Correction

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The authors wish to note, “The Western blot data in Fig. 4C (both Upper and Lower) were generated by performing a combined experiment using different samples. An error occurred while compiling the final Fig. 4 when IP: Bax 6A7 and IB: Bax poly bands were duplicated in a few lanes in both panels as they were transfected by common control shGFP RNA. Upon re-evaluation of the data, we identified the error in these two parts of Fig. 4C and, as a result, we have repeated this experiment and are providing a revised Fig. 4. The results of this repeat experiment do not change the conclusion of the published article.” The corrected figure and its legend appear below.
Fig. 4. Bim and NOXA play critical roles in Bax- and Bak-mediated cytotoxicity induced by mda-7/IL-24 and BI-97C1. (A) PC3 cells were infected with Ad.5/3-vec or Ad.5/3-mda-7 for 6 h and then treated with indicated concentrations of BI-97C1 for 48 h and Western blotting was performed. (B) PC3 cells transiently transfected with shGFP, shBim, or shNOXA (Inset) and treated as described in A. Cell death was determined using Trypan blue dye exclusion. SD (n = 3). *P < 0.01. (C) Upper and Lower: PC3 cells were treated as mentioned in B, after which cell lysates were subjected to determine Bax conformation analysis as indicated in Materials and Methods. (D) Upper: PC3 were treated as mentioned in A and Western blotting was performed. Lower: PC3 cells transiently transfected with pcDNA3.1 or pcDNA3.1DNPERK as described in Materials and Methods were treated as mentioned in A and cell death was determined using Trypan blue dye exclusion assay (Lower) and Western blotting (Upper) was performed. SD (n = 3). *Significantly lower vs. pcDNA3.1 transfected cells (P < 0.01).
Apogossypol derivative BI-97C1 (Sabutoclax) targeting Mcl-1 sensitizes prostate cancer cells to mda-7/IL-24–mediated toxicity


Limited options are available for treating patients with advanced prostate cancer (PC). Melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24), an IL-10 family cytokine, exhibits pleiotropic antitumor activities without adversely affecting normal cells. We previously demonstrated that suppression of the prosurvival Bcl-2 family member, myeloid cell leukemia-1 (Mcl-1), is required for mda-7/IL-24–mediated apoptosis of prostate carcinomas. Here we demonstrate that pharmacological inhibition of Mcl-1 expression with the unique Apogossypol derivative BI-97C1, also called Sabutoclax, is sufficient to sensitize prostate tumors to mda-7/IL-24–induced apoptosis, whereas ABT-737, which lacks efficacy against Mcl-1, fails to sensitize mda-7/IL-24–mediated cytotoxicity. A combination regimen of tropism-modified adenovirus delivered mda-7/IL-24 (Ad.S5.3/3mda-7) and BI-97C1 enhances cytotoxicity in human PC cells, including those resistant to mda-7/IL-24 or BI-97C1 alone. The combination regimen causes autophagy that facilitates NOXA- and Bim-induced and Bak/Bax-mediated mitochondrial apoptosis. Treatment with Ad.S5.3/3mda-7 and BI-97C1 significantly inhibits the growth of human PC xenografts in nude mice and spontaneously induced PC in Hi-myc transgenic mice. Tumor growth inhibition correlated with increased TUNEL staining and decreased Ki-67 expression in both PC xenografts and prostates of Hi-myc mice. These findings demonstrate that pharmacological inhibition of Mcl-1 with the Apogossypol derivative, BI-97C1, sensitizes human PC to mda-7/IL-24–mediated cytotoxicity, thus potentially augmenting the therapeutic benefit of this combinatorial approach toward PC.

Despite improvements in detection and treatment, prostate cancer remains the most common cancer and the second leading cause of cancer-related death in men in the United States (1). Because PC is a relatively slow-growing disease, repeated systemic gene therapy applications alone or in combination with antitumor chemotherapeutic agents over the lifespan of the patient may be required (1).

Melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24), a unique member of the IL-10-related cytokine family, exhibits nearly ubiquitous antitumor properties in vitro and in vivo (2–4), which culminated in its successful entry into the clinic where safety and clinical efficacy was achieved (5). Unique therapeutic aspects of adenovirus delivered mda-7/IL-24 (Ad.mda-7) lie in its selective induction of apoptosis in cancer cells, profound antitumor “bystander” effect exerted by the secreted MDA-7/IL-24 protein, and the ability of this unique cytokine to inhibit angiogenesis and provoke a potent antitumor immune response (2). Upon ectopic expression of mda-7/IL-24 by an adenovirus, MDA-7/IL-24 interacts with the endoplasmic reticulum (ER) chaperone protein BiP/GRP78 and initiates an unfolded protein response in tumor cells that promotes apoptosis (6). Recently, we reported that in PC, ovarian, and malignant gliomas, mda-7/IL-24–induced ER stress response produced apoptosis by suppressing expression of the antiapoptotic protein myeloid cell leukemia-1 (Mcl-1) (4, 7, 8).

In view of the essential roles of antitumor Bcl-2 family proteins in tumorigenesis and chemoresistance, efforts are focused on developing small molecule inhibitors of Bcl-2 family proteins as potential therapeutics for cancer (9). Unfortunately, due to the unique structure of Mcl-1 compared with Bcl-2 and Bcl-xL (9), currently used inhibitors such as ABT-737 or its clinical counterpart, ABT-263, display limited affinity for Mcl-1 (10) (Figs. S1 and S2). Using NMR binding assays and computational docking studies, we have recently identified a series of unique Apogossypol derivatives, compound 3 (BI-79D10) and compound 11 (BI-97C1; Sabutoclax), with pan-Bcl-2 inhibitor potency (11, 12). BI-79D10 binds to Bcl-xL, Bcl-2, and Mcl-1 with IC50 values of 190, 360, and 520 mM/L, respectively (13). BI-97C1 is an optically pure individual Apogossypol derivative that retains all of the properties of BI-79D10 along with superior in vitro and in vivo efficacy (11).

Because Mcl-1 is overexpressed in the majority of PCa (4), we hypothesized that suppressing Mcl-1 by treating human PC cells with BI-97C1 would sensitize them to mda-7/IL-24–mediated cytotoxicity. Our data suggest that treatment with the combination regimen of mda-7/IL-24 and BI-97C1 induces autophagy that facilitates apoptosis in association with up-regulation of NOXA, accumulation of Bim, and activation of Bak and Bax. Treatment with mda-7/IL-24 and BI-97C1 inhibited the growth of PC xenografts and suppressed PC development in an immunocompetent transgenic mouse model of PC.

Results Mcl-1 Promotes Resistance to mda-7/IL-24–Mediated Apoptosis of Human PC Cells. Genetic and pharmacological inhibitors of Mcl-1 were used to determine if suppression of Mcl-1 expression sensitizes PC cells to mda-7/IL-24–mediated cytotoxicity. To facilitate delivery of mda-7/IL-24 to PC cells, which frequently contain reduced levels of Coxsackie Adenovirus Receptors, we used a tropism-modified chimeric 5/3 adenovirus, Ad.S5.3/3mda-7 (14), siRNA-mediated reduction of Mcl-1 significantly sensitized PC3 cells to Ad.S5.3/3mda-7–induced apoptosis even at low doses (50 pfu/cell) (Fig. 1A). On the other hand, treatment of PC3 cells with BI-79D10 or BI-97C1 resulted in reduced viability with an...
IC\textsubscript{50} value of 750 nM for both compounds (Fig. 1B), whereas that of ABT-737 was 12 μM.

To evaluate the combinatorial effect of Mcl-1 inhibition and mda-7/IL-24, we treated PC3, DU-145, M2182, and normal SV40 T-antigen-immortalized prostate epithelial cells (P69) with a sublethal dose of Ad.5/3-mda-7 (50 pfu/cell) and BI-79D10 at either 350 nM (at which concentration it inhibits Bcl-2 and Bcl-x\textsubscript{L}) or 520 nM (at which level it inhibits Mcl-1) (13). Treatment of PC3, DU-145, or M2182 cells with 350 nM of BI-79D10 in combination with 50 pfu/cell of Ad.5/3-mda-7 had a minimal effect on promoting apoptosis, whereas infection with Ad.5/3-mda-7 (50 pfu/cell) and treatment with 520 nM of BI-79D10 profoundly promoted apoptosis (Fig. 1C). In contrast, the combination exerted little to no toxicity in normal P69 cells, indicating cancer-specific activity (Fig. 1C). Furthermore, the combination also showed a greater than additive induction of apoptosis in mda-7/IL-24-resistant DU-145 cells that stably overexpress Bcl-2, Bcl-x\textsubscript{L}, or Mcl-1 (Du-bcl-2, Du-bcl-x\textsubscript{L}, and Du-Mcl-1-8, respectively) (4, 15) (Fig. 1D). A similar enhanced induction of apoptosis was observed in these therapy-resistant PC cell lines after treatment with Ad.5/3-mda-7 and BI-97C1 (Fig. 2A and B).

