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Cytotoxic Xanthone Constituents of the Stem Bark of *Garcinia mangostana* (Mangosteen)

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Abstract

Bioassay-guided fractionation of a chloroform-soluble extract of *Garcinia mangostana* stem bark using the HT-29 human colon cancer cell line and an enzyme-based ELISA NF-κB assay, led to the isolation of a new xanthone, 11-hydroxy-3-*O*-methyl-1-isomangostin (**1**). The structure of **1** was elucidated by spectroscopic data analysis. In addition, ten other known compounds, 11-hydroxy-1-isomangostin (**2**), 11α-mangostanin (**3**), 3-isomangostin (**4**), α-mangostin (**5**), β-mangostin (**6**), garcinone D (**7**), 9-hydroxycalabaxanthone (**8**), 8-deoxygartanin (**9**), gartanin (**10**), and cratoxyxanthone (**11**), were isolated. Compounds **4**–**8** exhibited cytotoxicity against the HT-29 cell line with ED_{50} values of 4.9, 1.7, 1.7, 2.3, and 9.1 μM, respectively. In an ELISA NF-κB assay, compounds **5**–**7**, **9**, and **10** inhibited p65 activation with IC_{50} values of 15.9, 12.1, 3.2, 11.3, and 19.0 μM, respectively, and **6** showed p50 inhibitory activity with an IC_{50} value of 7.5 μM. α-Mangostin (**5**) was further tested in an in vivo hollow fiber assay, using HT-29, LNCaP, and MCF-7 cells, but it was found to be inactive at the highest dose tested (20 mg/kg).

Garcinia mangostana L. (Clusiaceae) is well-known in southeastern Asia for its pleasant-tasting fruits, commonly known as mangosteen, which is now used widely as a botanical dietary supplement in several countries.[1] Xanthones are the most characteristic secondary metabolite constituents of *G. mangostana* and over 80 compounds of this type have been isolated and characterized from the various parts of this plant.[1,2] The biological effects of the mangosteen xanthones are diverse, and include antioxidant, antibacterial, antifungal, antimalarial, anti-inflammatory, cytotoxic, and HIV-1 inhibitory activities.[1,2] Recent phytochemical investigations on the fruits of *G. mangostana* at The Ohio State University have resulted in the isolation of xanthones with antioxidant,[3] aromatase inhibitory,[4] and quinone reductase-inducing activities.[5]

As part of a collaborative project directed towards the discovery of novel natural product anticancer agents,[6] a $CHCl_3$-soluble extract of the stem bark of *G. mangostana* collected in

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Supporting Information Available: Data for in vivo hollow fiber evaluation of α-mangostin (**5**) against LNCaP and HT-29 cells, 1D and 2D NMR spectra for compound **1**, and tables of NMR data of the known compounds **2**–**11**. This material is available free of charge via the Internet at http://pubs.acs.org/jnp.

Indonesia showed cytotoxic activity against a “gatekeeper” HT-29 human colon cancer cell line with an ED_{50} value of 1.6 μg/mL. This extract also inhibited p50 and p65 activation with 57% and 67% inhibition at 50 μg/mL, respectively, in an ELISA NF-κB (nuclear factor-kappaB) assay. Therefore, it was subjected to bioactivity-guided fractionation, leading to the isolation of twelve xanthones, including a new compound (**1**). The structure elucidation of **1** and the biological evaluation of all compounds isolated are described herein.

