.16 (m, 3H), 7.06-6.94 (m, 5H), 6.80-6.75 (m, 2H), 3.46 (s, 3H), 3.30-3.05 (m, 18H), 2.83 (s, 3H), 2.71-2.64 (m, 4H), 2.50 (s, 3H), 1.77-1.73 (m, 2H), 1.20 (t, J = 7.6, 3H). MS (ESI): m/z 809.00 (M + H)⁺.

4-(3-(4-(4-(3-*tert*-Butylphenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-5-(4-chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (13): **13** was prepared from **35** and 3-*tert*-butylbenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.58-7.50 (m, 3H), 7.36-7.18 (m, 4H), 7.07-7.01 (m, 7H), 6.87-6.84 (m, 2H), 3.53-3.22 (m, 21H), 2.99 (t, J = 7.2 Hz, 2H), 2.88 (s, 3H), 2.38 (s, 3H), 1.85-1.81 (m, 2H), 1.17 (s, 9H). MS (ESI): m/z 837.00 (M + H)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-(3-(trifluoromethoxy)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (14): **14** was prepared from **35** and 3-trifluoromethoxybenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.70-7.43 (m, 4H), 7.29-7.20 (m, 3H), 7.10-7.01 (m, 7H), 6.87-6.85 (m, 2H), 3.52-3.25 (m, 21H), 2.98 (t, J = 7.3 Hz, 2H), 2.90 (s, 3H), 2.41 (s, 3H), 1.89-1.79 (m, 2H). MS (ESI): m/z 864.92 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-methoxyphenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (15): **15** was prepared from **35** and 3-methoxybenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.35-7.00 (m, 14H), 6.88-6.84 (m,

2H), 3.71 (s, 3H), 3.54-3.24 (m, 21H), 3.01 (t, $J = 6.7$ Hz, 2H), 2.91 (s, 3H), 2.40 (s, 3H), 1.89-1.82 (m, 2H). MS (ESI): m/z 811.00 ($M + H$)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-ethoxyphenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (16): 16 was prepared from 35 and 3-ethoxybenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.37-7.02 (m, 14H), 6.88-6.86 (m, 2H), 4.01-3.94 (m, 2H), 3.54-3.28 (m, 21H), 3.00 (t, $J = 7.1$ Hz, 2H), 2.93 (s, 3H), 2.44 (s, 3H), 1.92-1.82 (m, 2H), 1.34 (t, $J = 7.0$ Hz, 3H). MS (ESI): m/z 825.00 ($M + H$)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-isopropoxyphenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (17): 17 was prepared from 35 and 3-isopropoxybenzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 7.42-6.95 (m, 14H), 6.81-6.78 (m, 2H), 4.59-4.51 (m, 1H), 3.47 (s, 3H), 3.38-3.10 (m, 18H), 2.85 (s, 3H), 2.75 (t, $J = 7.3$ Hz, 2H), 2.50 (s, 3H), 1.77-1.75 (m, 2H), 1.28 (d, $J = 6.0$ Hz, 6H). MS (ESI): m/z 839.08 ($M + H$)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-(3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (18): 18 was prepared from 35 and 3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 8.29 (t, $J = 6.4$ Hz, 2H), 8.22 (s, 1H), 7.96 (t, $J = 7.9$ Hz, 1H), 7.37-7.15 (m, 5H), 7.04-7.00 (m, 5H), 6.83-6.79 (m, 2H), 3.46 (s, 3H), 3.40-3.22 (m, 18H), 2.88 (s, 3H), 2.82 (t, $J = 7.3$ Hz, 2H), 2.50 (s, 3H), 1.83-1.79 (m, 2H). MS (ESI): m/z 913.25 ($M + H$)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-4-(3-(4-(4-(3-(methylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (19): 19 was prepared from 35 and 3-(methylsulfonyl)benzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 8.12-8.02 (m, 3H), 7.74 (t, $J = 7.8$ Hz, 1H), 7.31-7.23 (m, 3H), 7.12-7.02 (m, 7H), 6.93-6.91 (m, 2H), 3.56-3.36 (m, 21H), 3.06-3.01 (m, 5H), 2.93 (s, 3H), 2.42 (s, 3H), 1.92-1.83 (m, 2H). MS (ESI): m/z 859.00 ($M + H$)⁺.

5-(4-Chlorophenyl)-1,2-dimethyl-N-(3-(4-ethylpiperazin-1-yl)propyl)-4-(3-(4-(4-(3-(methylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxamide (20): 20 was prepared from 35 and 3-(ethylsulfonyl)benzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 8.10-8.03 (m, 3H), 7.78 (t, $J = 7.9$ Hz, 1H), 7.34-7.24 (m, 3H), 7.16-7.05 (m, 7H), 6.92-6.89 (m, 2H), 3.54-3.29 (m, 21H), 3.14 (dd, $J = 14.8, 7.41$ Hz, 2H), 3.01 (t, $J = 7.2$ Hz, 2H), 2.94 (s, 3H), 2.46 (s, 3H), 1.90-1.85 (m, 2H), 1.09 (t, $J = 7.4$ Hz, 3H). MS (ESI): m/z 873.00 ($M + H$)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(3-(cyclopropylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-3-carboxamide (21): 21 was prepared from 35 and 3-(cyclopropyl)benzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CD₃OD): δ 8.09-8.02 (m, 3H), 7.80 (t, $J = 7.8$ Hz, 1H), 7.65-6.71 (m, 11H), 6.25 (s, 1H), 5.89 (d, $J = 1.7$ Hz, 1H), 3.46 (s, 3H), 3.37-3.23 (m, 17H), 2.92 (br, 1H), 2.79 (s, 3H), 2.60 (t, $J = 3.9$ Hz, 2H), 2.50 (s, 3H), 1.83 (s, 2H), 1.70-1.68 (m, 2H), 1.32 (s, 2H). MS (ESI): m/z 885.17 ($M + H$)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(4-fluoro-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-1H-pyrrole-3-carboxylic Acid (38): **38** was prepared from **34** and 4-fluoro-3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride according to general procedure I. ¹H NMR (300 MHz, CDCl₃): δ 8.29 (dd, *J* = 6.0, 2.0 Hz, 1H), 8.08-8.04 (m, 1H), 7.35-6.62 (m, 13H), 3.40 (s, 3H), 3.13-3.07 (m, 8H), 2.60 (s, 3H). MS (ESI): *m/z* 791.64 (M + H)⁺.

