A Dimeric Sesquiterpenoid from a Malaysian *Meiogyne* as a New Inhibitor of Bcl-xL/BakBH3 Domain Peptide Interaction[⊥]

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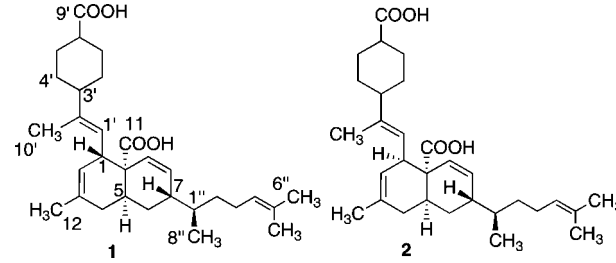
In an effort to find potent inhibitors of the antiapoptotic protein Bcl-xL, a systematic in vitro evaluation was undertaken on 1470 Malaysian plant extracts. The ethyl acetate extract obtained from the bark of *Meiogyne cylindrocarpa* was selected for its interaction with the Bcl-xL/Bak association. Bioassay-guided purification of this species led to the isolation of two new dimeric sesquiterpenoids (**1** and **2**) possessing an unprecedented substituted *cis*-decalin carbon skeleton. Meiogynin A (**1**) showed the strongest activity with a K_i of 10.8 ± 3.1 μM.

The antiapoptotic proteins, Bcl-xL and Bcl-2, play a crucial role in the regulation of cell death in many eukaryotic systems. They are especially involved in cancer development and resistance to treatment. For example, it has been shown that Bcl-xL is overexpressed in prostate cancer progression.[1] On the other hand, overexpression of Bcl-xL is also reported to confer a multidrug resistance phenotype.[2,3] Thus, Bcl-xL may serve as a molecular target in anticancer therapy, and agents that can down-regulate Bcl-xL expression or activity may be useful for cancer treatment or prevention.

The proteins of the Bcl-2 family are able to form heterodimers with apoptotic proteins such as Bak, and disruption of heterodimerization could be used to modulate cell death, reinstalling apoptosis in cancer cells. An affinity displacement assay based on Bcl-xL/Bak (BH3-domain) interaction has been developed using the fluorescence polarization assay, optimized and miniaturized for high-throughput screening according to Qian and co-workers.[4] This assay makes use of soluble Bcl-xL and fluorescein-tagged BH3 domain of the apoptotic protein Bak. Binding is measured as fluorescence polarization after competition between the ligand and the fluorescent BH3.

Biological screening was conducted on 1476 ethyl acetate extracts prepared from various parts of 670 Malaysian plants. The AcOEt bark extract of *Meiogyne cf. M. cylindrocarpa* (Burck) Heusden (Annonaceae)[5] was selected for its high potency as a modulating agent between Bcl-xL and Bak. In Malaysia, the Annonaceae family consists of 25 genera and 140 species.[6] The genus *Meiogyne* includes nine species distributed in Malesia from India to the Philippine Islands.[6] *M. cylindrocarpa*, the plant under study, was also known under the synonym *Mitrephora cylindrocarpa* Burck and *Polyaulax cylindrocarpa* (Burck) Backer. No phytochemical study has been carried out on these binomial identities. In the present report, we describe the isolation, structure elucidation, and biological activity of meiogynin A (**1**) and its 1-epimer (**2**). A biosynthetic pathway of meiogynin A (**1**) is also proposed.

Ground bark of *M. cylindrocarpa* was extracted by ethyl acetate to give a crude extract. One hundred milligrams of this extract was filtered on polyamide to remove tannins, and 15 mg of the filtered extract was fractionated on a semipreparative C_{18} column to give nine fractions according to a standardized method.[7] The bioassay used allowed us to identify one active fraction (F6, t_R from 42 to 50 min) that was used as a reference for further purification. A large amount of the extract (1.7 g) was then subjected to flash chromatography to give 11 fractions (FA to FK). Comparative study on analytical HPLC of the fractions with the reference fraction F6 allowed us to target those containing the active compounds. Subsequent preparative HPLC purification resulted in the isolation of compounds **1** and **2**.