Compound **1** was obtained as a yellow amorphous powder and produced a molecular ion peak at *m*/*z* 463.1729 $[M+Na]^+$ in the HRESITOFMS, corresponding to the sodiated elemental formula, $C_{25}H_{28}O_7Na$. The IR spectrum showed absorption bands at 3350 cm^{-1} for one or more hydroxy groups and at 1614 and 1456 cm^{-1} for aromatic groups.[7] The UV spectrum of **1** exhibited absorption maxima at 242, 254, and 303 nm, indicating the presence of a xanthone system.[7] The ^1H and ^{13}C NMR spectra of **1** were similar to those of the known compound, 11-hydroxy-1-isomangostin (**2**),[8] except for the presence of signals for a second methoxy group at δ_H 3.83 (3H, s, OCH_3-3) and δ_C 56.0 (CH_3, OCH_3-3). The positions of two methoxy groups were assigned C-3 and C-7 by the ^1H-^{13}C HMBC correlations between signals at δ_H 3.83 (3H, s, OCH_3) to δ_C 162.0 (C, C-3) and δ_H 3.78 (3H, s, OCH_3) to δ_C 142.9 (C, C-7), respectively. Further detailed analysis of the ^1H-^1H COSY, ^1H-^{13}C HSQC, and ^1H-^{13}C HMBC NMR data (Figure 1) allowed unambiguous assignments for all of the ^1H and ^{13}C NMR signals of **1**. The Mosher ester method was utilized in an attempt to determine an absolute configuration of **1.**[9] However, the reaction between the secondary hydroxy group at C-11 and the *R*- and *S*-MTPA-Cl reagents yielded a mixture of *S*- and *R*-MTPA esters and a mixture of *R*- and *S*-MTPA esters of **1**, respectively, indicating the presence of a racemic mixture, but not in a 1:1 mixtures due to the slightly positive specific rotation observed. Thus, the structure of the new compound **1** was elucidated as 11-hydroxy-3-*O*-methyl-1-isomangostin.

Compounds **2** and **3** exhibited NMR spectroscopic data identical to those of 11-hydroxy-1-isomangostin[8] and mangostanin.[10] To attempt to determine the absolute configuration of **2**, the Mosher ester method was performed,[9] but these reactions again produced evidence for the presence of a racemic mixture. As observed for **1**, the slightly positive specific rotation value of **2** could be also caused by an unequal ratio of 11*R* and 11*S* isomers present. Compound **3** was isolated previously from the fruits of *G. mangostana*, but its relative configuration has not been reported thus far. The energy-minimized stereostructure of **3** showed a dihedral angle of 26 between H-11 and H-10*β*, which corresponded to the coupling constant value with $^3J_{HH}$ = 8~9 Hz, from the Karplus correlation equation ($^3J_{HH} = A + B \cos \Phi + C \cos 2\Phi$; $A = 7$, $B = -1$, $C = 5$, Φ = dihedral angle).[7,11] The dihedral angle between H-11 and H-10*α* was calculated as 151° in the energy-minimized stereostructure of **3**, with the expected calculated coupling constant value $^3J_{HH}$ = 9~10 Hz.[7,11] These computational calculations were compared to the actual coupling constants observed in the ^1H NMR spectrum for **3** at δ_H 4.83 (1H, dd, *J* = 9.5, 8.2 Hz, H-11), 3.18 (1H, dd, *J* = 15.8, 8.2 Hz, H-10*β*), and 3.12 (1H, dd, *J* = 15.8, 9.5 Hz, H-10*α*). Based on these observations, the hydroxy group at C-11 was established as *α* (Figure 2). Therefore, compound **3** was determined as 11*α*-mangostanin.

Other known compounds were identified in the present investigation as 3-isomangostin (**4**),[9] *α*-mangostin (**5**),[12] *β*-mangostin (**6**),[13] garcinone D (**7**),[14] 9-hydroxycalabaxanthone (**8**),[15] 8-deoxygartanin (**9**),[16] gartanin (**10**),[17] cratoxyxanthone (**11**),[8] and mangostanol,[18] respectively, by comparison of their physical and spectroscopic data with those reported previously. Compounds **2**–**4**, **9**, and **10** were isolated as constituents of the stem bark part of this plant for the first time. In addition, cratoxyxanthone (**11**) has been isolated only from the bark of *Cratoxylum cochinchinense* (Lour.) Bl. (Clusiaceae) previously,[8] so this is the first report of its isolation from a plant of the genus *Garcinia*.

