Cytotoxic 3,4-*seco*-Cycloartane Triterpenes from *Gardenia sootepensis*

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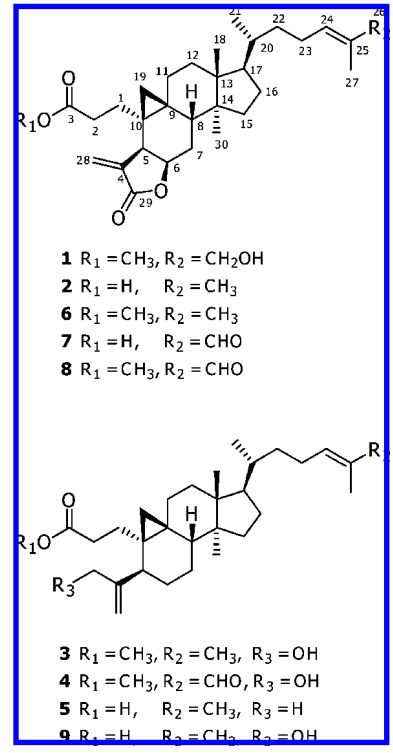
Five new 3,4-*seco*-cycloartanes, sootepins A−E (**1**−**5**), along with four known triterpenes (**6**−**9**), were isolated from the apical buds of *Gardenia sootepensis*. Their structures were elucidated on the basis of spectroscopic methods (1D and 2D NMR, HRESIMS, and X-ray crystallography), and the compounds were tested for in vitro cytotoxic activity against human breast (BT474), lung (CHAGO), liver (Hep-G2), gastric (KATO-3), and colon (SW-620) cancer cell lines. Generally, the compounds possessing an exomethylene γ-lactone ring showed broad cytotoxicity for all cell lines tested.

Plants belonging to the genus *Gardenia* have proven to be a rich source of cycloartane triterpenoids, some of which display interesting biological activities including cytotoxic and anti-HIV-1 effects.[1−5] Additionally, extracts of various species exhibiting anti-implantation and abortifacient effects[6] and antiulcer,[7] antibacterial,[8] diuretic,[9] analgesic,[9] hypertensive,[9] and larvicidal activity[10] have been reported. As part of our ongoing project on the discovery of natural anticancer agents, an EtOAc extract of the apical buds of *Gardenia sootepensis* Hutch. (Rubiaceae), distributed throughout northern parts of Thailand, was found to be cytotoxic. Investigation of the extract led to the isolation and identification of five new 3,4-*seco*-cycloartane triterpenes, sootepins A−E (**1**−**5**), and four known compounds (**6**−**9**). Compounds **1**−**9** were evaluated for cytotoxic activity against human breast (BT474), lung (CHAGO), liver (Hep-G2), gastric (KATO-3), and colon (SW-620) cancer cell lines.



Results and Discussion

The MeOH extract of fresh apical buds of *G. sootepensis* was partitioned between EtOAc and H_2O to afford an EtOAc extract, which was subjected to silica gel column chromatography using EtOAc−hexane mixtures of increasing polarity as eluent. Further purification by repeated normal column chromatography and preparative thin-layer chromatography gave five new 3,4-*seco*-cycloartanes (**1**−**5**) and four known compounds, tubiferolide methyl ester (**6**),[3] coronalolide (**7**),[1] coronalolide methyl ester (**8**),[1] and secaubryenol (**9**).[5] The structures of the known compounds were determined by comparison of their NMR spectroscopic data with those in the literature.

