Supplementary Material (ESI) for Chemical Communications

Profiling small molecule inhibitors against helix-receptor interactions: the Bcl-2 family inhibitor BH3I-1 potently inhibits p53/hDM2

Jason R. Porter,[a] Mark Helmers,[a] Ping Wang, [a] Jennifer L. Furman, [a] Stephen T. Joy, [b] Paramjit S. Arora* [b] and Indraneel Ghosh* [a]

Supporting Information

Experimental Methods

General Reagents and Small Molecule Inhibitors

All materials were obtained from Sigma-Aldrich unless otherwise noted. Restriction enzymes were obtained from NEB. BIM BH3 peptide was obtained from AnaSpec. BH3I-1 was from CalBiochem. ABT-737 was from Selleck Chemicals. (+)-Nutlin-3 and (-)-Nutlin-3, were from Cayman Chemical.

Cloning of Fusion Proteins and In Vitro Transcription

The fusion protein constructs used in this study are shown in Table above. DNA coding for NFluc(2-416) and CFluc(398-550) were generated by PCR with appropriate primers and subsequently cloned into the pETDuet-1 vector using standard techniques with verification by dideoxynucleotide sequencing. Fragments encoding helices were generated by over-lapping primer extension using standard techniques. Fragments encoding the receptors used in this study were generated by PCR from specific plasmids. Plasmids encoding hDM2, hDM4, Bcl-2, Bcl-x_L, Bcl-w, and BFL were obtained from Open Biosystems. p300 was provided by B. Z. Olenyuk. The fusion protein constructs were generated using standard cloning techniques and verified by dideoxynucleotide sequencing. The mRNA necessary for cell-free assays was generated as follows: PCR fragments corresponding to the desired fusion constructs were generated using a forward primer containing a T7 RNA polymerase promoter and a Kozak sequence and a reverse primer containing a 3' stem loop. The purified PCR products were subsequently used as templates for *in vitro* transcription using the RiboMAX Large Scale RNA Production System-T7 (Promega) following the manufacturer's protocols.

Table S1. Helix-NFluc and CFluc-Receptor constructs used in this study.

Receptor	Helix
CFluc-hDM2 (1-183)	BIM (142-161)-NFluc
CFluc-Bcl-2 (1-239)	p53(7-36)-NFluc
CFluc-Bcl-xL (1-233)	Hif-1α-CTAD-NFluc
CFluc-Bcl-w	CFluc-Hif-1α-CTAD*
CFluc-hDM4 (1-185)	
CFluc-p300 (323-423) or p300-NFluc	

*Note Hif-1α and p300 were used in both orientations with equivalent results for both interaction and inhibition experiments.

Cell-Free Interrogation of Protein-Protein Interactions

25 μL translations were carried out in treated rabbit reticulocyte lysate using 0.4 pmols of each CFluc-receptor mRNA and 2 pmols of each Helix-NFluc mRNA being analyzed and 0.5 μL of RNasin Plus (Promega). Translations were incubated at 30 °C for 90 minutes and assayed by combining 10 μL of translation solution with 40 μL of Steady-Glo Luciferase Assay System (Promega). Light emission was monitored 1 minute after Steady-Glo addition using a Turner Biosystems $20/20^n$ Luminometer with a 3 second delay and a 10 second integration time. Relative levels of luminescence were compared to a background translation containing no mRNA.

Cell-Free Interrogation of Peptide and Small-Molecule Inhibitors of Protein-Protein Interactions

To determine the inhibition of the interactions between p53 and hDM2 or hDM4 duplicate 25 μL translation reactions were carried out in Rabbit Reticulocyte Lysate (Promega) according to the manufacturer's protocol using 0.4 pmols of either CFluc-hDM2 or CFluc-hDM4 mRNA and 2 pmols of p53-NFluc mRNA and 0.5 μL of RNasin Plus (Promega) followed by an incubated at 30 °C for 90 minutes. Following incubation either (+)-nutlin-3 or (-)-nutlin-3 was added post-reassembly to a final concentration of 5 μM and allowed to incubate for 30 minutes at room temperature. Inhibition was assayed by combining 10 μL of translation solution with 40 μL of Steady-Glo Luciferase Assay System (Promega). Light emission was monitored 1 minute after Steady-Glo addition using a Turner Biosystems $20/20^n$ Luminometer with a 3 second delay and a 10 second integration time.

