Bioorganic & Medicinal Chemistry 19 (2011) 2790–2796

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

New 29-*nor*-cycloartanes with a 3,4-*seco*- and a novel 2,3-*seco*-structure from the leaves of *Sinocalycanthus chinensis*

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ARTICLE INFO

Article history:
Received 28 February 2011
Revised 22 March 2011
Accepted 23 March 2011
Available online 29 March 2011

Keywords:
Sinocalycanthus chinensis
Calycanthaceae
3,4-seco-29-nor-Cycloartane
2,3-seco-29-nor-Cycloartane
MDR

ABSTRACT

Eight new triterpenoids, including sinocalycanchinensins A–E (**1**–**5**) with a 3,4-*seco*-29-*nor*-cycloartane skeleton, sinocalycanchinensin F (**6**) possessing a novel 2,3-*seco*-29-*nor*-cycloartane skeleton, and 29-*nor*-cycloartanes, sinocalycanchinensins G and H (**7** and **8**), have been isolated from the leaves of *Sinocalycanthus chinensis*. Their structures were elucidated on the basis of spectroscopic examinations. The cytotoxicities of the isolated new triterpenes against a panel of human cancer cell lines, including multi-drug resistant (MDR) cancer cell lines, were also evaluated. Compound **5** demonstrated enhanced cytotoxicity against MDR KB cells in the presence of colchicine, although all the compounds showed moderate or no cytotoxicities.

1. Introduction

Sinocalycanthus chinensis Cheng et S.Y. Chang, the only representative in the genus *Sinocalycanthus* in the family Calycanthaceae, is native to Zhejiang of China. This plant is cultivated as an ornamental tree in China because of the attractive cream and yellow semi-double flowers, which appear on the terminal twigs from May to June.[1] The leaves of *S. chinensis* are used as a remedy for cold, cough, and wheeziness,[2] while the flowers and roots of this plant have been used for the treatment of stomachache.[3] However, there have been few reports of the constituents of *S. chinensis*.

In our chemical studies of medicinal plants aimed at searching for biologically active compounds, we have examined the leaves of *S. chinensis*, which has resulted in the isolation of eight new triterpenoids, including five 3,4-*seco*-29-*nor*-cycloartanes, sinocalycanchinensins A–E (**1**–**5**), a novel 2,3-*seco*-29-*nor*-cycloartane, sinocalycanchinensin F (**6**), and two 29-*nor*-cycloartanes, sinocalycanchinensins G and H (**7** and **8**), together with 14 known compounds. The cytotoxicities of the isolated new triterpenes against a panel of human cancer cell lines, including multi-drug resistant cancer cell lines, were also evaluated. In this paper, we describe the structure elucidation and cytotoxicities of these compounds.

2. Results and discussion

The leaves of *S. chinensis* were extracted with MeOH at room temperature. The MeOH extract was partitioned successively between EtOAc, BuOH, and water. The EtOAc-soluble portion was further partitioned between hexane and 90% MeOH. Repeated chromatographies of the 90% MeOH-soluble fraction over Sephadex LH-20, MCI gel CHP20P, YMC ODS-A, and purification by HPLC with Gel-Permeation Chromatography (GPC), ODS, and silica gel to afford eight new triterpenes (**1**–**8**), designated sinocalycanchinensins A–H (Fig. 1), together with 14 known compounds. The known compounds were identified as 8α-acetoxy-elemol,[4] 8α,11-elemodiol,[5] β-eudesmol,[6] cryptomeridiol,[6] 11-hydroxy-4α-methoxy-selinane,[7] β-sitosterol-3-*O*-β-D-glucoside,[8] kaempferol,[9] quercetin,[9] kaempferol-3-*O*-β-D-glucoside,[10] quercetin-3-*O*-β-D-glucoside,[10] scopoletin,[11] ferulic acid,[12] *p*-coumaric acid,[12] and isopropyl vanillate[13] by comparison of their physical and spectral data with those reported in the literature.

Sinocalycanchinensin A (**1**) was obtained as a white amorphous powder. The molecular formula of **1** was determined as $C_{29}H_{44}O_4$ by HRESIMS (*m*/*z* 479.3112 $[M+Na]^+$). The ^1H NMR spectrum showed a characteristic pair of doublet signals [δ_H 0.32, 0.49 (each 1H, d, J = 4.0 Hz)], ascribable to the cyclopropane methylene signals of a cycloartane-type triterpene. It also revealed the presence of a vinylic methyl group [δ_H 1.92 (3H, s)], two tertiary methyl groups [δ_H 0.94 and 0.98 (each 3H, s)], a secondary methyl group [δ_H 0.90 (3H, d, J = 6.0 Hz)], a vinyl group [δ_H 4.99 (1H, d, J = 10.0 Hz); 5.05 (1H, d, J = 17.2 Hz); 5.67 (1H, ddd, J = 8.8, 10.0, 17.2 Hz)], and a tri-substituted olefinic group [δ_H 6.10 (1H, t, J = 7.2 Hz)]. The ^{13}C NMR spectrum showed the signals of 29 carbon atoms: two carboxyl carbons, four olefinic carbons, including one quaternary, two methines, and a methylene, four methyl carbons, 11 methylene carbons, four methine carbons, and four quaternary carbons. The HMBC correlations of H_2-19 with C-1 and of

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doi:10.1016/j.bmc.2011.03.055

