Anacardic Acids from *Knema hookeriana* as Modulators of Bcl-xL/Bak and Mcl-1/Bid Interactions

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Supporting Information

**ABSTRACT:** Proteins of the Bcl-2 family are key targets in anticancer drug discovery. Disrupting the interaction between anti- and pro-apoptotic members of this protein family was the approach chosen in this study to restore apoptosis. Thus, a biological screening on the modulation of the Bcl-xL/Bak and Mcl-1/Bid interactions permitted the selection of *Knema hookeriana* for further phytochemical investigations. The ethyl acetate extract from the stem bark led to the isolation of six new compounds, three acetophenone derivatives (1−3) and three anacardic acid derivatives (4−6), along with four known anacardic acids (7−10) and two cardanol (11, 12). Their structures were elucidated by 1D and 2D NMR analysis in combination with HRMS experiments. The ability of these compounds to antagonize Bcl-xL/Bak and Mcl-1/Bid association was determined, using a protein−protein interaction assay, but only anacardic acid derivatives (4−10) exhibited significant binding properties, with *K*_i values ranging from 0.2 to 18 μM. Protein−ligand NMR experiments further revealed that anacardic acid 9, the most active compound, does not interact with the anti-apoptotic proteins Bcl-xL and Mcl-1 but instead interacts with pro-apoptotic protein Bid.

The Bcl-2 family of proteins, comprising both anti- and pro-apoptotic members, are key players in apoptosis. The anti-apoptotic proteins disable the pro-apoptotic ones by binding in a hydrophobic cleft through protein−protein interactions. The overexpression of anti-apoptotic proteins such as Bcl-xL and Mcl-1 plays a decisive role in cancer development and can be correlated with resistance to cancer therapeutics.† These proteins are considered to be challenging targets for the development of novel anticancer treatments, but it was shown that selective inhibition of Bcl-xL results in apoptosis “escape” through the Mcl-1 pathway.‡ Thus, identification of small molecules capable of binding to the hydrophobic cleft of both anti-apoptotic proteins Bcl-xL and Mcl-1, releasing the pro-apoptotic proteins such as Bax, Bad, or Bid, and ultimately restoring apoptosis, is a promising but challenging strategy in the fight against cancer.

Recently, an extensive biological screening with 9000 plant extracts was conducted to investigate small molecules modulating Bcl-xL/Bak interactions, leading to the isolation of new active compounds such as meiogynin A§ and kingianin G.¶ A second screening on Mcl-1/Bid interactions of the most active extracts on Bcl-xL/Bak interactions, along with 480 additional plant extracts led to the isolation of the dual inhibitors ferrugineic acids B, C, and J and a biologically active ethyl acetate extract from the *Knema hookeriana* Warb. (Myristicaceae).¶ The stem bark extract of this species displayed high-affinity binding to Bcl-xL and Mcl-1 (97% and 92% at 10 μg/mL, respectively) and prompted the search for secondary metabolites that regulate those anti-apoptotic proteins.

*Knema hookeriana* Warb. (Myristicaceae) is a tree that can be found in Indonesia, Malaysia, Singapore, and Thailand. Traditionally, the leaves of this plant are used as a stomach remedy, and the sap is also useful for dying casting nets and cloths.¶ Additionally, this plant is used as a preservative surface coating material for valuable wooden, porcelain, and metallic wares.¶ Only one chemical investigation to find antinematodal components has been published.¶ However, other *Knema*

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specimens have been employed as traditional medicines to treat sores, pimples, rheumatism, and cancer, and some of these activities have been verified in several studies. For example, all the compounds found in *Knema glomerata* exhibited moderate cytotoxic activities (ranging from 2 to 35 μM) against the three tumor cell lines A-549, MCF-7, and HT-29. According to previous phytochemical investigations, cardanol, acetylenes, flavonoids, acetophenones, lignans, acylphloroglucinols, acyclic sorcinols, and anacardic acids are the main compounds isolated from the *Knema* genus. The anacardic acids were found to be the most biologically active compounds in cancer assays, via inhibition of histone acetyltransferase and NFκB kinase, as well as reducing the expression of various gene products that mediate survival, such as Bcl-2 and Bcl-xL.