Cell-Free Interrogation of Bcl-2 Family Inhibitors:

Separate 25 μL translations were carried out in treated rabbit reticulocyte lysate using 0.4 pmols of either CFluc-Bcl-2 or CFluc-Bcl-x_L mRNA and 2 pmols of BIM-NFluc mRNA and 0.5 μL of RNasin Plus (Promega) followed by an incubated at 30 °C for 90 minutes. Following incubation 100 μM BIM peptide or DMSO was allowed to incubate with CFluc-receptor translations for 20 minutes at room temperature followed by the subsequent addition of an equal volume of BIM-NFluc translation and incubation for an additional 20 minutes. Samples were assayed for luciferase activity by combining 10 μL of translation solution with 40 μL of Steady-Glo Luciferase Assay System (Promega). Light emission was monitored 1 minute after Steady-Glo addition using a Turner Biosystems $20/20^n$ Luminometer with a 3 second delay and a 10 second integration time.

Cell-Free Interrogation of Inhibitor Specificity

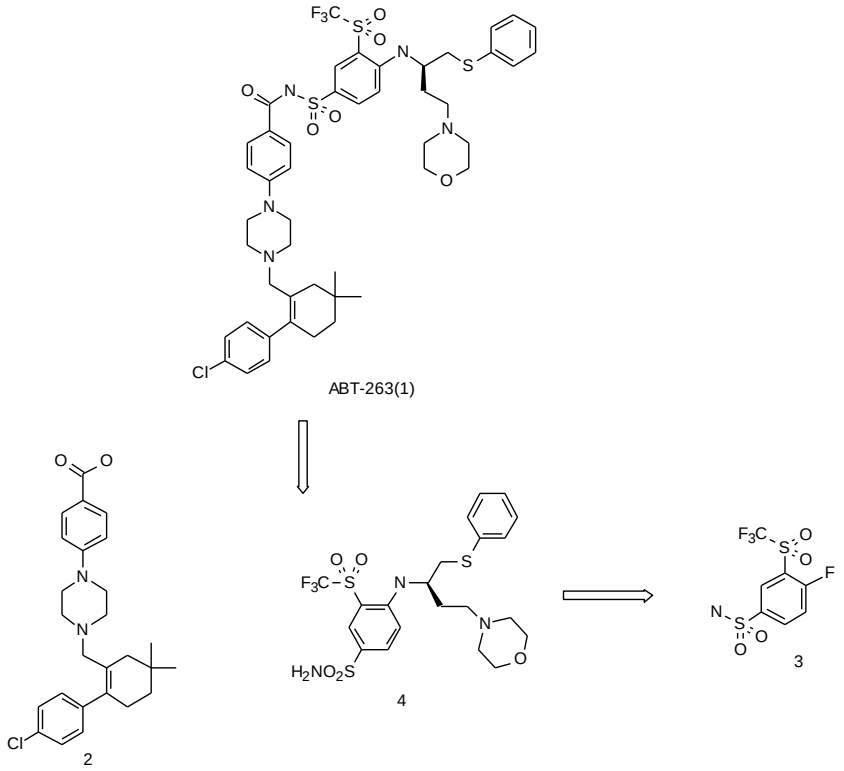
Separate 25 μL translations were carried out in treated rabbit reticulocyte lysate using 0.4 pmols of each CFluc-receptor mRNA and 2 pmols of each Helix-NFluc mRNA being analyzed, and 0.5 μL of RNasin Plus (Promega) followed by an incubated at 30 °C for 90 minutes. Following incubation 10 μL of each CFluc-receptor translation being analyzed was incubated with 100 μM of the small-molecule being analyzed or DMSO followed by a 20 minute incubation at room temperature. Subsequently, 10 μL of either BIM-NFluc (for Bcl-2 family interactions) or p53-NFluc (for hDM2 and hDM4 interactions) translation was added followed by an additional 20 minute incubation at room temperature. For tethered luciferase control inhibition 25 μL translations were carried out in treated rabbit reticulocyte lysate using 0.4 pmols mRNA and 0.5 μL of RNasin Plus (Promega) followed by 30 °C for 90 minutes. Following translation, inhibitors were incubated with tethered luciferase translation for 40 minutes at room temperature. Samples were assayed for luciferase activity by combining 10 μL of translation solution with 40 μL of Steady-Glo Luciferase Assay System (Promega). Light emission was monitored 1 minute after Steady-Glo addition using a Turner Biosystems $20/20^n$ Luminometer with a 3 second delay and a 10 second integration time. Relative levels of inhibition were determined by comparison with assays containing no inhibitor.