BI-97C1 is an optically purified small molecule suitable for clinical use (11). Induction of apoptosis was observed with as low as 10 pfu/cell of Ad.5/3-mda-7 in combination with BI-97C1 (Fig. 24). Similarly, BI-97C1 potently induced apoptosis of PC3 cells with an IC\textsubscript{50} of ~500 nM in combination with Ad.5/3-mda-7 (50 pfu/cell) (Fig. 2B). A time course analysis revealed that 48 h was required for the combination of Ad.5/3-mda-7 and BI-97C1 to significantly promote apoptosis of human PC cells (Fig. 2C).

Treatment of human PC cells with Ad.5/3-mda-7 and BI-97C1 resulted in PARP cleavage, activation of Caspase-3, cytosolic release of cytochrome c and Second mitochondrial-derived activator of caspase (Smac)/direct inhibitor of apoptosis protein-binding protein with low pi (DIABLO) as well as inactivation of cell proliferation signals, i.e., AKT and ERK (Fig. 2 D–F).

Bim and NOXA Activity Are Required for BI-97C1/mda-7-IL-24-Mediated Bak- and Bax-Dependent Apoptosis. To analyze the involvement of mitochondrial pathway of apoptosis (16), PC3 cells were treated with BI-97C1 (520 nM) and Ad.5/3-mda-7 (50 pfu/cell) and expression of Bak/Bax conformational changes (detected by immunoprecipitation using antibodies that specifically
recognize activated Bak or Bax) (16) and intracellular Bax localization were assessed. Treatment of PC3 with BI-97C1 and Ad.5/3-nda-7 moderately elevated expression of total Bax and Bak compared with either single agent alone (Fig. 3A). Treatment with the combination of BI-97C1 and Ad.5/3-nda-7 induced Bak or Bax conformational changes significantly greater than either agent alone (Fig. 3B). Similarly, combination treatment dramatically increased mitochondrial translocation of Bax (Fig. 3B) and Bak/Bax association (Fig. 3C). Transient shRNA-mediated knockdown of Bax and Bak promoted resistance to BI-97C1/nda-7/IL-24–mediated toxicity (Fig. 3D).

Considering the potential roles that the BH3-only proteins, NOXA and Bid, play in apoptosis induced by various stimuli (17), expression and/or phosphorylation of these proteins were examined following combination treatment in PC3 cells. After 48 h of combined treatment, we observed a significant increase in NOXA expression, Bid accumulation, and suppression of Bid and p-Bad expression compared with treatment with either agent alone (Fig. 4A). When PC3 cells were transiently transfected with shRNA against NOXA or Bim, a significant degree of resistance toward BI-97C1/nda-7/IL-24–mediated apoptosis was observed (Fig. 4B and C). Earlier, we reported that nda-7/IL-24 induces ER stress by activation of PKR-like ER-localized eIF2α kinase (PERK) (2). We observed that ER stress generated by nda-7/IL-24 was enhanced by combined treatment with BI-97C1 (Fig. 4D, Upper). Interestingly, inhibition of PERK by a dominant negative construct in PC3 cells protected them from cytotoxicity of the combination treatment accompanied by a reduction in Bid accumulation and PARP cleavage, but with minimal effects on elevated NOXA expression (Fig. 4D, Lower).