All compounds isolated in the present investigation were tested in vitro for their cytotoxic activity against the HT-29 human colon cancer cell line (Table 1). The major active compounds, *α*-mangostin (**5**) and *β*-mangostin (**6**), have been found cytotoxic against various human cancer cells,[19–22] including DLD-1 human colon cancer cells,[23] and compounds **4**, **7**, and **8** have been also reported for their cytotoxicity against epidermoid carcinoma (KB), breast cancer (BC-1), or small cell lung cancer (NCI-H187).[19] However, this is the first report of the evaluation of xanthones from *G. mangostana* for their cytotoxicity against the HT-29 colon cancer cell line. In an enzyme-based ELISA NF-κB assay, all compounds except for **3** and **4** and mangostanol were tested for their p50 (NF-κB1) and p65 (RelA) inhibitory activities, with *β*-mangostin (**6**) and garcinone D (**7**), respectively, being the most active substances found (Table 1). The major cytotoxic isolate, *α*-mangostin (**5**), was chosen for evaluation in an in vivo hollow fiber assay, which is used as a secondary bioassay in our drug discovery program to prioritize leads for subsequent analysis in traditional xenograft models.[24] However, compound **5** was found to be inactive against HT-29 and LNCaP (hormone-dependent human prostate cancer) cells implanted at the intraperitoneal (i.p.) site at doses of 2.5, 5, 10, and 20 mg/kg (Figure S1, Supporting Information). Therefore, on the basis of these results in the hollow fiber assay, *α*-mangostin (**5**) does not seem to be promising as a potential anticancer agent.

Experimental Section

General Experimental Procedures

The melting point was measured on a Fisher-Johns 12-144 melting point apparatus with a 12-142T thermometer (Fisher Scientific, Pittsburgh, PA), and is uncorrected. Optical rotations were measured with a Perkin-Elmer 343 automatic polarimeter. UV and IR spectra were obtained with a Shimadzu UV 160U spectrophotometer and Thermo Scientific Nicolet™ 6700 FT-IR spectrometer, respectively. 1D and 2D NMR experiments were performed on Bruker Avance DPX-300 and DRX-400 spectrometers with tetramethylsilane (TMS) as internal standard. Electrospray ionization (ESI) mass spectrometric analyses were performed with a 3-Tesla Finnigan FTMS-2000 Fourier Transform mass spectrometer. Silica gel (65–250 and 230–400 mesh, Sorbent Technologies, Atlanta, GA) and Sephadex LH-20 (Supelco, Bellefonte, PA) were used for column chromatography. Thin-layer chromatographic (TLC) analysis was performed on Silica G (silica gel, 0.2 mm layer thickness, Sorbent Technologies, Atlanta, GA) and RP-18 F_{254s} (Merck, Germany) TLC plates, with visualization under UV light (254 and 365 nm) and 10% (v/v) sulfuric acid spray followed by heating (120 °C, 2 min). A Sunfire PrepC_{18} column (150 mm × 19 mm i.d., Waters, Milford, MA) and a Sunfire guard column (5 μm, 10 mm × 19 mm i.d., Waters, Milford, MA) were used for preparative HPLC, along with a Waters system composed of a 600 controller, a 717 Plus autosampler, and a 2487 dual wavelength absorbance detector.

Plant Material

The stem bark of *G. mangostana* (400 g) was collected at Pangradin village, Jasinga, West Java, Indonesia, by S. R. in August, 2005, who also identified this plant. A voucher specimen (acquisition number 2285414) has been deposited in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, Illinois.