Synthesis of ABT-263

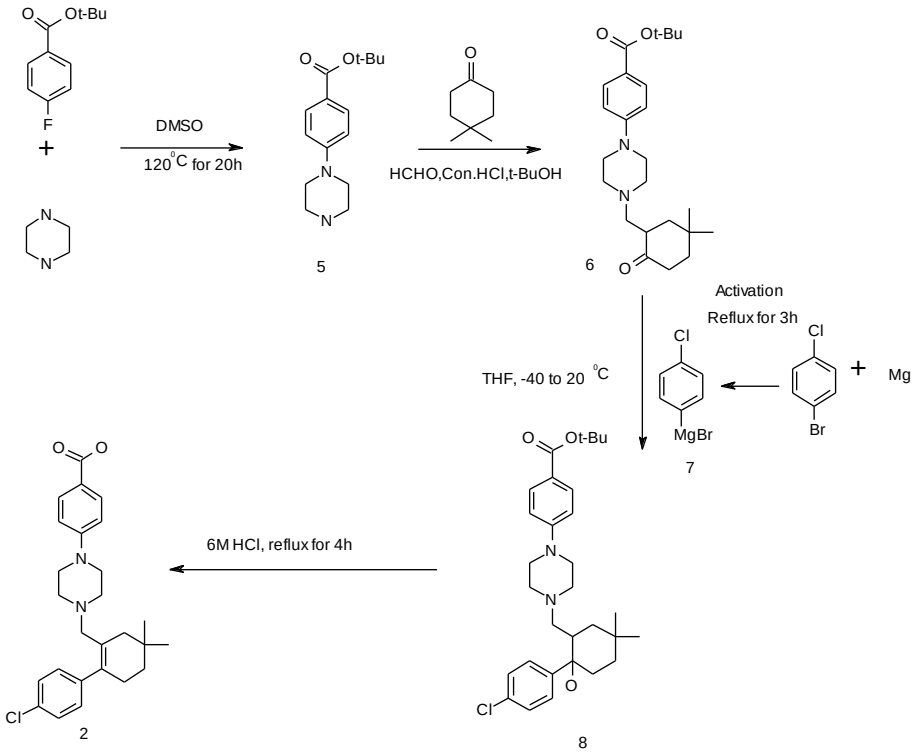
Synthesis of ABT-263 was carried out as described by Wang et al[1] with several minor modifications of the experimental conditions.

General Methods. Solvents and reagents were from Aldrich unless otherwise indicated and used without further purification. ^{1}H and ^{13}C NMR spectra were obtained on a Varian UNITY (300 MHz). Chemical shifts are reported as δ values (ppm) downfield relative to TMS. Mass spectra were performed on JEOL HX110A. Column chromatography was carried out on silica gel (250 mesh). Some compounds were purified by preparative HPLC on a Varian Symmetry C18 column using 0.1% TFA in CH_3CN/H_2O at a flow rate of 8 mL/min.

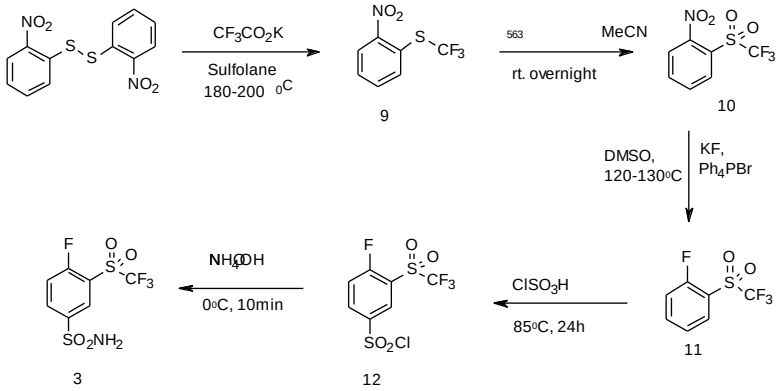
Scheme 1. Retrosynthesis of ABT-263 as previously described by Wang *et al.*[1]



