An Efficient Synthesis of ABT-263, a Novel Inhibitor of Antiapoptotic Bcl-2 Proteins

Guangjun Wang,[a,b,c] Hushan Zhang,[b] Jing Zhou,[b] Chengyong Ha,[a] Duanqing Pei,[b] Ke Ding*[b]

[a] Key Laboratory of Cellulose and Lignocellulosics Chemistry, Guangzhou Institute of Chemistry, Chinese Academy of Sciences, Guangzhou 510650, Guangdong, P. R. of China

[b] Laboratory of Regenerative Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, International Business Incubator A-3, Guangzhou Science Park, Guangzhou 510663, Guangdong, P. R. of China
Fax +86(20)32290485; E-mail: ding_ke@gibh.ac.cn

[c] Graduate School of the Chinese Academy of Sciences, Beijing 100039, Beijing, P. R. of China

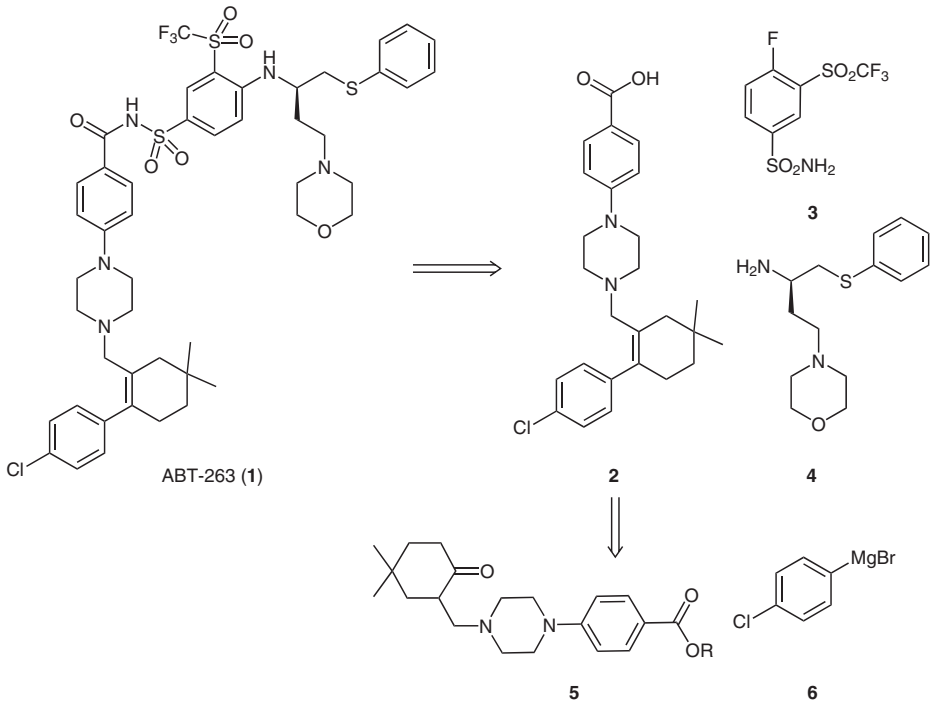
Received 27 February 2008; revised 14 April 2008

Abstract: ABT-263, a newly developed Bcl-2 inhibitor, was efficiently synthesized. The key intermediates 4-(4-{[2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl]methyl}piperazin-1-yl)benzoic acid and 4-fluoro-3-[(trifluoromethyl)sulfonyl]benzenesulfonamide were efficiently prepared by a three-component Mannich reaction and by nucleophilic fluorination of 1-nitro-2-[(trifluoromethyl)sulfonyl]benzene as the key steps, respectively. Our work may lay a foundation for a new process development of this promising anticancer drug candidate.

Key words: Bcl-2 family protein inhibitor, anticancer drugs, ABT-263, synthesis, sulfonamides

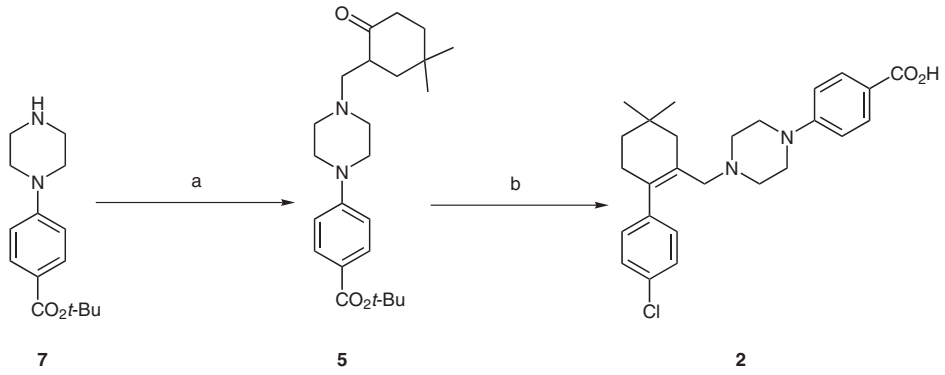
The Bcl-2 (B-cell lymphoma 2) family proteins are fundamental regulators of apoptosis. They comprise proapoptotic proteins such as Bak, Bax, Bim, and Bad, and antiapoptotic members such as Bcl-2, Bcl-x_L, Bcl-w, and so forth.[1–3] Antiapoptotic Bcl-2 proteins are overexpressed in a variety of human cancers, and such overexpression contributes greatly to cancer-cell resistance to current therapeutic agents.[4–12] Consequently, these proteins are considered to be promising molecular targets for new anticancer drug development. Much effort has been devoted to designing and synthesizing small-molecule inhibitors of Bcl-2, Bcl-x_L, Bcl-w, and other antiapoptotic Bcl-2 family proteins.[13–27]