Extraction and Isolation

The dried stem bark of *G. mangostana* (400 g) was extracted with MeOH (3 × 1 L) overnight at room temperature. The solvent was evaporated in vacuo to afford a MeOH extract (75 g), which was then suspended in MeOH-H_2O (9:1, 1 L), and partitioned with hexane (3 × 1 L). To the defatted residue, which was dried in vacuo, was added 10% MeOH in H_2O (1 L), and then this was partitioned with $CHCl_3$ (3 × 1 L). The $CHCl_3$-soluble layer was washed with 1% aqueous NaCl (3 × 1 L) to provide a partially detannified $CHCl_3$ extract (14 g). This $CHCl_3$

extract was subjected to silica gel column chromatography (CC; φ 3.5 cm; 60–250 mesh, 200 g), using gradient mixtures of MeOH in $CHCl_3$ (0→1%) as mobile phases, affording nine fractions (FI - FIX). These fractions were evaluated against the HT-29 cell line, and, of these fractions, FI - FV were found to be active (ED_{50} <10 μg/mL). Compounds **6** (300 mg, 0.075% w/w) and **5** (2.5 g, 0.625% w/w) were isolated from fraction FI and FII, respectively, by precipitation in MeOH. The residual portion of fraction FI (464 mg), eluted with 100% CH_2Cl_2 from the first separation, was subjected to silica gel CC (φ 2.5 cm; 230–400 mesh, 50 g), with hexanes-EtOAc (4:1→1:1) as solvent system, yielding **8** (8.0 mg, 0.002% w/w). Fraction FII (3.3 g, after partial removal of α-mangostin), also eluted with 100% CH_2Cl_2 from the first purification step, was separated by silica gel CC (φ 3 cm; 230–400 mesh, 100 g), using gradient mixtures of hexanes-EtOAc-MeOH (20:10:1→10:10:1) for elution, affording seven sub-fractions. Sub-fraction FII-1 (100 mg), eluted with hexanes-EtOAc-MeOH (20:10:1), was further purified by preparative HPLC, using an isocratic mixture of MeOH-H_2O (8:2, 8 mL/min) as solvent system, to afford **9** (t_R 12.1 min, 3.1 mg, 0.00077% w/w) and **10** (t_R 19.5 min, 2.2 mg, 0.00055% w/w). Fraction FIII (987 mg), eluted with 0.1% MeOH in CH_2Cl_2 from the first separation, was subjected again to silica gel CC (φ 2.5 cm; 230–400 mesh, 50 g), using gradient mixtures of MeOH in $CHCl_3$ (1→10%) as mobile phases, providing ten sub-fractions. Passage over Sephadex LH-20 of the third fraction (210 mg) from this column, which was eluted with 1% MeOH in $CHCl_3$, using 100% MeOH, afforded mangostanol (2.2 mg, 0.00055% w/w). Fraction FIV (1.1 g), eluted with 0.15% MeOH in CH_2Cl_2 from the first separation, was separated by silica gel CC (φ 3 cm; 230–400 mesh, 70 g), using a gradient solvent system of CH_2Cl_2-acetone (99:1→9:1), providing 16 sub-fractions. Sub-fraction 10 (100 mg), eluted with CH_2Cl_2-acetone (95:5), was subjected to Sephadex LH-20 chromatography using 100% MeOH as solvent, yielding **11** (15.5 mg, 0.0038% w/w). The fourth fraction (10 mg) from this Sephadex LH-20 column was purified by preparative HPLC, using an isocratic mixture of CH_3CN-H_2O (9:1, 8 mL/min) as solvent system, to afford **3** (t_R 18.5 min, 3.4 mg, 0.00085% w/w). The combined sub-fractions 11 and 12 (97 mg), eluted with CH_2Cl_2-acetone (95:5), were subjected to passage over Sephadex LH-20 (100% MeOH), providing four sub-fractions. The first sub-fraction (20.1 mg) from this separation was purified by preparative HPLC using an isocratic mixture of MeOH-H_2O (85:15, 5 mL/min) as solvent system, to obtain **1** (t_R 11.8 min, 5.1 mg, 0.00127%). Combined sub-fractions 13 and 14 (162 mg), eluted with CH_2Cl_2-acetone (95:5), were also separated by Sephadex LH-20 CC (100% MeOH), affording **7** (28.2 mg, 0.007% w/w) and **4** (3.2 mg, 0.0008% w/w). Fraction FV (242 mg), eluted with 0.2% MeOH in CH_2Cl_2 from the first separation, was subjected to Sephadex LH-20 CC, using 100% MeOH as solvent system, and then purified by preparative HPLC using an isocratic mixture of CH_3CN-H_2O (9:1, 8 mL/min) as solvent system, furnishing **2** (t_R 9.2 min, 3.9 mg, 0.00097% w/w).