Compound **1** was isolated as a white, amorphous powder, $[\alpha]_D$ −281 (*c* 0.12, CH_2Cl_2). The HRESIMS indicated a $[M + Na]^+$ ion peak at *m/z* 491.3134, which suggested a molecular formula of $C_{30}H_{44}O_4Na$ (calcd 491.3137). The IR spectrum of **1** showed absorption bands for hydroxy and carbonyl groups at 2927 and 1694 cm^{-1}, respectively. Analysis of the ^1H and ^{13}C NMR spectra suggested a triterpenoid-type compound possessing four double bonds and two carbonyl groups (Table 1). In the ^1H NMR spectrum, resonances for five methyls at δ_H 1.66 (3H, s, Me-12), 1.65 (3H, s, Me-10′), 1.71 (3H, s, Me-6″), 1.63 (3H, s, Me-7″), and 0.78 (3H, d, J = 6.5 Hz, Me-8″), five vinylic protons at δ_H 5.17 (1H, brd, J = 5.0 Hz, H-2), 5.65 (1H, dd, J = 10.1, 2.5 Hz, H-8), 5.59 (1H, d, J = 10.1 Hz, H-9), 4.96 (1H, d, J = 10.6 Hz, H-1′), and 5.12 (1H, t, J = 7.0, H-4″), four methine protons at δ_H 3.18 (1H, dd, J = 10.6, 5.0 Hz, H-1), 2.56 (1H, m, H-5), 2.35 (1H, m, H-7), and 2.26 (1H, tt, J = 12.1, 3.5 Hz, H-6′), and 14 aliphatic protons at δ_H 1.13−2.02 (14H, m) were observed. The ^{13}C NMR spectrum, with carbon multiplicities determined by a DEPT135 experiment, confirmed the presence of two carboxyl groups at δ_C 180.8 and 182.2 (C-11 and C-9′, respectively), eight olefinic carbons (five methines and three quaternary), five methyl, eight methylene, and six methine groups, and two quaternary carbons, which accounted for all degrees of unsaturation given by the molecular formula. The COSY and HMBC spectra revealed the presence of three main spin systems, which help distinguish a tetrasubstituted decalin moiety

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Table 1. NMR Spectroscopic Data (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR, $CDCl_3$) for Compounds **1** and **2**

	1		**2**	
position	δ_C, mult.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	43.3	3.18 dd (10.6, 5.0)	41.1	3.50 d (10.6)
2	120.1	5.17 brd (5.0)	121.8	5.00 brs
3	133.5		134.6	
4	31.6	1.87/1.91 m	31.6	1.88/1.91 m
5	28.0	2.56 m	34.5	2.58 m
6	25.4	1.51/1.79 m	26.3	1.50/1.72 m
7	36.1	2.35 m	36.5	2.39 m
8	133.7	5.65 dd (10.1, 2.5)	134.6	5.75 brd (11.0)
9	129.8	5.59 d (10.1)	124.5	5.73 brd (11.0)
10	48.6		50.6	
11	180.8		181.8	
12	23.4	1.66 s	23.1	1.57 s
1′	123.2	4.96 d (10.6)	121.8	5.25 d (10.6)
2′	140.1		142.3	
3′	46.3	1.81 m	46.5	1.83 m
4′ax/eq	30.1[a]	1.13/1.78[a] m	30.5[a]	1.26/1.66[a] m
5′ax/eq	28.7	1.50/1.95 m	28.7	1.50/1.95 m
6′	42.9	2.26 tt (12.1, 3.5)	42.2	2.27 tt (11.7, 3.0)
7′ax/eq	29.3	1.42/1.99 m	29.1	1.44/2.03 m
8′ax/eq	30.7[a]	1.26/1.67[a] m	30.9 [a]	1.21/1.78[a] m
9′	182.2		182.8	
10′	14.7	1.65 s	14.7	1.65 s
1″	36.4	1.57 m	36.4	1.58 m
2″	34.1	1.19/1.33 m	34.0	1.19/1.36 m
3″	26.0	1.93/2.02 m	25.9	1.92/2.02 m
4″	124.7	5.12 t (7.0)	124.6	5.10 t (7.1)
5″	131.2		131.2	
6″	25.7	1.71 s	25.9	1.69 s
7″	17.7	1.63 s	17.6	1.60 s
8″	15.3	0.78 d (6.5)	15.5	0.80 d (6.8)