General Procedure II

(R)-4-(azetidin-1-yl)-1-(phenylthio)butan-2-amine (37a): A solution of **36** (1.0 g 2.3 mmol), azetidine (394 mg, 6.9 mmol), EDCI (662 mg, 3.5 mmol), and HOBt (466 mg, 3.5 mmol) in DCM (12 mL) was stirred for 4 h at room temperature until no **36** was observed by TLC, then diluted with DCM (100 mL), washed sequentially with 1M HCl (50 mL), H₂O (50 mL), and brine (20 mL), dried (MgSO₄), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 50% EtOAc/hexanes to provide **(R)-(9H-fluoren-9-yl)methyl 4-(azetidin-1-yl)-4-oxo-1-(phenylthio)butan-2-ylcarbamate**, 957 mg (88%). The amide was dissolved in MeCN (20 mL) and treated with Et₂NH (2.1 mL, 20 mmol). The solution was stirred for 2 h at room temperature until no starting material was observed by TLC and concentrated. The concentrate was flash chromatographed on silica gel with 5% MeOH/CH₂Cl₂ to provide **(R)-3-amino-1-(azetidin-1-yl)-4-(phenylthio)butan-1-one**, 497 mg (98%). A mixture of this free amine and 1M BH₃ in THF (5 mL) was stirred for 16 h at room temperature, treated with MeOH (1.5 mL) and concentrated HCl (0.5 mL), stirred at 80°C for 3 h, cooled to room temperature, adjusted to pH 10 with 4M Na₂CO₃, diluted with CH₂Cl₂ (150 mL), washed with H₂O (50 mL) and brine (10 mL), dried (MgSO₄), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 15% MeOH/CH₂Cl₂ to provide **37a**, 445 mg (95%). ¹H NMR (300 MHz, D₂O): δ 7.53 (d, *J* = 7.2 Hz, 2H), 7.49-7.38 (m, 3H), 4.32-4.18 (m, 2H), 4.03-3.82 (m, 2H), 3.54-3.15 (m, 5H), 2.61-2.51 (m, 1H), 2.44-2.39 (m, 1H), 2.07-1.90 (m, 2H). ¹³C NMR (75 MHz, D₂O): δ 132.99, 131.13, 129.78, 128.10, 54.91, 54.77, 50.64, 48.53, 35.92, 26.45, 15.82. MS (ESI): *m/z* 237.66 (M + H)⁺.

(R)-1-(Phenylthio)-4-(pyrrolidin-1-yl)butan-2-amine (37b): **37b** was prepared from **36** and pyrrolidine according to general procedure II. ¹H NMR (300 MHz, D₂O): δ 7.55 (d, *J* = 7.2 Hz, 2H), 7.46-7.37 (m, 3H), 3.54-3.48 (m, 3H), 3.39-3.05 (m, 4H), 3.02-2.89 (m, 1H), 2.87-2.68 (m, 1H), 2.25-1.94 (m, 6H). ¹³C NMR (75 MHz, D₂O): δ 133.02, 131.13, 129.78, 128.09, 54.38, 54.08, 50.74, 48.77, 35.85, 27.70, 22.58. MS (ESI): *m/z* 251.26 (M + H)⁺.

(R)-1-(Phenylthio)-4-(piperidin-1-yl)butan-2-amine (37c): **37c** was prepared from **36** and piperidine according to general procedure II. ¹H NMR (300 MHz, D₂O): δ 7.59 (d, *J* = 7.3 Hz, 2H), 7.50-7.39 (m, 3H), 3.50-3.00 (m, 7H), 2.90-2.72 (m, 2H), 2.30-2.07 (m, 2H), 2.01-1.58 (m, 5H), 1.54-1.35 (m, 1H). ¹³C NMR (75 MHz, D₂O): δ 133.02, 131.20, 129.78, 128.11, 53.52, 53.19, 52.68, 48.88, 35.88, 25.89, 22.74, 20.93. MS (ESI): *m/z* 265.38 (M + H)⁺.

(R)-1-(3-Amino-4-(phenylthio)butyl)azetidin-3-ol (37d): **37d** was prepared from **36** and azetidin-3-ol according to general procedure II. ¹H NMR (300 MHz, D₂O): δ 7.55 (d, *J* = 7.1 Hz, 2H), 7.50-7.39 (m, 3H), 4.72-4.63 (m, 1H), 4.51-3.69 (m, 4H), 3.51-3.43 (m, 1H), 3.38-3.20 (m, 4H), 2.08-1.90 (m, 2H). ¹³C NMR (75 MHz, D₂O): δ 132.92, 131.13, 129.78, 128.15, 63.27, 62.52, 58.73, 50.71, 48.44, 35.93, 26.66. MS (ESI): *m/z* 253.60 (M + H)⁺.