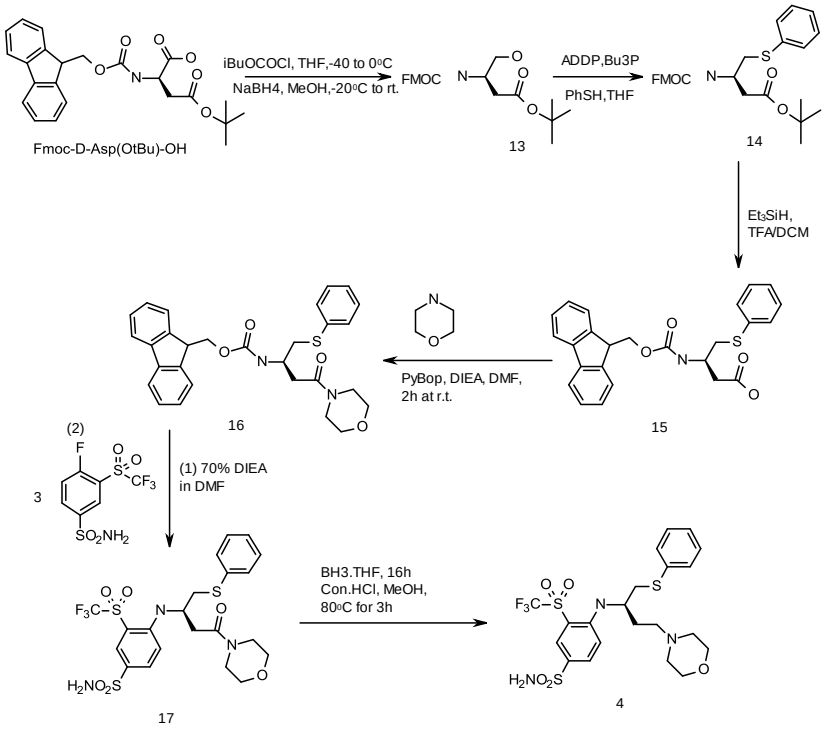
Scheme 2. Synthesis of **2** as described.[1-3]



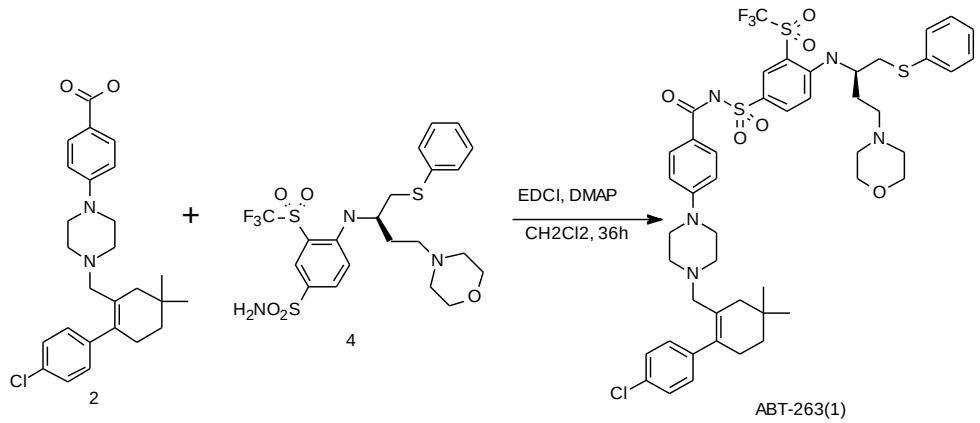
Scheme 3. Synthesis of **3** as previously described.[1, 4, 5]



Scheme 4. Synthesis of the building block **4** as previously described.[6-8]



Scheme 5. Synthesis of **1**, **ABT-263,** as described by Wang.[1, 9]



Synthetic protocols are essentially as described by Wang *et al*.[1]

Synthesis of Tert-Butyl 4-Piperazin-1-ylbenzoate (5): Tert-Butyl 4-fluorobenzoate (2.5 g, 12.7 mmol) was added to a solution of piperazine (3.74 g, 43.4 mmol) in DMSO (10 mL) at RT. The mixture was stirred at 120ºC for 20 h and subsequently poured into a brine solution (50 mL), extracted with EtOAc (3×25 mL), dried (Na_2SO_4), filtered, and concentrated. The concentrate was purified by column chromatography on silica gel (CH_2Cl_2-CH_3OH, 5:1) to provide 3.25 g (97%) of pale yellow **5**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.84 (d, 2H), 6.81 (d, 2H), 3.24 (t, 4H), 3.00 (t, 4H), 1.55 (s, 9H). ESI-MS: m/z = 263.0 $[MH]^+$.

