Journal of Medicinal Chemistry

Article

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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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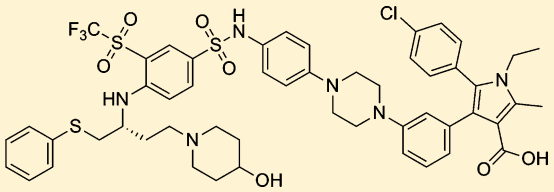
ABSTRACT: Bcl-2 and Bcl-xL antiapoptotic proteins are attractive cancer therapeutic targets. We have previously reported the design of 4,5-diphenyl-1*H*-pyrrole-3-carboxylic acids as a class of potent Bcl-2/Bcl-xL inhibitors. In the present study, we report our structure-based optimization for this class 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 proapoptotic and antiapoptotic members.[5−7] Proapoptotic 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 prosurvival function is not completely understood, it is clear that these proteins inhibit apoptosis by directly binding to and sequestering their prodeath 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 antiapoptotic 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 proapoptotic 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 antiapoptotic function and promote apoptosis in tumor cells with high expression of these proteins. In the past 10 years, a number of classes of nonpeptide, 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 leukemia[37] but has a very limited single-agent activity in patients with small cell lung cancer.[38]

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

Received: July 13, 2012

dx.doi.org/10.1021/jm3010306 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

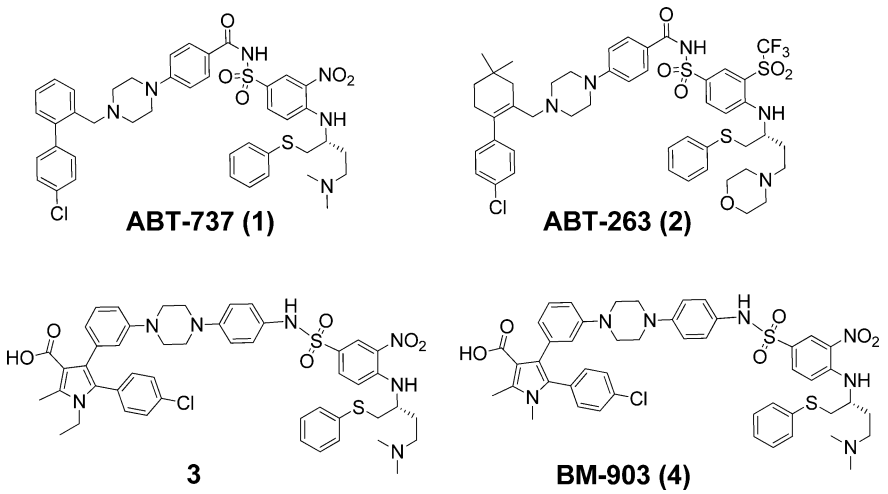


Figure 1. Chemical structures of previously reported representative potent Bcl-2/Bcl-xL inhibitors.

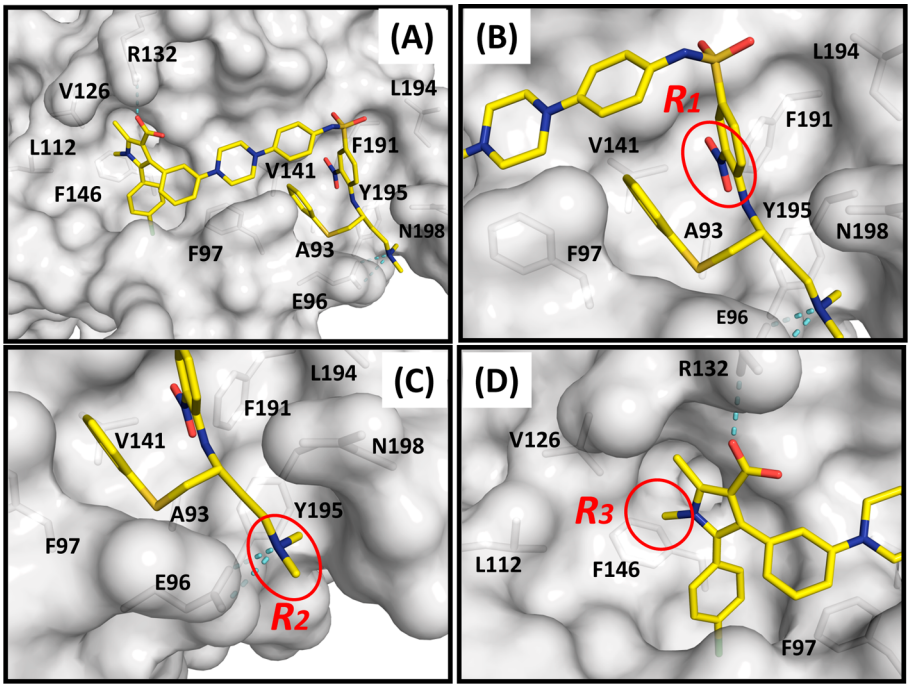


Figure 2. (A–D). Co-crystal structure of compound **4** (BM-903) complexed with Bcl-xL. Compound **4** is colored in yellow, and the surface representation of Bcl-xL is shown. Residues interact with the nitro-phenyl, and the thio-phenyl groups on the compounds are labeled. Hydrogen bonds are depicted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

compounds based upon a cocrystal structure (Figure 2) of Bcl-xL complexed with compound **4** (BM-903), an analogue of compound 3. 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 cocrystal 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. Because **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.