(R)-1-(3-Amino-4-(phenylthio)butyl)piperidin-4-ol (37e): **37e** was prepared from **36** and piperidin-4-ol according to general procedure II. ¹H NMR (300 MHz, D₂O): δ 7.51 (d, *J* = 3.5 Hz, 2H), 7.41-7.33 (m, 3H), 4.09-3.77 (m, 1H), 3.46-2.69 (m, 9H), 2.12-2.08 (m, 3H),

1.87 (br, 2H), 1.69-1.57 (m, 1H). ^{13}C NMR (75 MHz, D_2O): δ 132.99, 131.18, 129.76, 128.04, 64.59, 52.58, 51.38, 48.87, 47.89, 35.88, 30.88, 28.85, 26.16. MS (ESI): m/z 281.46 ($\text{M} + \text{H}$) $^+$.

(R)-1-(3-Amino-4-(phenylthio)butyl)-4-methylpiperidin-4-ol (37f): **37f** was prepared from **36** and 4-methylpiperidin-4-ol according to general procedure II. ^1H NMR (300 MHz, D_2O): δ 7.44 (d, $J = 6.8$ Hz, 2H), 7.40-7.34 (m, 3H), 3.55-3.47 (m, 1H), 3.39-2.81 (m, 8H), 2.67-2.04 (m, 2H), 1.81-1.76 (m, 4H), 1.27 (s, 3H). ^{13}C NMR (75 MHz, D_2O): δ 133.09, 131.26, 129.81, 128.15, 65.02, 52.64, 49.10, 48.96, 48.56, 35.84, 34.62, 28.45, 25.88. MS (ESI): m/z 295.36 ($\text{M} + \text{H}$) $^+$.

General Procedure III

(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl) piperazin-1-yl)phenyl)-1,2-dimethyl-1H-pyrrole-3-carboxylic Acid (22): To a solution of **38** (79 mg, 0.1 mmol) and (R)-N₁,N₁-dimethyl-4-(phenylthio)butane-1,3-diamine (44 mg, 0.2 mmol) in DMF (1 mL) was added DIPEA (0.5 mL). The solution was stirred for 4 hours at room temperature until no **38** was observed by TLC. The reaction mixture was concentrated *in vacuo* and the residue was purified by HPLC to give the pure product **22** (trifluoroacetic acid salt, 81 mg, yield 81%). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. ^1H NMR (300 MHz, CD_3OD): δ 7.84 (d, $J = 2.0$ Hz, 1H), 7.70 (dd, $J = 9.1, 2.2$ Hz, 1H), 7.26-6.96 (m, 16H), 6.86-6.78 (m, 2H), 3.98-3.91 (m, 1H), 3.42-3.31 (m, 11H), 3.20-3.02 (m, 4H), 2.80 (s, 6H), 2.57 (s, 3H), 2.25-1.98 (m, 2H). MS (ESI): m/z 996.30 ($\text{M} + \text{H}$) $^+$.

(R)-5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(4-(4-morpholino-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxylic Acid (23): **23** was prepared from **38** and (R)-4-morpholino-1-(phenylthio)butan-2-amine according to general procedure III. ^1H NMR (300 MHz, CD_3OD): δ 7.93 (d, $J = 2.1$ Hz, 1H), 7.74 (dd, $J = 9.2, 2.22$ Hz, 1H), 7.44-6.89 (m, 17H), 6.83 (d, $J = 9.4$ Hz, 1H), 4.03-3.98 (m, 3H), 3.78-3.71 (m, 2H), 3.56-3.36 (m, 11H), 3.25-3.09 (m, 8H), 2.64 (s, 3H), 2.32-2.09 (m, 2H). MS (ESI): m/z 1037.80 ($\text{M} + \text{H}$) $^+$.

(R)-4-(3-(4-(4-(4-(Azetidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-5-(4-chlorophenyl)-1,2-dimethyl-1H-pyrrole-3-carboxylic Acid (24): **24** was prepared from **38** and **37a** according to general procedure III. ^1H NMR (300 MHz, CD_3OD): δ 7.90 (d, $J = 2.0$ Hz, 1H), 7.60 (dd, $J = 9.1, 2.0$ Hz, 1H), 7.31-7.26 (m, 4H), 7.24-6.96 (m, 13H), 6.88-6.82 (m, 1H), 4.25-4.15 (m, 2H), 4.03-3.97 (m, 3H), 3.43 (s, 3H), 3.40-3.11 (m, 12H), 2.67 (s, 3H), 2.54-2.35 (m, 2H), 2.07-1.85 (m, 2H). MS (ESI): m/z 1007.08 ($\text{M} + \text{H}$) $^+$.

(R)-5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(4-(1-(phenylthio)-4-(pyrrolidin-1-yl)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxylic Acid (25): **25** was prepared from **38** and **37b** according to general procedure III. ^1H NMR (300 MHz, CD_3OD): δ 7.95 (d, $J = 1.9$ Hz, 1H), 7.74 (dd, $J = 9.1, 2.1$ Hz, 1H), 7.30-7.01 (m, 14H), 6.84 (d, $J = 9.2$ Hz, 1H), 6.58-6.42 (m, 3H), 3.99 (br, 1H), 3.80-3.53 (m, 2H), 3.46 (s, 3H), 3.44-2.95 (m, 14H), 2.61 (s, 3H), 2.29-1.68 (m, 6H). MS (ESI): m/z 1021.75 ($\text{M} + \text{H}$) $^+$.

(R)-5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(4-(4-(1-(phenylthio)-4-(piperidin-1-yl)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxylic Acid (26): 26 was prepared from 38 and 37c according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 8.36 (d, *J* = 2.2 Hz, 1H), 7.61 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.20-6.92 (m, 15H), 6.53-6.38 (m, 3H), 4.12 (br, 1H), 3.42 (s, 3H), 3.36-3.17 (m, 11H), 2.88 (s, 5H), 2.61 (s, 3H), 2.29-2.19 (m, 8H). MS (ESI): *m/z* 1035.92 (M + H)⁺.