Sootepin A (**1**) was obtained as colorless crystals. Its molecular formula was determined as $C_{31}H_{46}O_5$ from the HRESIMS ion at *m/z* 499.3427 $[M + H]^+$, indicating nine degrees of unsaturation. The ^1H NMR spectrum displayed a pair of doublets at δ_H 0.16 and 0.41 ($J = 4.8$ Hz), characteristic of the C-19 methylene protons of the cyclopropane ring of a cycloartane triterpene,[11−15] an olefinic proton, one vinylic methyl group, two tertiary methyl groups, one secondary methyl group, and one methoxy methyl. A pair of doublets at δ_H 5.73 and 6.33 ($J = 1.6$ Hz) were ascribed to H-28a and H-28b in the exocyclic methylene γ-lactone ring, and signals of the β- and γ-methine protons of the lactone ring appeared at δ_H 3.23 (H-5) and 4.74 (H-6), respectively. In addition, the lactonization of C-4 onto C-6 was confirmed by the HMBC correlations observed from H-6 to C-29 (δ_C 170.8) (Figure 1). The ^{13}C and HSQC spectra revealed the presence of 31 nonequivalent carbons including two carbonyl carbons, four sp^2 carbons (two quaternary C, one CH, and one CH_2), and 26 sp^3 carbons (four quaternary C, five CH, 13 CH_2, four CH_3, and one $-OCH_3$). These ^1H and ^{13}C spectra were closely related to those previously reported for tubiferolide methyl ester (**6**),[3] except for the marked differences in chemical shift values corresponding to the side chain at C-26. In the ^1H NMR spectrum of **1**, the signal attributable to an oxygen-bearing methylene at δ_H 3.99 replaced those corresponding to the methyl signal of **6** at δ_H 1.68. The relative stereochemistry of **1** was assigned on the basis of a NOESY experiment (Figure 2). Observation of a strong NOESY cross-peak between H-5 and H-6 permitted the assignment of a relative 5,6-*cis*-configuration. Additionally, the compound exhibited NOEs between H-8 and H_3-18, H-8 and H-19b, H-6 and H_3-30, H-17 and H_3-30, and H-17 and H_3-21. These were in good agreement with the relative configurations at C-5, C-6, C-8, C-9, C-10, C-13, C-14, and C-17 long-established for the cycloartane core. Finally, the relative configu-

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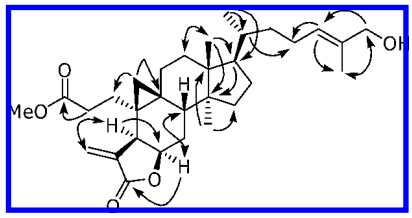

Figure 1. Key HMBC of **1**.

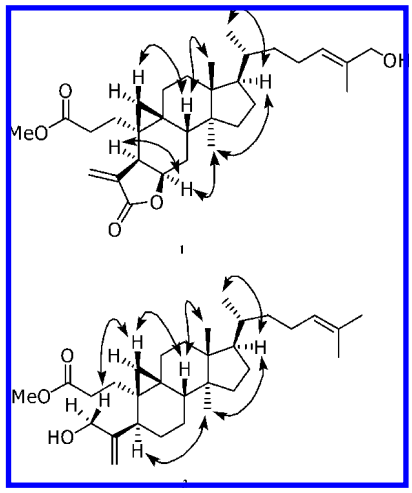

Figure 2. Key NOESY correlations of **1** and **3**.

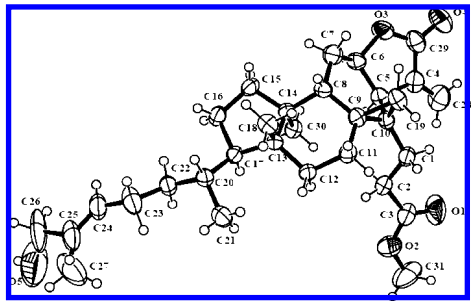

Figure 3. ORTEP drawing of **1** with atom labeling.

ration of compound **1** was confirmed by X-ray diffraction analysis as shown in Figure 3.

Sootepin B (**2**) was isolated as a light yellow, amorphous solid and had the molecular formula $C_{30}H_{44}O_4$, as established by HRESIMS (*m*/*z* 469.3315 $[M + H]^+$). In the ^1H NMR spectrum, typical signals for a cyclopropane methylene proton appeared as two doublets at δ_H 0.16 and 0.43 ($J = 5.2$ Hz), and its NMR data were almost the same those of tubiferolide methyl ester (**6**), except for the absence of a methoxy group at C-3 in **6**. The relative configuration of **2** was assigned to be the same as that of **1** and **6** by comparing their NMR data and NOESY correlations of H-5/H-6, H-6/H_3-30, H-8/H_3-18, H-8/H-19b, and H-17/H_3-21.