11-Hydroxy-3-*O*-methyl-1-isomangostin (1)—yellow amorphous powder; mp 130–132 °C; $[\alpha]_D^{25}$ +9.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 242 (4.43), 252 (4.39), 303 (4.18) nm; IR (film) ν_{max} 3350, 2931, 1614, 1456, 1374, 1272, 1208, 1181, 1138, 1115 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.76 (1H, s, H-5), 6.29 (1H, s, H-4), 5.33 (1H, t, *J* = 6.4 Hz, H-16), 4.07 (2H, t, *J* = 6.4 Hz, H-15), 3.85 (1H, t, *J* = 4.9 Hz, H-11), 3.83 (3H, s, OCH_3-3), 3.78 (3H, s, OCH_3-7), 2.84 (1H, dd, *J* = 17.3, 4.9 Hz, H-10β), 2.69 (1H, dd, *J* = 17.3, 4.9 Hz, H-10α), 1.80 (3H, s, H-19), 1.65 (3H, s, H-18), 1.48 (3H, s, H-14), 1.36 (3H, s, H-13); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.9 (C, C-9), 162.0 (C, C-3), 157.4 (C, C-4a), 154.6 (C, C-6), 154.3 (C, C-5a), 153.7 (C, C-1), 142.9 (C, C-7), 137.2 (C, C-8), 131.4 (C, C-17), 124.3 (CH, C-16), 115.3 (C, C-8a), 108.0 (C, C-9a), 104.0 (C, C-2), 101.4 (CH, C-5), 90.7 (CH, C-4), 78.2 (C, C-12), 68.8 (CH, C-11), 61.9 (CH_3, OCH_3-7), 56.0 (CH_3, OCH_3-3), 26.6 (CH_2, C-15), 26.5 (CH_2, C-10), 26.1 (CH_3, C-18), 24.7 (CH_3, C-13), 22.6 (CH_3, C-14), 18.5 (CH_3, C-19); ESIMS (positive mode) *m*/*z* 463.17 $[M+Na]^+$ (100), 322.80 (10), 172.89 (40); HRESITOFMS (positive mode) *m*/*z* 463.1729 $[M+Na]^+$ (calcd for $C_{25}H_{28}O_7$, 463.1733).

11-Hydroxy-1-isomangostin (2)—$[\alpha]_D^{25}$ +11.4 (*c* 0.1, MeOH).

11*α*-Mangostanin (3)—$[\alpha]_D^{25}$ −1.75 (*c* 0.1, MeOH).

Cratoxyxanthone (11)—$[\alpha]_D^{25}$ +0.06 (*c* 0.13, MeOH).

Cytotoxicity Assay

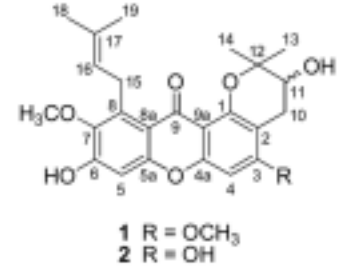
Cytotoxic potential against HT-29 was determined using a established protocol.[25] Camptothecin was employed as the positive control (ED_{50} = 0.06 μM).

Enzyme-based ELISA NF-κB Assay

The NF-κB p65 and p50 inhibitory activity assay was conducted according to a published protocol.[26,27] Rocaglamide was used as the positive control and exhibited IC_{50} values of 0.08 and 2.0 μM in this assay.

