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New bioactive aromatic compounds from *Vismia guianensis*

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Abstract

Five benzophenones, vismiaguianones A–E, and two benzocoumarins, vismiaguianins A and B were isolated from the $CHCl_3$ extract of the roots of *Vismia guianensis* by bioassay-directed fractionation using the DNA strand-scission assay and KB cell line. Of the isolates obtained, vismiaguianone B exhibited DNA strand-scission activity, whereas vismiaguianones D and E and vismiaguianin A were found to be significantly cytotoxic. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Vismia guianensis*; Guttiferae; Roots; Prenylated benzophenones; Vismiaguianones A–E; Benzocoumarins; Vismiaguianins A and B; DNA strand-scission activity; Cytotoxic activity

1. Introduction

As a part of an ongoing collaborative search for novel antineoplastic agents of plant origin, the roots of *Vismia guianensis* (Aubl.) Choisy (Guttiferae) were subjected to detailed phytochemical investigation, since a chloroform-soluble extract exhibited cytotoxic activity against the KB (human oral epidermoid carcinoma) cell line. The leaves of *V. guianensis* have been used as a blood tonic for children in the form of a decoction with another plant *Canthium glabriflorum* (Macfoy and Sama, 1983). Anthranoids (Grosse et al., 1997), quinoids (Gonzales et al., 1980; delle Monache et al., 1980a; Botta et al., 1986) and xanthones (Botta et al., 1986) have been reported previously as constituents of *V. guianensis*. In the present investigation, prenylated benzophenones and benzocoumarins have been found for the first time from *V. guianensis*, although prenylated benzophenones have been isolated from a related species, *V. cayennensis* (delle Monache et al., 1980b; Fuller et al., 1999).

Five new benzophenones, namely, vismiaguianones A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), and two new benzocoumarins, vismiaguianins A (**6**) and B (**7**), were isolated from the roots of *V. guianensis* by bioassay-directed fractionation monitored by cytotoxicity toward the KB cell line. Among these, vismiaguianone B (**2**) exhibited moderate DNA strand-scission activity, and vismiaguianones D (**4**) and E (**5**), and vismiaguianin A (**6**) were found to have moderate cytotoxicity, whereas the other compounds were found to be inactive in both assays. We report herein the isolation and structure elucidation of the new compounds **1**–**7** as well as their biological activity.

2. Results and discussion

The molecular formula of **1** was established as $C_{23}H_{26}O_5$ by HR-EIMS which showed a molecular ion

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peak at m/z 382.1784. The IR spectrum of **1** showed absorption bands at 3355 cm^{-1} for one or more hydroxyl groups and at 1610 cm^{-1} for a conjugated carbonyl group. A carbonyl group appeared at δ_C 200.3 in the ^{13}C NMR spectrum of **1**. This carbonyl group (C-13) was hydrogen-bonded with a hydroxyl group at C-2 as evidenced by a proton signal at δ_H 12.60. Signals at δ_H 7.46/δ_C 127.3, 7.37/127.5, 7.44/130.1 and δ_C 143.0 indicated a mono-substituted benzene ring (ring A), which was attached to the carbonyl carbon at C-13 according to a HMBC correlation of H-8(12)/C-13. These characteristics indicated a benzophenone skeleton (Botta et al., 1986; Fuller et al., 1999). The second benzene ring (ring B) was fully substituted by two hydroxyls, one prenyl, and one hydroxy-dimethyl-dihydropyran ring. The ^1H and ^{13}C NMR spectra of compound **1** displayed characteristic signals for a prenyl group at δ_H 3.45/δ_C 21.8, 5.32/121.8, 1.86/25.9, 1.81/17.9 and δ_C 143.0. This prenyl group was located at C-3, as determined by the HMBC correlations of H-1′/C-3, H-1′/C-2, and H-1′/C-4, as shown in Fig. 1. The signals for a hydroxy-dimethyl-dihydropyran ring (ring C) appeared at δ_H 2.56, 2.78/δ_C 25.7, 3.61/68.7, 0.85/21.1, and 0.94/23.5, and a signal for C-3″ overlapped with the solvent signal. The positioning of ring C in **1** was based on two major considerations. First, the H-1″ proton belonging to this ring showed a two-bond connectivity with C-5 and a three-bond connectivity with C-6 in the HMBC spectrum of **1**. Second, the proton signal observed at δ_H 12.60 for a hydrogen-bonded proton in the ^1H NMR spectrum of **1** would not be present if there were two hydroxyl groups that hydrogen-bonded to one carbonyl oxygen of the benzophenone moiety (delle Monache et al., 1984; Seo et al., 1999). Therefore, ring C was positioned at C-5 and C-6 which would permit only one hydroxyl group (OH-2) to undergo hydrogen-bonding with the carbonyl group. Stereochemistry at C-2″ in **1** has not been determined. Based on the analysis of the above data, structure **1** was assigned to the new compound, vismiaguianone A (see Table 1).