Sootepin C (**3**), obtained as a colorless oil, had a molecular formula of $C_{31}H_{50}O_3$ as determined by HRESIMS (*m*/*z* 471.3840 $[M + H]^+$), suggesting seven degrees of unsaturation. The ^1H NMR spectrum also showed typical signals associated with a 3,4-*seco*-cycloartane triterpene, including two tertiary methyl singlets at δ_H 0.92 and 0.96, one secondary methyl doublet at δ_H 0.88 ($J = 6.4$ Hz), and a characteristic pair of doublets at δ_H 0.46 and 0.71 ($J = 4.3$ Hz), assigned to the C-19 methylene protons in the cyclopropane ring. Allylic coupling observed in the COSY spectrum between a two-proton broad singlet at δ_H 4.13 accounting for a primary alcoholic group and two broad singlets of a terminal alkene at δ_H 5.07 and 5.09 was suggestive of the structure of a 29-hydroxy-3,4-*seco*-cycloartane. Both ^1H and ^{13}C NMR data of **3** were virtually identical to those previously reported for secaubryenol (**9**),[5] with the only difference being the appearance of a three-proton singlet of a methoxy group at δ_H 3.64. Consequently, the structure of this derivative was established as **3**. The same NOESY correlations of H-8/H_3-18, H-8/H-19b, H-17/H_3-21, and H-17/H_3-30 as for **9** gave evidence for the relative configuration of **3** at C-5, C-8, C-9, C-10, C-13, C-14, C-17, and C-20 (Figure 2).

Sootepin D (**4**) was obtained as a colorless oil, and its molecular formula $C_{31}H_{48}O_4$ was deduced from the HRESIMS (*m*/*z* 485.3628 $[M + H]^+$), 14 mass units more than that of **3**. Comparison of the ^1H and ^{13}C NMR spectra of **4** with those of **3** revealed them to be very similar, with the only difference being the appearance of a singlet due to an aldehyde group at δ_H 9.39 in the ^1H NMR spectrum, coupled in the HSQC spectrum to a newly appearing aldehyde carbonyl carbon at δ_C 195.5, while a vinylic methyl signal at δ_H 1.68 and at δ_C 17.6 had disappeared. The relative configuration of **4** was assigned to be the same as that of **3** on the basis of NOESY correlations H-8/H_3-18, H-8/H-19b, H-17/H_3-21, and H-17/H_3-30. Thus, the structure of this new compound was established as **4**.

Sootepin E (**5**) was isolated as a colorless oil. Its molecular formula, $C_{30}H_{48}O_2$, was determined on the basis of HRESIMS at *m*/*z* 441.3731 $[M + H]^+$. The ^1H and ^{13}C NMR data of **5** were also similar to those of **3**. The NMR spectrum showed the presence of an additional vinylic methyl at δ_H 1.68 and at δ_C 19.7, while the signals of oxygen-bearing methylene (C-29) at δ_H 4.13 and at δ_C 64.7 as for **3** had disappeared. This indicated that the OH-29 in **3** was replaced by H-29. There was no three-proton singlet characteristic of a methoxy group observed. Thus, the structure of this derivative was depicted as **5**. The relative configuration of **5** was established to be the same as that of **3** and **4** on the basis of the NOESY correlations.

The cytotoxicity of compounds **1**−**9** was tested in vitro against five human tumor cell lines (Table 3). All compounds possessing an exomethylene γ-lactone ring system (**1**–**2** and **6**–**7**) exhibited broad cytotoxic activity for all cell lines tested, except that compound **8** was only moderately cytotoxic to KATO-3, SW-620, and Hep-G2 cells. Compounds **3**, **4**, and **9**, which do not contain an exomethylene γ-lactone ring, showed no significant activity or were inactive, while compound **5** showed broad activity for all five cell lines. Compound **5** has the same structure as **3**, except for the absence of an OH group at C-29 and an OCH_3 at C-1. Thus, this result revealed that these additional groups reduce the cytotoxic activity of compounds in this type. Compounds **1** and **5** were more cytotoxic than the positive control, doxorubicin.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter using a sodium lamp at wavelength 589 nm, and UV data were recorded on a Shimadzu UV-160 spectrophotometer. Melting points were measured using a Fisher-Johns melting point apparatus. IR spectra were recorded on a Perkin-Elmer model 1760X Fourier transform infrared spectrophotometer. HRESIMS spectra were obtained using a Bruker micrOTOF mass spectrometer. The NMR spectra were recorded on a Varian YH400 spectrometer at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR using TMS (trimethylsilane) as internal standard.