Synthesis of Tert-Butyl 4-[1] benzoate (6): Concentrated HCl (0.4 mL) was added to a suspension of paraformaldehyde (60 mg, 2 mmol), 4,4-dimethylcyclohexanone (0.3 g, 2.4 mmol), and **5** (0.524 g, 2 mmol) in t-BuOH (20 mL). The resulting mixture was refluxed for 3 h, cooled to room temperature. Anhydrous ether was added into the reaction mixture and allowed to stand overnight at 4ºC following which the white precipitate was filtered. The residue was dissolved and purified again by column chromatography on silica gel (CH_2Cl_2-CH_3OH, 5:1) to provide 0.38g (47.5%) of **6**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88 (d, 2H), 6.86 (d, 2H), 3.29 (m, 4H), 2.86 (m, 2H), 2.68 (m, 1H), 2.60(m, 4H), 2.27 (m, 2H), 1.98 (m, 2H), 1.75 (m, 2H), 1.57 (s, 9H), 1.40 (m, 2H), 1.24 (s, 3H), 1.04 (s, 3H). ESI-MS: m/z = 401.1 $[MH]^+$.

Synthesis of Tert-Butyl 4-(4-{2-(4-Chlorophenyl)2-hydroxy-5,5-dimethyl-cyclo hexyl}meth-yl)piperazin-1-yl)benzoate (8): A Grignard reagent, p-chlorophenyl magnesium bromide, was generated in situ by reacting 4-bromochlorobenzene (2.18 g, 11.4 mmol), Mg (0.3 g, 12.35 mmol) and I_2 (ca. 1 mg) in anhydrous THF (10 mL) and refluxing the mixture for 3 h under argon. 1 mL Grignard solution (1 mol/L) was added drop-wise to a solution of **6** (130 mg, 0.3 mmol) in THF (2 mL) at - 20ºC. The temperature was allowed to rise to room temperature over 3 h, and the resulting mixture was stirred overnight. Saturated NH_4Cl (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (320 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, CH_2Cl_2/CH_3OH, 40:1) to obtain 74 mg (48.2%) of **8**. ESI-MS: m/z =513.4 $[MH]^+$.

Synthesis of 4-(4-{[2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl]methyl} piperazin-1-yl) benzoic Acid (2): Compound **8** (71.8 mg, 0.14 mmol) was refluxed in 6 M HCl (4 mL, 2 mL conc. HCl and 2 mL dioxane) for 6 h, then was adjusted to pH 6 by the addition of sat. aq $NaHCO_3$. The solution was extracted with EtOAc (3×10 mL) and dried (Na_2SO_4), filtered and concentrated. The concentrate was chromotographed on silica gel (CH_2Cl_2/CH_3OH, 20:1) to provide 61.2 mg (88.4%) of **2**. ESI-MS: m/z = 439.1 $[MH]^+$.

Synthesis of 1-Nitro-2-[(trifluoromethyl)sulfonyl] benzene (9): 1,2-bis(2-Nitro phenyl) disulfide (6.16 g, 20 mmol) and CF_3CO_2K (6.08 g, 40 mmol) were dissolved in sulfolane (5 mL), and the resulting mixture was heated to 180ºC. The distilled yellow oil was collected under reduced pressure at 210ºC to give **9** (7.4 g) as a crude product, which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): = 8.10 (d, 1H), 7.80 (d, 1H), 7.66 (t, 1H), 7.51 (t, 1H). ^{19}F NMR (300 MHz, $CDCl_3$): δ= - 78 (CF3). EI-MS: m/z = 222.99 $[M]^+$.

Synthesis of 10: H_5IO_6 (4.787 g, 10 mmol) was added to acetonitrile (60 mL) by vigorous stirring at room temperature for 30 min following which CrO_3 (100 mg, 1 mmol, 10 mol %) was adding to the solution. The mixture was stirred at room temperature for 5 min to give an orange solution (with some remaining white solid.) To this solution, **9** was added (2.23 g, 10 mmol, with CH_3CN 5 mL) at room temperature. The reaction resulted in a white precipitate immediately. The mixture was stirred overnight, filtered, concentrated, dissolved in EtOAc, washed with saturated aqueous Na_2SO_3 (the solution turned green from orange), and finally washed twice with brine, dried over Na_2SO_4 and concentrated. The residue was purified by chromatography on silica gel (PE/EtOAc, 5:1) to provide **10**. EI-MS: m/z = 254.98 $[M]^+$.

Synthesis of 4-Fluoro-3-[(trifluoromethyl)sulfonyl] benzene (3): Freshly dried KF (290.5 mg, 5 mmol), Ph_4PBr (524.1 mg, 1.25 mmol), and **10** (637.5 mg, 2.5 mmol) were suspended in anhydrous DMSO (10 mL), and the resulting mixture was stirred at 130ºC for 15 min. After the mixture had been cooled to room temperature, H_2O was added and the mixture was extracted, concentrated, and purified by silica gel (PE/EtOAc, 10:1). The mixture **11** was obtained, which was used for the next step without further purification.