Because 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

dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX–XXX

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). Similarly to **3** and **4**, compound **22** has no appreciable binding to Mcl-1 at concentrations as high as 5 μM, 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**.[24,27,39] Compound **22** potently inhibits cell growth in the H1417 and H146 cancer cell lines, with IC_{50} values of 151 and 98 nM, respectively. The binding and cellular data thus indicated that **22** is a promising lead compound for further modifications.

The cocrystal 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 cocrystal 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 μM.

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 IC_{50} values of 21 and 22 nM, respectively, in these two cancer cell lines, compound **31** has IC_{50} 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.

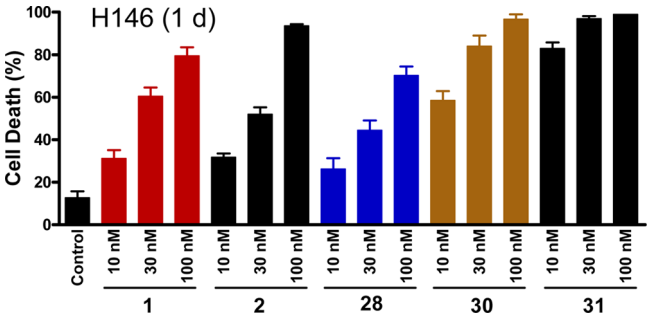


Figure 3. Induction of cell death by compounds **1**, **2**, **28**, **30**, and **31** in the H146 cell line. Cells were treated with different concentrations of the compounds for 24 h. Cell viability was determined using a trypan blue exclusion assay.

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 h 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

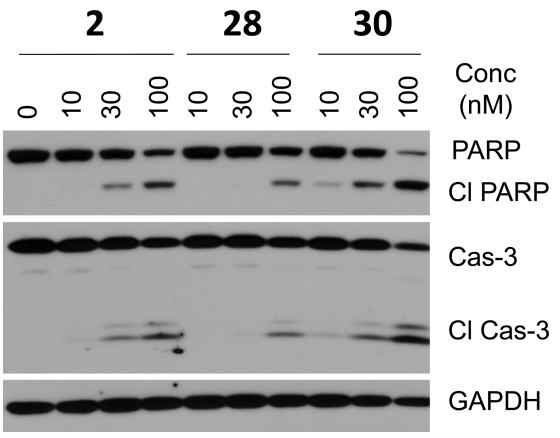


Figure 4. Western blot analysis of biochemical markers of apoptosis in H146 cancer cells treated with compounds **2**, **28**, and **30**. H146 cancer cells were treated with indicated concentrations of each compound for 24 h. PARP, cleaved PARP (Cl PARP), caspase-3, and cleaved caspase-3 (Cl Cas-3) were probed with specific antibodies. GAPDH was used as the loading control.

dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX–XXX

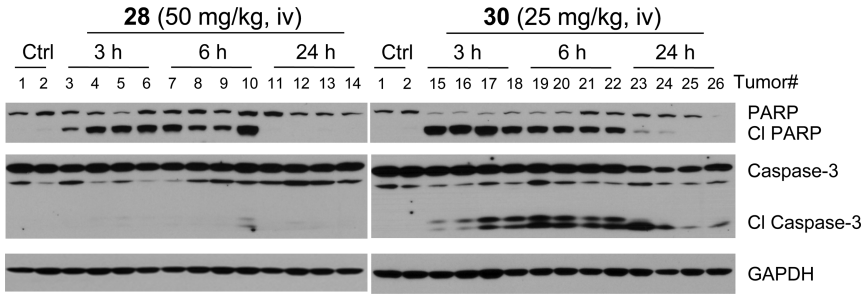


Figure 5. Induction of cleavage of PARP and caspase-3 in H146 xenograft tumors by compounds **28** and **30**. SCID mice bearing H146 xenograft tumors (100−200 mm^3) were treated with vehicle control, single dose of **28** (50 mg/kg, iv) or **30** (25 mg/kg, iv). Mice were sacrificed at indicated time-point and tumors were removed for Western blot analysis of cleavage of PARP and caspase-3. Cl PARP, cleaved PARP; Cl Caspase-3, cleaved Caspase-3.

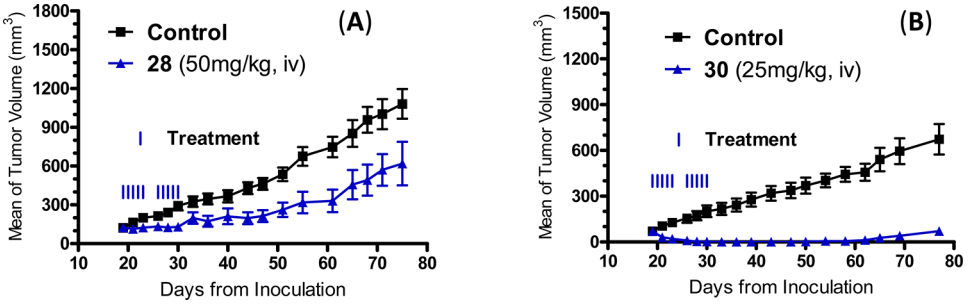


Figure 6. Antitumor activity of compounds **28** and **30** in H146 xenograft tumors model in two separate experiments. SCID mice bearing xenograft tumors (~100 mm^3) were treated with vehicle control, compound **28** at **50** mg/kg (iv) or compound **30** at 25 mg/kg, (iv), daily, 5 days a week for two weeks.