In ongoing efforts aimed at the search for plant-derived pro-apoptotic agents by their binding to Bcl-xL and Mcl-1, the isolation and structural characterization of six new compounds, three acetylenone derivatives (1–3) and three anacardic acid derivatives (4–6), and four known anacardic acids (7–10) and two cardanols (11, 12) from *K. hookeriana* were delineated in this study. These metabolites were evaluated for modulating the interaction potential of the anti-apoptotic proteins with pro-apoptotic proteins using a protein–protein interaction bioassay. Binding mechanisms of compound 9 with Bcl-xL and Mcl-1 and then with Bid were also scrutinized through protein interaction bioassay.

## RESULTS AND DISCUSSION

Preparative C18 HPLC applications of the ethyl acetate extract of the *K. hookeriana* stem bark yielded 12 phenolic compounds (Figure 1).

The HRESIMS data of compound 1 exhibiting a protonated [M + H]+ ion at m/z 307.2270 (calcd [M + H]+, m/z 307.2268) together with its 13C NMR data led to its molecular formula being established as C19H30O3, suggesting the presence of a tetrasubstituted aromatic ring comprising 10 methylene groups, instead of 12 for kneglomeratanone B. The superimposable resonances at δH 2.81 and δC 115.5) and C-5 (δC 110.8). The correlation with the acetylenone protons (δH 2.62) to C-1 supported the presence of a tetrasubstituted aromatic ring comprising 10 methylene groups, instead of 12 for kneglomeratanone B. Thus, compound 1 (khoquerianone A) was identified as 2,4-dihydroxy-6-undecylacetophenone.

Compounds 2 and 3 were respectively assigned the molecular formulas C19H28O3 and C21H32O3, on the basis of the superimposable resonances at δH 2.81 and δC 115.5) and C-5 (δC 110.8). The correlation with the acetylenone protons (δH 2.62) to C-1 supported the presence of a tetrasubstituted aromatic ring comprising 10 methylene groups, instead of 12 for kneglomeratanone B. Thus, compound 1 (khoquerianone A) was identified as 2,4-dihydroxy-6-undecylacetophenone.

An alkyl chain was connected to the aromatic ring at C-6 (δC 148.1) based on the HMBC correlations between H-1′ (δH 8.1, δC 148.1) and C-1 (δC 115.5) and C-5 (δC 110.8). The correlation from the acetylenone protons (δH 2.62) to C-1 supported the location of the acetyl group at C-1. The 1D and 2D NMR data of compound 1 displayed similarities with those of kneglomeratanone B, indicating the same resaccharonone moiety, substituted with an aliphatic side chain comprising 10 methylene groups, instead of 12 for kneglomeratanone B. Thus, compound 1 (khoquerianone A) was identified as 2,4-dihydroxy-6-undecylacetophenone.

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acetophenone and (Z)-2,4-dihydroxy-6-(undec-6-en-1-yl)-aceto-phenone and (Z)-2,4-dihydroxy-6-(tridec-8-en-1-yl)-aceto-phenone, respectively, and assigned the trivial names knoeke-rianic acid (B) and C (3).

The HRESIMS data of 5 showed a protonated [M + H]+ ion at \( m/z \) 291.1958 (calcld [M + H]+, 291.1955). In conjunction with the 13C NMR data, the molecular formula of 5 was established as C18H26O3, indicating six indices of hydrogen deficiency. The spectroscopic data of compound 5 were similar to those of anacardic acids and compound 4, which was indicative of 5 being structurally based on the anacardic acid scaffold possessing one double bond in the side chain. The Z configuration and position of the olefinic functionality at C-6’ were established as for compounds 2 and 3. Thus, compound 5 was identified as (Z)-2-hydroxy-6-(undec-6-en-1-yl)benzoic acid and given the trivial name knoeke-rianic acid B (5).