ABT-263 (**1**, Scheme 1), developed by Abbott Laboratories, is one of the most promising orally bioavailable small-molecule inhibitors of Bcl-2 family proteins.[26,27] It has a $K_i \leq 1$ nM against Bcl-2, Bcl-x_L, and Bcl-w, and exhibits submicromolar activity against a variety of human

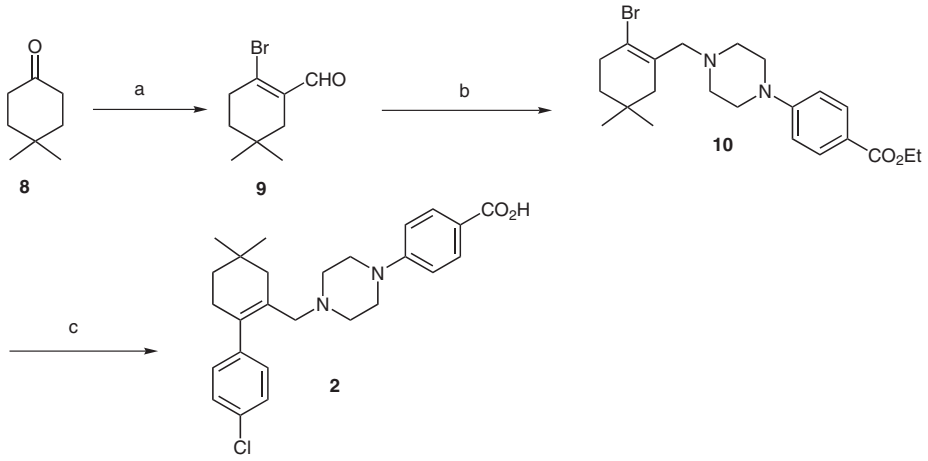


Scheme 1 Retrosynthetic analysis of ABT-263.

SYNTHESIS 2008, No. 15, pp 2398–2404
Advanced online publication: 11.06.2008
DOI: 10.1055/s-2008-1067129; Art ID: F04408SS



Scheme 2 Synthesis of building block **2**. *Reagents and conditions*: (a) 4,4-dimethylcyclohexanone, HCHO, HCl, *t*-BuOH, reflux (53%); (b) 1. 4-ClC_6H_4MgBr (**6**), THF, –40 to 20 °C; 2. 6 M aq HCl, reflux (74%).



Scheme 3 Abbott's reported method for the synthesis of building block **2**.[28] *Reagents and conditions*: (a) $CHCl_3$, DMF, Br_3P; (b) ethyl 4-(piperazin-1-yl)benzoate, $NaBH_3CN$; (c) 1. 4-$ClC_6H_4B(OH)_2$, $[PdCl_2(PPh_3)_2]$; 2. LiOH.

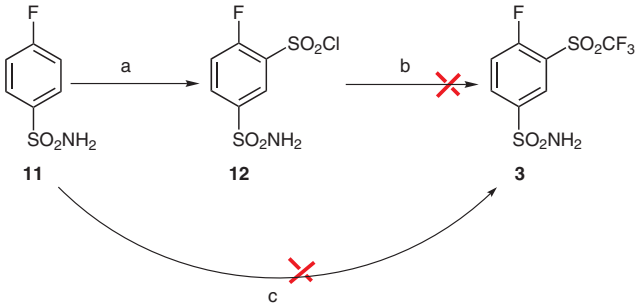
cancer cells *in vitro*.[26] ABT-263 (**1**) is currently in phase I/IIa clinical trials for treatment of small cell lung cancer, nonhematological malignancies, and so forth.[26,27] Due to its superpotency and selectivity, ABT-263 (**1**) provides an important small-molecular tool for the further investigation of biological functions of antiapoptotic Bcl-2 family proteins in different cell types. The chemical structure of ABT-263 (**1**) has been released. Most recently, a general synthetic method for ABT-263 (**1**) and its related derivatives has been provided in a disclosed patent application, but no experimental details were given.[28] As a part of our chemical biology program, we aimed to synthesize ABT-263 (**1**) as a chemical tool to study the potential regulating function of Bcl-2 family proteins in the differentiation, self-renewal, and/or cell cycle of embryonic stem cells. Herein, we report a new efficient synthesis of this molecule.

A retrosynthetic analysis of ABT-263 (**1**) revealed that the molecule could be synthesized through three key building blocks **2**, **3**, and **4** (Scheme 1). Intermediate **4** is readily prepared by a previously reported procedure.[23,29] Our effort mainly focused on the preparation of building blocks **2** and **3**.