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 (iv) dosing 5 days a week for 2 weeks were well tolerated in SCID mice and the animals had less than 10% 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. On the basis of 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 iv 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 while both **28** and **30** effectively induce robust cleavage of PARP in H146 tumor tissues at 3 and 6 h time points, **30** is much more effective than **28** in induction of cleavage of caspase-3 (Figure 5). These data suggested that while both compounds induce apoptosis in tumor tissues, **30** is clearly more effective than **28**.

On the basis of 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 achieved rapid and complete tumor regression. Of seven mice treated with **30**, all mice were still 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 Tables 1 and 2 is shown in Scheme 1. Compound **34**, which was prepared according to a previously reported method,[39] 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 Et_2NH 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

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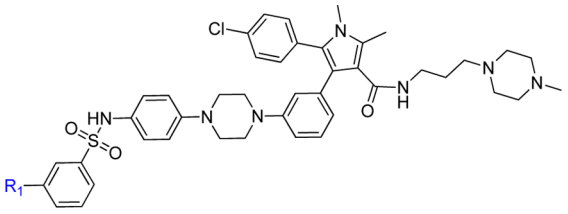
Table 1. Structure−Activity Relationships of the Nitro Group Replacements



	R_1	Bcl-2		Bcl-xL	
		IC_{50} (nM)	K_i (nM)	IC50 (nM)	K_i (nM)
5	O_2N–	117±38	31.3±9.7	131±41	37.7±12.4
6	H–	2200±830	569±215	3732±832	1132±252
7	F–	977±204	253±53	1307±256	395±78
8	Cl–	660±180	171±47	1060±412	320±125
9	Br–	255±56	65.8±14.4	499±102	150±31
10	F_3C–	582±221	151±57	647±173	195±52
11	H_3C–	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
14	F_3C–O–	763±229	197±59	1646±722	498±219
15	H_3C–O–	802±28	207±8	2163±383	655±116
16	–O–	1624±130	420±34	3646±597	1106±182
17	–O–	2021±380	522±99	1052±278	317±84
18	F_3C–S(=O)(=O)–	153±54	39.2±14.1	136±22	39.0±6.7
19	H_3C–S(=O)(=O)–	2543±66	658±171	1920±221	581±67
20	–S(=O)(=O)–	828±266	214±69	954±215	288±65
21	–S(=O)(=O)–	844±166	218±43	1035±427	312±130

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 IC_{50} 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

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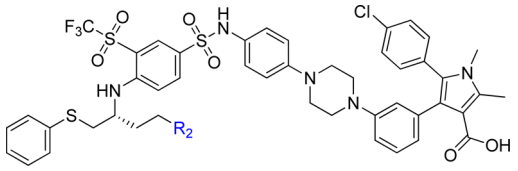
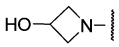
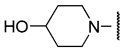
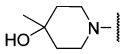
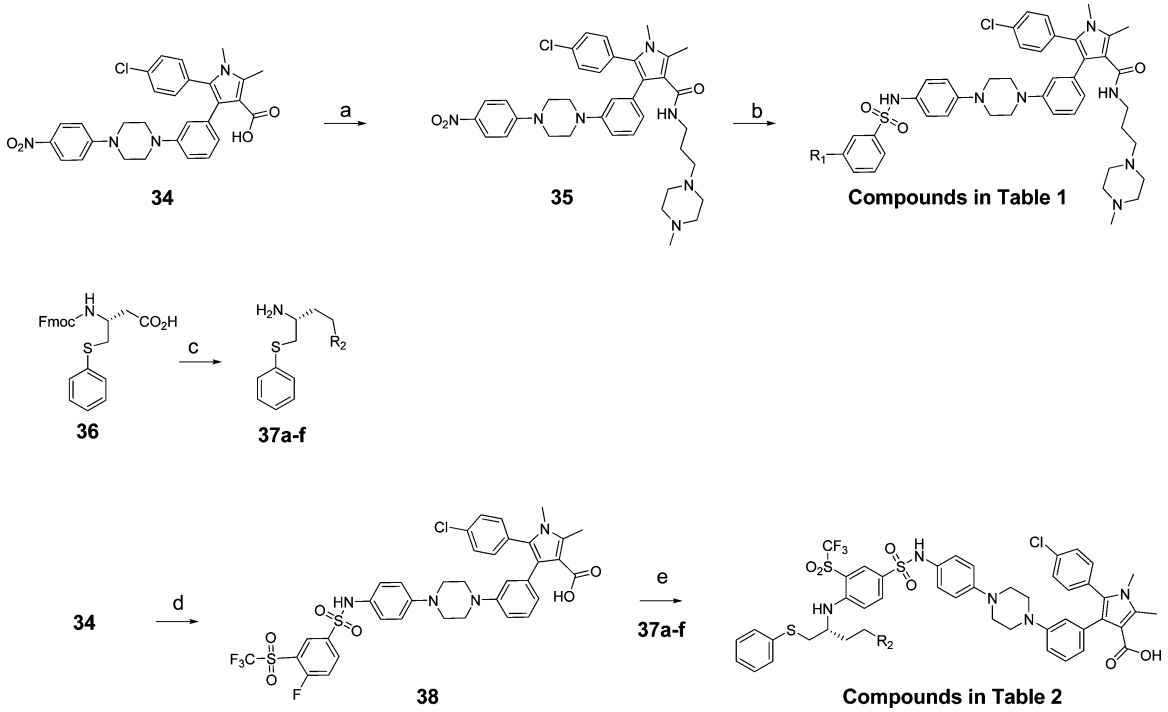
Table 2. Structure–Activity Relationships of the Tail Soluble Group