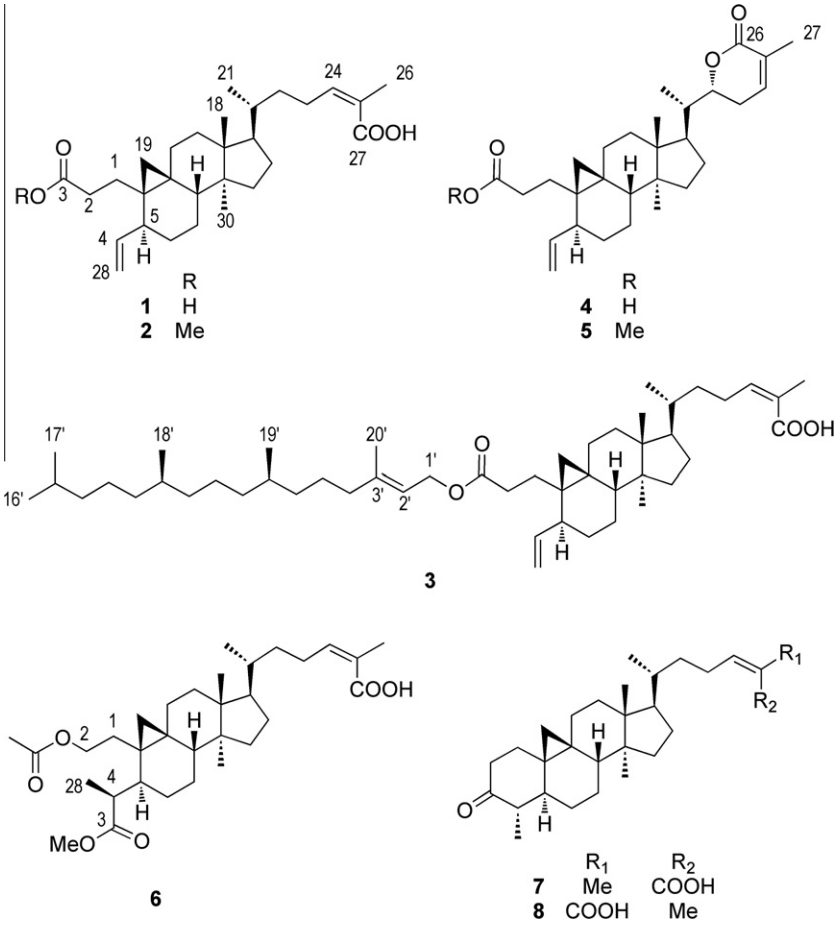

Figure 1. Structures of sinocalycanchinensins A–F (**1**–**8**).

H_2-2 with the carboxyl carbon at δ_C 180.4, together with the connectivity of C-1 and C-2 shown by the ^1H–^1H COSY spectrum, implied the presence of a 3,4-*seco*-structure.[14] In addition, the HMBC correlations of the proton signals of the vinyl group with C-5 as well as the olefinic proton [δ_H 5.67 (1H, ddd, J = 8.8, 10.0, 17.2 Hz)] with C-6 indicated the absence of Me-29. These spectral data indicated the presence of a 29-*nor*-3,4-*seco*-cycloartane skeleton,[15,16] which were further supported by the following HMBC correlations: H_2-1 with C-5, C-10 and C-19; H-8 with C-9 and C-14; H_2-11 with C-9 and C-19; Me-18 with C-12, C-13, and C-17; Me-30 with C-13, C-14, and C-15. In contrast, the olefinic proton signal at δ_H 6.10 displayed HMBC correlations with the carboxyl carbon (δ_C 173.6) and the methyl carbon (δ_C 20.5) as well as C-22 (δ_C 35.8), indicating that the double bond is present at C-24 and C-25, and the methyl group and the carboxylic acid group are attached at C-25. The geometry of the C24(25) double bond was assigned as *Z* from the NOESY correlation of H-24 with the vinyl methyl group. Thus, the planar structure of **1** was assigned. The configuration of H-5 was assigned as α from the NOESY correlation of H-19 with H-4 as well as H-5 with Me-30. Further examination of the NOESY spectrum indicated that the configurations in **1** were consistent with those of the cycloartane-type skeleton from the following key NOESY correlations: H-19/H-8, H-8/H-18, and H-18/H-20 on the β-face; and Me-30/H-17, and H-17/Me-21 on the α-face (Fig. 2). On the basis of the examination, the structure of **1** was characterized as shown.

The ^1H and ^{13}C NMR spectra of sinocalycanchinensin B (**2**) were quite similar to those of **1** except for the observation of a methoxy signal [δ_H 3.65 (3H, s); δ_C 51.5], suggesting **2** to be a methyl ester of **1**. This result was further supported by the HRESIMS, which showed an $[M+Na]^+$ peak at *m*/*z* 493.3280, corresponding to a molecular formula of $C_{30}H_{46}O_4$ (*m*/*z* 493.3294). The location of the methyl ester group was assigned to be C-3 from the HMBC correlation of the OMe signal with the carboxyl carbon signal at δ_C 174.4, which also had the HMBC correlation from H_2-1. The NOESY correlations observed in **2** indicated with the presence of the same configurations seen in **1**. From this spectral examination, the structure of **2** was assigned as shown.

Sinocalycanchinensin C (**3**) was obtained as a colorless oil and shown to possess a molecular formula of $C_{49}H_{82}O_4$ by HRESIMS (*m*/*z* 733.6187, $[M+Na]^+$). The ^1H and ^{13}C NMR spectra of **3** were closely correlated with those of **2**. It also showed signals due to a trisubstituted double bond [δ_H 5.33 (1H, t, J = 7.2 Hz); δ_C 118.2, 142.6], an oxymethylene [δ_H 4.58 (2H, d, J = 7.2 Hz); δ_C 61.3], a vinyl methyl group [δ_H 1.69 (3H, s); δ_C 16.4], four secondary methyl groups [δ_H 0.85, 0.86, 0.88 × 2 (each 3H, d, J = 7.2 Hz); δ_C 19.7, 19.8, 22.6, 22.7], nine sp^3 methylene carbons [δ_C 24.5, 24.8, 25.1, 36.7, 37.3, 37.4 (2C), 39.4, 39.9], and three sp^3 methine carbons [δ_C 28.0, 32.7, 32.8], but the methoxyl signal seen in **2** was absent. In the HMBC spectrum of **3**, the oxymethylene proton signal showed a correlation with the C-3 carboxyl carbon and the olefinic carbons [δ_C 118.2, 142.6], which in turn displayed correlations with the vinyl methyl signal. In contrast, two of the secondary methyls showed HMBC correlations with the same methine and methylene carbons, whereas the other two displayed HMBC correlations with two methylene carbons in each case. From this spectral observation, **3** was presumed to be a diterpene alcohol ester of **1**. Alkaline hydrolysis of **3** gave **2** and an alcohol, which was identified as