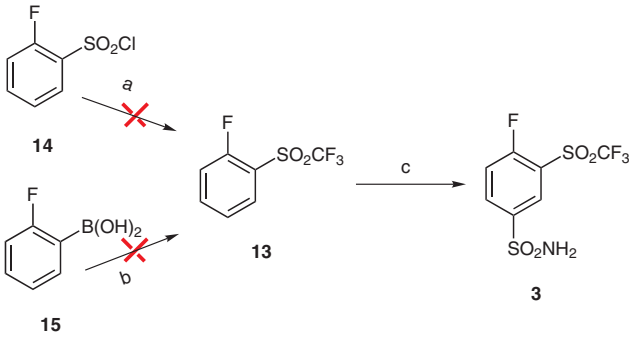
From the chemical structure, it is clear that compound **2** might be synthesized by a direct condensation of Grignard reagent **6** with intermediate **5** (Scheme 1). And it should be possible to prepare intermediate **5** by a three-component Mannich addition of a 4-(piperazin-1-yl)benzoic acid ester with 4, 4-dimethylcyclohexanone and formaldehyde (Scheme 2).

Firstly, compound **7** was prepared in 95% yield by the nucleophilic substitution of *tert*-butyl 4-fluorobenzoate with piperazine at 120 °C.[30] A three-component Mannich reaction of compound **7** with formaldehyde and 4,4-dimethylcyclohexanone was then successfully carried out to produce compound **5** in 53% yield (Scheme 2). Compound **5** reacted with Grignard reagent **6** to give a condensation product, which was treated in a one-pot dehydration and deprotection procedure in a six-molar hydrochloric acid solution to give building block **2** in 74% yield (over two steps) (Scheme 2).

In the disclosed patent application,[28] a different strategy for the synthesis of intermediate **2** was described, namely a one-pot bromination–carbonylation and a Suzuki coupling reaction as the key steps (Scheme 3). However, there were no experimental details and isolated yields provided.



Scheme 4 Attempts at introducing a (trifluoromethyl)sulfonyl group onto a phenyl ring. *Reagents and conditions*: (a) $ClSO_3H$, reflux; (b) CF_3CO_2Na, CuI, DMF, 170 °C; (c) $(CF_3SO_2)_2O$ or CF_3SO_2Cl.



Scheme 5 Alternative attempts to synthesize compound **3**. *Reagents and conditions*: (a) CF_3CO_2Na, CuI; (b) CF_3SO_2Cl, $PdCl_2$; (c) 1. $ClSO_3H$; 2. NH_4OH.

Building block **3** seemed structurally very simple. Yet the preparation of this compound cost us much effort, due to the difficulty of introducing a (trifluoromethyl)sulfonyl group onto the phenyl ring (Scheme 4). Initially, we planned to prepare intermediate **3** through a direct Friedel–Crafts reaction of 4-fluorobenzenesulfonamide (**11**) with trifluoromethanesulfonyl chloride or trifluoromethanesulfonic anhydride (Scheme 4). Unfortunately, no desired product was obtained, although a variety of Lewis acids were tested under different conditions.

Recently, a highly efficient method was reported for the preparation of a [(trifluoromethyl)sulfonyl]aryl group from an aromatic sulfonyl chloride by treatment with sodium trifluoroacetate in the presence of copper(I) iodide as the catalyst.[31] We therefore tried to apply this method to the synthesis of key intermediate **3** (Scheme 4). Although 3-(aminosulfonyl)-6-fluorobenzenesulfonyl chloride (**12**) was obtained in up to 85% yield by treatment of 4-fluorobenzenesulfonamide (**11**) with sulfurochloridic acid, it could not be converted into intermediate **3** under the reported conditions (Scheme 4).

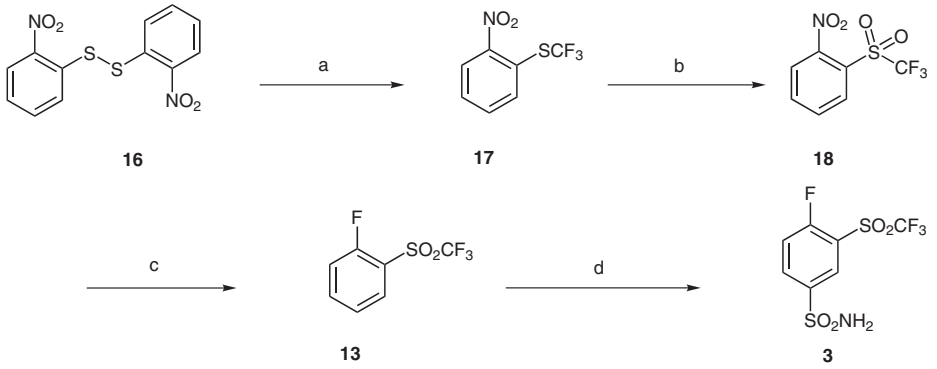
Because of the high challenge of directly introducing a (trifluoromethyl)sulfonyl group, we explored other strategies to synthesize key building block **3**. If we could obtain 1-fluoro-2-[(trifluoromethyl)sulfonyl]benzene (**13**) as an alternative, we would be able to prepare intermediate **3** from **13** by chlorosulfonylation followed by sulfonamidation with ammonium hydroxide (Scheme 5). We therefore first treated 2-fluorobenzenesulfonyl chloride (**14**) with sodium trifluoroacetate in *N*,*N*-dimethylformamide at 180 °C in the presence of copper(I) iodide (Scheme 5). However, instead of compound **13**, an unidentified product was obtained; this might be due to the high reactivity of the fluoride moiety. Other methods such as palladium-catalyzed coupling of 2-fluorophenylboronic acid (**15**) with potassium trifluoromethanesulfinate or trifluoromethanesulfonyl chloride were also explored,[32] but none of these attempts yielded the desired product **13** (Scheme 5).