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	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
29		18.6±0.9	4.6±0.3	21.8±5.6	4.5±1.0	179±11	171±26

Scheme 1. Synthesis of Target Compounds in Tables 1 and 2



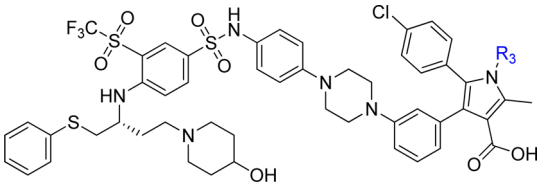
Reagents and conditions: (a) 3-(4-methyl-piperazin-1-yl)-propylamine, EDCI, DMAP, CH_2Cl_2, room temp; (b) (i) Pd/C, H_2, MeOH, room temp, (ii) sulfonyl chlorides, Py, 0 °C; (c) (i) amines, EDCI, HOBT, CH_2Cl_2, room temp, (ii) Et_2NH, CH_3CN, room temp; (iii) BH_3, THF, room temp; (d) (i) Pd/C, H_2, MeOH, room temp, (ii) 4-fluoro-3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride, Py, 0 °C; (e) DIPEA, DMF, room temp.

C18 reverse phase semipreparative HPLC column with solvent A (0.1% of TFA in H_2O) and solvent B (0.1% of TFA in CH_3CN) as eluents. All the target compounds have purities of >95% based upon UPLC analysis. Compounds **1** (ABT-737)[24] 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 CH_2Cl_2 (10 mL) was stirred for 3 h until no **34** was observed by TLC. The mixture was diluted

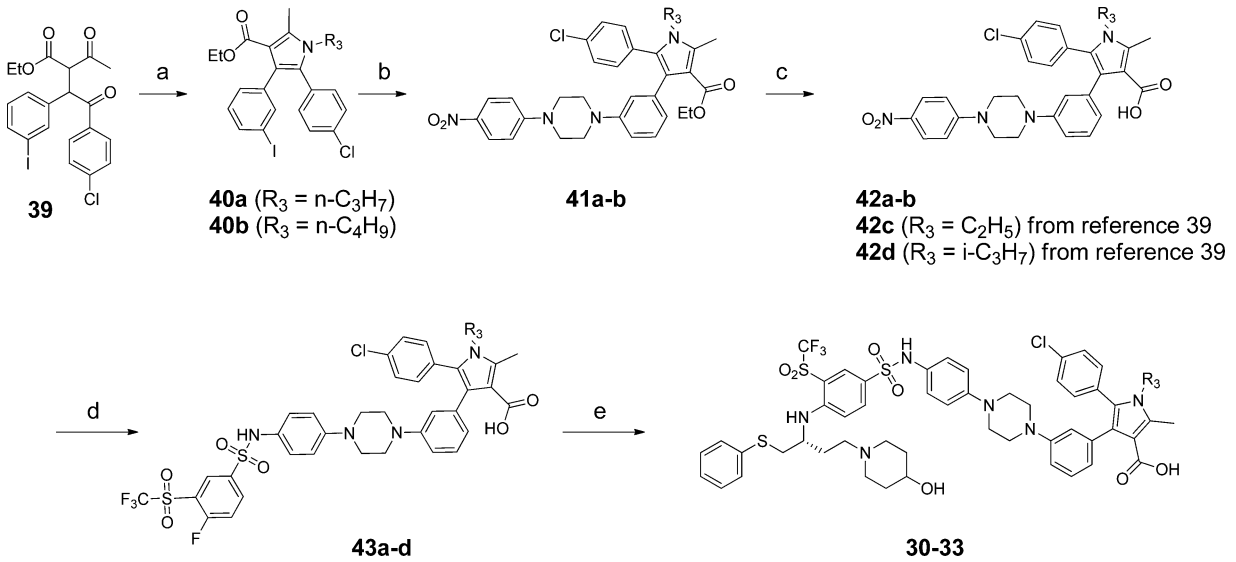
dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX–XXX

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
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

Scheme 2. Synthesis of Compounds 30−33 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.