[a] Assignments are interchangeable.

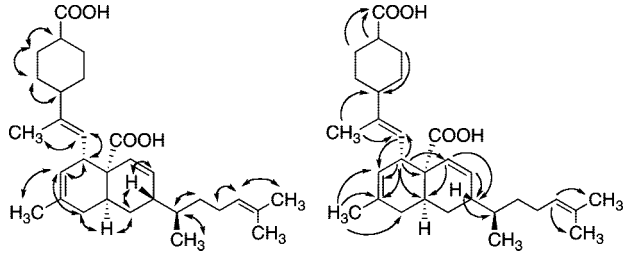

Figure 1. Key COSY (left) and HMBC (right) correlations for meiogynin A (**1**).

bearing two double bonds, a 4-(prop-1-en-2-yl)cyclohexanecarboxylic acid moiety, and a 2-methylhept-2-ene side chain (Figure 1). A careful examination of the COSY spectrum allowed the determination of the axial and equatorial protons for the cyclohexane ring (Table 1). This was confirmed in the ROESY spectrum by observation of cross-peaks between the protons at δ_H 1.13, 2.26, and 1.26 (H-4′$_{ax}$, H6′$_{ax}$, and H-8′$_{ax}$) on one side and at δ_H 1.81, 1.50, and 1.42 (H-3′$_{ax}$, H5′$_{ax}$, and H-7′$_{ax}$) on the other side (Figure 2). The large coupling constant observed for H-6′ (δ_H 2.26, tt, J = 12.1, 3.5 Hz) confirmed the equatorial location of the carboxylic acid at C-6′. The substitutions of the *cis*-decalin ring by the aliphatic side chains at positions 1 and 7 were deduced from the correlations displayed in the HMBC spectrum, between H-1 and C-2, C-1′, and C-2′ (δ_C 120.1, 123.2, and 140.1, respectively) on one hand and from H-7 to C-1″ (δ_C 36.4) and from H-8 and H-9 to C-7 (δ_C 36.1) on the other hand. The relative configuration of the asymmetric carbons was determined using a ROESY experiment from the following evidence. A *cis*-junction for the decalin ring system could be deduced from correlations between H-1 and H-9, H-5 and H-1′, and H-7 and H-4. The geometry of the C1′−C2′ double bond was assigned as *E* by observation of a cross-peak between H-1′ and H-3′ (δ_H 4.96 and 1.81, respectively). The relative configuration of the methyl at C-1″ was elucidated by conformational analysis (see below). All these data allowed assigning the structure of compound **1** with the relative configuration 1*R**,5*S**,7*S**,10*S**,1″*R**. Compound **1** was named meiogynin A.

Compound **2** was isolated as a white, amorphous powder. The HRESIMS showed the same ion peak at *m*/*z* 491.3129 as compound **1**, indicating the same formula, $C_{38}H_{50}O_4Na$ ([M + Na]$^+$, calcd *m*/*z* 491.3137). The IR and ^{1}H and ^{13}C NMR spectroscopic data were almost identical to those of **1** (Table 1). Only slight differences were observed for the chemical shifts of the *cis*-decalin moiety and the vinylic CH-1′. The HMBC and COSY spectra did not show any noticeable differences with those of **1**, but a careful analysis of the ROESY spectrum (Figure 2) and conformational analysis (see below) allowed us to suggest that compound **2** is the epimer at C-1 of meiogynin A and possesses the relative configuration 1*S**,5*S**,7*S**,10*S**,1″*R**. The sole presence of a strong correlation between H-1 (δ_H 3.50, d, J = 10.6 Hz) and H-5 (δ_H 2.58) in the ROESY spectrum suggested a β-position at C-1 for the aliphatic side chain, instead of the α-position for **1**. Other correlations allowed proposing the structure of compound **2**, which was named 1-*epi*-meiogynin A.