Plant Material. Aerial parts of *G. sootepensis* were collected from Kampangpech Province, Thailand, in November 2008. A voucher specimen (BKF 156377) has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

Extraction and Isolation. The fresh apical buds (93.11 g) of *G. sootepensis* were extracted with MeOH (500 mL × 2, each 2 days) at room temperature. After removing the solvent *in vacuo*, the combined MeOH crude extract was suspended in H_2O (250 mL), then partitioned with EtOAc (200 mL × 3), to afford a EtOAc crude extract (43.47 g). This crude extract was chromatographed on a silica gel column eluted with a gradient of hexane−CH_2Cl_2 (from 1:0 to 0:1) to yield seven fractions (I−VII). Fraction IV (3.27 g) was subjected to silica gel column chromatography (CC) and eluted with a gradient system of

Table 1. ^{1}H NMR Data of Compounds **1**−**5** ($CDCl_3$, 400 MHz, δ in ppm, J in Hz)

position	1	2	3	4	5
1	2.24 m	2.26 m	2.10 m	1.68 m	2.06 m
	1.61 m	1.59 m	1.38 m	1.36 m	1.37 m
2	2.48 m	2.53 m	2.50 m	2.51 m	2.54 m
		2.46 m	2.28 m	2.28 m	2.30 m
5	3.23 d (8.0)	3.23 d (8.3)	2.50 m	2.50 m	2.42 m
6	4.74 dd (7.1, 7.3)	4.74 dd (6.9, 7.8)	1.68 m	2.14 m	1.47 m
			1.05 m	1.00 m	1.08 m
7	1.61 m	1.80 m	1.68 m	1.33 m	1.35 m
	1.78 m	1.54 m	1.27 m	1.08 m	1.08 m
8	2.12 m	2.13 br t (5.5)	1.52 m	1.53 m	1.60 m
11	1.78 m	1.80 m	2.12 m	2.12 m	2.09 m
	1.65 m	1.54 m	1.55 m	1.21 m	1.26 m
12	1.66 m	1.59 m	1.68 m	1.66 m	1.65 m
15	1.32 m	1.33 m	1.27 m	1.30 m	1.37 m
					1.28 m
16	1.32 m	1.91 m	1.28 m	1.92 m	1.89 m
	1.93 m	1.33 m		1.30 m	1.28 m
17	1.61 m	1.59 m	1.60 m	1.66 m	1.60 m
18	0.90 s	0.92 br s	0.96 br s	0.97 s	0.95 br s
19	0.16 d (4.8)	0.43 d (5.2)	0.71 d (4.3)	0.72 d (4.3)	0.73 d (4.1)
	0.41 d (4.8)	0.16 d (5.2)	0.46 d (4.3)	0.48 d (4.3)	0.40 d (4.1)
20	1.06 m	1.42 m	1.68 m	1.49 m	1.28 m
21	0.83 br s	0.88 d (6.5)	0.88 d (6.4)	0.93 br s	0.88 d (6.3)
22	1.53 m	1.42 m	1.52 m	1.58 m	1.48 m
	1.23 m	1.03 m	1.03 m	1.21 m	1.05 m
23	2.12 m	2.02 m	2.03 m	2.40 m	2.05 m
	1.91 m	1.83 m	1.86 m	2.27 m	1.89 m
24	5.38 br t (6.5)	5.08 t (6.8)	5.10 m	6.49 t (7.0)	5.10 t (7.1)
26	3.99 s	1.67 br s	1.68 m	9.39 s	1.68 br s
27	1.66 s	1.59 br s	1.60 m	1.75 s	1.60 br s
28	5.73 d (1.6)	6.33 d (2.1)	5.09 br s	5.10 br s	4.81 br s
	6.33 d (1.6)	5.72 d (1.7)	5.07 br s	5.10 br s	4.73 br s
29			4.13 br s	4.13 br s	1.68 br s
30	0.88 s	0.90 br s	0.92 br s	0.93 s	0.92 br s
OMe	3.68 s		3.64 s	3.64 s	