with EtOAc (200 mL), washed sequentially with 1 M HCl (50 mL), H_2O (50 mL), and brine (20 mL), dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 5% MeOH/CH_2Cl_2 to provide 1.15 g (86%) of **35**. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 equiv 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 h until no aniline was observed by TLC. H_2O (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 Na_2SO_4, 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. ^{1}H NMR (300 MHz, CD_3OD): 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 $(M + H)^+$.

dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX−XXX

*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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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-(trifluoromethyl)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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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-trifluoromethoxylbenzene-1-sulfonyl chloride according to general procedure I. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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-isopropoxylbenzene-1-sulfonyl chloride according to general procedure I. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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. ^{1}H NMR (300 MHz, CD_3OD): 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)phenylsulfonamido)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. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (dd, J = 6.0, 2.0 Hz, 1H), 8.08−8.04 (m, 1H), 7.35−6.62

dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX−XXX

(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 and then diluted with DCM (100 mL), washed sequentially with 1 M HCl (50 mL), H_2O (50 mL), and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 50% EtOAc/hexanes to provide (*R*)-(9*H*-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 Et_2NH (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/CH_2Cl_2 to provide (*R*)-3-amino-1-(azetidin-1-yl)-4-(phenylthio)butan-1-one, 497 mg (98%). A mixture of this free amine and 1 M BH_3 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 4 M Na_2CO_3, diluted with CH_2Cl_2 (150 mL), washed with H_2O (50 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The concentrate was flash chromatographed on silica gel with 15% MeOH/CH_2Cl_2 to provide **37a**, 445 mg (95%). ^{1}H NMR (300 MHz, D_2O), 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). ^{13}C NMR (75 MHz, D_2O): 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. ^{1}H NMR (300 MHz, D_2O): 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). ^{13}C NMR (75 MHz, D_2O): 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. ^{1}H NMR (300 MHz, D_2O): 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). ^{13}C NMR (75 MHz, D_2O): 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. ^{1}H NMR (300 MHz, D_2O): 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), 2.08−190 (m, 2H). ^{13}C NMR (75 MHz, D_2O): 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. ^{1}H NMR (300 MHz, D_2O): 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 $(M + H)^+$.

*(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. ^{1}H 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 $(M + H)^+$.

General Procedure III. *(R)-5-(4-Chlorophenyl)-4-(3-(4-(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*)-N_1,N_1-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 h 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. ^{1}H 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 $(M + H)^+$.

*(R)-5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(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. ^{1}H 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 $(M + H)^+$.

*(R)-4-(3-(4-(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. ^{1}H 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 $(M + H)^+$.

*(R)-5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(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. ^{1}H 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 $(M + H)^+$.

*(R)-5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(4-(4-(1-(phenylthio)-4-(piperidin-1-yl)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)-phenyl)-1H-pyrrole-3-carboxylic Acid (**26**).* **26** was prepared from **38** and **37c** according to general procedure III. ^{1}H NMR (300 MHz, CD_3OD): 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)-phenylsulfonamido)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. ^{1}H NMR (300 MHz, CD_3OD): 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)-phenylsulfonamido)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. ^{1}H 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). ^{13}C NMR (75 MHz, CD_3OD): 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 $(M + H)^+$.

dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX−XXX

*(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-hydroxy-4-methylpiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)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. ^{1}H NMR (300 MHz, CD_3OD): 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 1 M HCl (50 mL) and 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 10% EtOAc/hexane to provide 1.89 g of **40a** (91%). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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. ^{1}H 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%). ^{1}H 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 $(M + H)^+$.

*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. ^{1}H 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 $(M + H)^+$.

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 h until no **41a** was observed by TLC. After cooling, the reaction was neutralized with 1 M 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). ^{1}H 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 $(M + H)^+$.

*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. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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)phenylsulfonamido)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. ^{1}H NMR (300 MHz, $CDCl_3$): 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)phenylsulfonamido)phenyl)piperazin-1-yl)-phenyl)-2-methyl-1H-pyrrole-3-carboxylic Acid (**43b**).* **43b** was prepared from **42b** according to general procedure I. ^{1}H NMR (300 MHz, $CDCl_3$): 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)phenylsulfonamido)phenyl)piperazin-1-yl)-phenyl)-2-methyl-1H-pyrrole-3-carboxylic Acid (**43c**).* **43c** was prepared from **42c**[39] according to general procedure I. ^{1}H NMR (300 MHz, $CDCl_3$): 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)phenylsulfonamido)phenyl)piperazin-1-yl)-phenyl)-1-isopropyl-2-methyl-1H-pyrrole-3-carboxylic Acid (**43d**).* **43d** was prepared from **42d**[39] according to general procedure I. ^{1}H NMR (300 MHz, $CDCl_3$): 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-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)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. ^{1}H NMR (300 MHz, CD_3OD): 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). ^{13}C NMR (75 MHz, CD_3OD): 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 $(M + H)^+$.

dx.doi.org/10.1021/jm3010306 | J. Med. Chem. XXXX, XXX, XXX−XXX

*(R)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)-phenylsulfonamido)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. ^{1}H NMR (300 MHz, CD_3OD): 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-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)-phenylsulfonamido)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. ^{1}H 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-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)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. ^{1}H NMR (300 M Hz, CD_3OD): 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 for Bcl-2, Bcl-xL, and Mcl-1 Proteins. The binding assays for Bcl-2, Bcl-xL and Mcl-1 proteins were the same as those described in our previous publications.[39,40]

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 μmol/L phenylmethylsulfonyl fluoride and one 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 and 110 mm^3, mice were randomized into different groups, eight mice per group, with a mean tumor volume of 70 mm^3. 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 ± 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.