As mentioned above, the relative configuration of compounds **1** and **2** was confirmed by conformational study (see Figure 2 and Experimental Section). The conformational analysis revealed that the *cis*-decalin exists in only one conformation for both compounds **1** and **2**. The relevant dihedral angles are presented in Table 2, indicating a "half-chair-sofa" conformation for the *cis*-decalin moiety. However, many subconformers exist due to the presence of the two side chains for both compounds. According to experimental data of the ROESY spectrum, the distances H-1″−H-7, H-1″−H-8, and CH_3-8″−H-8 must be small (<2.8 Å) for both compounds **1** and **2**. This suggests an *R** configuration at C1″ for a 1*R**,5*S**,7*S**,10*S** (**1**) or 1*S**,5*S**,7*S**,10*S** (**2**) *cis*-decalin moiety (Figure 2). A value of −60° for the dihedral angle C-8″−C-1″−C-7−C8 is found in this case. In contrast, a C1″ *S* configuration relative to the same *cis*-decalin would imply a value of 180° for the C-8″−C-1″−C-7−C8 dihedral angle, leading to a CH_3-8″−H-8 distance greater than 4 Å. Conformational analysis revealed also that two positions of the cyclohexane moiety may exist due to the rotation around the C1′−C3′ axis, characterized by a distance of 2.2 or 3.7 Å for H-1′−H-3′. The conformers for compounds **1** and **2** depicted in Figure 2 were obtained from the experimental data of the ROESY spectrum, which impose a short distance (<2.8 Å) for H-1′−H-3′. These two conformers have shown a small energetic difference of 1.4 kJ/mol (0.3 kcal/mol) with the corresponding most stable conformation, which in turn would have a distance of 3.7 Å for H-1′−H-3′. The results obtained from this conformational analysis are in agreement with the relative configuration assigned for compounds **1** and **2**.

The biosynthesis of compounds **1** and **2** could involve an enzymatic catalysis of a Diels−Alder reaction[8] between two sesquiterpene units. For example, a bisabolatriene acid subunit such as **3** and a zingiberene-type unit such as **4** may be linked "head to tail" through a [4+2] intermolecular Diels−Alder reaction to give meiogynin **1** (Figure 3). Compound **3** could be formed by oxidation and dehydration of α-bisabolol. This hypothetic biogenesis was supported by the presence of α-bisabolol in the bark of *M. cylindrocarpa*. This sesquiterpene was firmly identified by GC-MS analysis of the essential oil obtained by hydrodistillation. Analysis of the fragments obtained by EI showed the presence of ions at *m*/*z* 204, 119, 109, and 69, in agreement with a reference spectrum of α-bisabolol (U.S. National Institute of Standards and Technology (NIST) mass spectral library (Version 1.6d, 1998)).

The binding affinity of compounds **1** and **2** was evaluated by competition between Bcl-xL and the fluorescent-tagged BH3 domain of the protein Bak, as described.[4] Results are given by the K_i value, the inhibition constant of a compound determined at equilibrium with a reference ligand. Compounds **1** and **2** exhibited K_i values of 10.8 ± 3.1 and >100 μM, respectively, indicating