**Induction of Autophagy by the Combination of nda-7/IL-24 and BI-97C1 Precedes Apoptosis.** Autophagy is usually initiated in response to increased metabolic requirements during cellular stress induced by therapeutic treatments (18). For that reason, we evaluated autophagy in response to treatment with nda-7/IL-24 and BI-97C1. Induction of LC3 in autophagic vacuoles was determined by transient transfection of PC3 cells with a baculovirus-expressing RFP fused with LC3. Treatment with BI-97C1 or nda-7/IL-24 alone resulted in a significant increase in the LC3-RFP signal and LC3-II expression that was further augmented by combination treatment (Fig. 5A). Because nda-7/IL-24 selectively targets transformed cells, we transfected MEFs with a human pDNA3.1-H-ras construct. Human H-ras transfected Bax−/− MEFs were resistant to nda-7/IL-24 + BI-97C1–induced cytotoxicity compared with wild-type MEFs, indicating that cell death induced by the combination is due to apoptosis, not autophagy (Fig. 5B). On the other hand, we also detected a slight induction of autophagy in Bax−/− MEFs compared with wild-type MEFs following combination treatment (Fig. S3C). These results suggest that autophagy induced by the combination treatment might protect cells from initial stress followed by a switch to apoptosis at a later time point. Furthermore, the increase in LC3-RFP signal and expression of LC3-II was maximum at 24 h and decreased by 48 h of post-treatment, whereas apoptosis, detected by TUNEL assay and PARP cleavage, was evident at 48 h, but not at 24 h post-treatment (Fig. 5C and G). Notably, we observed that down-regulation of the essential autophagy genes Beclin-1 or ATG5 (to a lesser extent) (but not ATG7) by shRNA reduced the cytotoxicity of the combination effect (Fig. 5D). This finding was supported by the observation that 24 h after combination treatment, the interaction between Mc-I and Beclin-1 was disrupted (Fig. 5E). The switch between autophagy and apoptosis is a complex process that is currently poorly understood (18). Previously, it was found that in a cellular model system where autophagy precedes apoptosis, IL-3 depletion of Ba/F3 cells caused caspase-mediated cleavage of Beclin-1 and PI3KC3 (18). Interestingly, cleavage of Beclin-1 during apoptosis not only inactivates its autophagic function, but also generates a Beclin-1-derived fragment that translocated to the mitochondria and enhanced the release of cytochrome c and hence augmented apoptosis (18). We also observed that 48 h after combined treatment, activation of caspase-3 was associated with cleavage of Beclin-1 (Fig. 5F). When caspase-3 activity was inhibited using a pharmacological inhibitor, PC3 cells developed resistance toward the cytotoxicity induced by the combination of Ad.5/3-nda-7 and BI-97C1 (Fig. 5F).

**Combination Treatment with nda-7/IL-24 and BI-97C1 Potentiates Inhibition of Human Prostate Tumor Growth in Vivo in Immune Deficient and Immune Competent Animals.** To test efficacy in vivo, we performed studies that used two animal models, one using immune deficient athymic nude mice xenografted with human prostate tumor cells (M2182) (11) and one using a spontaneous immunocompetent transgenic mouse model of PC (the Hi-Myc mouse) (19). Initial studies were performed in nude mice bearing...
s.c. M2182 human prostate tumors established on both flanks that stably express luciferase (M2182-Luc). Mice were treated when tumor size was ∼100 mm³, using a suboptimal dose of 0.5 × 10⁸ pfu/injection of Ad.5/3-vec or Ad.5/3-mda-7 (seven injections). BI-97C1 was injected i.p. (11) either at 1 mg/kg or 3 mg/kg 3 times per week for the 3-wk duration of the study (nine injections). Tumor growth was markedly inhibited by combination treatment compared with treatment with either agent alone (Fig. 6 A). Combined treatment of Ad.5/3-mda-7 and BI-97C1 (3 mg/kg) significantly reduced Ki-67 expression and increased TUNEL signal compared with treatment with either agent alone (Fig. 6 B). BI-97C1 was observed to be nontoxic to athymic nude mice (NCRnu/nu) when injected i.p. (up to 10 mg/kg) or when injected i.v. (25 mg/kg) (Fig. S4 and Tables S1 and S2).