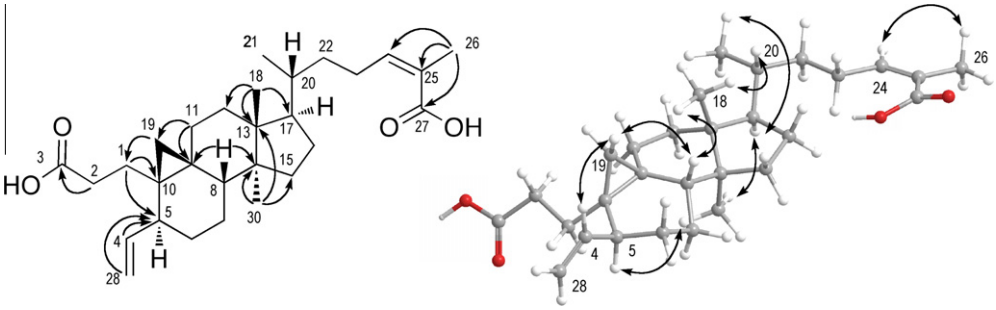

Figure 2. Key HMBC (left) and NOESY (right) of sinocalycanchinensin A.

phytol[17] by comparison of physical and spectral data with those of the authentic sample. The geometry of the phytol moiety in **3** was assigned as *E* from the NOESY correlation of H_2-1′ and Me-20′. Based on these examinations, the structure of **3** was characterized as shown.

The ^{1}H and ^{13}C NMR spectra of sinocalycanchinensin D (**4**) resembled those of **1**, indicating that **4** was also a 29-*nor*-3,4-*seco*-cycloartane-type triterpene. This was also supported by the following HMBC correlations: H_2-1 with C-5, C-10 and C-19; H_2-2 with C-3; H-8 with C-9 and C-14; H_2-11 with C-9 and C-19; Me-18 with C-12, C-13, and C-17; Me-30 with C-13, C-14, and C-15; Me-27 with C-24, C-25, and C-26. In addition, the existence of an oxymethine [δ_H 4.48 (1H, dt, J = 3.6, 13.2 Hz); δ_C 80.6] was also indicated, and this oxymethine was assigned to be C-22 from the HMBC correlations of the carbon resonance for this oxymethine with Me-21 and H-24. The presence of a δ-lactone structure was suggested from the ^{13}C resonance of C-26 (δ_C 166.5). This was further supported by HRESIMS, which gave an $[M+Na]^+$ ion peak at m/z 491.3152, corresponding to a molecular formula of $C_{30}H_{44}O_4$. The NOESY correlations of H-4/H-19, H-19/H-8, H-8/H-18, and H-18/H-20 on the β-face, as well as the NOESY correlations of H-5/Me-30, Me-30/H-17, and H-17/Me-21 on the α-face, suggested that the configurations in **5** were identical to those in the cycloartane-type triterpenes (Fig. 3). The absolute configuration at C-22 of the lactone moiety was elucidated as *R* based on the positive cotton effect ($\Delta\varepsilon$ + 3.65 at 253 nm) in the CD spectrum of **4**.[18] On the basis of this examination, the structure of **4** was elucidated as shown.

The ^{1}H and ^{13}C NMR spectra of sinocalycanchinensin E (**5**) were quite similar to those of **4** except for the appearance of a methoxyl signal (δ_H 3.64) and an upfield shift of the carboxyl carbon resonance [δ_C 174.2 (−4.8 ppm)], suggesting that **5** was a methyl ester of **4**. The molecular formula of **5**, confirmed as $C_{30}H_{44}O_4$ by HRESIMS, was also consistent with this observation. The location of the methyl ester group was assigned to be C-3 from the HMBC correlation of the OMe signal with the C-3 carboxyl carbon signal at δ_C 172.7. The structural confirmation was obtained by the treatment of **4** with trimethylsilyldiazomethane, which yielded a product identical to **5**. On the basis of these observations, **5** was characterized as shown.

Sinocalycanchinensin F (**6**) was obtained as a white amorphous powder, and its molecular formula was assigned as $C_{32}H_{50}O_6$ by HRESIMS (m/z 553.3504, $[M+Na]^+$). The ^{1}H and ^{13}C NMR spectra were similar to those of **1**, showing signals of the cyclopropyl methylene group [δ_H 0.39, 0.48 (each 1H, d, J = 4.4 Hz); δ_C 29.6], two tertiary methyl groups [δ_H 0.94, 0.95 (each 3H, s); δ_C 18.2, 19.4], a secondary methyl group [δ_H 0.90 (3H, d, J = 6.0 Hz); δ_C 18.1], a trisubstituted double bond [δ_H 6.09 (1H, t, J = 7.2 Hz); δ_C 125.7, 147.1], and a carboxyl carbon (δ_C 172.4). Signals corresponding to the vinyl group found in **1** were absent, while those for an oxymethylene group [δ_H 4.12, 4.22 (each 1H, m); δ_C 62.8], a methoxy group [δ_H 3.68 (3H, s), δ_C 51.6], an acetyl group [δ_H 2.06 (3H, s); δ_C 21.1, 171.2], and a secondary methyl group [δ_H 1.08 (3H, d, J = 6.8 Hz), δ_C 11.1] were observed. The oxymethylene was assigned to C-2 from the HMBC correlation of these proton signals with C-1 and C-10. These proton signals also exhibited an HMBC correlation with the acetyl carbonyl carbon, indicating the location of the acetyl group at C-2. In contrast, the secondary methyl signal showed HMBC correlations with C-5 and the carboxyl carbon at δ_C 177.0, which in turn coupled with the methoxyl proton signal, suggesting the presence of a 1-carbomehoxy-ethyl group at C-5. The other HMBC correlations were good agreement of the presence of 2,3-*seco*-29-*nor*-cycloartane strucutre: H_2-1 with C-10 and C-19; H-8 with C-14; H-5 with C-10; H_2-11 with C-8, C-9 and C-19; Me-18 with C-12, C-13, and C-17; Me-30 with C-13, C-14, and C-15 (Fig. 4). The NOESY correlations of Me-26 and H-24 indicated the geometry of the C24(25) double bond to be *Z*. The sterochemistry of C-4 was assigned to be *S** from the NOESY correlations of H-4 and H-2β as well as Me-28 and H-6β. The following NOESY correlations indicated that the configurations in **6** were identical with those in cycloartane-type triterpene: H-4/H-19, H-19/H-8, H-8/H-18, and H-18/H-20 on the β-face, and the NOESY correlations of H-5/Me-30, Me-30/H-17, and H-17/Me-21 on the α-face (Fig. 4). From the spectral evidence described above, sinocalycanchinesin F (**6**) was elucidated as a 29-*nor*-2,3-*seco*-cycloartane-type triterpene, as shown.