Compound **2** showed a molecular ion peak at m/z 382.1784 by HR-EIMS, corresponding to the elemental formula $C_{23}H_{26}O_5$. The IR spectrum displayed absorption bands at 3495 cm^{-1} for one or more hydroxyl groups and at 1640 cm^{-1} for a conjugated carbonyl group. Compound **2** showed ^1H and ^{13}C NMR spectral data similar to those of compound **1** except for the signals for ring C. Resonances at δ_H 2.98, 3.07/δ_C 27.3, 4.72/91.2, 1.33/25.7, 1.21/24.0 and δ_C 72.0 indicated the presence of a dihydrofuran ring with a hydroxyisopropyl group substituent in **2**. The presence of two ^1H NMR spectroscopic signals at δ_H 9.82 and 8.14 for weakly hydrogen-bonded hydroxyl protons indicated that the two hydroxyl groups were hydrogen-bonded to the carbonyl group of the benzophenone moiety (delle Monache et al., 1984; Seo et al., 1999). Thus, two hydroxyl functionalities were assigned to C-2 and C-6 in ring B, and ring C was fused to ring B at C-4 and C-5. Therefore, structure **2** was assigned to the new compound, vismiaguianone B.

Compound **3** showed a molecular ion peak at m/z 382.1784 by HR-EIMS, corresponding to the molecular formula $C_{23}H_{26}O_5$. The IR spectrum displayed absorption bands at 3560 cm^{-1} for one or more hydroxyl groups and at 1631 cm^{-1} for a conjugated carbonyl group. The ^1H and ^{13}C NMR spectral data of **3** exhibited signals similar to those of **1** and **2**. Signals at δ_H 2.86, 2.93/δ_C 27.1, 4.40/91.3, 0.92/25.3, 1.05/23.4, and δ_C 71.5 indicated the presence of a dihydrofuran ring with a hydroxyisopropyl group similar to that in compound **2**. The only structural difference between compounds **2** and **3** was in the position of ring C. In the ^1H NMR spectrum of **3**, only one hydrogen-bonded hydroxyl proton appeared at δ_H 12.71, indicating that ring C was attached to C-5 and C-6. In addition, slightly more shielded chemical shift values for H-2″, H-4″ and H-5″ in the ^1H NMR spectrum of **3**, compared to the analogous data for **2**, maybe attributed to the ring current effect of benzene ring A (Silverstein et al., 1991). This provided further evidence for the positions of attachment of ring C in **3**. Therefore, structure **3** was assigned to the new compound, vismiaguianone C.

Compound **4** gave a molecular ion peak at m/z 428.1618 by HR-EIMS, corresponding to the elemental formula $C_{27}H_{24}O_5$. The IR spectrum showed absorption bands at 3496 cm^{-1} for a hydroxyl group and at 1773 cm^{-1} for the carbonyl of a lactone ring. The ^1H and ^{13}C NMR spectra of compound **4** displayed characteristic signals for a benzophenone skeleton similar to compounds **1**–**3**, with the ^{13}C NMR signal of the carbonyl group appearing at δ_C 198.9. A mono-substituted phenyl ring conjugated to the carbonyl group, was indicated by the observation of signals at δ_H 7.66/δ_C 128.6, 7.50/129.5, 7.58/133.2, and δ_C 139.8 in the ^1H and ^{13}C NMR spectra of **4**. The second benzene ring of the benzophenone skeleton (ring B) was

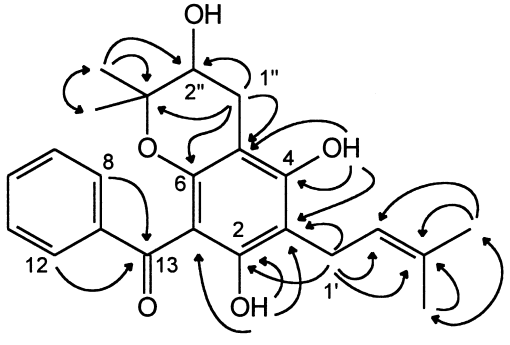


Fig. 1. HMBC correlations for compound **1**.