Next, we tested the efficacy of Ad.5/3-mda-7 and BI-97C1 in a spontaneous model of PC, the Hi-Myc mouse (19). For optimal therapeutic outcome for adenocarcinoma, we began treatment at 22 wk of age. The ability to systemically deliver adenoviruses is
The combination regimen of \textit{mda-7/IL-24} and BI-97C1 additively inhibits prostate tumor growth in athymic and immunocompetent mice and a hypothetical model of the combination regimen. (A) Athymic nude mice bearing M2812 xenografts stably expressing luciferase in both right and left flanks were treated as indicated in Materials and Methods. Tumor growth was visualized by bioluminescence measured by the Xenogen imaging system. (B) At the end of the study in A, tumors were harvested and stained for Ki-67, \textit{mda-7/IL-24} and TUNEL. (C) Left: The prostatic region of male Hi-Myc mice were sonoporated for 10 min following tail-vein injection of the indicated complement-treated MB/Ad complexes and treated as described in Materials and Methods. At the end of the experiment, the mice were killed and the prostates were harvested, weighed, and photographed. Right: Hi-Myc mice were treated as described above and proliferative index was measured by Ki-67 staining of paraffin-embedded sections of prostate from treated mice as described in D. SD (n = 7). (D) Hi-Myc mice were treated as described in C. Paraffin-embedded sections obtained from the prostate were analyzed by immunohistochemistry using anti-Ki-67 and anti-MDA-7/IL-24 antibodies. Apoptosis in the prostate sections was detected by TUNEL assay. (E) Hypothetical model of action for the combination regimen of \textit{mda-7/IL-24} and BI-97C1 (Sabutoclax) in PC cells.

Discussion

Even though \textit{mda-7/IL-24} can induce apoptosis in diverse human cancers, it is apparent that some subsets of cancer cells are relatively resistant to this therapy (2). One mode of resistance to \textit{mda-7/IL-24}–based therapies occurs through elevated expression of either Mcl-1 or Bcl-x\textsubscript{L}, which promotes resistance to \textit{mda-7/IL-24}–induced apoptosis (4, 15). Exposure of PC cells to \textit{mda-7/IL-24} and BI-97C1 cooperate to induce Bim–dependent apoptosis. Because the Bcl-2/Bcl-x\textsubscript{L}, or Mcl-1, to \textit{mda-7/IL-24}–dependent apoptosis. Because the Bcl-2/Bcl-x\textsubscript{L} inhibitor ABT-737/ABT-263 does not inhibit Mcl-1, treatment with ABT-737 failed to sensitize PC cells to \textit{mda-7/IL-24}–induced apoptosis (Fig. 5.3 A and B).

Based on the present data, a hypothetical model is proposed by which \textit{mda-7/IL-24} and BI-97C1 cooperate to induce Bim- and NOXA-dependent intrinsic apoptosis (Fig. 6E). Although the mechanism by which Bim promotes apoptosis remains unclear, it has been shown that up-regulation of Bim results in the release of proapoptotic multidomain Bcl-2 family members Bak and Bax. Once activated, Bak and Bax permeabilize the mitochondrial membrane, thereby facilitating the release of apoptotic inducing factors such as cytochrome \textit{c}, apoptosis inducing factor, and Smac/DIABLO (16). Evidence for direct activation of Bak by Bim also exists (22). Similarly, inhibition of Mcl-1 by treatment with BI-97C1 results in up-regulation of NOXA, which may lead to not only activation of Bak (16), but also the ability of Mcl-1 to efficiently sequester Bim (23). Alternatively, activation of Bak/Bax by BI-97C1 and \textit{mda-7/IL-24} may inhibit Bcl-2 and Bcl-x\textsubscript{L}. It is important to note that in the present combination studies, the concentration of BI-97C1 used was 520 nM, which also inhibits Bcl-2 and Bcl-x\textsubscript{L}. Our data also suggest that at early times, autophagy induction by the combination may provide an initial cytoprotective response, based on evidence that knocking down Beclin-1 or ATG5 expression protected cells from \textit{mda-7/IL-24}/BI-97C1 toxicity. On the other hand, at later times (e.g., 48 h), caspase-mediated cleavage of Beclin-1 may instead augment apoptosis rendered by the combination regimen (Fig. 6E). In summary, the present study highlights the noteworthy potential of a combinatorial approach involving \textit{mda-7/IL-24}, a broad-acting anticancer gene, and BI-97C1, which targets Mcl-1, to sensitize PC to \textit{mda-7/IL-24}–mediated cytotoxicity, thereby enhancing therapeutic efficacy. Based on the safety profile of the individual agents and the fact that \textit{mda-7/IL-24} is now in the
Materials and Methods
Cells, Stable Clones, Culture Conditions, and Adenoviral Constructs. P69 and human PC cells M2182, DU-145, and PC3, wild type, and Bax−/−/Bak−/− MEF were cultured as described (15). DU-145 clones stably overexpressing Bcl-2, Bcl-xL, Mcl-1, and M2182-Luc were generated as described (4, 15, 11).