The HRESIMS data of compound 6 producing a protonated molecular ion at \( m/z \) 327.1974 (calcld [M + H]+, 291.1955) along with 13C NMR analysis permitted its molecular formula to be assigned as C17H22O4, indicating six indices of hydrogen deficiency. The spectroscopic data of compound 6 were similar to those of anacardic acids and compound 5, which was indicative of 6 being structurally based on the anacardic acid scaffold possessing one double bond in the side chain. The Z configuration and position of the olefinic functionality at C-6’ were established as for compounds 2 and 3. Thus, compound 6 was identified as 2-hydroxy-6-(tridec-8-en-1-yl)benzoic acid and given the trivial name knoeke-rianic acid C (6).

The structures of the seven other compounds were elucidated by comparing their observed and reported spectroscopic data and ozonolysis followed by reduction using dimethyl sulfide for compounds possessing acyclic double bonds. Their structures were assigned as the 3-tridecylphenol (11), (Z)-3-(tridec-8-en-1-yl)phenol (12), anagigantic acid (7), (Z)-2-hydroxy-6-(tridec-8-en-1-yl)benzoic acid (8), 2-hydroxy-6-tridecylbenzoic acid (9), and (Z)-2-hydroxy-6-(pentadec-10-en-1-yl)benzoic acid (10). In addition, compound 4, knoeke-rianic acid A, was previously synthesized but never reported as a natural product.

The ability of compounds 1–12 to antagonize Bcl-xL/Bak and Mcl-1/Bid association was determined using a fluorescence polarization assay adapted from Qian and co-workers (Table 1, Figure S27, Supporting Information). The principle of this biological test is based on the competition of interaction between a small-molecule inhibitor and a fluorescent pro-apoptotic peptide (BH3 domain of BAK protein or BID protein) with the anti-apoptotic proteins Bcl-xL and Mcl-1. Results are given by the Ki value, the concentration corresponding to 50% of the binding of the labeled reference compound, and corrected for experimental conditions according to Cheng and Prusoff.

None of the compounds with a cardanol (11, 12) or resacetophenone (1–3) skeleton were able to displace Bcl-xL/Bak and Mcl-1/Bid interactions. On the contrary, anacardic acid derivatives (4–10) are highly potent antagonists of both Bcl-xL/Bak and Mcl-1/Bid association. Therefore, the acid functional group seems essential for inducing activity that is modulated by the length of the side chain. Compounds with a C13 chain (8, 9) are active at a submicromolar range, while compounds with a C8 (6), C9 (4), C11 (5, 7), or C15 (10) chain have binding affinities in the micromolar range. However, the binding activity does not seem to be influenced by the degree of unsaturation or the presence of a terminal phenyl group. In addition, despite their significant binding affinities (these are some of the most potent natural compounds reported so far), they were not found to be cytotoxic on the HCT-116 cell line.

Protein–ligand NMR experiments were carried out in order to confirm that the promising activities of anacardic acid derivatives were due to a direct binding to Bcl-xL and Mcl-1. Thus, HSQC 1H–13C experiments of Bid (pro-apoptotic partner of Mcl-1) were performed alone or in combination with 1.5 equiv of compound 9, the most potent compound. The protein HN resonances were not disturbed upon addition of the ligand, indicating that compound 9 does not bind to these anti-apoptotic proteins (Figures S25 and S26, Supporting Information). In addition, 1D 1H NMR and 2D 1H–13C TOCSY experiments of Bid (pro-apoptotic partner of Mcl-1) alone (at 5 mM: see Figure S26, Supporting Information) or in combination with increasing amounts (0.25, 0.5, 1.0, 2.0, and 5.0 equiv) of compound 9 were investigated. The 1H NMR spectra (Figure 3) clearly show that the broadness of the Bid signals increases significantly and proportionally to the concentration of 9 (at higher concentrations, compound 9 precipitates in the NMR tube). These results indicate either a nonspecific interaction between Bid and 9, via the formation of micelles, or a direct interaction of high affinity between both partners.