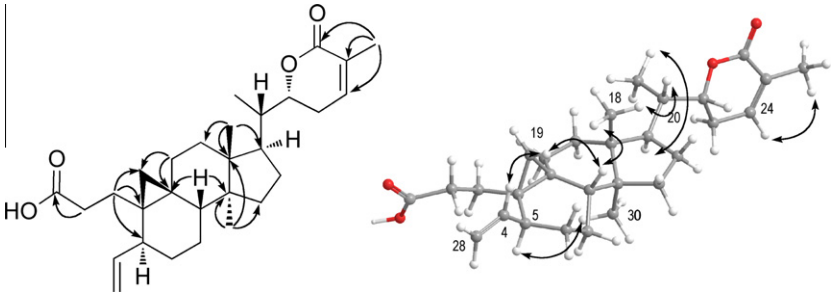

Figure 3. Key HMBC (left) and NOESY (right) of sinocalycanchinensin D (**4**).

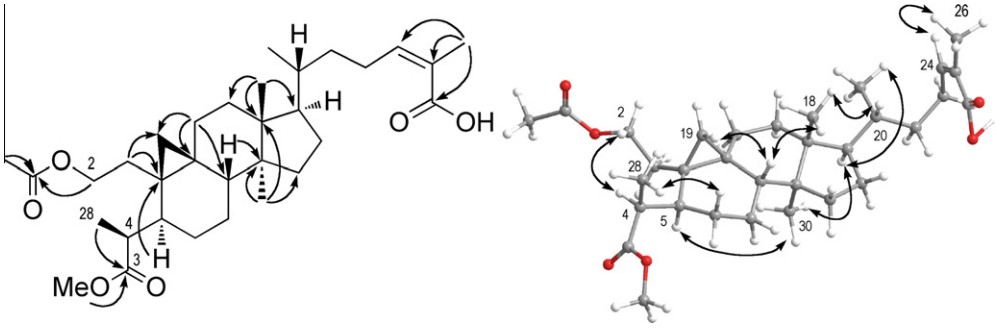

Figure 4. Key HMBC (left) and NOESY (right) of sinocalycanchinensin F.

Sinocalycanchinensins G (**7**) and H (**8**) were obtained as a white amorphous powder, and both were shown to possess the same molecular formula, $C_{29}H_{44}O_3$, by HRESIMS (*m*/*z* 463.3175, $[M+Na]^+$ in **7**; *m*/*z* 463.3188, $[M+Na]^+$ in **8**). The ^1H and ^{13}C NMR spectra of **7** and **8** were quite similar to each other, and were closely correlated with those of **1**. However, both of them showed the absence of the vinyl group and one of the carboxylic acid groups, but instead exhibited the presence of a secondary methyl group and a carbonyl group. The HMBC correlations of the secondary methyl signal with C-3 and the carbonyl carbon resonance in each case indicated the assignment of the carbonyl group to be C-3 and the location of the secondary methyl group to be C-4 in **7** and **8**. In contrast, the carbonyl resonance also had an HMBC correlation with the methylene proton resonances assignable to H_2-2, which further coupled with C-10, thus indicating the presence of a 29-*nor*-3-oxo-cycloartane structure in each case. Compounds **7** and **8** were considered to be geometric isomers based on the carbon resonance for the vinyl methyls being different. The geometries of the double bond in **7** and **8** were assigned as *Z* and *E*, respectively, from the following NOESY correlations: Me-26/H-24 in **7**; Me-27/H2-23 in **8**. The configuration of H-4 was also assigned as β from the NOESY correlation of H-19 and H-4. The following NOESY correlations were also consistent with the presence of the cycloartane-type skeleton in **7** and **8**: H-4/H-19, H-19/H-8, H-8/H-18, and H-18/H-20 on the β-face, and the NOESY correlations of H-5/Me-30, Me-30/H-17, and H-17/Me-21 on the α-face. Based on this evidence, the structures of **7** and **8** were concluded to be as shown.

Eight new triterpenoids, including five 3,4-*seco*-29-*nor*-cycloartanes, a novel 2,3-*seco*-29-*nor*-cycloartane, and two 29-*nor*-cycloartanes, have been isolated from the leaves of *S. chinensis*. Compound **5** represents the first example of a 29-*nor*-2,3-*seco*-cycloartane triterpene. In contrast, 29-*nor*-3,4-*seco*-cycloartane triterpenes have been reported only from the aerial part of *Antirhea acutata* (Rubiaceae), and this is the second example of the isolation of this type of triterpene. In addition, sinocalycanchinensin C (**3**) is the first example of a phytol ester of triterpene. Phytol, a component of chlorophylls, is known to be derived from a mevalonate-independent pathway, while cycloartanes are a metabolite of the mevalonate pathway.[19] Sinocalycanchinensin C is therefore a biosynthetically interesting compound, as it is considered to be derived from the products of both mevalonate and mevalonate-independent pathways.

3. Bioassay data

Compounds **1**–**8** were examined for their cytotoxic activity against a panel of human cancer cell lines, namely, the KB (human epidermoid carcinoma of nasopharynx), K562 (leukemia), and MCF-7 (breast carcinoma) cell lines, as well as multidrug-resistant (MDR) human cancer cell lines, including KB-C2 (colchicine-resistant KB) and K562/Adr (doxorubicin-resistant K562) (Table 1). Compound **8** showed weak cytotoxicities against all the tested cell lines, with IC_{50} values ranging from 14.8 to 18.8 μg/mL, while the cytotoxicities of compound **7** were slightly less potent than those of **8**. Compound **6** also showed similar cytotoxicities against the tested cell lines except for MCF-7. In contrast, compound **3** was not a cytoxic compound, having IC_{50} values >100 μg/mL against all the tested cell lines. Compound **5** was also a weak cytotoxic compound, but it showed significant enhanced cytotoxicity against KB-C2 cells in the presence of colchicine with an IC_{50} value of 1.51 μg/mL. Since colchicine had no effect on the growth of KB-C2 cells at this concentration level, it was suggested that compound **5** might show some MDR-reversing effects. Overall, 3,4-*seco*-cycloartanes were less cytotoxic than the others.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-2200 polarimeter. NMR spectra were recorded on a Bruker AVANCE-400 instrument (^1H NMR: 400 MHz, ^{13}C NMR:100 MHz) using TMS as an internal standard. HRESIMS were obtained on a Waters LCT Premier. CD spectra were run on a CD-J600 spectropolarimeter (JASCO). Column chromatography: silica gel 60 N (KANTO CHEMICAL, 63–210 μm), Sephadex LH-20 (GE Healthcare Bioscience), ODS (YMC ODS A), MCI gel CHP20P (MITSUBISHI CHEMICAL CORPORATION), and Toyopearl HW-40C (TOSHO); HPLC: gel permeation chromatography (Asahipak, GS-310 2G, MeOH), silica gel (KANTO CHENICAL, Mightysil Si 60 φ20 × 250 mm), octadecyl silane (KANTO CHEMICAL, Mightysil RP-18 GP φ20 × 250 mm).