Hollow Fiber Assay

α-Mangostin (**5**) was evaluated in the in vivo hollow fiber model, using HT-29, LNCaP, and MCF-7 cells, according to a procedure described in the literature.[28,29] Paclitaxel was used as the positive control for this experiment at a dose of 20 mg/kg.



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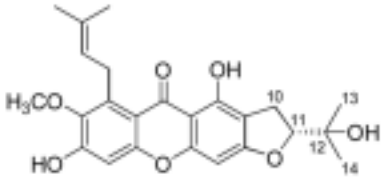

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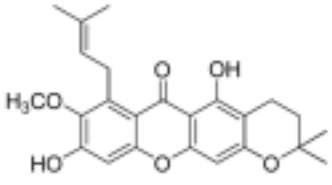

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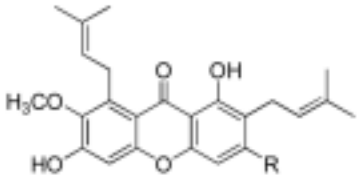

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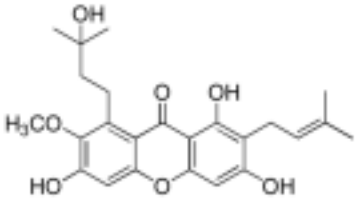

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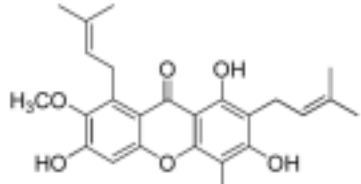

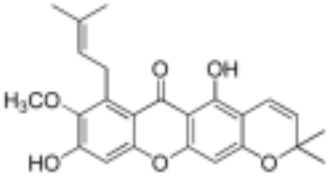

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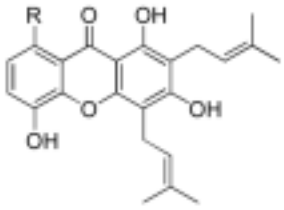

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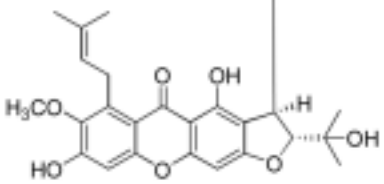