<table>
<thead>
<tr>
<th>compound</th>
<th>Bcl-xL/Bak ( K_i ) (( \mu \text{M} ))</th>
<th>Mcl-1/Bid ( K_i ) (( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;23</td>
<td>&gt;33</td>
</tr>
<tr>
<td>2</td>
<td>&gt;23</td>
<td>&gt;33</td>
</tr>
<tr>
<td>3</td>
<td>&gt;23</td>
<td>&gt;33</td>
</tr>
<tr>
<td>4</td>
<td>&gt;23</td>
<td>17.7 ± 3.1</td>
</tr>
<tr>
<td>5</td>
<td>3.2 ± 0.1</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>16.3 ± 0.5</td>
<td>3.7 ± 2.0</td>
</tr>
<tr>
<td>7</td>
<td>1.2 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td>5.7 ± 0.6</td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td>11</td>
<td>&gt;23</td>
<td>&gt;33</td>
</tr>
<tr>
<td>12</td>
<td>&gt;23</td>
<td>&gt;33</td>
</tr>
<tr>
<td>meiogynine A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 0.3</td>
<td>8.6 ± 0.5</td>
</tr>
<tr>
<td>ABT-737&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57 ± 10 ( \text{nM} )</td>
<td>&gt;33</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reference compounds. <sup>b</sup>Results are expressed in IC<sub>50</sub> values.
From the Bid assignment and these TOCSY experiments (Figure S26, Supporting Information), it is evident that 9 interacts specifically with the last five residues of the Bid C-terminal, namely, Asp16, Ser17, Met18, Asp19, and Arg20 (Figure 5).

In conclusion, the chemical study of K. hookeriana led to the identification of three new resacetophenones (1−3), two new anacardic acid derivatives (5, 6), an anacardic acid (4) obtained naturally for the first time, four known anacardic acids, and two known cardanol. Of this alkylphenol series, only the anacardic acids exhibited strong disruption of the anti- (Bcl-xL, Mcl-1) protein Bid and not for anti-apoptotic proteins Bcl-xL and Mcl-1 was not achieved, but anacardic acids were identified for the first time as modulators of Bcl-xL/Bak and Mcl-1/Bid interactions and could be used as chemical tools for studying these protein−protein interactions.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a Perkin-Elmer Lambda 5 spectrophotometer. IR spectra were performed on a PerkinElmer Spectrum 100 FT-IR spectrometer. NMR spectra were recorded in CDCl3-d6 on a Bruker 600 MHz instrument (Avance 600) using a 1.7 mm microprobe for compound 2, on a Bruker 500 MHz instrument (Avance 500) for plant extracts, fractions, and compounds 4 and 11, and on a Bruker 300 MHz instrument (Avance 300) for compounds 1, 3, 5, 6−10, and 12. The assignment and Chemical Shift Perturbation (CSP) experiments of the Bid peptide were recorded on a Bruker Avance III 600US2 spectrometer, equipped with a 5 mm z-gradient TCI (H/C/N) cryoprobe. TOCSY and NOESY data were processed using TOPSIN and analyzed using Mestrenova.

The titration experiments of the Bcl-xL with 9 were performed on a Bruker Avance III 800US2 spectrometer, equipped with a 5 mm z-gradient TCI (H/C/N) cryoprobe. The 1H−15N HMQC data were processed using TOPSIN and analyzed using SPARKY.

HRESIMS spectra and LC chromatograms were obtained on a UPLC Acquity (Waters) using a BEH C18 2.1 × 50 mm, 1.7 μm column coupled with an LCT Premier XE (Waters) TOF spectrometer. Kromasil analytical and preparative C18 columns (250 × 4.6 mm and 250 × 21.2 mm i.d.; 5 μm; Thermo) were used for HPLC separations using a Dionex autopurification system equipped with a Gilson 215 liquid handler, a Dionex UltiMate HPG-3200BX binary pump (Thermo Scientific), a UV−vis diode array detector (200−600 nm, Dionex UVD340U), and a PL-ELS 1000 ELSD Polymer Laboratory detector. All solvents were purchased from SDS (France); analytical and preparative plates (Si gel 60 F254), from Merck (France).