Beaumont and Clark reported on the synthesis of 1-fluoro-2-[(trifluoromethyl)sulfonyl]benzene (**13**) in high yield by the fluorination of 1-nitro-2-[(trifluoromethyl)sulfonyl]benzene (**18**) (Scheme 6).[33] Thus, if we could develop an efficient synthetic method for compound **18**, access to compound **3** should be easy. On the basis of this consideration, an alternative approach was designed for the synthesis of intermediate **3** (Scheme 6). First, commercially available compound **16** was heated with potassium trifluoroacetate at 180–230 °C; this gave pure **17** directly after distillation in a yield of 70% (Scheme 6).[34] However, the oxidation of **17** gave **18** in only 17% yield when chromium(VI) oxide was utilized as the oxidant at 100 °C.[33] Although other strong oxidants such as potassium dichromate, potassium permanganate, and sodium periodate were also tested, the oxidation yield could be improved with none of these agents. Fortunately, when periodic acid was combined with a catalytic amount of chromium(VI) oxide, the oxidization yield improved to 80%, even when the reaction was carried out at room temperature (Scheme 6). With compound **18** in hand, compound **13** was readily prepared by Beaumont and Clark's procedure (Scheme 6).[33] Compound **13** was then chlorosulfonated with chlorosulfuric acid at 85 °C for 24 hours, and the resulting product was treated with ammonia at 0 °C for a few minutes to yield the important building block **3** in a combined yield of 65% over two steps (Scheme 6). {It is important that during the last step, the mixture must be neutralized with acid before any workup, because otherwise 4-amino-3-[(trifluoromethyl)sulfonyl]benzenesulfonamide will be obtained.}

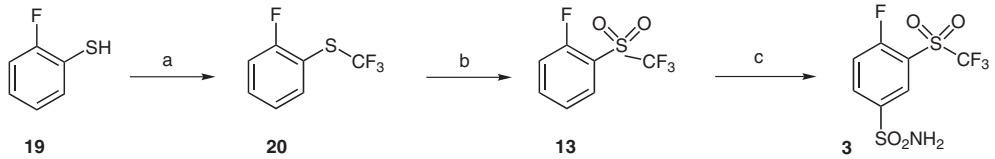
Scientists at Abbott Laboratories revealed a different procedure in the disclosed patent application[29] for the synthesis of building block **3**; for this, direct trifluoromethylation of 2-fluorobenzenethiol (**19**) was used as the key step (Scheme 7). But no experimental details or isolated yields were provided.

With all three building blocks **2**, **3**, and **4** available, target molecule ABT-263 (**1**) was readily prepared by the procedure outlined in Scheme 8. Briefly, compound **21** was obtained by a simple nucleophilic substitution reaction between compounds **3** and **4**. Coupling of **21** with intermediate **2** produced the target molecule ABT-263 (**1**) in 84% yield (Scheme 8).

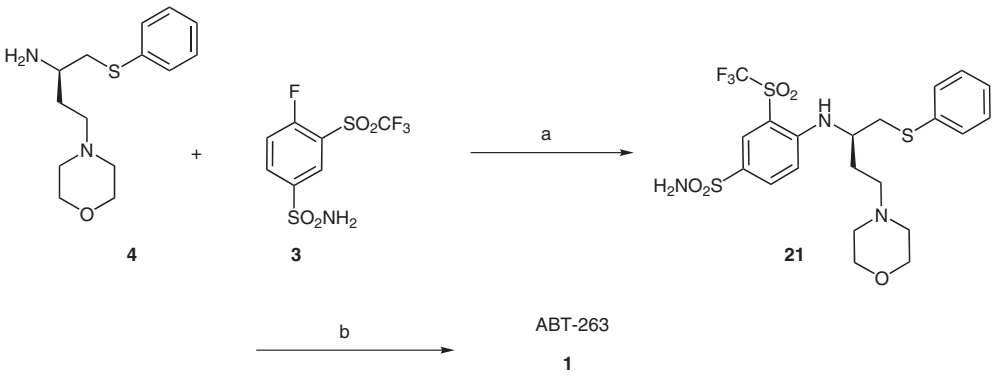
In summary, an alternative efficient synthesis of Bcl-2 family protein inhibitor ABT-263 (**1**) is described. Key in-



Scheme 6 Synthesis of compound **3**. *Reagents and conditions*: (a) CF_3CO_2K, sulfolane, 180–230 °C (70%); (b) H_5IO_6, CrO_3, MeCN, r.t. (80%; 55% from **16**); (c) KF, Ph_4PBr, DMSO, 130 °C (85%); (d) 1. $ClSO_3H$, 85 °C; 2. NH_4OH, 0 °C (65%).



Scheme 7 Synthetic method for the synthesis of **3** in the disclosed patent application.[29] *Reagents and conditions*: (a) CF_3I; (b) $RuCl_3$, $NaIO_4$; (c) 1. $ClSO_3H$; 2. NH_4OH.