observed as being fully substituted by two hydroxyls, one prenyl and one lactone ring. Signals due to the two hydrogen-bonded hydroxyl groups appeared at δ_H 9.69 and 7.91, similar to those in compound **2**. Therefore, the positions of the hydroxyl groups in ring B were determined as C-2 and C-6, respectively (delle Monache et al., 1984; Seo et al., 1999). The two hydroxyl protons showed a common three-bond connectivity with C-1 at δ_C 107.5 in the HMBC spectrum of **4**, providing further evidence for their relative positions. Signals for a prenyl group appeared at δ_H 3.42/δ_C 22.3, 5.20/121.4, 1.77/18.3, 1.70/26.2 and δ_C 134.9. The prenyl group was positioned at C-5 from the HMBC correlations of H-1′ with C-4 (three-bond), C-5 (two-bond) and C-6 (three-bond). The HMBC correlations of the methine proton, H-1″ of the lactone ring with C-3 (two-bond) and C-4 (three-bond) and the three-bond connectivity between the methylene protons of H-2″ and C-3 indicated that the lactone ring was fused to C-3 and C-4 in compound **4**. Another phenyl group resonating at δ_H 7.16/δ_C 127.1, 7.27/129.4, 7.23/127.8 and δ_C 166.9 was placed at C-1″ from the HMBC correlations of H-1″/C-4″, C-5″(9″), and H-2″/C-4″. Stereochemistry at C-1″ could not be determined since the two methylene protons of the H-2″ appeared as a singlet at δ_H 3.09, which is different from that of compound **5** (in infra). Therefore, structure **4** was assigned to the new compound, vismiaguianone D (see Table 2).

The elemental formula of **5** was determined as $C_{27}H_{24}O_5$ by HR-EIMS (observed m/z 428.1619). Compound **5** exhibited an almost identical IR spectrum to that of **4**. The ^1H and ^{13}C NMR spectral data of **5** were similar to those of the isomeric **4**, which differ from **5** only in the position of the phenyl lactone ring. In the HMBC spectrum of **5**, H-1″ showed cross peaks with C-3 (two-bond), and C-2 (three-bond), indicating that the lactone ring is attached to C-2 and C-3. The two protons of the H-2″ methylene functionality at δ_H 2.90 (1H, *d*, $J = 2.6$ Hz) and δ_H 2.92 (1H, *d*, $J = 5.7$ Hz), were deduced as *trans* and *cis* relative to H-1″, respectively, from their coupling constants, although the absolute configuration at C-1″ could not be determined. Two possible energy-minimized stereostructures, C-1″*R* and C-1″*S* of **5** were proposed by the molecular modeling program (HyperChem™ 4.0). In both molecular models, dihedral angles between the protons of H-1″/H-2″ *trans* (C-1″*R*, 78.4°; C-1″*S*, 76.3°) and H-1″/H-2″ *cis* (C-1″*R*, 41.1°; C-1″*S*, 43.0°) were calculated and inserted into the Karplus corre-