■ ASSOCIATED CONTENT

Accession Codes

Coordinates for Bcl-xL complexed with **4** were deposited into the Protein Data Bank with accession number 3SPF.

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Author Contributions

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Notes

The authors declare the following competing financial interest(s): Dr. Wang is a co-founder of Ascentage, which has licensed the Bcl-2/Bcl-xL inhibitors described in the manuscript. Dr. Wang serves as an officer in Ascentage and owns stocks in Ascentage.

■ ACKNOWLEDGMENTS

This research was supported in part by a grant from the National Cancer Institute, National Institutes of Health (U19CA113317). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract no. DE-AC02-06CH11357. Use of the LS-CAT Sector 21 was supported by the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor for the support of this research program (grant 085P1000817).

■ ABBREVIATIONS USED

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, *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole hydrate; DIPEA, *N*,*N*-diisopropylethylamine; FPA, fluorescence polarization assay

■ REFERENCES

(1) Lowe, S. W.; Lin, A. W. Apoptosis in cancer. *Carcinogenesis* **2000**, *21*, 485−495.

(2) Reed, J. C. Apoptosis-based therapies. *Nature Rev. Drug. Discovery* **2002**, *1*, 111−121.

(3) Hanahan, D.; Weinberg, R. A. The hallmarks of cancer. *Cell* **2000**, *100*, 57−70.

(4) Nicholson, D. W. From bench to clinic with apoptosis-based therapeutic agents. *Nature* **2000**, *407*, 810−816.

(5) Adams, J. M.; Cory, S. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. *Curr. Opin. Immunol.* **2007**, *19*, 488−496.

(6) Youle, R. J.; Strasser, A. The BCL-2 protein family: opposing activities that mediate cell death. *Nature Rev. Mol. Cell Biol.* **2008**, *9*, 47−59.

(7) van Delft, M. F.; Huang, D. C. How the Bcl-2 family of proteins interact to regulate apoptosis. *Cell Res.* **2006**, *16*, 203−213.

(8) Cory, S.; Adams, J. M. The Bcl2 family: regulators of the cellular life-or-death switch. *Nature Rev. Cancer* **2002**, *2*, 647−656.

(9) Willis, S. N.; Fletcher, J. I.; Kaufmann, T.; van Delft, M. F.; Chen, L.; Czabotar, P. E.; Ierino, H.; Lee, E. F.; Fairlie, W. D.; Bouillet, P.; Strasser, A.; Kluck, R. M.; Adams, J. M.; Huang, D. C. Apoptosis initiated