11 (320 mg) was suspended in $ClSO_3H$ (1 mL) at 0 ºC, and the resulting mixture was heated under strirring at 95ºC for 18 h, and slowly cooled to room temperature. H_2O (10 mL) was added drop wise to quench the reaction, and the mixture was extracted with EtOAc (2×50mL). The organic phases were combined, dried (Na_2SO_4) and concentrated. The mixture **12** was used for the next step without further purification.

NH_4OH (2 mL) was added to the mixture above at 0ºC, and the resulting solution was stirred for 10 min. The mixture was neutralized with 2 M HCl at 0ºC, 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). The product was purified by HPLC again [0-100% (40min), 0.1% TFA, ACN/H2O] to provide 120 mg of **3**. ^{1}H NMR (300 MHz, DMSO-d6): δ= 8.46 (m, 1H), 8.41 (m, 1H), 7.95 (t, 1H), 7.79 (s, 2H). ^{19}F NMR (300 MHz, $CDCl_3$): δ= -78.58 (CF3), -101.24 (F). EI-MS: m/z =306.96 $[M]^+$.

Synthesis of (R)-3-(9H-Fluoren-9-yloxycarbonylamino)-4-hydroxybutyric Acid tert-Butyl Ester (13): A solution of Fmoc-D-Asp(OtBu)-OH (1.5 g, 3.63 mmol) and DIPEA (0.77 mL) in THF (17 mL) at -40ºC was treated with isobutyl chloroformate (0.517 mL, 4.02 mmol), warmed to 0ºC over 30 min, cooled to -20ºC, and $NaBH_4$ (0.273 g, 7.27 mmol) in MeOH (10 mL) was added drop wise. The reaction was gradually warmed to room temperature over 2 h, diluted with EtOAc (33 mL), washed with water and brine, dried (Na_2SO_4), filtered, concentrated, and purified by silica gel to provide 1.35 g (94%) of **13**. ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.75 (d, 2H), 7.58 (d, 2H), 7.37 (dd, 2H), 7.26 (dd, 2H), 5.48 (m, 1H), 4.39 (br, 2H), 4.21 (t, 1H), 4.02 (m, 1H), 3.72 (m, 2H), 2.56 (m, 2H), 2.45 (m, 1H), 1.45 (s, 9H).

Synthesis of (R)-3-(9H-Fluoren-9-yloxycarbonylamino)-4-phenylsulfanyl-butyric Acid tert- Butyl Ester (14): A solution of Bu_3P (0.4 mL, 1.62 mmol) was added to 1,1'-(azodicarbonyl) dipiperidine (420 mg, 1.66 mmol) in anhydrous THF (10 mL) at 0ºC and stirred for 30 min, when it turned colorless from yellow. Then **13** (310 mg, 0.78 mmol) and thiophenol (0.17 mL, 1.66 mmol) was added to the reaction mixture under Argon, stirred for 24 h, and concentrated. The concentrated was chromatographed on silica gel (EtOAc/hexanes, 1:10 to 1:4) to provide 265.5 mg (69.5%) of **14**. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.77 (d, 2H), 7.55 (d, 2H), 7.39 (m, 4H), 7.30 (m, 4H), 7.19 (m, 1H), 5.48 (m, 1H), 4.34 (br, 2H), 4.19 (t, 1H), 4.11 (m, 1H), 3.24 (m, 1H), 3.10 (m, 1H), 2.60 (m, 2H), 1.43 (s, 9H). ESI-MS: m/z = 512.1 $[MNa]^+$.

Synthesis of 15: Compound **14** (0.2764 g, 0.565 mmole) was treated with Triethylsilane (0.291 mL, 2.5 mmol) and TFA (0.966 mL, 13 mmol) in CH_2Cl_2 (2.05 mL) for 60 min, and concentrated. The concentrate was chromatographed by silica gel (EtOAc) to provide 200 mg (82%) of **15**. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.74 (d, 2H), 7.54 (d, 2H), 7.39 (m, 4H), 7.29 (m, 4H), 7.19 (m, 1H), 5.43 (m, 1H), 4.36 (br, 2H), 4.17 (m, 1H), 3.96 (m, 1H), 3.24 (m, 1H), 3.08 (m, 1H), 2.77 (m, 2H). ESI-MS: m/z = 433.9 $[MH]^+$.