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Chin YW, Kinghorn AD. Mini-Rev Org Chem 2008;5:355–364.
2. Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M. Phytother Res 2009;23:1047–1065. [PubMed: 19172667]
3. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD. J Agric Food Chem 2006;54:2077–2082. [PubMed: 16536578]
4. Balunas MJ, Su B, Brueggemeier RW, Kinghorn AD. J Nat Prod 2008;71:1161–1166. [PubMed: 18558747]
5. Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD. Phytochemistry 2008;69:754–758. [PubMed: 17991497]
6. Kinghorn AD, Carcache de Blanco EJ, Chai H-B, Orjala J, Farnsworth NR, Soejarto DD, Oberlies NH, Wani MC, Kroll DJ, Pearce CJ, Swanson SM, Kramer RA, Rose WC, Emanuel S, Vite GD, Jarjoura D, Cope FO. Pure Appl Chem 2009;81:1051–1063. [PubMed: 20046887]
7. Pavia, DL.; Lampman, GM.; Kriz, GS. Introduction to Spectroscopy. Thomson Learning, Ltd; London: 2001.
8. Sia GL, Bennett GJ, Harrison LJ, Sim KY. Phytochemistry 1995;38:1521–1528.
9. (a) Dale JA, Mosher HS. J Am Chem Soc 1972;95:512–519. (b) Ohtani I, Kusumi T, Kashman Y, Kakisawa H. J Am Chem Soc 1991;113:4902–4906. (c) Su BN, Park EJ, Mbwambo ZH, Santarsiero BD, Mesecar AD, Fong HHS, Pezzuto JM, Kinghorn AD. J Nat Prod 2002;65:1278–1282. [PubMed: 12350147]
10. Nilar, Harrison LJ. Phytochemistry 2002;60:541–548. [PubMed: 12052521] . In this reference, the compound number of mangostanin should be **15** rather than the erroneous **13**.
11. Crews, P.; Rodriguez, J.; Jaspars, M. Organic Structure Analysis. Oxford University Press; New York: 1998.
12. Sen AK, Sarkar KK, Mazumder PC, Banerji N, Uusuori R, Hase TA. Phytochemistry 1982;21:1747–1750.
13. Likhitwitayawuid K, Phadungcharoen T, Krungkrai J. Planta Med 1998;64:70–72. [PubMed: 9491769]
14. Bennett GJ, Harrison LJ, Sia GL, Sim KY. Phytochemistry 1993;32:1245–1251.
15. Sen AK, Sarkar KK, Mazumder PC, Banerji N, Uusvuori R, Hase TA. Phytochemistry 1980;19:2223–2225.
16. Nguyen LHD, Vo HT, Pham HD, Connolly JD, Harrison LJ. Phytochemistry 2003;63:467–470. [PubMed: 12770600]
17. Govindachari TR, Kalyanaraman PS, Muthukumaraswamy N, Pai BR. Tetrahedron 1971;27:3919–3926.
18. Chairungsrilerd N, Takeuchi K, Ohizumi Y, Nozoe S, Ohta T. Phytochemistry 1996;43:1099–1102.
19. Suksamrarn S, Komutiban O, Ratananukul P, Chimnoi N, Lartpornmatulee N, Suksamrarn A. Chem Pharm Bull 2006;54:301–305. [PubMed: 16508181]
20. Ee GCL, Daud S, Izzaddin SA, Rahmani M. J Asian Nat Prod Res 2008;10:481–485. [PubMed: 18464092]
21. Laphookhieo S, Syers JK, Kiattansakul R, Chantrapromma K. Chem Pharm Bull 2006;54:745–747. [PubMed: 16651783]
22. Matsumoto K, Akao Y, Yi H, Ohguchi K, Ito T, Tanaka T, Kobayashi E, Iinuma M, Nozawa Y. Bioorg Med Chem 2004;12:5799–5806. [PubMed: 15498656]
23. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Int J Mol Sci 2008;9:355–370. [PubMed: 19325754]

24. Mi Q, Pezzuto JM, Farnsworth NR, Wani MC, Kinghorn AD, Swanson SM. J Nat Prod 2009;72:573–580. [PubMed: 19161316]

25. Likhitwitayawuid K, Angerhofer CK, Cordell GA, Pezzuto JM, Ruangrungsi N. J Nat Prod 1993;56:30–38. [PubMed: 8450319]

26. Renard P, Ernest I, Houbion A, Art M, Le Calvez H, Raes M, Remacle J. Nucleic Acids Res 2001;29:e21. [PubMed: 11160941]

27. Salim AA, Pawlus AD, Chai HB, Farnsworth NR, Kinghorn AD, Carcache-Blanco EJ. Bioorg Med Chem Lett 2007;17:109–112. [PubMed: 17055270]

28. Mi Q, Cui B, Silva GL, Lantvit DD, Lim E, Chai H, Hollingshead MG, Mayo JG, Kinghorn AD, Pezzuto JM. Cancer Lett 2002;184:13–20. [PubMed: 12104043]

29. Hollingshead MG, Alley MC, Camalier RF, Abbott BJ, Mayo JG, Malspeis L, Grever MR. Life Sci 1995;57:131–41. [PubMed: 7603295]