(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(3-hydroxyazetidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-1H-pyrrole-3-carboxylic Acid (27): 27 was prepared from 38 and 37d according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.89 (d, *J* = 1.9 Hz, 1H), 7.75 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.36-7.09 (m, 13H), 7.01-6.66 (m, 5H), 4.03 (br, 1H), 3.81-3.50 (m, 2H), 3.44 (s, 3H), 3.40-2.89 (m, 15H), 2.62 (s, 3H), 2.28-1.73 (m, 2H). MS (ESI): *m/z* 1024.00 (M + H)⁺.

(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-1H-pyrrole-3-carboxylic Acid (28): 28 was prepared from 38 and 37e according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.86 (s, 1H), 7.71 (d, *J* = 9.2 Hz, 1H), 7.27-7.25 (m, 4H), 7.20-6.79 (m, 14H), 4.03-3.75 (m, 2H), 3.49-3.31 (m, 13H), 3.14-2.89 (m, 6H), 2.58 (s, 3H), 2.26-1.88 (m, 5H), 1.67-1.63 (m, 1H). ¹³C NMR (75 MHz, CD₃OD): δ 167.60, 151.31, 146.91, 144.09, 137.99, 137.13, 136.10, 134.72, 133.49, 133.40, 132.64, 131.05, 130.84, 130.39, 128.98, 128.37, 128.14, 127.35, 126.82, 123.13, 122.17, 121.99, 117.62, 116.34, 113.93, 110.08, 108.62, 64.34, 59.71, 53.60, 52.62, 51.06, 50.69, 48.51, 37.97, 31.40, 30.84, 29.54, 28.24, 27.94, 10.71. MS (ESI): *m/z* 1051.30 (M + H)⁺.

(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(4-hydroxy-4-methylpiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-1H-pyrrole-3-carboxylic acid (29): 29 was prepared from 38 and 37f according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.79 (d, *J* = 1.6 Hz, 1H), 7.66 (dd, *J* = 9.1, 1.7 Hz, 1H), 7.20-6.75 (m, 18H), 4.03-3.88 (m, 1H), 3.45-3.33 (m, 10H), 3.25-2.95 (m, 9H), 2.51 (s, 3H), 2.22-1.93 (m, 2H), 1.80-1.66 (m, 4H), 0.97 (s, 3H). MS (ESI) *m/z* 1066.16 (M + H)⁺.

General Procedure IV

Ethyl 5-(4-chlorophenyl)-4-(3-iodophenyl)-2-methyl-1-propyl-1H-pyrrole-3-carboxylate (40a): To the solution of 39 (2.0 g, 4.1 mmol) in MeOH (20 mL) was added propylamine (1.0 mL, 12.3 mmol) and the solution was stirred for 6 h at room temperature until no starting material was observed by TLC. The solution was acidified with 1M HCl (50 mL) and extracted with EtOAc (2 × 50 mL). The combined EtOAc solutions were washed with brine (50 mL), dried (Na₂SO₄) and concentrated *in vacuo*. The concentrate was flash chromatographed on silica gel with 10% EtOAc/hexane to provide 1.89 g of 40a (91%). ¹H NMR (300 MHz, CDCl₃): δ 7.52 (t, *J* = 1.6 Hz, 1H), 7.46-7.42 (m, 1H), 7.28-7.24 (m, 2H), 7.08-6.94 (m, 3H), 6.84 (t, *J* = 7.8 Hz, 1H), 4.08 (dd, *J* = 14.3, 7.1 Hz, 2H), 3.73 (t, *J* = 7.7 Hz, 2H), 2.61 (s, 3H), 1.58-1.46 (m, 2H), 1.04 (t, *J* = 7.1 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 165.60, 139.89, 138.20, 136.00, 134.64, 133.88, 132.60, 130.30, 130.17, 129.77, 128.79, 128.56, 122.57, 111.21, 92.91, 59.30, 45.83, 24.01, 14.03, 11.69, 11.07.

Ethyl 1-butyl-5-(4-chlorophenyl)-4-(3-iodophenyl)-2-methyl-1H-pyrrole-3-carboxylate (40b): **40b** was prepared from **39** and butylamine according to general procedure IV. ^1H NMR (300 MHz, CDCl_3): δ 7.52 (s, 1H), 7.43 (d, $J = 7.9$ Hz, 1H), 7.26 (d, $J = 8.4$ Hz, 2H), 7.06 (d, $J = 8.4$ Hz, 2H), 6.95 (d, $J = 8.1$ Hz, 1H), 6.84 (t, $J = 7.7$ Hz, 1H), 4.07 (dd, $J = 14.4$, 7.3 Hz, 2H), 3.76 (t, $J = 7.6$ Hz, 2H), 2.62 (s, 3H), 1.53-1.43 (m, 2H), 1.22-1.09 (m, 2H), 1.04 (t, $J = 7.1$ Hz, 3H), 0.78 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 165.60, 139.90, 138.21, 135.96, 134.63, 133.88, 132.62, 130.29, 130.14, 129.78, 128.79, 128.55, 122.56, 111.21, 92.92, 59.30, 44.05, 32.77, 19.79, 14.04, 13.55, 11.70.