when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* **2007**, *315*, 856−859.
(10) Green, D. R.; Evan, G. I. A matter of life and death. *Cancer Cell* **2002**, *1*, 19−30.
(11) Amundson, S. A.; Myers, T. G.; Scudiero, D.; Kitada, S.; Reed, J. C.; Fornace, A. J., Jr. An informatics approach identifying markers of chemosensitivity in human cancer cell lines. *Cancer Res.* **2000**, *60*, 6101−6110.
(12) Petros, A. M.; Medek, A.; Nettesheim, D. G.; Kim, D. H.; Yoon, H. S.; Swift, K.; Matayoshi, E. D.; Oltersdorf, T.; Fesik, S. W. Solution structure of the antiapoptotic protein bcl-2. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3012−3017.
(13) Sattler, M.; Liang, H.; Nettesheim, D.; Meadows, R. P.; Harlan, J. E.; Eberstadt, M.; Yoon, H. S.; Shuker, S. B.; Chang, B. S.; Minn, A. J.; Thompson, C. B.; Fesik, S. W. Structure of Bcl-xL−Bak peptide complex: recognition between regulators of apoptosis. *Science* **1997**, *275*, 983−986.
(14) Petros, A. M.; Nettesheim, D. G.; Wang, Y.; Olejniczak, E. T.; Meadows, R. P.; Mack, J.; Swift, K.; Matayoshi, E. D.; Zhang, H.; Thompson, C. B.; Fesik, S. W. Rationale for Bcl-xL/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. *Protein Sci.* **2000**, *9*, 2528−2534.
(15) Wang, J. L.; Liu, D.; Zhang, Z. J.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 7124−7129.
(16) Degterev, A.; Lugovskoy, A.; Cardone, M.; Mulley, B.; Wagner, G.; Mitchison, T.; Yuan, J. Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. *Nature Cell Biol.* **2001**, *3*, 173−182.
(17) Tzung, S. P.; Kim, K. M.; Basanez, G.; Giedt, C. D.; Simon, J.; Zimmerberg, J.; Zhang, K. Y.; Hockenbery, D. M. Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. *Nature Cell Biol.* **2001**, *3*, 183−191.
(18) Enyedy, I. J.; Ling, Y.; Nacro, K.; Tomita, Y.; Wu, X.; Cao, Y.; Guo, R.; Li, B.; Zhu, X.; Huang, Y.; Long, Y. Q.; Roller, P. P.; Yang, D.; Wang, S. Discovery of small-molecule inhibitors of Bcl-2 through structure-based computer screening. *J. Med. Chem.* **2001**, *44*, 4313−4324.
(19) Kutzki, O.; Park, H. S.; Ernst, J. T.; Orner, B. P.; Yin, H.; Hamilton, A. D. Development of a potent Bcl-x(L) antagonist based on alpha-helix mimicry. *J. Am. Chem. Soc.* **2002**, *124*, 11838−11839.
(20) Wang, G.; Nikolovska-Coleska, Z.; Yang, C. Y.; Wang, R.; Tang, G.; Guo, J.; Shangary, S.; Qiu, S.; Gao, W.; Yang, D.; Meagher, J.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Abaan, H. O.; Tomita, Y.; Wang, S. Structure-based design of potent small-molecule inhibitors of anti-apoptotic Bcl-2 proteins. *J. Med. Chem.* **2006**, *49*, 6139−6142.
(21) Tang, G.; Ding, K.; Nikolovska-Coleska, Z.; Yang, C. Y.; Qiu, S.; Shangary, S.; Wang, R.; Guo, J.; Gao, W.; Meagher, J.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Wang, S. Structure-based design of flavonoid compounds as a new class of small-molecule inhibitors of the anti-apoptotic Bcl-2 proteins. *J. Med. Chem.* **2007**, *50*, 3163−3166.
(22) Tang, G.; Yang, C. Y.; Nikolovska-Coleska, Z.; Guo, J.; Qiu, S.; Wang, R.; Gao, W.; Wang, G.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Wang, S. Pyrogallol-based molecules as potent inhibitors of the antiapoptotic Bcl-2 proteins. *J. Med. Chem.* **2007**, *50*, 1723−1726.
(23) Tang, G.; Nikolovska-Coleska, Z.; Qiu, S.; Yang, C. Y.; Guo, J.; Wang, S. Acylpyrogallols as inhibitors of antiapoptotic Bcl-2 proteins. *J. Med. Chem.* **2008**, *51*, 717−720.
(24) Oltersdorf, T.; Elmore, S. W.; Shoemaker, A. R.; Armstrong, R. C.; Augeri, D. J.; Belli, B. A.; Bruncko, M.; Deckwerth, T. L.; Dinges, J.; Hajduk, P. J.; Joseph, M. K.; Kitada, S.; Korsmeyer, S. J.; Kunzer, A. R.; Letai, A.; Li, C.; Mitten, M. J.; Nettesheim, D. G.; Ng, S.; Nimmer, P. M.; O'Connor, J. M.; Oleksijew, A.; Petros, A. M.; Reed, J. C.; Shen, W.; Tahir, S. K.; Thompson, C. B.; Tomaselli, K. J.; Wang, B.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **2005**, *435*, 677−681.
(25) Petros, A. M.; Dinges, J.; Augeri, D. J.; Baumeister, S. A.; Betebenner, D. A.; Bures, M. G.; Elmore, S. W.; Hajduk, P. J.; Joseph, M. K.; Landis, S. K.; Nettesheim, D. G.; Rosenberg, S. H.; Shen, W.; Thomas, S.; Wang, X.; Zanze, I.; Zhang, H.; Fesik, S. W. Discovery of a potent inhibitor of the antiapoptotic protein Bcl-xL from NMR and parallel synthesis. *J. Med. Chem.* **2006**, *49*, 656−663.
(26) Bruncko, M.; Oost, T. K.; Belli, B. A.; Ding, H.; Joseph, M. K.; Kunzer, A.; Martineau, D.; McClellan, W. J.; Mitten, M.; Ng, S. C.; Nimmer, P. M.; Oltersdorf, T.; Park, C. M.; Petros, A. M.; Shoemaker, A. R.; Song, X.; Wang, X.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL. *J. Med. Chem.* **2007**, *50*, 641−662.