Plant Material. The bark of K. hookeriana Warb. was collected at Gunung Bujang Melaka, Perak, Malaysia, in April 1996. The plant was identified by T. Leong Eng, botanist at the University of Malaya. A voucher specimen (KL-4584) has been deposited at the Herbarium of the Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation. The air-dried bark and stem (100 g) of K. hookeriana were extracted with EtOAc (3 × 300 mL) to yield a crude extract (2.5 g) after concentration in vacuo at 40 °C. A portion (350 mg) of the extract was subjected to preparative HPLC (Kromasil C18 column, 250 × 21.2 mm, 5 μm, at 21 mL/min) using a gradient of MeCN−H2O (8:2 to 9:1) plus 0.1% formic acid to afford three fractions (F1, F7, and F14) and compounds 1 (6 mg), 2 (4 mg), 4 (2 mg), 5 (25 mg), 6 (3 mg), 7 (96 mg), 8 (74 mg), 9 (31 mg), 10 (13 mg), 11 (4 mg), and 12 (3 mg). Fraction F7 (5 mg) was purified by preparative TLC (Merck, Si gel 60 F254, 2 mm, n-heptane−EtOAc, 8:2) to obtain compound 3 (3 mg).
Figure 4. Effect of the addition of 9 on the chemical shifts of Bid protons.

**Khookerianone A (1):** colorless gum; UV (CHCl₃) λ_{max} (log ε) 329 (3.65) nm; IR (neat) ν max 3135, 2922, 2853, 1615, 1588, 1454, 1360, 1259, 1160 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 13.00 (1H, s, OH-2), 6.24 (1H, d, J = 2.5 Hz, H-5), 6.22 (1H, d, J = 2.5 Hz, H-3), 5.70 (1H, br s, OH-4), 5.33 (2H, m, H-8', H-9'), 2.81 (2H, t, J = 7.7 Hz, H-1'), 2.62 (3H, s, COCH₃), 1.99−2.00 (2H, m, H-6', H-10'), 1.57 (2H, m, H-7', H-11'), 0.87 (3H, t, J = 6.6 Hz, H-3); ¹³C NMR (CDCl₃, 75 MHz) δ 204.5 (CO), 166.1 (C-2), 161.0 (C-1), 115.5 (C-1'), 110.8 (C-5), 101.9 (C-3), 36.6 (C-1'), 32.5 (COCH₃), 32.4 (C-2'), 32.1 (C-9'), 29.5−29.9 (C-3−C-8'), 22.9 (C-10'), 14.3 (C-11'); HRESIMS m/z [M + H⁺] 307.2270 (calcd for C₁₉H₃₁O₃ 307.2268).

**Khookerianone B (2):** yellowish oil; UV (CHCl₃) λ_{max} (log ε) 294 (3.60) nm; IR (neat) ν max 3335, 2915, 2850, 1617, 1590 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) δ 12.98 (1H, s, OH-2), 6.23 (1H, d, J = 2.4 Hz, H-5), 6.22 (1H, d, J = 2.4 Hz, H-3), 5.66 (1H, br s, OH-4), 5.33 (2H, m, H-6', H-7'), 2.82 (2H, t, J = 8.0 Hz, H-1'), 2.63 (3H, s, COCH₃), 2.00 (2H, m, H-5', H-8'), 1.58 (2H, m, H-7', H-9'), 1.30 (8H, m, H-3', H-4', H-9', H-10'); ¹³C NMR (CDCl₃, 150 MHz) δ 204.5 (CO), 166.3 (C-2), 161.0 (C-4), 148.0 (C-6), 129.5−130.5 (C-6', C-7'), 115.6 (C-1), 110.7 (C-5), 101.9 (C-3), 36.5 (C-1'), 32.5 (COCH₃), 32.4 (C-2'), 32.1 (C-9'), 29.6−29.9 (C-3−C-4'), 27.2−27.3 (C-5−C-8'), 22.6 (C-10'), 14.2 (C-11'); HRESIMS m/z [M + H⁺] 333.2421 (calcd for C₂₁H₃₅O₃ 333.2424).