General Procedure V

Ethyl 5-(4-chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl) piperazin-1-yl)phenyl)-1-propyl-1H-pyrrole-3-carboxylate (41a): Ester **40a** (1.52 g, 3.0 mmol), 1-(4-nitrophenyl)piperazine (1.87 g, 9.0 mmol), CuI (57 mg, 0.3 mmol), L-proline (173 mg, 1.5 mmol) and K_2CO_3 (1.24 g, 9.0 mmol) were dissolved in 30 mL of DMSO. This solution was stirred for 8 h under a nitrogen atmosphere at 80°C until no **40a** was observed by TLC. The reaction mixture was cooled, saturated NH_4Cl solution (50 mL) was added and the solution extracted with EtOAc (2×50 mL). The combined EtOAc solutions were washed with brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The concentrate was flash chromatographed on silica gel with 40% EtOAc/hexane to provide 1.44 g of **41a** (82%). ^1H NMR (300 MHz, CDCl_3): δ 8.14-8.09 (m, 2H), 7.26-7.06 (m, 5H), 6.86-6.62 (m, 5H), 4.09 (dd, $J = 14.2$, 7.08 Hz, 2H), 3.75 (t, $J = 7.7$ Hz, 2H), 3.48 (t, $J = 4.9$ Hz, 4H), 3.15 (t, $J = 5.3$ Hz, 4H), 2.62 (s, 3H), 1.61-1.48 (m, 2H), 1.05 (t, $J = 7.1$ Hz, 3H), 0.76 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 165.88, 154.75, 149.55, 138.55, 136.55, 135.45, 133.55, 132.68, 131.00, 129.94, 128.43, 127.88, 125.93, 124.32, 123.39, 119.32, 114.08, 112.69, 111.39, 59.26, 49.01, 46.90, 45.86, 24.06, 14.06, 11.7, 11.08. MS (ESI): m/z 587.50 ($\text{M} + \text{H}$) $^+$.

Ethyl 1-butyl-5-(4-chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxylate (41b): **41b** was prepared from **40b** according to general procedure V. ^1H NMR (300 MHz, CDCl_3): δ 8.11 (d, $J = 9.3$ Hz, 2H), 7.26-7.06 (m, 5H), 6.84-6.63 (m, 5H), 4.09 (dd, $J = 14.3$, 7.1 Hz, 2H), 3.79 (t, $J = 7.6$ Hz, 2H), 3.48 (t, $J = 4.7$ Hz, 4H), 3.14 (t, $J = 5.3$ Hz, 4H), 2.62 (s, 3H), 1.55-1.45 (m, 2H), 1.22-1.10 (m, 2H), 1.05 (t, $J = 7.1$ Hz, 3H), 0.79 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 165.88, 154.75, 149.54, 138.52, 136.55, 135.41, 133.54, 132.71, 130.98, 129.91, 128.42, 127.88, 125.93, 124.31, 123.38, 119.33, 114.07, 112.69, 111.38, 59.26, 49.00, 46.89, 44.06, 32.82, 19.79, 14.07, 13.55, 11.74. MS (ESI): m/z 601.82 ($\text{M} + \text{H}$) $^+$.

General Procedure VI

5-(4-Chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1-propyl-1H-pyrrole-3-carboxylic acid (42a): To a solution of **41a** (1.00 g, 1.7 mmol) in 30 mL of 1:1:1 dioxane, EtOH, and H_2O was added NaOH (680 mg, 17.0 mmol) and the solution was refluxed for 20 hours until no **41a** was observed by TLC. After cooling the reaction was neutralized with 1M HCl and the compound was extracted with EtOAc. The EtOAc solution was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* to produce 900 mg of compound **42a** as a white solid (95% yield, used for next steps directly without purification). ^1H NMR (300 MHz, CDCl_3): δ 8.13 (d, $J = 9.3$ Hz, 2H), 7.26 (d, $J = 8.2$ Hz, 2H), 7.09-7.03 (m, 3H), 6.84-6.64 (m, 5H), 3.74 (t, $J = 7.5$ Hz, 2H), 3.48 (t, $J = 4.5$ Hz, 4H), 3.15 (t, $J = 5.2$ Hz, 4H), 2.62 (s, 3H), 1.57-1.50 (m, 2H), 0.76 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.73, 154.75, 149.50, 138.56, 137.41, 135.73, 133.77, 132.67, 130.70, 130.50, 128.51, 128.14, 125.93, 124.55, 123.26, 119.87, 114.30, 112.67, 109.84, 48.92, 46.79, 45.94, 23.98, 12.09, 11.04. MS (ESI): m/z 559.17 ($\text{M} + \text{H}$) $^+$.

1-Butyl-5-(4-chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1H-pyrrole-3-carboxylic Acid (42b): 42b was prepared from 41b according to general procedure VI. ¹H NMR (300 MHz, CDCl₃): δ 8.11 (d, *J* = 9.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 7.03 (t, *J* = 7.7 Hz, 1H), 6.82-6.63 (m, 5H), 3.78 (t, *J* = 8.0 Hz, 2H), 3.44 (t, *J* = 4.8 Hz, 4H), 3.13 (t, *J* = 5.4 Hz, 4H), 2.61 (s, 3H), 1.54-1.44 (m, 2H), 1.20-1.10 (m, 2H), 0.79 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 170.66, 154.77, 149.44, 138.47, 137.36, 135.79, 133.74, 132.74, 130.75, 130.52, 128.49, 128.03, 125.92, 124.68, 123.29, 119.99, 114.22, 112.65, 109.95, 48.94, 46.75, 44.15, 32.73, 19.78, 13.51, 12.13. MS (ESI): *m/z* 573.80 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(4-fluoro-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1-propyl-1H-pyrrole-3-carboxylic Acid (43a): 43a was prepared from 42a according to general procedure I. ¹H NMR (300 MHz, CDCl₃): δ 8.31 (dd, *J* = 5.9, 1.8 Hz, 1H), 8.09-8.04 (m, 1H), 7.37 (t, *J* = 8.9 Hz, 1H), 7.25-6.64 (m, 12H), 3.75 (t, *J* = 7.4 Hz, 2H), 3.18-3.11 (m, 8H), 2.64 (s, 3H), 1.58-1.51 (m, 2H), 0.77 (t, *J* = 7.3 Hz, 3H). MS (ESI): *m/z* 819.00 (M + H)⁺.