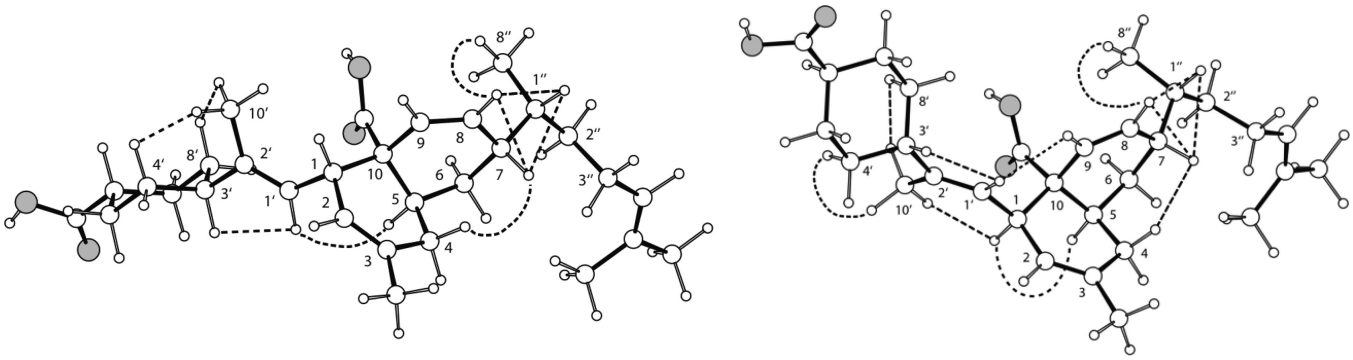

Figure 2. Minimized structures of **1** (left) and **2** (right) and selected ROESY correlations (dotted lines).

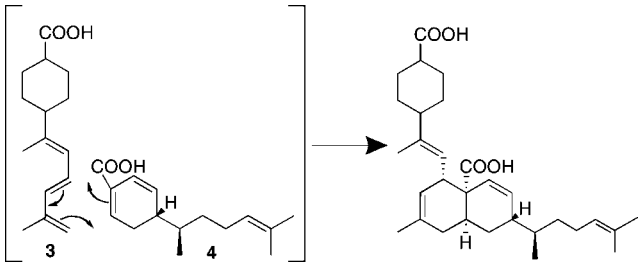

Figure 3. Hypothetical biosynthetic pathway proposed for meiogynin A (**1**).

Table 2. Dihedral Angles for the *cis*-Decalin Moiety

cis-decalin	half-chair
C1−C2−C3−C4	2°
C2−C3−C4−C5	−13°
C3−C4−C5−C10	40°
C4−C5−C10−C1	−56°
C5−C10−C1−C2	44°
C10−C1−C2−C3	−18°
cis-decalin	sofa
C5−C6−C7−C8	−26°
C6−C7−C8−C9	−1°
C7−C8−C9−C10	−3°
C8−C9−C10−C5	32°
C9−C10−C5−C6	−58°
C10−C5−C6−C7	58°

that meiogynin A **1** acts as an antagonist to the Bcl-xL/Bak association. Meiogynin A also showed cytotoxic activity for the KB cancer cell line with an IC_{50} value of 4.0 μM.

In conclusion, bioguided purification of *M. cylindrocarpa* led to the isolation of two new dimeric sesquiterpenoids. This is the first report on the isolation of terpenoids having a *cis*-decalin moiety substituted with a carboxylic acid. Furthermore, an in vitro binding affinity assay on Bcl-xL/Bak association revealed that meiogynin A (**1**) exhibited potent activity. A biogenetic pathway involving a Diels−Alder reaction has been proposed. The biomimetic synthesis of meiogynin A analogues, relying on this reaction, is currently under study.