Table 1
Cytotoxicity (IC_{50} in μg/mL) of compounds **1**–**8** against human cancer cell lines and multidrug-resistant human cancer cell lines [a] with or without colchicine (for KB-C2) in vitro

	KB	KB-C2	KB-C2 (+col.[b])	MCF-7	K562	K562/Adr
1	30.7	30.4	33.4	61.6	29.9	38.9
2	20.9	22.2	18.9	35.7	16.5	17.6
3	>100	>100	>100	>100	>100	
4	31.7	26.4	25.6	39.2	16.7	7.0
5	62.2	49.2	1.51	>100	22.4	20.5
6	16.6	21.7	17.5	33.8	13.2	19.5
7	19.9	21.6	21.4	23.7	14.8	7.3
8	18.6	18.8	17.2	18.6	14.8	19.4
Daunorubicin	0.45	7.9	11.6	0.033	1.49	8.5

[a] Cell lines: KB (human epidermoid carcinoma of the nasopharynx), KB-C2 (multidrug-resistant KB), K562 (leukemia), and MCF-7 (breast carcinoma).
[b] 2.5 μM colchicine.

4.2. Plant material

The leaves of *S. chinensis* Cheng et S.Y. Chang, cultivated in the Godzu-Branch Herbal Garden of the Niigata University of Pharmacy and Applied Life Sciences (NUPALS), Agano city, Niigata, were collected in September 2004. The plant was identified by Professor Yasumasa Ikeshiro of the NUPALS, and a voucher specimen (NUPAL090104) has been deposited in the herbarium of the NUPALS.

4.3. Extraction and isolation

The fresh leaves of *S. chinensis* (7.6 kg) were extracted with MeOH (3 × 54 L) at room temperature. The MeOH extracts were concentrated under reduced pressure to give a residue (880 g) that was partitioned between EtOAc and H_2O. After removal of the solvent of the EtOAc layer, the extracts were further partitioned between *n*-hexane and 90%MeOH, giving an *n*-hexane-soluble fraction (79 g) and a 90% MeOH-soluble fraction (77 g). The 90% MeOH-soluble fraction was subjected to chromatography over Sephadex LH-20 with EtOH to give four fractions (frs. 1–4). Fraction 1 (44.4 g) was rechromatographed over Sephadex LH-20 [H_2O–MeOH (2:3→0:1)] to afford a further 10 fractions (frs. 1.1–1.10). Fr. 1.3 was rechromatographed over MCI gel CHP20P [H_2O–MeOH (3:2→0:1)] to furnish 11 fractions (frs. 1.3.1–1.3.11). Fraction 1.3.5 was further fractionated by Toyopearl HW-40C chromatography [H_2O–MeOH (1:1 → 0:1)] into eight fractions (frs. 1.3.5.1–1.3.5.8). Scopoletin was crystallized from fr. 1.3.5.3 (MeOH) to give a pure sample (50 mg). Fr. 1.3.5.5 was purified by GPC on HPLC to afford ferulic acid (49 mg). Silica gel chromatography of fr. 1.3.5.7 with $CHCl_3$–MeOH (20:1) gave *p*-coumaric acid (29 mg). Fraction 1.3.7 was fractionated by YMC ODS-A [H_2O–MeOH (3:7 → 0:1)] into further seven fractions (frs. 1.3.7.1–1.3.7.7). Silica gel column chromatography of fr. 1.3.7.2 with *n*-hexane-EtOAc (4:1) furnished isopropyl vanillate (6 mg). Frs. 1.3.7.4 was chromatographed over silica gel [*n*-hexane-acetone (9:1)] to afford 8α,11-elemodiol (134 mg) and cryptomeridiol (21 mg), while silica gel column chromatography of fr. 1.3.7.6 with *n*-hexane-acetone (9:1) yielded 11-hydroxy-4α-methoxy-selinane (20 mg). Fr. 1.3.8 was repeatedly chromatographed over Toyopearl HW-40C [H_2O–MeOH (3:2 → 0:1)], YMC ODS-A [H_2O–MeOH (2:3 → 0:1)], and silica gel [*n*-hexane-acetone (9:1)] to give 8α-acetoxy-elemol (79 mg). Fraction 1.4 was further fractionated by MCI gel CHP20P chromatography [H_2O–MeOH (3:2 → 0:1)] into 11 fractions (frs. 1.4.1–1.4.11). Fraction 1.4.4 was repeatedly chromatographed over YMC ODS-A [H_2O–MeOH (3:7 → 0:1)] and silica gel [*n*-hexane-acetone (4:1)] to afford **4** (12 mg). Repeated chromatography of fraction 1.4.10 over YMC ODS-A [H_2O–MeOH (3:7 → 0:1)], silica gel [*n*-hexane-acetone (9:1)], and GPC on HPLC (MeOH) gave **6** (12 mg). Repeated chromatography of fr. 1.4.9 over YMC ODS-A [H_2O–MeOH (3:7 → 0:1)] and silica gel [*n*-hexane-EtOAc (9:1)] furnished β-eudesmol (13 mg). Fr. 1.5 was fractionated by YMC ODS-A chromatography [H_2O–MeOH (3:2 → 0:1)] into 17 fractions (frs. 1.5.1–1.5.17). Fr. 1.5.11 was further chromatographed over Toyoperal HW-40C [H_2O–MeOH (2:3 → 0:1)] to afford a further five fractions (frs. 1.5.11.1–1.5.11.5). Repeated chromatography of fr. 1.5.11.3 over Sephadex LH-20 (acetone), silica gel [*n*-hexane-acetone (9:1)], and GPC on HPLC furnished **6** (8 mg). Fr. 1.5.11.5 was applied on a silica gel column with *n*-hexane-acetone (9:1) to afford **1** (162 mg) and **5** (31 mg). Fr. 1.5.13 was repeatedly chromatographed over Sephadex LH-20 (acetone), and silica gel [*n*-hexane-EtOAc (9:1)], and then purified by silica gel HPLC [$CHCl_3$–MeOH (50:1)] to give **8** (4 mg). Fraction 1.6 was repeatedly chromatographed over YMC ODS-A [H_2O–MeOH (2:3 → 0:1)] and silica gel [*n*-hexane-EtOAc (20:1)], and then purified by Mightysil RP-18 on HPLC to give **2** (101 mg) and **7** (61 mg). Fr. 1.8 was rechromatographed over MCI gel CHP20P [H_2O–MeOH (3:2 → 0:1)] to furnish 10 fractions (frs. 1.8.1–1.8.10). Fraction 1.8.8 was repeatedly chromatographed over YMC ODS-A [H_2O–MeOH (2:3 → 0:1)], Toyopearl HW-40C [H_2O–MeOH (9:1)], and silica gel [*n*-hexane-EtOAc (20:1)] to give **3** (16 mg). Crystallization of fr. 1.8.6 furnish β-sitosterol-3-*O*-β-D-glucoside (73 mg). Fr. 2 (18.6 g) was rechromatographed over Sephadex LH-20 (70% MeOH) to give eight fractions (frs. 2.1–2.8). Crystallization of fr. 2.7 (MeOH) afforded kaempferol (3.0 g). MCI gel CHP20P chromatography of fr. 2.4 with H_2O–MeOH (7:3 → 0:1) and then crystallization furnished kaempferol-3-*O*-β-D-glucoside (577 mg). Fr. 3 (1.0 g) was fractionated by Sephadex LH-20 [H_2O–MeOH (2:3 → 0:1)] into a further ten fractions (frs. 3.1–3.10). Quercetin was crystallized from fr. 3.9