1-Butyl-5-(4-chlorophenyl)-4-(3-(4-(4-(4-fluoro-3-(trifluoromethylsulfonyl)phenyl)sulfon amido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carboxylic Acid (43b): 43b was prepared from 42b according to general procedure I. ¹H NMR (300 MHz, CDCl₃): δ 8.31 (dd, *J* = 5.9, 2.2 Hz, 1H), 8.09-8.04 (m, 1H), 7.37 (t, *J* = 8.8 Hz, 1H), 7.26-6.64 (m, 12H), 3.78 (t, *J* = 8.0 Hz, 2H), 3.21-3.08 (m, 8H), 2.64 (s, 3H), 1.55-1.45 (m, 2H), 1.23-1.10 (m, 2H), 0.79 (t, *J* = 7.2 Hz, 3H). MS (ESI): *m/z* 833.52 (M + H)⁺.

5-(4-Chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-fluoro-3-(trifluoromethylsulfonyl)phenyl)sulfon amido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carboxylic Acid (43c): 43c was prepared from 42c³⁹ according to general procedure I. ¹H NMR (300 MHz, CDCl₃): δ 8.32 (dd, *J* = 6.0, 2.0 Hz, 1H), 8.08-8.03 (m, 1H), 7.35 (t, *J* = 8.9 Hz, 1H), 7.26-6.64 (m, 12H), 3.83 (dd, *J* = 14.0, 6.9 Hz, 2H), 3.17-3.08 (m, 8H), 2.64 (s, 3H), 1.63 (t, *J* = 7.1 Hz, 3H). MS (ESI): *m/z* 805.66 (M + H)⁺.

5-(4-Chlorophenyl)-4-(3-(4-(4-(4-fluoro-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-2-methyl-1H-pyrrole-3-carboxylic Acid (43d): 43d was prepared from 42d³⁹ according to general procedure I. ¹H NMR (300 MHz, CDCl₃): δ 8.30 (dd, *J* = 5.9, 1.6 Hz, 1H), 8.10-8.07 (m, 1H), 7.35 (t, *J* = 8.9 Hz, 1H), 7.26-6.68 (m, 12H), 4.42-4.33 (m, 1H), 3.18-3.13 (m, 8H), 2.73 (s, 3H), 1.41 (d, *J* = 10.0 Hz, 6H). MS (ESI): *m/z* 819.52 (M + H)⁺.

(R)-5-(4-Chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio) butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl) phenyl)-2-methyl-1H-pyrrole-3-carboxylic Acid (30): 30 was prepared from 43c and 37e according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.86 (s, 1H), 7.71 (d, *J* = 9.1 Hz, 1H), 7.29-6.96 (m, 16H), 6.87-6.79 (m, 2H), 4.04-3.77 (m, 4H), 3.49-3.28 (m, 8H), 3.17-2.94 (m, 8H), 2.60 (s, 3H), 2.05-1.69 (m, 6H), 1.10 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (75 MHz, CD₃OD): δ 169.03, 152.67, 148.36, 145.43, 139.23, 137.46, 137.39, 136.08, 135.09, 134.77, 134.28, 132.37, 132.02, 131.76, 131.61, 130.35, 129.66, 129.54, 128.73, 128.19, 124.95, 124.51, 123.26, 118.97, 117.66, 115.29, 111.72, 109.98, 61.10, 53.98, 52.42, 52.05, 51.22, 50.93, 40.10, 39.34, 32.77, 30.90, 28.19, 27.32, 25.25, 25.06, 24.62, 24.57, 16.14, 13.88, 11.87. MS (ESI) *m/z* 1066.26 (M + H)⁺.

(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-2-methyl-1H-pyrrole-3-carboxylic Acid (31): 31 was prepared from 43d and 37e according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.84 (d, *J* = 1.8 Hz, 1H), 7.70 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.26-6.95 (m, 17H), 6.80 (d, *J* = 9.2 Hz, 1H), 4.42-4.33 (m, 1H), 4.02-3.73 (m, 2H), 3.48-3.31 (m, 10H), 3.25-2.88 (m, 6H), 2.67 (s, 3H), 2.20-1.87 (m, 5H), 1.66-1.62 (m, 1H), 1.38 (d, *J* = 7.1 Hz, 6H). MS (ESI) *m/z* 1080.30 (M + H)⁺.

(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1-propyl-1H-pyrrole-3-carboxylic Acid (32): 32 was prepared from 43a and 37e according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.91 (d, *J* = 1.8 Hz, 1H), 7.78 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.46-7.03 (m, 17H), 6.87 (d, *J* = 9.1 Hz, 1H), 4.02-4.00 (m, 2H), 3.86 (t, *J* = 7.6 Hz, 2H), 3.51-2.95 (m, 16H), 2.65 (s, 3H), 2.27-2.11 (m, 3H), 1.93 (br, 2H), 1.72-1.63 (m, 1H), 1.61-1.51 (m, 2H), 0.75 (t, *J* = 5.1 Hz, 3H). MS (ESI): *m/z* 1080.28 (M + H)⁺.