**Khookerianone acid B (5):** colorless gum; UV (CHCl₃) λ_{max} (log ε) 311 (3.55), 243 (3.70) nm; IR (neat) ν max 2923, 2851, 1617, 1603, 1576, 1444, 1297, 1216 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 10.62 (1H, s, OH-2), 7.34 (1H, t, J = 7.9 Hz, H-4), 6.85 (1H, dd, J = 7.9 Hz, 1.1 Hz, H-3), 6.75 (1H, dd, J = 7.9 Hz, 1.1 Hz, H-5), 5.34 (2H, m, H-6', H-7'), 2.96 (2H, t, J = 8.0 Hz, H-1'), 2.00 (2H, m, H-5', H-8'), 1.59 (2H, m, H-2'), 1.30−1.36 (8H, m, H-3', H-4', H-9', H-10'); ¹³C NMR (CDCl₃, 75 MHz) δ 176.0 (COOH), 163.8 (C-2), 147.9 (C-6), 135.6 (C-4), 129.9−130.2 (C-6', C-7'), 123.0 (C-3), 116.1 (C-5), 110.7 (C-1), 36.7 (C-1'), 32.2 (C-2'), 32.1 (C-9'), 29.6−29.8 (C-3−C-4'), 27.1−27.4 (C-7', C-10'), 22.6 (C-12'), 14.2 (C-13'); HRESIMS m/z [M + H⁺] 333.2424 (calcd for C₂₁H₃₅O₃ 333.2424).
Khookerianic acid C (6): brown powder; UV (CHCl₃) λmax (log ε) 308 (3.46), 242 (3.60) nm; IR (neat) νmax 3450, 2920, 2850, 1651, 1600, 1590 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) 11.07 (1H, s, OH-2), 7.37 (1H, t, J = 7.8 Hz, H-4), 7.29 (2H, m, H-2″, H-6″), 7.20 (3H, m, H-3″−H-5″), 6.89 (1H, dd, J = 7.8, 1.0 Hz, H-3), 6.79 (1H, dd, J = 7.8, 1.0 Hz, H-5), 2.98 (2H, t, J = 7.6 Hz, H-1′), 2.62 (2H, t, J = 7.6 Hz, H-8′), 1.62 (4H, m, H-2′, H-7′), 1.35 (8H, m, H-3′−H-6′); ¹³C NMR (CDCl₃, 125 MHz) δ 174.9 (C-OOH), 163.9 (C-2), 147.8 (C-6), 143.1 (C-1″), 135.6 (C-4), 128.6 (C-3″, C-5″), 128.4 (C-2″, C-6″), 125.8 (C-4″), 122.9 (C-5), 116.1 (C-3), 110.5 (C-1), 36.7 (C-1′), 36.2 (C-8′), 32.3 (C-2′), 31.7 (C-7′), 29.5−30.0 (C-3′−C-6′); HRESIMS m/z [M + H]+ 327.1974 (calcd for C₂₁H₂₇O₃ 327.1955).

Ozonolysis of Compounds 2, 3, 5, 8, 10, and 12. A portion of each compound (2 to 6 mg) was dissolved in CH₂Cl₂, and the solution was cooled to −78 °C. Ozone gas, generated by a BMT 802x ozone generator, flow rate 0.2 mL/min, was passed into the solution until the color turned blue. An excess of dimethylsulfoxide was added, and the reaction mixture was stirred for 4 h at room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in MeOH and injected in UPLC/HRESIMS.

Biological Assays. The modulations of the interaction between Bcl-xL/Bak and between Mcl-1/Bid were evaluated by competition against a fluorescent-labeled reference compound (fluorescent-tagged BH3 domain of the protein Bak or Bid), as described earlier. Meiogynine A and ABT-737 were used as positive controls. Results are expressed as IC₅₀; the concentration corresponding to 50% of such inhibition, and corrected for experimental conditions according to the Kenakin rearranged equation. Assays were run in triplicate. The cytotoxic activities of the compounds were evaluated against the cancer cell line HCT-116 (human colon carcinoma). Cytotoxicity assays were performed according to a published procedure.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00915.

¹H and ¹³C NMR spectra for compounds 1−12, HSQC ¹H−¹³C NMR spectra of Bcl-xL and Mcl-1 with compound 9, proton assignments for Bid peptide, as well as additional CSP experiments (PDF)

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Notes
The authors declare no competing financial interest.

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