Scheme 8 A final synthesis of ABT-263. *Reagents and conditions*: (a) DIPEA, DMSO, r.t. (87%); (b) benzoic acid **2**, EDCI, DMAP, CH_2Cl_2 (84%).

termediates 4-(4-{[2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl]methyl}piperazin-1-yl)benzoic acid (**2**) and 4-fluoro-3-[(trifluoromethyl)sulfonyl]benzenesulfonamide (**3**) were efficiently synthesized by use of a three-component Mannich reaction and nucleophilic fluorination of 1-nitro-2-[(trifluoromethyl)sulfonyl]benzene (**18**) as the key steps, respectively. Our work may lay a foundation for a new process development of this promising anticancer drug candidate. Furthermore, this work provided us with an important chemical tool for studying the potential regulating function of Bcl-2 family proteins in the differentiation, self-renewal, and/or cell cycle of stem cells.

All the starting materials and chemical reagents were obtained from commercial suppliers and were used without further purification. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400M spectrometer; $CDCl_3$ or DMSO-d_6 were used as solvents and TMS as an internal standard. Mass spectra were recorded on an Agilent 1200 LC-MS or HP5989A spectrometer. HRMS was carried out on a Finnigan MAT-95 spectrometer from the Shanghai Institute of Materia Medica (CAS).

tert-Butyl 4-Piperazin-1-ylbenzoate (7)

tert-Butyl 4-fluorobenzoate (4.9 g, 25 mmol) was added to a soln of piperazine (6.4 g, 75 mmol) in DMSO (20 mL) at r.t. The mixture was stirred at 120 °C for 20 h, and was then poured into a brine soln (100 mL). The resulting mixture was extracted with EtOAc (3 × 50 mL) and the organic phase was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2–MeOH, 5:1); this gave **7**.

Yield: 6.3 g (95%); white solid.

^1H NMR (400 MHz, $CDCl_3$): δ = 7.86 (d, *J* = 8.8 Hz, 2 H), 6.84 (d, *J* = 8.8 Hz, 2 H), 3.27 (t, *J* = 4.8 Hz, 4 H), 3.03 (t, *J* = 4.8 Hz, 4 H), 1.56 (s, 9 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.9, 154.3, 130.9, 121.9, 131.8, 80.1, 48.7, 45.8, 28.3.

ESI-MS: *m*/*z* = 263.3 $[M + H]^+$.

***tert*-Butyl 4-{4-[(5,5-Dimethyl-2-oxocyclohexyl)methyl]piperazin-1-yl}benzoate (5)**

Concd HCl (0.5 mL) was added to a suspension of paraformaldehyde (60 mg, 20 mmol), 4,4-dimethylcyclohexanone (1.89 g, 15 mmol), and **7** (2.62 g, 10 mmol) in *t*-BuOH (20 mL). The resulting mixture was refluxed for 3 h with vigorous stirring. The mixture was then concentrated to 10 mL under reduced pressure, and poured into sat. $NaHCO_3$ (20 mL). The mixture was extracted with EtOAc (3 × 20 mL). The organic phases were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, PE–EtOAc, 5:1); this gave **5**.

Yield: 2.12 g (53%); white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84 (d, *J* = 8.4 Hz, 2 H), 6.82 (d, *J* = 8.8 Hz, 2 H), 3.29 (m, 4 H), 2.82–2.86 (m, 1 H), 2.59–2.69 (m, 1 H), 2.60 (m, 2 H), 2.52 (m, 3 H), 2.22–2.25 (m, 2 H), 1.93–1.98 (m, 1 H), 1.73 (m, 1 H), 1.66 (m, 1 H), 1.55 (s, 9 H), 1.35 (m, 2 H), 1.28 (s, 3 H), 1.06 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 212.7, 165.9, 153.9, 130.9, 121.8, 113.7, 80.0, 57.2, 53.2, 47.6, 45.7, 43.8, 40.2, 38.6, 31.4, 30.8, 28.3, 24.5.

ESI-MS: *m*/*z* = 424.1 $[M + Na]^+$.

***tert*-Butyl 4-(4-{[2-(4-Chlorophenyl)-2-hydroxy-5,5-dimethylcyclohexyl]methyl}piperazin-1-yl)benzoate**

A Grignard soln prepared from 1-bromo-4-chlorobenzene (1.15 g, 6 mmol) and Mg (0.22 g, 9 mmol) in THF (20 mL) was added dropwise to a soln of **5** (1.2 g, 3 mmol) in THF (20 mL), at –30 °C. The temperature was allowed to rise to r.t. over 3 h, and the resulting mixture was stirred overnight. Then sat. NH_4Cl (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (3 × 20 mL). The organic phases were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, PE–EtOAc, 10:1); this gave *tert*-butyl 4-(4-{[2-(4-Chlorophenyl)-2-hydroxy-5,5-dimethylcyclohexyl]methyl}piperazin-1-yl)benzoate.

Yield: 1.34 g (87%); white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, *J* = 8.8 Hz, 2 H), 7.45 (d, *J* = 7.6 Hz, 2 H), 7.31 (d, *J* = 8.0 Hz, 2 H), 6.79 (d, *J* = 4.8 Hz, 2 H), 6.65 (s, 1 H), 3.25 (s, 4 H), 2.64 (s, 2 H), 2.43 (d, *J* = 13.6 Hz, 1 H), 2.25–2.28 (m, 2 H), 2.18 (d, *J* = 14.0 Hz, 1 H), 1.99–2.09 (m, 2 H), 1.73 (d, *J* = 5.6 Hz, 2 H), 1.64 (d, *J* = 9.6 Hz, 1 H), 1.56 (s, 9 H), 1.24 (d, *J* = 9.6 Hz, 2 H), 0.96 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.8, 153.6, 148.5, 131.9, 130.9, 128.0, 126.4, 122.3, 113.8, 80.2, 77.3, 60.1, 54.9, 48.0, 40.3, 40.1, 37.7, 34.6, 33.1, 30.9, 28.3, 23.8.