Synthesis of 16: A solution of **15** (120 mg, 0.277 mmol) in DMF (2.78 mL), PyBOP (180 mg, 0.333 mmol), and DIEA (97 µL, 0.554mmol) was activated for 30 min, then morpholine (24.11 µL, 0.277 mmol) was added to the mixture and stirred for 3 h. The reaction mixture was purified directly on silica gel (EtOAc/hexanes, 4:1) to provide 96.6 mg (69.4%) of **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.74 (d, 2H), 7.58 (d, 2H), 7.39 (m, 4H), 7.32 (m, 4H), 7.17 (m, 1H), 6.17 (m, 1H), 4.36 (m, 2H), 4.20 (m, 1H), 4.08 (m, 1H), 3.53 (m, 6H), 3.33 (m, 4H), 2.82 (m, 1H), 2.53 (m, 1H). ESI-MS: m/z = 503.2 $[MH]^+$.

Synthesis of Compound 17: A mixture of **16** (20 mg, 0.071 mmol), **3** (32 mg, 0.104 mmol), and DIPEA (1.4 mL) were stirred for 36 h and subsequently diluted with EtOAc (10 mL), washed with water (10 mL), brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The concentrate was purfied on silica gel (EtOAc/ hexanes, 3:1) to provide 17. ESI-MS: m/z =566.1 $[M-H]^-$

Synthesis of Compound 4: A mixture of **17** (40 mg, 0.071 mmol) and 1 M BH_3 in THF (1 mL) was stirred for 24 h. Then treated with MeOH (1 mL) , conc.HCl (0.4 mL), and stirred at 80ºC for 3 h, cooled to room temperature, adjusted to pH 10 with saturated Na_2CO_3, diluted with EtOAc (10 mL), washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. The concentrate was purified on silica gel (EtOAc) to provide **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.95 (s, 1H), 7.81 (d, 2H), 7.31 (m, 4H), 7.26 (m, 2H), 7.19 (t, 2H), 7.02 (d, 1H), 6.88 (d, 1H), 4.09 (m, 1H), 3.48 (br, 4H), 3.31 (m, 2H), 2.29 (br, 4H), 2.16 (br, 2H), 1.92 (m, 1H), 1.74 (m, 1H). ESI-MS: m/z = 554.1 $[MH]^+$. $[\alpha]_D^{26}$ = -83 (C 0.27, CH_2Cl_2).

Synthesis of 4-(4-{[2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl]methyl}piperazin-1-yl)-N-[{4-morpholino-1-(phenylsulfanyl)butan-2-yl}amino}-3-[(trifluoromethyl)sulfonyl]phenyl]sulfonul] benzamide (ABT-263, 1): Compound **4** (11.4 mg, 0.0206 mmol) was added to a solution of **2** (30 mg, 0.0685 mmol), EDCI (30 mg, 0.156 mmol), and DMAP (13 mg, 0.106 mmol) in CH_2Cl_2 (0.25 mL) at room temperature. The resulting

mixture was stirred for 36 h and water was added. The organic phase was washed with sat. aq NH_4Cl (2×5 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by chromatography (silica gel, CH_2Cl_2/CH_3OH, 40:1) to provide 9.8 mg (48.9%) of ABT-263. ^1H NMR (300 MHz, DMSO): δ= 8.10 (d, 1H), 7.91 (dd, 1H), 7.70 (d, 2H), 7.27 (m, 9H), 6.94 (d, 1H), 6.84 (d, 2H), 5.78 (s, 1H), 4.04 (m, 1H), 3.52 (m, 4H), 3.30 (m, 6H), 2.62 (s, 2H), 2.39 (m, 12H), 1.93 (m, 3H), 1.79 (m, 1H), 1.43 (m, 2H), 0.98 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ= 154.4, 152.0, 141.2, 138.1, 135.4, 134.8, 132.3, 131.4, 130.0, 129.5, 128.5, 128.3, 127.9, 127.7, 121.2, 118.5, 113.7, 113.1, 109.0, 66.9, 62.9, 54.7, 53.8, 53.1, 50.9, 47.2, 42.1, 40.1, 39.3, 32.9, 31.6, 30.2, 29.5, 26.7, 25.6.
ESI-HRMS: m/z $[MH]^+$ calculated for C47H56ClF3N5O6S3: 974.3028; found: 974.3034. $[\alpha]_D^{26}$ = -69.3 (C 0.38, CH_2Cl_2).