Table 2
^{1}H NMR data for compounds **1**–**8** in $CDCl_3$[a]

Position	1	2	3[b]	4	5	6	7	8
4	5.67 (ddd, 8.8, 10.0, 17.2)	5.66 (ddd, 8.8, 8.8, 17.6)	5.67 (ddd, 8.4, 10.0, 17.2)	5.66 (ddd, 8.8, 10.0, 17.6)	5.65 (ddd, 8.4, 10.0, 17.2)	2.92 (m)	2.24 (m)	2.22 (m)
18	0.98 (s)	0.97 (s)	0.98 (3H, s)	1.02 (s)	1.01 (s)	0.95 (s)	1.01 (s)	1.02 (s)
19	0.32 (d, 4.0))	0.31 (d, 4.4)	0.32 (d, 4.0)	0.34 (d, 4.4)	0.31 (d, 4.4)	0.39 (d, 4.4)	0.41 (d, 4.0)	0.41 (d, 4.0)
	0.49 (d, 4.0	0.47 (d, 4.4)	0.47 (d, 4.0)	0.50 (d, 4.4)	0.48 (d, 4.4)	0.64 (d, 4.4)	0.63 (d, 4.0)	0.64 (d, 4.0)
21	0.90 (d, 6.0)	0.90 (d, 7.2)	0.91 (d, 6.4)	0.98 (d, 6.4)	0.96 (d, 6.4)	0.90 (d, 6.0)	0.92 (d, 6.0)	0.94 (d, 6.8)
22	1.17 (m)	1.15 (m)	1.17 (m)	4.48 (dt, 3.6, 13.2)	4.46 (dt, 3.6, 13.2)	1.14 (m)	1.17 (m)	1.20 (m)
	1.55 (m)	1.55 (m)	1.56 (m)			1.55 (m)	1.56 (m)	1.58 (m)
24	6.10 (t, 7.2)	6.09 (t, 7.2)	6.10 (t, 6.8)	6.61 (brd, 6.4)	6.60 (brd, 6.4)	6.09 (t, 7.2)	6.11 (t, 6.8)	6.91 (t, 7.2)
26	1.92 (s)	1.92 (s)	1.93 (s)	—	—	1.93 (s)	1.93 (s)	
27	—	—	—	1.92 (s)	1.91 (s)	–	–	1.86 (s)
28	4.99 (d, 10.0), 5.05 (d, 17.2)	4.98 (d, 10.0), 5.04 (d, 17.2)	4.98 (dd, 2.0, 10.0), 5.04 (dd, 2.0, 17.2)	4.99 (dd, 2.0, 10.0), 5.05 (dd, 2.0, 17.6)	4.97 (dd, 1.6, 10.0), 5.03 (dd, 1.6, 17.2)	1.08 (d, 6.8)	1.00 (d, 6.8)	1.00 (d, 6.4)
30	0.94 (s)	0.93 (s)	0.93 (3H, s)	0.93 (s)	0.92 (s)	0.94 (s)	0.92 (s)	0.92 (s)
OMe		3.65 (s)			3.64 (s)	3.68 (s)		
OAc						2.06 (s)		

[a] δ (ppm); 400 MHz.
[b] For phytol moiety: 0.85 (3H, d, *J* = 7.2 Hz, Me-19′), 0.86 (3H, d, *J* = 7.2 Hz, Me-18′), 0.88 (6H, d, *J* = 7.2 Hz, Me-16′ and Me-17′), 1.69 (3H, s, Me-20′), 2.01 (2H, t, *J* = 7.6 H, H_2-4′), 4.58 (2H, d, *J* = 7.2 Hz, H_2-1′), 5.33 (1H, t, *J* = 6.0 Hz, H-3′).