(27) Park, C. M.; Bruncko, M.; Adickes, J.; Bauch, J.; Ding, H.; Kunzer, A.; Marsh, K. C.; Nimmer, P.; Shoemaker, A. R.; Song, X.; Tahir, S. K.; Tse, C.; Wang, X.; Wendt, M. D.; Yang, X.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. Discovery of an orally bioavailable small molecule inhibitor of prosurvival B-cell lymphoma 2 proteins. *J. Med. Chem.* **2008**, *51*, 6902−6915.
(28) Wendt, M. D.; Shen, W.; Kunzer, A.; McClellan, W. J.; Bruncko, M.; Oost, T. K.; Ding, H.; Joseph, M. K.; Zhang, H.; Nimmer, P. M.; Ng, S. C.; Shoemaker, A. R.; Petros, A. M.; Oleksijew, A.; Marsh, K.; Bauch, J.; Oltersdorf, T.; Belli, B. A.; Martineau, D.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. Discovery and structure−activity relationship of antagonists of B-cell lymphoma 2 family proteins with chemopotentiation activity in vitro and in vivo. *J. Med. Chem.* **2006**, *49*, 1165−1181.
(29) Park, C. M.; Oie, T.; Petros, A. M.; Zhang, H.; Nimmer, P. M.; Henry, R. F.; Elmore, S. W. Design, synthesis, and computational studies of inhibitors of Bcl-XL. *J. Am. Chem. Soc.* **2006**, *128*, 16206−16212.
(30) Shoemaker, A. R.; Oleksijew, A.; Bauch, J.; Belli, B. A.; Borre, T.; Bruncko, M.; Deckwirth, T.; Frost, D. J.; Jarvis, K.; Joseph, M. K.; Marsh, K.; McClellan, W.; Nellans, H.; Ng, S.; Nimmer, P.; O'Connor, J. M.; Oltersdorf, T.; Qing, W.; Shen, W.; Stavropoulos, J.; Tahir, S. K.; Wang, B.; Warner, R.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. A small-molecule inhibitor of Bcl-XL potentiates the activity of cytotoxic drugs in vitro and in vivo. *Cancer Res.* **2006**, *66*, 8731−8739.
(31) Shoemaker, A. R.; Mitten, M. J.; Adickes, J.; Ackler, S.; Refici, M.; Ferguson, D.; Oleksijew, A.; O'Connor, J. M.; Wang, B.; Frost, D. J.; Bauch, J.; Marsh, K.; Tahir, S. K.; Yang, X.; Tse, C.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. *Clin. Cancer Res.* **2008**, *14*, 3268−3277.
(32) Tse, C.; Shoemaker, A. R.; Adickes, J.; Anderson, M. G.; Chen, J.; Jin, S.; Johnson, E. F.; Marsh, K. C.; Mitten, M. J.; Nimmer, P.; Roberts, L.; Tahir, S. K.; Xiao, Y.; Yang, X.; Zhang, H.; Fesik, S.; Rosenberg, S. H.; Elmore, S. W. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* **2008**, *68*, 3421−3428.
(33) Vogler, M.; Dinsdale, D.; Dyer, M. J.; Cohen, G. M. Bcl-2 inhibitors: small molecules with a big impact on cancer therapy. *Cell Death Differ.* **2009**, *16*, 360−367.
(34) Chonghaile, T. N.; Letai, A. Mimicking the BH3 domain to kill cancer cells. *Oncogene* **2008**, *27* (Suppl1), S149−S157.
(35) Kang, M. H.; Reynolds, C. P. Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. *Clin. Cancer Res.* **2009**, *15*, 1126−1132.
(36) Petros, A. M.; Huth, J. R.; Oost, T.; Park, C. M.; Ding, H.; Wang, X.; Zhang, H.; Nimmer, P.; Mendoza, R.; Sun, C.; Mack, J.; Walter, K.; Dorwin, S.; Gramling, E.; Ladror, U.; Rosenberg, S. H.; Elmore, S. W.; Fesik, S. W.; Hajduk, P. J. Discovery of a potent and selective Bcl-2 inhibitor using SAR by NMR. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6587−6591.
(37) Roberts, A. W.; Seymour, J. F.; Brown, J. R.; Wierda, W. G.; Kipps, T. J.; Khaw, S. L.; Carney, D. A.; He, S., Z.; Huang, D. C.; Xiong, H.; Cui, Y.; Busman, T. A.; McKeegan, E. M.; Krivoshik, A. P.; Enschede, S. H.; Humerickhouse, R. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J. Clin. Oncol.* **2012**, *5*, 488−496.
(38) Rudin, C. M.; Hann, C. L.; Garon, E. B.; Ribeiro de Oliveira, M.; Bonomi, P. D.; Camidge, D. R.; Chu, Q.; Giaccone, G.; Khaira, D.; Ramalingam, S. S.; Ranson, M. R.; Dive, C.; McKeegan, E. M.; Chyla, B.

dx.doi.org/10.1021/jm3010306 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

J.; Dowell, B. L.; Chakravartty, A.; Nolan, C. E.; Rudersdorf, N.; Busman, T. A.; Mabry, M. H.; Krivoshik, A. P.; Humerickhouse, R. A.; Shapiro, G. I.; Gandhi, L. Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. *Clin. Cancer Res.* **2012**, *18*, 3163−3169.

(39) Zhou, H.; Aguilar, A.; Chen, J.; Bai, L.; Liu, L.; Meagher, J. L.; Yang, C. Y.; McEachern, D.; Cong, X.; Stuckey, J. A.; Wang, S. Structure-Based Design of Potent Bcl-2/Bcl-xL Inhibitors with Strong in Vivo Antitumor Activity. *J. Med. Chem.* **2012**, *55*, 6149−6161.

(40) Zhou, H.; Chen, J.; Meagher, J. L.; Yang, C. Y.; Aguilar, A.; Liu, L.; Bai, L.; Cong, X.; Cai, Q.; Fang, X.; Stuckey, J. A.; Wang, S. Design of Bcl-2 and Bcl-xL Inhibitors with Subnanomolar Binding Affinities Based upon a New Scaffold. *J. Med. Chem.* **2012**, *55*, 4664−4682.