ESI-MS: *m*/*z* = 513.3 $[M + H]^+$.

4-(4-{[2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl]methyl}piperazin-1-yl)benzoic Acid (2)

The *tert*-butyl 4-(4-{[2-(4-chlorophenyl)-2-hydroxy-5,5-dimethylcyclohexyl]methyl}piperazin-1-yl)benzoate obtained above (1.03 g, 2 mmol) was refluxed in 6 M HCl (10 mL) for 4 h. After the soln was adjusted to pH 6 by the addition of sat. aq $NaHCO_3$, the mixture was extracted with EtOAc (3 × 20 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, PE–EtOAc, 2:1); this gave **2**.

Yield: 0.75 g (85%); white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, *J* = 8.8 Hz, 2 H), 7.18 (d, *J* = 8.4 Hz, 2 H), 6.90 (d, *J* = 8.4 Hz, 2 H), 6.72 (d, *J* = 8.8 Hz, 2 H), 3.21 (d, *J* = 4.8 Hz, 4 H), 2.78 (s, 2 H), 2.32 (s, 4 H), 2.17 (s, 2 H), 1.95 (s, 2 H), 1.37 (t, *J* = 6.4 Hz, 2 H), 0.89 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 154.5, 141.8, 135.3, 132.1, 131.8, 129.7, 129.0, 128.4, 119.1, 113.4, 60.5, 52.3, 47.1, 41.5, 35.6, 30.9, 28.9, 28.1.

ESI-MS: *m*/*z* = 439.0 $[M + H]^+$.

HRMS–FAB: *m*/*z* $[M + H]^+$ calcd for $C_{26}H_{32}ClN_2O_2$: 439.2147; found: 439.2138.

1-Nitro-2-[(trifluoromethyl)sulfonyl]benzene (18)

Disulfide **16** (6.16 g, 20 mmol) and CF_3CO_2K (6.08 g, 40 mmol) were dissolved in sulfolane (4 mL), and the resulting mixture was heated to 180 °C. After the release of CO_2 at 180 °C, the reaction temperature was increased to 230 °C, and the distilled yellow oil was collected; this gave **17** as a crude product, which was used for the next step without further purification.

CrO_3 (30 mg, 3 mmol) was added to a suspension of H_5IO_6 (13.68 g, 60 mmol) in MeCN (20 mL), and the resulting mixture was stirred for 30 min. Then the crude product **17** obtained above was added to the mixture, which was then stirred overnight. After the organic solvent was removed, sat. aq Na_2SO_3 was added at 0 °C until the mixture became a clear blue soln. The resulting soln was extracted with EtOAc (3 × 50 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, PE–EtOAc, 10:1); this gave **18**.

Yield: 2.81 g (55%, over 2 steps); yellow oil.

^{1}H NMR (400 MHz,$CDCl_3$): δ = 8.22 (d, *J* = 7.6 Hz, 1 H), 8.01 (m, 1 H), 7.91 (m, 2 H).

MS (EI): *m*/*z* = 255 $[M]^+$.

1-Fluoro-2-[(trifluoromethyl)sulfonyl]benzene (13)

Freshly dried KF (0.58 g, 10 mmol), Ph_4PBr (1.05 g, 2.5 mmol), and **18** (1.28 g, 5 mmol) were suspended in anhyd DMSO (10 mL), and the resulting mixture was stirred at 130 °C for 15 min. After the mixture had been cooled to r.t., H_2O was added and the mixture was extracted with EtOAc (3 × 100 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, PE–EtOAc, 30:1); this gave **13**.

Yield: 969 mg (85%); yellow oil.

^{19}F NMR (400 MHz, $CDCl_3$): δ = –78.39 (CF_3), –103.81 (F).

MS (EI): *m*/*z* = 228 $[M]^+$.

4-Fluoro-3-[(trifluoromethyl)sulfonyl]benzenesulfonamide (3)

Compound **13** (456 mg, 2.0 mmol) was suspended in $ClSO_3H$ (1 mL) at 0 °C, and the resulting mixture was heated under stirring at 90 °C for 24 h, and subsequently slowly cooled to r.t. H_2O (10 mL) was added carefully to quench the reaction, and the mixture was extracted with EtOAc (3 × 50 mL). The organic phases were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, PE–EtOAc, 30:1); this gave 4-fluoro-3-[(trifluoromethyl)sulfonyl]benzenesulfonyl chloride.

NH_4OH (2 mL) was added to the thus obtained 4-fluoro-3-[(trifluoromethyl)sulfonyl]benzenesulfonyl chloride at 0 °C, and the resulting soln was stirred for 5 min at 0 °C. Then the mixture was neutralized with 2 M HCl at 0 °C, and extracted with EtOAc (3 × 100 mL). The organic phases were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, PE–EtOAc, 2:1); this gave **3**.

Yield: 400 mg (65%, over 2 steps); white solid.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.46 (m, 1 H), 8.42 (m, 1 H), 7.97 (dd, *J* = 8.8 Hz, 1 H), 7.81 (s, 2 H).

MS (EI): *m*/*z* = 307 $[M]^+$.