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Experimental information for Fluorescence Polarization Experiment:

MDM-2 concentration: 617 nM
p53flu concentration: 15 nM
BH3I-1 Concentration range: 800 µM to 391 nM
Total solution volume in each well: 60 µL
Buffer: 1x PBS, 5 mM EDTA, 0.5 mM DTT, 0.1% pluronic acid

Method: Add 48 uL of each BH3I-1 concentration to the appropriate wells. Incubate 75 µL 6.17 µM MDM-2 in buffer with 75 µL 150 nM p53flu for 45 minutes. Add 12 µL p53flu/MDM-2 mixture to each well. Incubate 45 minutes before reading.

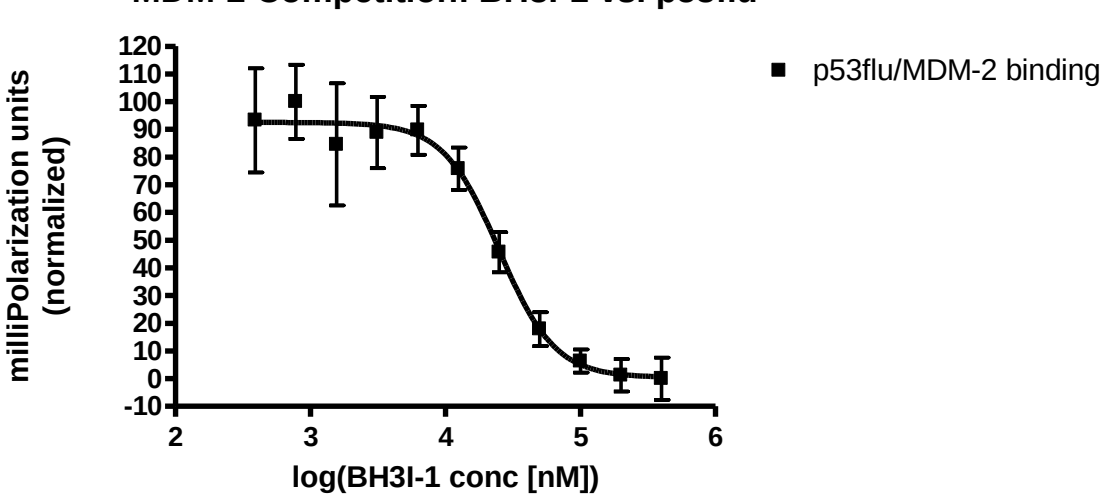
Curve Fitting:

	BH3I-1 Raw Data	BH3I-1 Normalized Data
Sigmoidal dose-response (variable slope)		
Best-fit values		
BOTTOM	6.345	0.4106
TOP	83.72	92.53
LOGEC50	4.398	4.398
HILLSLOPE	-2.109	-2.109
EC50	24996	24996
Std. Error		
BOTTOM	6.250	7.440
TOP	4.455	5.303
LOGEC50	0.08933	0.08933
HILLSLOPE	0.8126	0.8126
95% Confidence Intervals		
BOTTOM	-6.436 to 19.13	-14.80 to 15.63
TOP	74.61 to 92.83	81.68 to 103.4
LOGEC50	4.215 to 4.581	4.215 to 4.581
HILLSLOPE	-3.771 to -0.4473	-3.771 to -0.4473
EC50	16413 to 38068	16413 to 38068
Goodness of Fit		
Degrees of Freedom	29	29
R^2	0.8407	0.8407

Absolute Sum of Squares	6727	9534
Sy.x	15.23	18.13
Data		
Number of X values	11	11
Number of Y replicates	3	3
Total number of values	33	33
Number of missing values	0	0

BH3I-1 Normalized

log(BH3I-1 conc [nM])	p53flu/MDM-2 binding Trial 1	p53flu/MDM-2 binding Trial 2	p53flu/MDM-2 binding Trial 3
2.591760	128.5714	64.28571	86.90476
2.892790	126.1905	82.14286	91.66666
3.193820	126.1905	51.19048	76.19048
3.494850	113.0952	84.52381	69.04762
3.795880	107.1429	78.57143	83.33334
4.096910	85.71429	60.71429	80.95238
4.397940	57.14286	47.61905	32.14286
4.698970	14.28571	29.76191	9.523809
5.000000	14.28571	4.761905	0.000
5.301030	2.380952	10.71429	-9.523809
5.602060	11.90476	2.380952	-14.28571



K_d = 5.34 μM