Table 3
^{13}C NMR data for compounds **1**–**8** in $CDCl_3$[a]

Position	1	2	3[b]	4	5	6	7	8
1	28.6	28.8	28.8	28.6	28.8	31.3	32.9	32.9
2	31.8	31.7	31.9	31.6	31.7	62.8	40.9	40.9
3	180.4	174.4	174.0	179.0	174.2	177.0	213.7	213.7
4	142.8	142.9	142.9	142.7	142.7	41.4	50.0	50.0
5	42.3	42.2	42.2	42.3	42.2	38.8	46.1	46.1
6	29.0	29.0	29.1	28.9	28.9	22.8	25.9	25.9
7	24.5	24.6	24.6	24.6	24.5	24.9	25.2	25.2
8	48.0	48.1	48.2	48.2	48.1	48.2	47.1	47.1
9	23.7	23.7	23.7	23.5	23.5	21.9	25.0	24.9
10	27.6	27.7	27.7	27.7	27.7	25.6	29.3	29.3
11	27.2	27.1	27.2	27.1	27.0	27.6	27.2	27.2
12	33.0	33.0	33.1	32.9	32.9	33.0	32.8	32.8
13	45.1	45.1	45.1	45.6	45.6	45.1	45.4	45.5
14	48.9	48.9	48.9	48.5	48.5	48.9	48.8	48.8
15	35.7	35.8	35.8	35.8	35.8	35.6	35.4	35.4
16	28.1	28.1	28.1	27.0	27.0	28.1	28.1	28.1
17	52.1	52.2	52.3	48.0	48.1	52.2	52.2	52.2
18	18.3	18.3	18.4	18.1	18.1	18.2	17.9	17.9
19	28.1	28.1	28.2	28.1	28.0	29.6	27.0	26.9
20	36.0	36.0	36.0	39.1	39.1	36.0	36.0	36.0
21	18.1	18.1	18.1	13.0	13.0	18.1	18.1	18.1
22	35.8	35.8	35.9	80.6	80.5	35.8	35.8	34.8
23	26.8	26.9	27.0	23.6	23.5	26.9	26.9	25.9
24	147.3	147.3	147.3	139.4	139.3	147.1	147.4	145.6
25	125.8	125.8	125.7	17.0	16.9	125.7	125.7	126.6
26	20.5	20.5	20.5	128.3	128.3	20.6	20.5	172.5
27	173.6	173.4	173.2	166.6	166.5	172.4	172.7	12.0
28	114.1	114.1	114.1	114.3	114.1	11.1	10.7	10.7
30	19.4	19.4	19.5	19.6	19.5	19.4	19.1	19.2
OMe		51.5			51.4	51.6		
OAc						21.1		
						171.2		

[a] δ (ppm); 100 MHz.
[b] For phytol moiety: 61.3 (C-1′), 118.2 (C-2′), 142.6 (C-3′), 39.9 (C-4′), 25.1 (C-5′), 36.7 (C-6′), 32.7 (C-7′), 37.4 (C-8′), 24.5 (C-9′), 37.4 (C-10′), 32.8 (C-11′), 37.3 (C-12′), 24.8 (C-13′), 39.4 (C-14′), 28.0 (C-15′), 22.6 (C-16′), 22.7 (C-17′), 19.8 (C-18′), 19.7 (C-19′),16.4 (C-20′).

(MeOH) to give a pure sample (214 mg). Fr. 3.4 was chromatographed over MCI gel CHP20P [H_2O–MeOH (7:3 → 0:1)] to afford quercetin-3-*O*-β-D-glucoside (19 mg).

4.4. Sinocalycanchinensin A (1)

Amorphous powder; $[\alpha]_D^{20}$ +51.1 (*c* 6.2, $CHCl_3$); HRESIMS: *m*/*z* 479.3112 $[M+Na]^+$ (Calcd for $C_{29}H_{44}O_4$, 479.3137); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3.

4.5. Sinocalycanchinensin B (2)

Colorless oil; $[\alpha]_D^{20}$ +57.9 (*c* 2.9, $CHCl_3$); HRESIMS: *m*/*z* 493.3280, $[M+Na]^+$ (Calcd for $C_{30}H_{46}O_4Na$, 493.3294); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3.

4.6. Sinocalycanchinensin C (3)

Colorless oil; $[\alpha]_D^{20}$ +38.6 (*c* 1.6, $CHCl_3$); HRESIMS: *m*/*z* 733.6187 $[M-H]^-$ (Calcd for $C_{49}H_{82}O_4$, 733.6135); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3.

4.7. Alkaline Hydrolysis of 3

A solution of **3** (6 mg) in 1% NaOMe–MeOH (2 mL) was heated at 60 °C with stirring for 1 day. The reaction mixture was neutralized with ion-exchange resin (Dowex 50WX8), filtered, and concentrated under reduced pressure. The residue was chromatographed over silica gel [*n*-hexane-acetone (9:1)] to give phytol (1.2 mg) as a colorless oil; $[\alpha]_D^{20}$ +0.7 (*c* 0.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85, 0.86 (each 3H, d, *J* = 6.6 Hz, Me-18 and Me-19), 0.88 (6H, d, *J* = 6.5 Hz, Me-16 and Me-17), 1.68 (3H, s, Me-20), 2.00 (2H, br t, *J* = 6.8 Hz, H_2-4), 4.16 (2H, d, *J* = 7.2 Hz, H_2-1), 5.42 (1H, tq, *J* = 6.8, 1.2 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2 (C-20), 19.7 (C-19), 19.7 (C-18), 22.6 (C-16), 22.7 (C-17), 24.5 (C-9), 24.8 (C-13), 25.1 (C-5), 28.0 (C-15), 32.7 (C-11), 32.8 (C-7), 36.6 (C-6), 37.3 (C-), 37.4 (C-), 37.4 (C-), 39.4 (C-14), 39.9 (C-4), 59.4 (C-1), 123.1 (C-2), 140.3 (C-3), and sinocalycanchinensin B (**2**) (2.4 mg).

4.8. Sinocalycanchinensin D (4)

Amorphous powder; $[\alpha]_D^{20}$ +97.0 (*c* 1.0, $CHCl_3$); HRESIMS: *m*/*z* 477.2982 $[M+Na]^+$ (Calcd for $C_{29}H_{42}O_4$, 477.2981); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; CD (MeOH; 2.0×10^{-4} M, $\Delta\varepsilon$) λ_{max} CD: 253 (+3.65).

4.9. Sinocalycanchinensin E (5)

Colorless oil; $[\alpha]_D^{20}$ +77.8 (*c* 2.4, $CHCl_3$); HRESIMS: *m*/*z* 491.3152 $[M+Na]^+$ (Calcd for $C_{30}H_{44}O_4$, 491.3137); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; CD (MeOH; 2.0×10^{-4} M, $\Delta\varepsilon$) λ_{max} CD: 253 (+3.63).