HRMS (EI): *m*/*z* $[M]^+$ calcd for $C_7H_5F_4NO_4S_2$: 306.9591; found: 306.9599.

4-{[(*R*)-4-Morpholino-1-(phenylsulfanyl)butan-2-yl]amino}-3-[(trifluoromethyl)sulfonyl]benzenesulfonamide (21)

Compounds **4** (266 mg, 1.0 mmol), **3** (307 mg, 1.0 mmol), and DIPEA (0.5 mL) were dissolved in DMSO (5 mL), and the resulting mixture was stirred at r.t. for 16 h. EtOAc (100 mL) was added to dilute the mixture, which was then washed sequentially with 3 M HCl (30 mL), H_2O (30 mL), and brine (2 × 30 mL). The organic phase was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, CH_2Cl_2–MeOH, 20:1); this gave **21**.

Yield: 482 mg (87%); white solid; $[\alpha]_D^{20}$ –27.8 (*c* 0.3, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1 H), 7.95 (d, *J* = 8.4 Hz, 1 H), 7.39 (d, *J* = 7.6 Hz, 2 H), 7.28–7.32 (m, 2 H), 7.01 (d, *J* = 8.4 Hz, 1 H), 6.83 (d, *J* = 8.4 Hz, 1 H), 5.85 (s, 2 H), 4.07 (s, 1 H), 3.85 (br, 4 H), 3.09–3.18 (m, 2 H), 2.75–2.79 (br, 4 H), 2.20–2.23 (m, 1 H), 1.98–2.01 (m, 1 H), 1.42–1.54 (m, 1 H), 1.24–1.27 (m, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.1, 136.2, 134.6, 132.9, 131.2, 130.3, 129.4, 127.5, 121.7, 118.4, 114.1, 108.9, 65.0, 54.3, 52.7, 50.7, 38.9, 28.4.

ESI-MS: *m*/*z* = 554.1 $[M + H]^+$.

ESI-HRMS: *m*/*z* $[M + H]^+$ calcd for $C_{21}H_{27}F_3N_3O_5S_3$: 554.1065; found: 554.1060.

4-(4-{[2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl]methyl}piperazin-1-yl)-*N*-[(4-{[(*R*)-4-morpholino-1-(phenylsulfanyl)butan-2-yl]amino}-3-[(trifluoromethyl)sulfonyl]phenyl)sulfonyl]benzamide (ABT-263; 1)

Compound **21** (305 mg, 0.55 mmol) was added to a soln of **2** (219 mg, 0.5 mmol), EDCI (195 mg, 1.0 mmol), and DMAP (122 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at r.t. The resulting mixture was stirred for 36 h and then partitioned between H_2O (50 mL) and CH_2Cl_2 (100 mL). The organic phase was washed with sat. aq NH_4Cl (2 × 30 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, CH_2Cl_2–MeOH, 40:1); this gave ABT-263 (**1**).

Yield: 408 mg (84%); pale yellow solid; $[\alpha]_D^{20}$ –59 (*c* 0.28, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34 (s, 1 H), 8.06 (d, *J* = 8.8 Hz, 1 H), 7.73 (d, *J* = 8.4 Hz, 2 H), 7.35 (d, *J* = 7.2 Hz, 2 H), 7.28 (d, *J* = 7.2 Hz, 2 H), 7.23 (d, *J* = 5.6 Hz, 2 H), 7.15 (d, *J* = 8.4 Hz, 2 H), 6.99 (m, 1 H), 6.76 (d, *J* = 8.4 Hz, 2 H), 6.58 (d, *J* = 9.2 Hz, 1 H), 5.79 (s, 1 H), 3.89 (m, 1 H), 3.72 (s, 4 H), 3.36 (br, 3 H), 2.63–2.72 (m, 1 H), 2.40–2.63 (m, 8 H), 2.15–2.25 (m, 3 H), 1.85–2.04 (m, 4 H), 1.73 (m, 1 H), 1.32–1.53 (m, 3 H), 1.25 (s, 4 H), 0.89 (s, 3 H), 0.87 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.0, 151.7, 140.8, 137.9, 135.1, 134.6, 132.2, 131.1, 129.9, 129.3, 129.2, 128.4, 127.8, 127.4, 121.7, 118.5, 113.6, 112.9, 108.8, 66.3, 62.6, 54.5, 53.4, 52.9, 50.7, 46.7, 41.8, 39.8, 39.0, 32.6, 31.3, 29.8, 29.7, 29.3, 28.0, 25.4.

ESI-MS: *m*/*z* = 974.0 $[M + H]^+$, 996.0 $[M + Na]^+$.

ESI-HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{47}H_{55}ClF_3N_5NaO_6S_3$: 996.2853; found: 996.2876.

Acknowledgment

We thank the Chinese Academy of Sciences (grant KSCX2-YW-R-27), Natural Science Foundation of Guangdong province, and Guangzhou Institute of Biomedicine and Health for their financial support.