Assessment of Cell Viability, Cell Death, and Apoptosis. Apoptotic cells were identified and quantified by Annexin V-fluorescein isothiocyanate staining and DAPI staining. A total of 90,000 cells were analyzed per condition. To confirm that Bcl-2, Bcl-xL, Mcl-1, and A20 were overexpressed in each of the stably transfected clones, Western blot analysis was performed using Mcl-1, BiP/GRP78, Bak, Phosho-PERK, α-actin, and Bcl-xL antibodies.

Transient Transfections. Transfections were carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol with different plasmids for 24 h before treatment. 100 nm SMARTpool Mcl-1, Bcl-xL siRNAs, or control siRNA (scControl) (Dharmacon), siATG5, siBeclin-1, or siATG7 (Santa Cruz Biotechnology) (24, 25).

Immunoprecipitation and Immunoblotting. Immunoprecipitation and immunoblotting were performed as previously described (4). The primary antibodies used in this study were as follows: Mcl-1, BiP/GRP78, Smo/DIABLO, GADD153, cytokrome c, NOXA (Santa Cruz Biotechnology), PARP, Bcl-xL, and p-Akt (Thr-183/Tyr-202). The secondary antibodies were horseradish peroxidase-conjugated goat anti-mouse and anti-rabbit IgG (Santa Cruz Biotechnology).

Detection of Bak and Bak Activation. Detection of Bak and Bak activation was performed by flow cytometry as described (16). For Bak translocation studies, mitochondrial and cytosolic fractions were prepared by using the mitochondria fractionation kit (Active Motif) according to the manufacturer's protocol.

Human PC Xenografts. M2182-Luc (1 × 106) cells were injected s.c. in the left and right flank of male athymic nude mice as described (11). After establishing visible tumors of ~100 mm3, intratumoral injections of Ad-S3/3 or Ad-S3/3-mda-7 were given to the tumors on the left flank at a dose of 0.5 × 107 pfu in 100 μL. The injections were given 3 times the first week and then 2 times/wk for two more weeks for a total of seven injections. BI-97C1 (dissolved in ethanol/Cremophor EL/saline = 10:10:80) was injected i.p. at a subtoxic level (11) either at 1 mg/kg or 3 mg/kg 3 times per week throughout the study (total nine injections).

Preparation of MBs, Ultrasound Platform, and UTMD. Hi-Myc (19) mice were obtained from the Mouse Repository at NIC and genotyping was done based on the primer information available in NIC. Preparation of MBs followed by UTMD for delivery of mda-7/IL-24 expressing Ads has been described (20). The mice were divided into four groups: (i) Ad-S3/3-vec, (ii) Ad-S3/3-vec + BI-97C1, (iii) Ad-S3/3-mda-7, and (iv) Ad-S3/3-mda-7 + BI-97C1. Ultrasound (sonoporation) was performed (20) in the ventral side of mice in the posttatic area. BI-97C1 was administered i.p. at a dose level of 3 mg/kg 3 times per week for the duration of the study (total nine injections).

Immunohistochemical Staining. For immunohistochemical analysis, formalin-fixed and paraffin-embedded specimens of 3- to 4-μm thickness were sectioned and stained with anti-MDA-7/IL-24 and K-67 as described (4).

Monitoring Autophagy. For detection of autophagy, PC3 cells were infected with BacMam LC3B-RFP baculovirus reagent (PremoTM Autophagy Sensor Kit, Invitrogen). For staining cytoskeleton, MAP4-GFP (Cellular Lights MAP4-GFP, Invitrogen) transduction reagents were used and after treatment samples were analyzed by confocal laser-scanning microscopy (Zeiss 510 Meta confocal imaging system).

Molecular Modeling and NMR Studies. A 15N-labeled Mcl-1 sample was prepared and NMR measurements of Mcl-1 and compound interactions were carried out using a series of 1H-15N HSQC spectra as described (9). Molecular modeling studies were conducted on a Linux workstation and a 64 3.2-GHz CPUS Linux cluster. Docking studies were performed using the crystal structure of Mcl-1 in complex with a BH3 mimetic ligand (Protein Data Bank code 2NLT).

Statistical Analysis. Data are presented as the mean ± SD and analyzed for statistical significance using one-way ANOVA followed by Newman-Keuls test as a post hoc test. P < 0.05 was considered significant.

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