Table 1
NMR spectral data of compounds **1**–**3**

Position	**1**		**2**		**3**	
	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C
1		105.3		104.8		101.3
2		161.4		161.2		161.7
3		105.3		103.7		105.8
4		160.6		165.8		158.8
5		97.9		104.8		104.4
6		153.5		154.5		160.5
7		143.0		140.1		141.5
8 and 12	7.46 (2H, *brd*, 7.5)	127.3	7.62 (2H, *brd*, 7.5)	127.7	7.52 (2H, *dd*, 7.1, 1.4)	127.3
9 and 11	7.37 (2H, *brt*, 7.5)	127.5	7.52 (2H, *brt*, 7.5)	129.3	7.43 (2H, *brt*, 7.1)	127.7
10	7.44 (1H, *brd*, 7.5)	130.1	7.58 (1H, *brd*, 7.5)	132.1	7.47 (2H, *brd*, 7.1)	130.7
13		200.3		197.2		198.7
1′	3.45 (2H, *d*, 7.2)	21.8	3.25 (2H, *d*, 7.2)	22.1	3.43 (2H, *d*, 7.1)	21.8
2′	5.32 (1H, *t*, 7.2)	121.8	5.23 (*brt*, 7.2)	121.8	5.32 (1H, *brt*, 7.1)	121.8
3′		143.0		132.7		136.1
4′	1.86 (3H, *s*)	25.9	1.69 (3H, *brs*)	25.7	1.80 (3H, *s*)	25.8
5′	1.81 (3H, *d*, 1.2)	17.9	1.75 (3H, *brs*)	17.8	1.86 (3H, *s*)	17.9
1″	2.56 (1H, *dd*, 16.8, 5.4)	25.7	2.98 (1H, *dd*, 15.6, 8.0)	27.3	2.86 (1H, *dd*, 14.7, 9.3)	27.1
	2.78 (1H, *dd*, 16.8, 5.1)		3.07 (1H, *dd*, 15.6, 9.2)		2.93 (1H, *dd*, 14.8, 9.3)	
2″	3.61	68.7	4.72 (1H, *dd*, 9.2, 8.0)	91.2	4.40 (1H, *t*, 9.3)	91.3
3″		*[b]		72.0		71.5
4″	0.85 (3H, *s*)	21.1	1.33 (3H, *s*)	25.7	0.92 (3H, *s*)	25.3
5″	0.94 (3H, *s*)	23.5	1.21 (3H, *s*)	24.0	1.05 (3H, *s*)	23.4
OH-2	12.60 (1H, *s*)		9.82 (1H, *s*)		12.71 (1H, *s*)	
OH-4	6.35 (1H, *s*)					
OH-6			8.14 (1H, *s*)			

[a] Intensities, multiplicities and J values (Hz) shown in parentheses.
[b] Overlapped with $CDCl_3$.

lation graph, and the expected coupling constants were found to be consistent with the real values. Therefore, structure **5** was assigned to the new compound, vismiaguianone E.

The elemental formula of compound **6** was established as $C_{20}H_{18}O_5$ by HR-EIMS (observed m/z 338.1157). The IR spectrum showed absorption bands at 3405 cm^{-1} for a hydroxyl and 1718 cm^{-1} for a conjugated carbonyl functionality. The ^1H and ^{13}C NMR spectra of **6** showed one aromatic methoxyl signal and one aromatic methyl signal at δ_H 3.88/δ_C 55.8 and 2.69/24.2, respectively. Two *meta*-coupled aromatic protons appeared at δ_H 6.56 (*d*, 2.3)/δ_C 99.5 and 6.60 (*d*, 2.3)/103.3. Another aromatic proton, long-range coupled with the aromatic methyl group, was observed at δ_H 7.16 (*d*, 0.8)/δ_C 126.0. The presence of these aromatic functionalities was confirmed by the ten aromatic carbons present in the ^{13}C NMR spectrum of **6** (Table 3), and suggested a naphthalene skeleton in the molecule of **6**. The ^{13}C NMR signal for a carboxylic carbonyl group appeared at δ_C 163.2. A dimethylpyran ring was detected at δ_H 6.51/δ_C 117.3, 5.47/125.1, 1.59/28.8 and δ_C 81.1. The relative positions of these functionalities were decided by detailed analysis of the HMBC correlations as shown in Fig. 2. Therefore, structure **6** was assigned to the new compound, vismiaguianin A.

Compound **7** gave a molecular ion peak at m/z 356.1255 by HR-EIMS, corresponding to the molecular formula $C_{20}H_{20}O_6$. The structural skeleton of **7** was deduced by comparison with the ^1H and ^{13}C NMR spectral data of **6**. A dihydrofuran ring with a hydroxyisopropyl group was shown by signals at δ_H 3.15, 3.46/δ_C 27.1 (C-1′), 4.95/93.3 (C-2′), 1.36/25.9 (C-4′), 1.51/26.1 (C-5′), and δ_C 70.6 (C-3′) in the ^1H and ^{13}C NMR spectral of **7**. Therefore, structure **7** was assigned to the new compound, vismiaguianin B. To the best of our knowledge, the benzocoumarin skeleton of structures of **6** and **7**, has not previously been