(R)-1-Butyl-5-(4-chlorophenyl)-4-(3-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenyl)sulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carboxylic acid (33): 33 was prepared from 43b and 37e according to general procedure III. ¹H NMR (300 MHz, CD₃OD): δ 7.91 (s, 1H), 7.73 (d, *J* = 8.6 Hz, 1H), 7.33-6.81 (m, 18H), 4.08-3.79 (m, 4H), 3.53-2.94 (m, 16), 2.63 (s, 3H), 2.33-2.19 (m, 1H), 2.17-2.04 (m, 2H), 1.98-1.87 (m, 2H), 1.75-1.58 (m, 1H), 1.54-1.44 (m, 2H), 1.23-1.11 (m, 2H), 0.78 (t, *J* = 7.3 Hz, 3H). MS (ESI): *m/z* 1094.36 (M + H)⁺.

Fluorescence polarization based binding assays

Details of the expression and purification of Bcl-2, Bcl-xL and Mcl-1 proteins and determination of *K_d* values of fluorescent probes to proteins are provided in the SI. IC₅₀ and *K_i* values for the interaction with Bcl-2/Bcl-xL of our designed compounds and reference compounds were determined in competitive binding experiments, in which an inhibitor in serial dilutions was allowed to compete with a fixed concentration of a fluorescent probe for a fixed concentration of a protein. Mixtures of 5 μl of the tested compound in DMSO and 120 μl of pre-incubated protein/probe complex in the assay buffer were added to assay plates and incubated at room temperature for 2 h with gentle shaking. The final concentrations of the protein and probe were 1.5 nM and 1 nM for the Bcl-2 assay, 10 nM and 2 nM for the Bcl-xL assay, and 20 nM and 2 nM for the Mcl-1 assay, respectively. Controls containing protein/probe complex only (equivalent to 0% inhibition) or free probe only (equivalent to 100% inhibition), were included in each assay plate. FP values were measured as described above. IC₅₀ values were determined by nonlinear regression fitting of the competition curves. The *K_i* value of a compound to a protein was calculated using the equation described previously⁴¹ based upon the measured IC₅₀ value, the *K_d* value of the probe to the protein, and the concentration of the protein and probe in the competitive assays. *K_i* values were also calculated using a published equation.⁴² The values obtained from both equations were found to be in excellent agreement.

Cell death assay

Cell death assays were performed using trypan blue staining. Cells were treated with the indicated compounds. At the end of treatment, cells were collected and stained with trypan

blue. Cells that stained blue or the morphologically unhealthy cells were scored as dead cells. At least 100 cells were counted for each sample.

Western blotting

Cells were lysed using radioimmunoprecipitation assay lysis buffer (PBS containing 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS) supplemented with 1 μ mol/L phenylmethylsulfonyl fluoride and 1 protease inhibitor cocktail tablet per 10 mL on ice for 20 min, and lysates were then cleared by centrifugation before protein concentration determination using the Bio-Rad protein assay kit according to the manufacturer's instructions. Proteins were electrophoresed onto 4–20% SDS-PAGE gels (Invitrogen) and transferred onto polyvinylidenedifluoride membranes. Following blocking in 5% milk, membranes were incubated with a specific primary antibody, washed, and incubated with horseradish peroxidase–linked secondary antibody (Amersham). The signals were visualized with the chemiluminescent horseradish peroxidase antibody detection reagent (Denville Scientific). Rabbit antibodies against PARP and caspase-3 are from Cell Signaling Technology. Rabbit anti-GAPDH is from Santa Cruz Biotechnology.

Efficacy studies

When tumors reached tumor volumes between 40–110 mm³, mice were randomized into different groups, 8 mice per group, with a mean tumor volume of 70 mm³. Mice were treated with compound **28** at 50 mg/kg, or compound **30** at 25 mg/kg, intravenously, daily, 5 days a week for 2 weeks, or vehicle control. Tumor sizes and animal weights were measured 3 times a week during the treatment and twice a week after the treatment. Data are presented as mean tumor volumes $\pm$ SEM. Statistical analyses were performed by two-way ANOVA and unpaired two-tailed t test, using Prism (version 4.0, GraphPad, La Jolla, CA). $P < 0.05$ was considered statistically significant. The efficacy experiment was performed under the guidelines of the University of Michigan Committee for Use and Care of Animals.

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Abbreviations

Bcl-2	B-cell lymphoma 2
Bax	Bcl-2 related protein X
Bak	Bcl-2 antagonist/killer
Bok	Bcl-2-related ovarian killer protein
Bad	Bcl-2 antagonist of cell death
Bid	BH3 interacting death domain
Bim	Bcl-2 interacting mediator
Bik	bcl-2 interacting killer
Puma	p53 upregulated modulator of apoptosis
Bcl-xL	B-cell lymphoma x long

BH	Bcl homology
PARP	poly(ADP-ribose) polymerase
SCID	severe combined immunodeficiency
EDCI	<i>N</i> -(3-Dimethylaminopropyl)- <i>N</i> '-ethylcarbodiimide hydrochloride
HOBT	1-Hydroxybenzotriazole hydrate
DIPEA	<i>N,N</i> -Diisopropylethylamine
FPA	fluorescence polarization assay

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Figure 1.
Chemical structures of previously reported representative potent Bcl-2/Bcl-xL inhibitors.

Figure 2.
(A)–(D). Co-crystal structure of compound **4** (BM-903) complexed with Bcl-xL. Compound **4** is colored in yellow and the surface representation of Bcl-xL is shown. Residues interact with the nitro-phenyl and the thio-phenyl groups on the compounds are labeled. Hydrogen bonds are depicted in dash cyan lines. The modifications are highlighted in (B)–(D) with a red cycle.

Figure 3.

Induction of cell death by compounds **1**, **2**, **28**, **30** and **31** in the H146 cell line. Cells were treated with different concentrations of the compounds for 24 hr. Cell viability was determined using a trypan blue exclusion assay.

Figure 4.