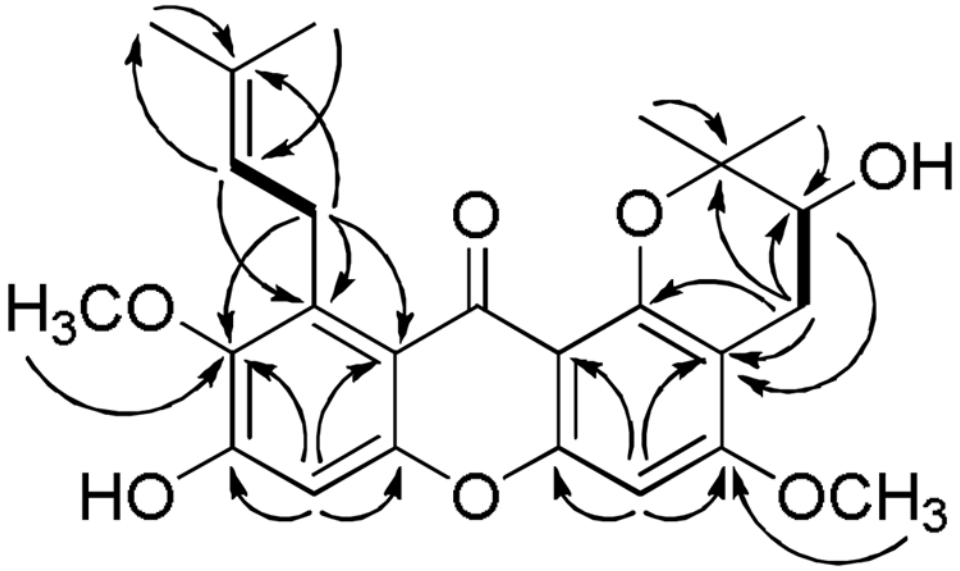

Figure 1.
Important ^{1}H-^{1}H COSY (▬) and ^{1}H-^{13}C HMBC (→) correlations of compound **1**.

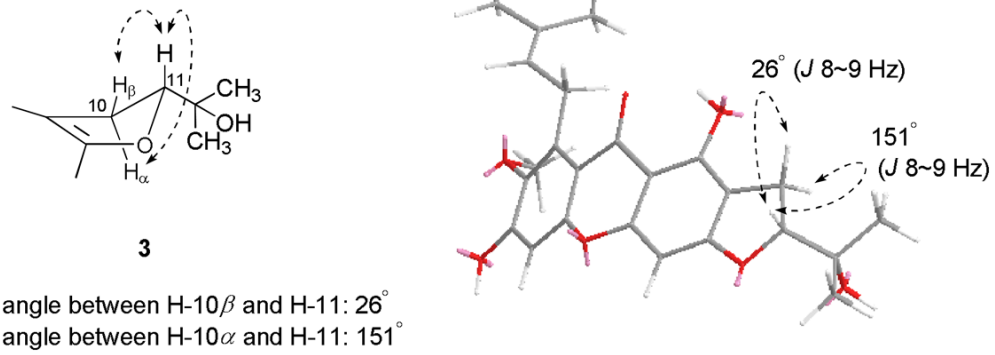

Figure 2.
Dihedral angles in the energy-minimized stereostructure of compound **3**.

Table 1

In Vitro Activity of Compounds **4–10** in Cytotoxicity (HT-29 Cell Line) and ELISA NF-κB (p65 and p50) Assays.

compound	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**camptothecin**[d]	**rocaglamide**[d]
cytotoxicity[a]	4.9	1.7	1.7	2.3	9.1	>10	>10	0.06	-
NF-κB (p65)[b]	NT[c]	15.9	12.1	3.2	>20	11.3	19.0	-	0.08
NF-κB (p50)[b]	NT[c]	>20	7.5	>20	>20	>20	>20	-	2.0

[a] Compounds **1**–**3** and **11** were inactive (ED_{50} >10 μM).

[b] Compounds **1**, **2**, and **11** were inactive (IC_{50} >20 μM).

[c] Compounds **3** and **4** were not tested in the NF-κB assays because of the limited amounts available.

[d] Positive control substances.

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