Experimental Section

General Experimental Procedures. Optical rotations were measured at 25 °C on a JASCO P1010 polarimeter. The UV spectra were recorded on a Perkin-Elmer Lamba 5 spectrophotometer. IR spectra were obtained on a Nicolet FTIR 205 spectrophotometer. Specific rotation was obtained in CH_2Cl_2 with a JASCO P-1010 polarimeter. The NMR spectra were recorded on a Bruker 600 MHz (Avance 600) spectrometer with $CDCl_3$ as solvent. HRESIMS were run on a MALDI-TOF spectrometer (Voyager-De STR; Perspective Biosystems). Kromasil analytical, semipreparative, and preparative C_{18} columns (250 × 4.5 mm; 250 × 10 mm and 250 × 21.2 mm i.d., 5 μm Thermo) were used for preparative HPLC separations using a Waters autopurification system equipped with a binary pump (Waters 2525), a UV−vis diode array detector (190−600 nm, Waters 2996), and a PL-ELS 1000 ELSD Polymer Laboratory detector. GC-MS analyses were performed on a Thermofisher Trace 2000 system equipped with an Equity-5 fused capillary column (marque Supelco, 30 m × 0.25 mm × 0.25 μm). Analytical TLC plates (Si gel 60 F 254) were purchased from SDS (France). Polyamide DC 6 and a polyamide cartridge were purchased from Macherey-Nagel (Chromabond PA, 1 g). A Versapack C_{18} Cartridge (40 × 75 mm) was purchased from Serlabo Technologies. All other chemicals and solvents were of analytical grade and purchased from SDS (France).

Plant Material. Trunk bark of *Meiogyne cylindrocarpa* was collected in the dense rain forest of Mersing, Johor (Malaysia), in November 1994, and the identification of *Meiogyne cf. M. cylindrocarpa* was proposed by Dr. J. A. Kessler (Leiden). A voucher specimen (KL-4405) was deposited at the herbarium of the Forest Research Institute, Kepong, Malaysia, and at the National Herbarium of the Nederland, Leiden.

Extraction and Isolation. Trunk bark (80 g) was extracted with EtOAc (3 × 250 mL) at 40 °C. The extract was concentrated in vacuo at 40 °C to yield 1.8 g. A 100 mg aliquot of the crude extract was dissolved in a 1:1 mixture of AcOEt−MeOH and filtered on a polyamide cartridge before being tested at 10 μg mL^{-1} on the Bcl-xL protein in vitro. The filtered extract was then fractionated on a semipreparative C_{18} column, according to a standardized method previously detailed.[7] Fraction 6 was shown to possess binding affinity for Bcl-xL.

The remaining EtOAc extract (1.7 g) was submitted to flash chromatography on a reversed-phase column (Versapack C_{18} cartridge, 40 × 75 mm) using a step gradient mobile phase (solvent: A (MeCN)−B (H_2O) 20−100% A for 70 min; flow rate 10 mL/min) to give 11 fractions (FA to FK). Fraction I (436 mg) was submitted to HPLC (nine injections of 48 mg each in 1.0 mL of a 60:40 MeCN−H_2O mixture), on a preparative Kromasil RP-18 column (C_{18} Kromasil, 250 × 21.2 mm, i.d. 5 μm, Waters) using an isocratic mobile phase consisting of 75:25 MeCN−H_2O + 0.1% trifluoroacetic acid, flow rate 21 mL/min, to give meiogynin A (**1**) (80 mg, t_R 63.9 min) and 1-*epi*-meiogynin A (**2**) (7 mg, t_R 59.3 min).

Meiogynin A (1): white, amorphous powder; $[\alpha]_D$ −281 (*c* 0.12, CH_2Cl_2); UV ($CHCl_3$) λ_{max} (log ϵ) 240 (3.20) nm; IR ν_{max} (ns) 2927, 1694 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 1); HRESIMS [M + Na] *m*/*z* 491.3134 (calcd for $C_{30}H_{44}O_4Na$, 491.3137).

1-*epi*-Meiogynin A (2): white, amorphous powder; $[\alpha]_D$ +5 (*c* 0.17, CH_2Cl_2); UV ($CHCl_3$) λ_{max} (log ϵ) 240 (3.73) nm; IR ν_{max} (ns) 2924, 1691 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Table 1); HRESIMS [M + Na] *m*/*z* 491.3129 (calcd for $C_{30}H_{44}O_4Na$, 491.3137).