EtOAc−hexane to give 11 subfractions (IVa−IVk). Subfractions IVf and IVi were combined and then subjected to CC over silica gel using a mixture of EtOAc−hexane (1:3) to afford **3** (21 mg), **1** (125 mg), and **5** (24 mg). Subfraction IVk was separated on a silica gel column (EtOAc−hexane, 1:2) to yield **8** (450 mg). Fraction V was rechromatographed on a silica gel column using EtOAc−hexane (1:3) to furnish **5** (170 mg), **6** (170 mg), and a second crop of **8** (1.75 g). Fraction VI was separated into four fractions (VIa−VId) by column chromatography over silica gel (EtOAc−hexane, 1:1), and VId was further purified by a silica gel column eluting with acetone−hexane (1:2) to yield **7** (14 mg) and **2** (30 mg). Fraction VII was subjected to silica gel CC eluted with a gradient system of acetone−hexane (from 1:9 to 1:4) to give 13 fractions (VIIa−VIIm). VIIc were further purified by preparative TLC (acetone−benzene, 1:9) to yield **4** (23 mg). VIIi afforded secauryenol **9** (48 mg) after recrystallization from acetone−hexane.

Sootepin A (1): colorless crystals; mp 143−144 °C; $[\alpha]^{25}_D$ +173.0 (c 0.1, MeOH); UV (MeOH) ν_{max} (log ϵ) 210 (3.84); IR (KBr) ν_{max} 3566, 3503, 2952, 1755, 1733, 1459, 1378, 1266, 1145, 1002 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS m/z 499.3427 (calcd for $C_{31}H_{46}O_5$ + H, 499.3424).

X-ray Crystallographic Analysis of Sootepin A (1). Crystal data: colorless crystal; $C_{31}H_{46}O_5$, M_r = 498.68, triclinic, $P1$, a = 7.3556(2) Å, b = 7.4296(2) Å, c = 14.2899(4) Å, α = 81.7620(10)°, β = 82.9370(10)°, γ = 65.0020(10)°, Z = 1, and V = 698.77(3) Å^3, Mo Kα radiation, λ = 0.71073 Å. The intensity data were collected at 293 K to a maximum 2θ value of 50.92°. Of the 7425 reflections collected, 4618 were unique, 329 parameters (R_{int} = 0.0232). The crystal structure was solved by direct methods and using the SHELXS97[16] program. Refinements were made by full-matrix least-squares on all F^2 data using SHELXL97[17] to final R values [$I > 2\sigma(I)$] of R_1 = 0.0454, wR_2 = 0.1219 and goodness of fit on F^2 = 1.010. All non-hydrogen atoms were anisotropically refined. All hydrogen atoms were added at calculated positions and refined using a rigid model. Crystallographic data, excluding structure factors, have been deposited at the Cambridge Crystallographic Data Centre under the deposition number CCDC

Table 2. ^{13}C NMR Data of Compounds **1**−**5** ($CDCl_3$, 400 MHz, δ in ppm)