Western blot analysis of biochemical markers of apoptosis in H146 cancer cells treated with compounds **2**, **28** and **30**. H146 cancer cells were treated with indicated concentrations of each compound for 24 hr. PARP, cleaved PARP (CI PARP), caspase-3 and cleaved caspase-3 (CI Cas-3) were probed with specific antibodies. GAPDH was used as the loading control.

Figure 5.

Induction of cleavage of PARP and caspase-3 in H146 xenograft tumors by compounds **28** and **30**. SCID mice bearing H146 xenograft tumors (100–200 mm³) were treated with vehicle control, single dose of **28** (50 mg/kg, *i.v.*) or **30** (25 mg/kg, *i.v.*). Mice were sacrificed at indicated time-point and tumors were removed for western blot analysis of cleavage of PARP and caspase-3. CI PARP, cleaved PARP; CI Caspase-3, cleaved Caspase-3.

Figure 6.

Antitumor activity of compounds **28** and **30** in H146 xenograft tumor model. SCID mice bearing xenograft tumors (100 mm³) were treated with vehicle control, compound **28** at 50 mg/kg, *i.v.* or compound **30** at 25 mg/kg, *i.v.*, daily, 5 days a week for two weeks. The tumor growth inhibition for both compounds was statistically highly significant with $p = 0.0033$ for compound **28** versus vehicle control and $p = 0.0006$ for compound **30** versus vehicle control, respectively, when the mean tumor volume reached 750 mm³ in the vehicle treated group in both experiments.

Scheme 1.

Synthesis of target compounds in Table 1 and Table 2.

Reagents and conditions: (a) 3-(4-Methyl-piperazin-1-yl)-propylamine, EDCI, DMAP, CH₂Cl₂, room temp; (b) (i) Pd/C, H₂, MeOH, room temp; (ii) Sulfonyl chlorides, Py, 0 °C; (c) (i) amines, EDCI, HOBT, CH₂Cl₂, room temp; (ii) Et₂NH, CH₃CN, room temp; (iii) BH₃, THF, room temp; (d) (i) Pd/C, H₂, MeOH, room temp; (ii) 4-fluoro-3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride, Py, 0 °C; (e) DIPEA, DMF, room temp.

Scheme 2.

Synthesis of target compounds in Table 3.

Reagents and conditions: (a) amines, MeOH, room temp; (b) 1-(4-nitrophenyl)piperazine, CuI, L-proline, K_2CO_3 , 100 °C; (c) excess NaOH, 1:1:1 Dioxane, EtOH, H_2O , reflux; (d) (i) Pd/C, H_2 , MeOH, room temp; (ii) 4-fluoro-3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride, Py, 0 °C; (e) **37e**, DIPEA, DMF, room temp.

Table 1

Structure-activity relationships of the nitro group replacements.

R ₁	Bd-2			Bd-xL		
	IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)
5	117±38	31.3±9.7	131±41	37.7±12.4		
6	2200±830	569±215	3732±832	1132±252		
7	977±204	253±53	1307±256	395±78		
8	660±180	171±47	1060±412	320±125		
9	255±56	65.8±14.4	499±102	150±31		
10	582±221	151±57	647±173	195±52		
11	689±151	178±39	1988±492	602±149		
12	621±27	160±7	1951±594	591±181		
13	1908±195	494±51	744±225	224±68

	R ₁	Bcl-2			Bcl-xL		
		IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)
14		763±229	197±59	1646±722	498±219		
15		802±28	207±8	2163±383	655±116		
16		1624±130	420±34	3646±597	1106±182		
17		2021±380	522±99	1052±278	317±84		
18		153±54	39.2±14.1	136±22	39.0±6.7		
19		2543±66	658±171	1920±221	581±67		
20		828±266	214±69	954±215	288±65

R ₁	Bcl-2		Bcl-xL	
	IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)
21	844±166	218±43	1035±427	312±130

Table 2

Structure-activity relationships of the tail soluble group.

ID	R ₂	Bcl-2		Bcl-xL		IC ₅₀ (cell growth assay) [nM]		
		IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)	H1417	H1416	H146
22		9.2±4.1	2.1±1.0	5.1±1.0	< 1	151±7	98 ±17	
23		55.2±19.9	14.1±5.2	10.8±2.2	1.1±0.4	677±82	476±7	
24		7.5±2.6	1.7±0.7	16.5±2.9	2.8±0.5	126±5	86±12	
25		8.3±2.6	1.8±0.3	10.7±2.2	1.1±0.4	106±25	87±5	
26		15.6±2.2	3.8±0.6	12.1±2.6	1.5±0.5	171±12	133±6	
27		7.7±1.9	1.7±0.5	10.5±1.8	1.0±0.3	299±11	241±19	
28		10.2±1.7	2.4±0.4	7.0±2.2	< 1	125±5	108±15

ID	R ₂	Bel-2		Bel-xL		IC ₅₀ (cell growth assay) [nM]	
		IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)	H1417	H146
29		18.6±0.9	4.6±0.3	21.8±5.6	4.5±1.0	179±11	171±26

Table 3

Modifications of the *N*-methyl site in compound 28.

ID	R ₃	Bcl-2		Bcl-xL		IC ₅₀ (cell growth assay) [nM]		
		IC ₅₀ (nM)	K _i (nM)	IC ₅₀ (nM)	K _i (nM)	H1417	H146	H146
28		10.2±1.7	2.4±0.4	7.0±2.2	<1	125±5	108±15	
30		5.4±0.9	1.2±0.2	6.0±1.4	<1	21±1	22±5	
31		4.0±0.9	0.8±0.2	3.9±0.5	<1	9±1	13±415	
32		30.1±10.3	7.5±2.4	8.2±2.1	<1	105±8	128±48	
33		78.2±22.5	20.0±5.6	6.3±2.4	<1	97±28	128±21	