Computational Procedure. One thousand conformations of compounds **1** and **2** were generated by the random search Monte Carlo method[9] and optimized by the PRCG minimization method[10] using the Macromodel (version 5.5) program[11] with the MM2 force field[12] and GB/SA chloroform solvation. The search was carried out on blocks of 100 Monte Carlo steps until no additional conformation was found to be of lower energy than the current minimum. Duplicated conformations as well as those that had chirality changes were discarded. From these conformational searches, all the possible conformations within 12.5 kJ/mol (3 kcal/mol) from the global minimum were analyzed.

GC-MS Analysis of Essential Oil of *M. cylindrocarpa.* A sample of the bark (50 g) of *M. cylindrocarpa* was dried at room temperature for 10 days and then submitted to hydrodistillation for 7 h, in a Clevenger-type apparatus. The oil (100 μL) was dried over anhydrous Na_2SO_4 and stored at 4 °C. The GC-MS analysis of the essential oil was performed in the EI mode at 70 eV with the following program: injector temperature 280 °C, oven temperature 80−280 °C at a rate of 10 °C/min, carrier gas helium (1 mL/min), injection volume 1 μL in CH_2Cl_2. The identification of α-bisabolol was based by comparison of the mass spectra pattern with the U.S. National Institute of Standards and Technology (NIST) mass spectral library (Version 1.6d, 1998).

Biological Assay. The binding affinity of compounds **1** and **2** was evaluated on Bcl-xL by competition against a fluorescent-labeled reference compound (fluorescent-tagged BH3 domain of the protein Bak), as described by Qian et al.[4] The unlabeled peptide Bak(BH3) was used as a positive control (K_i = 666 ± 36 nM). Results are expressed as binding activity, i.e., percentage of inhibition of the binding of labeled reference compound, or as K_i, the concentration corresponding to 50% of such inhibition, and corrected for experimental conditions according to Cheng and Prusoff.[13] The human KB tumor cell line, mouth epidermoid carcinoma, was originally obtained from ATCC (Manassas, VA). The cytotoxicity assays were performed according to a published procedure.[14] Taxotere was used as a control compound.

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References and Notes

(1) Castilla, C.; Congregado, B.; Chinchon, D.; Torrubia, F. J.; Japon, M. A.; Saez, C. *Endocrinology* **2006**, *147*, 4960–4967.
(2) Minn, A. J.; Rudin, C. M.; Boise, L. H.; Thompson, C. B. *Blood* **1995**, *86*, 1903–1910.
(3) Kharbanda, S.; Pandey, P.; Schofield, L.; Israels, S.; Roncinske, R.; Yoshida, K.; Bharti, A.; Yuan, Z. M.; Saxena, S.; Weichselbaum, R.; Nalin, C.; Kufe, D. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6939–6942.
(4) Qian, J.; Voorbach, M. J.; Huth, J. R.; Coen, M. L.; Zhang, H.; Ng, S.-C.; Comess, K. M.; Petros, A. M.; Rosenberg, S. H.; Warrior, U.; Burns, D. J. *Anal. Biochem.* **2004**, *328*, 131–138.
(5) Dr. P. J. A. Kessler (National Herbarium of the Netherlands, Leiden), personal communication.
(6) Whitmore, T. C. In *Tree Flora of Malaya, A Manual for the Forester*; Whitmore, T. C., Ed.; Longman: Hong Kong, 1972; Vol. 1, pp 61−99.
(7) Bousserouel, H.; Litaudon, M.; Morleo, B.; Martin, M. T.; Thoison, O.; Nosjean, O.; Boutin, J. A.; Renard, P.; Sevenet, T. *Tetrahedron* **2005**, *61*, 845–851.
(8) Oikawa, H.; Tokiwano, H. *Nat. Prod. Rep.* **2004**, *21*, 321–352.
(9) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.
(10) Polak, E.; Ribiere, G. *Rev. Fr. Inf. Rech. Oper.* **1969**, 35–44.
(11) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
(12) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.
(13) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(14) Tempête, C.; Werner, G. H.; Favre, F.; Rojas, A.; Langlois, N. *Eur. J. Med. Chem.* **1995**, *30*, 647–650.

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