position	1	2	3	4	5
1	30.9	31.0	28.9	28.9	28.8
2	31.2	31.2	31.6	31.6	31.3
3	173.5	178.6	174.6	174.5	179.7
4	139.1	139.1	152.5	152.4	149.5
5	39.0	39.0	42.1	42.1	45.9
6	74.5	74.6	29.0	28.9	27.7
7	27.2	27.2	25.3	25.3	25.0
8	38.3	38.3	47.9	47.9	47.7
9	25.1	25.1	21.8	21.8	21.4
10	28.2	28.1	27.4	28.3	27.0
11	26.5	26.6	26.9	26.9	26.9
12	33.0	33.0	33.0	33.0	33.0
13	45.7	45.7	45.1	45.1	45.1
14	48.6	48.6	48.8	48.9	48.9
15	34.8	34.8	35.7	35.6	35.9
16	27.7	27.7	25.1	28.1	28.1
17	51.4	51.4	52.2	52.1	52.2
18	15.9	15.8	18.2	18.0	18.0
19	23.0	23.0	30.2	30.2	30.0
20	35.9	35.9	35.8	36.0	35.6
21	18.4	18.4	18.2	18.0	18.2
22	35.9	36.3	36.3	34.7	36.3
23	24.5	24.9	24.9	26.0	24.9
24	126.8	125.0	125.2	155.6	125.2
25	134.4	131.1	130.9	139.1	130.9
26	69.0	25.7	25.7	195.5	25.7
27	13.6	17.7	17.6	9.2	17.6
28	123.1	123.2	110.4	110.5	111.5
29	170.8	170.9	64.7	64.7	19.7
30	20.1	20.1	19.3	19.4	19.3
OMe	51.8		51.6	51.6	

Table 3. Cytotoxic Data for Compounds **1**−**9**

	IC_{50} (μg/mL)/cell line				
compound	BT-474	KATO-3	CHAGO	SW-620	Hep-G2
1	5.92	2.10	3.97	1.80	2.90
2	4.98	3.68	5.24	5.57	5.86
3	>10	7.79	>10	>10	3.49
4	>10	7.35	>10	>10	>10
5	6.07	1.91	5.20	4.22	3.14
6	5.33	4.90	4.08	5.78	3.41
7	6.59	5.85	5.42	4.98	6.41
8	>10	8.64	>10	6.19	6.80
9	>10	>10	>10	>10	>10
doxorubicin	8.92	5.45	3.98	>10	3.36

730211. Copies can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-0-1223-226033, e-mail: deposit@ccdc.cam.ac.uk).

Sootepin B (2): light yellow, amorphous solid; mp 132−133 °C; $[\alpha]^{25}_D$ +167.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.11); IR (KBr) ν_{max} 3530, 3424, 2942, 1744, 1708, 1450, 1377, 1279 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* 469.3315 (calcd for $C_{30}H_{44}O_4$ + H, 469.3318).

Sootepin C (3): colorless oil; $[\alpha]^{25}_D$ +173.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.46); IR (KBr) ν_{max} 3436, 2934, 1742, 1450, 1370, 1266, 1159 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* 471.3840 (calcd for $C_{31}H_{50}O_3$ + H, 471.3838).

Sootepin D (4): colorless oil; $[\alpha]^{25}_D$ +58.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (4.08); IR (KBr) ν_{max} 3428, 2938, 2868, 1724, 1683, 1450, 1372, 1164, 1062 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* 485.3628 (calcd for $C_{31}H_{48}O_4$ + H, 485.3631).

Sootepin E (5): colorless oil; $[\alpha]^{25}_D$ +97.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.77); IR (KBr) ν_{max} 3443, 2936, 1708, 1457, 1370, 1300, 1209 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2; HRESIMS *m*/*z* 441.3731 (calcd for $C_{30}H_{48}O_2$ + H, 441.3733).

In Vitro Cytotoxicity Bioassays.[18,19] All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000 cells per well with compounds added from DMSO-diluted stock. After three days in culture, attached cells were stained with MTT (3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium] bromide). The absorbency at 540 nm was measured using a microplate reader after solubilizing the bound dye. The mean IC_{50} is the concentration of agent that inhibited cell growth by 50% under the experimental conditions and was the average from at least six independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: human breast ductol carcinoma ATCC No. HTB 20 (BT474), undifferentiated lung carcinoma (CHAGO), liver hepatoblastoma (Hep-G2), gastric carcinoma ATCC No. HTB 103 (KATO-3), and colon adenocarcinoma ATCC No. CCL 227 (SW-620). All cell lines were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, and cultured in RPMI-1640 supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 5% fetal bovine serum, and 100 μg/mL kanamycin.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**5** are available free of charge via the Internet at http://pubs.acs.org.

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