4.10. Methylation of 4

A solution of **4** (5 mg) in MeOH (2 mL) was treated with was treated with trimethylsilyldiazomethane ($TMSCHN_2$, 0.5 mL in hexane) at room temperature for 1 h with stirring. After removal of solvent by evaporation, the residue was purified by prep. TLC (silica gel, $CHCl_3$–MeOH, 97:3) to afford a product, which was shown to be identical with **4** by spectral comparison.

4.11. Sinocalycanchinensin F (6)

Colorless oil; $[\alpha]_D^{20}$ +55.4 (*c* 0.4, $CHCl_3$); HRESIMS: *m*/*z* 553.3504 $[M+Na]^+$ (Calcd for $C_{32}H_{50}O_6$, 553.3505); ^{1}H NMR data, see Table 2;^{13}C NMR data, see Table 3.

4.12. Sinocalycanchinensin G (7)

White amorphous powder; $[\alpha]_D^{20}$ +40.3 (*c* 5.2, $CHCl_3$); HRESIMS: *m*/*z* 463.3175 $[M+Na]^+$ (Calcd for $C_{29}H_{44}O_3$, 463.3188); ^{1}H NMR data, see Table 2;^{13}C NMR data, see Table 3.

4.13. Sinocalycanchinensin H (8)

White amorphous powder; $[\alpha]_D^{20}$ +39.6 (*c* 0.4, $CHCl_3$); HRESIMS: *m*/*z* 463.3188 $[M+Na]^+$ (Calcd for $C_{32}H_{50}O_6$, 463.3188); ^{1}H NMR data, see Table 3;^{13}C NMR data, see Table 2.

4.14. Cell lines and cell culture

KB (human epidermoid carcinoma of the nasopharynx), MCF-7 (breast carcinoma), K562 (leukemia), and K562/Adr (multidrug-resistant human erythromyelogenous leukemia) cells were obtained from the Cell Resource Center for Biomedical Research (Tohoku University). Multidrug-resistant human epidermoid carcinoma KB-C2 cells were kindly provided by Professor Shin-ichi Akiyama (Kagoshima University, Japan). KB cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS). KB-C2 cells were maintained in DMEM medium in the presence of 10% FBS and 5 μg/mL colchicine. MCF-7 and K562 cells were cultured in RPMI1640 supplemented with 10% FBS. K562/Adr (doxorubicin-resistant K562 cell line) cells were cultured in RPMI1640 medium containing 10% FBS and

0.5 μM doxorubicin. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2-95% air.

4.15. Cytotoxicity assays

Cells in exponential growth were trypsinized, dispersed in a single cell suspension, and dispensed in 100 μL volumes into 96-well plates. For each assay, 1×10^4 cells/well for K562 and K562/Adr, 5×10^3 cells/well for KB and KB-C2, or 5×10^3 cells for MCF7 were inoculated in 100 mL medium containing 10% FBS and incubated for 24 h. Test samples were dissolved in small amounts of DMSO and diluted in the appropriate culture medium (final concentration of DMSO <0.5%). After removal of pre-incubated culture medium, 100 μL of medium containing various concentrations of each test compound were added and further incubated for 48 h. Cell proliferaion was determined by a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[20] IC_{50} values are defined as the concentration of each test samples that reduced absorbance to 50% of vehicle-treated controls.

Acknowledgments

We thank Professor Yasumasa Ikeshiro (Niigata University of Pharmacy and Applied Life Sciences) for the identification of *Sinocalycanthus chinensis*.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.055.

References and notes

1. Ye, Q.; Qiu, Y.; Quo, Y.; Chen, J.; Yang, S.; Zhao, M.; Fu, C. *J. Zhejiang Univ. Sci. B* **2006**, *7*, 868.
2. Li, J.; Jin, Z. *Biochem. Syst. Ecol.* **2006**, *34*, 725.
3. *Chinese Materia Medica*; Jiangsu New Medical College, Ed.; Shanghai People's Publishing House: Shanghai, 1977; p 1829.
4. San, F. A.; Medarde, M.; Lopez, J. L.; Miguel del Corral, J. M.; Puebla, P.; Barrero, A. F. *Phytochemistry* **1988**, *27*, 2241.
5. Mata, R.; Navarrete, A.; Alvarez, L.; Pereda-Miranda, R.; Delgado, G.; Romo de Vivar, A. *Phytochemistry* **1987**, *26*, 191.
6. Cornwell, C. P.; Reddy, N.; Leach, D. N.; Wyllie, S. G. *Flavour Fragr. J* **2000**, *15*, 421.
7. Kaneko, N.; Hasegawa, S.; Hirose, Y. *Phytochemistry* **1985**, *24*, 185.
8. Kojima, H.; Sato, N.; Hatano, A.; Ogura, H. *Phytochemistry* **1990**, *29*, 2351.
9. Satake, T.; Kamiya, K.; An, Y.; Oishi, T.; Yamamoto, J. *Biol. Pharm. Bull.* **2007**, *30*, 935.
10. Wang, K.; Yang, C.; Zhang, Y. *Food Chem.* **2007**, *101*, 365.
11. Sibanda, S.; Ndengu, B.; Multari, G.; Pompi, V.; Galeffi, C. *Phytochemistry* **1989**, *28*, 1550.
12. Ternai, B.; Markham, K. R. *Tetrahedron* **1976**, *32*, 565.
13. Poonsakdi, P.; Cheryklin, P.; Niyomtham, N.; Bertoni, D. R.; Ruchirawat, S. *Org. Lett.* **2007**, *9*, 2637. Supplementary data.
14. Cabrera, G. M.; Gallo, M.; Seldes, A. M. *J. Nat. Prod.* **1996**, *59*, 343.
15. Lee, D.; Cuendet, M.; Axelrod, F.; Chavez, P. I.; Fong, H. H. S.; Pezzutoa, J. M.; Kinghorn, A. D. *Tetrahedron* **2001**, *57*, 7107.
16. Lee, D.; Park, E. J.; Cuendet, M.; Axelrod, F.; Chavez, P. I.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1565.
17. Brown, G. D. *Phytochemistry* **1994**, *36*, 1553.
18. Wang, W.; Liu, J.; Ma, X.; Yang, M.; Wang, W.; Xu, Z.; Liu, P.; Guo, D. *Helv. Chim. Acta* **2006**, *89*, 1888.
19. Michel Rohmer *Nat. Prod. Rep.* **1999**, *16*, 565.
20. Mosmann, T. *J. Immunol. Methods.* **1983**, *65*, 55.