References

(1) Adams, J. M.; Cory, S. *Science* **1998**, *281*, 1322.
(2) Cory, S.; Adams, J. M. *Nat. Rev. Cancer* **2002**, *2*, 647.
(3) Minn, A. J.; Swain, R. E.; Ma, A.; Thompson, C. B. *Adv. Immunol.* **1998**, *70*, 245.
(4) Van D, M. F.; Huang, D. C. *Cell Res.* **2006**, *16*, 203.
(5) Thompson, C. B. *Science* **1995**, *267*, 1456.
(6) Reed, J. C. *Adv. Pharmacol.* **1997**, *41*, 501.
(7) Danial, N. N.; Korsmeyer, S. J. *Cell* **2004**, *116*, 205.
(8) Nicholson, D. W. *Nature* **2000**, *407*, 810.
(9) Green, D. R.; Evan, G. I. *Cancer Cell* **2002**, *1*, 19.
(10) Amundson, S. A.; Myers, T. G.; Scudiero, D.; Kitada, S.; Reed, J. C.; Fornace, A. J. Jr. *Cancer Res.* **2000**, *60*, 6101.
(11) Danial, N. N. *Clin. Cancer Res.* **2007**, *13*, 7254.
(12) Adams, J. M.; Cory, S. *Oncogene* **2007**, *26*, 1324.
(13) For reviews, see: (a) Beauparlant, P.; Shore, G. C. *Cell Eng.* **2004**, *4*, 223. (b) Hangary, S.; Johnson, D. E. *Leukemia* **2003**, *17*, 1470. (c) Huang, Z. W. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 565. (d) Wang, S.; Yang, D.; Lippman, M. E. *Semin. Oncol.* **2003**, *30*, 133. (e) Manion, M. K.; Fry, J.; Schwartz, P. S.; Hockenbery, D. M. *Curr. Opin. Investig. Drugs* **2006**, *7*, 1077. (f) Reed, J. C.; Zhai, D.; Hyer, M.; Welsh, K. *Drug Discovery Res.* **2007**, 408.
(14) Degterev, A.; Lugovskoy, A.; Cardone, M.; Mulley, B.; Wagner, G.; Mitchison, T.; Yuan, J. *Nat. Cell Biol.* **2001**, *3*, 173.
(15) Wang, J. L.; Liu, D.; Zhang, Z. J.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7124.
(16) Tzung, S. P.; Kim, K. M.; Basanez, G.; Giedt, C. D.; Simon, J.; Zimmerberg, J.; Zhang, K. Y.; Hockenbery, D. M. *Nat. Cell Biol.* **2001**, *3*, 183.
(17) Kitada, S.; Leone, M.; Sareth, S.; Zhai, D.; Reed, J. C.; Pellecchia, M. *J. Med. Chem.* **2003**, *46*, 4259.
(18) 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. *Nature* **2005**, *435*, 677.
(19) 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. *J. Med. Chem.* **2007**, *50*, 3163.
(20) Xing, C.; Saleh, M.; Pitot, H.; Hartung, J.; Holmlund, J.; AlbertLoBuglio, A.; Forero, A. P. *The 2005 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery Biology and Clinical Applications, Nov 14–18, 2005, Philadelphia, Pennsylvania*; American Association for Cancer Research: Philadelphia, PA, **2005**, Abstract C89.
(21) Zhang, Z.; Jin, L.; Qian, X.; Wei, M.; Wang, Y.; Wang, J.; Yang, Y.; Xu, Q.; Xu, Y.; Liu, F. *ChemBioChem* **2007**, *8*, 113.
(22) 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. *J. Med. Chem.* **2006**, *49*, 656.
(23) Wendt, M. D.; Wang, S.; Kunzer, A.; McClellan, W. J.; Bruncko, M.; Oost, T. K.; Joseph, M. K.; 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. *J. Med. Chem.* **2006**, *49*, 1165.
(24) 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. *J. Med. Chem.* **2007**, *50*, 641.
(25) Park, C. M.; Oie, T.; Petros, A. M.; Zhang, H.; Nimmer, P. M.; Henry, R. F.; Elmore, S. W. *J. Am. Chem. Soc.* **2006**, *128*, 16206.
(26) Park C. M.; *Abstracts*, 41st Western Regional Meeting of the American Chemical Society; San Diego, **2007**.
(27) Lock, R.; Carol, H.; Houghton, P. J.; Morton, C. L.; Kolb, E. A.; Gorlick, R.; Reynolds, C. P.; Maris, J. M.; Keir, S. T.; Wu, J.; Smith, M. A. *Pediatr. Blood Cancer* **2008**, *50*, 1181.
(28) Bruncko, M.; Ding, H.; Elmore, S. W.; Kunzer, A. R.; Lynch, C. L.; McClellan, W. J.; Park, C. M.; Song, X.; Wang, X. US 27135A1, **2007**; *Chem. Abstr.* **2007**, *146*, 229049.
(29) Steven, W.; Bruncko, M.; Park, C. M. US 272744, **2005**; *Chem. Abstr.* **2005**, *144*, 36377.
(30) Dai, K.; Minoru, I.; Mikio, Y.; Shoichi, M.; Mitsugu, H.; Kiyoaki, K.; Keiichi, A. *Bioorg. Med. Chem.* **2006**, *14*, 2089.
(31) Ying, C.; Chun, C. *J. Fluorine Chem.* **2005**, *126*, 937.
(32) Bandgar, B. P.; Bettigeri, S. V.; Phopase, J. *Org. Lett.* **2004**, *6*, 2105.
(33) Beaumont, A. J.; Clark, J. H. *J. Fluorine Chem.* **1991**, *52*, 295.
(34) Popkova, V. Y.; Marhold, A. EP 109892, **1999**; *Chem. Abstr.* **1999**, *132*, 3248.