Table 2
^1H and ^{13}C NMR spectral data of compounds **4** and **5**

Position	**4**		**5**	
	δ_H[a]	δ_C	δ_H[a]	δ_C
1		107.5		137.6
2		157.0		151.2
3		105.6		104.8
4		155.8		158.3
5		109.1		109.8
6		158.2		160.9
7		139.8		141.0
8 and 12	7.66 (2H, *b d*, 7.7)	128.6	7.59 (2H, *brd*, 7.2)	128.0
9 and 11	7.50 (2H, *brt*, 7.7)	129.5	7.42 (2H, *t*, 7.2)	128.1
10	7.58 (1H, *b d*, 7.7)	133.2	7.50 (1H, *d*, 7.2)	132.0
13		198.9		198.8
1′	3.42 (2H, *t*, 6.3)	22.3	3.40 (1H, *dd*, 8.0, 16.8) 3.56 (1H, *dd*, 6.5, 16.8)	22.1
2′	5.20 (1H, *tt*, 6.3, 1.0)	121.4	5.28 (1H, *dd*, 6.5, 8.0)	120.6
3′		134.9		137.6
4′	1.77 (3H, *s*)	18.3	1.81 (3H, *s*)	18.0
5′	1.70 (3H, *d*, 1.0)	26.2	1.78 (3H, *s*)	25.8
1″	4.64 (1H, *t*, 4.5)	35.1	4.54 (1H, *dd*, 2.6, 5.7)	34.8
2″	3.05 (2H, *d*, 4.5)	37.0	2.90 (1H, *d*, 2.6) 2.92 (1H, *d*, 5.7)	36.9
3″		166.9		165.0
4″		141.4		140.6
5″ and 9″	7.16 (2H, *brd*, 6.9)	127.1	7.07 (2H, *brd*, 6.7)	126.7
6″ and 8″	7.27 (2H, *brt*, 6.9)	129.4	7.26 (2H, *brt*, 6.7)	129.0
7″	7.23 (1H, *brd*, 6.9)	127.8	7.21 (1H, *brd*, 6.7)	127.5
OH-2	9.69 (1H, *s*)			
OH-6	7.91 (1H, *s*)			

[a] Intensities, multiplicities and *J* values (Hz) shown in parentheses.

Table 3
^1H and ^{13}C NMR spectral data of compounds **6** and **7**

Position	**6**		**7**	
	δ_H[a]	δ_C	δ_H[a]	δ_C
2		163.2		169.3
3		98.5		100.4
4		158.8		158.9
4a		108.4		106.3
5		132.9		132.2
6	7.16 (1H, *d*, 0.8)	126.0	7.18 (1H, *s*)	124.6
6a		137.9		138.6
7	6.56 (1H, *d*, 2.3)	99.5	6.75 (1H, *dd*, 2.2)	99.3
8		161.8		161.5
9	6.60 (1H, *d*, 2.3)	103.3	6.84 (1H, *dd*, 2.2)	103.2
10		156.1		157.0
10a		107.1		108.0
10b		153.8		155.3
11	3.88 (3H, *s*)	55.8	3.78 (3H, *s*)	55.4
12	2.69 (3H, *d*, 0.8)	24.2	2.61 (3H, *s*)	21.8
1′	6.51 (1H, *d*, 9.0)	117.3	(a) 3.15 (1H, *dd*, 10.5, 14.8) (b) 3.46 (1H, *dd*, 8.1, 10.5)	27.1
2′	5.47 (1H, *d*, 9.0)	125.1	4.95 (1H, *dd*, 8.1, 10.4)	93.3
3′		81.1		70.6
4′	1.59 (3H, *s*)	28.8	1.36 (3H, *s*)	25.9
5′	1.59 (3H, *s*)	28.8	1.51 (3H, *s*)	26.1
OH-10	8.89 (1H, *s*)			

[a] Intensities, multiplicities and *J* values (Hz) shown in parentheses.

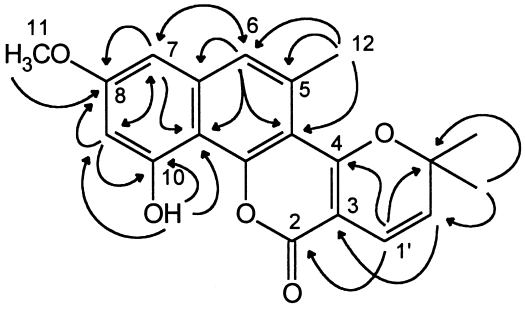


Fig. 2. HMBC correlations for compound **6**.

reported from natural sources, although synthetic benzocoumarin has been reported (Harvey et al., 1988)

All the ^{1}H and ^{13}C NMR signals of **1**–**7** were assigned unambiguously by detailed analysis of the HMQC and HMBC spectra of each compound.

Compounds **1**–**7** were tested for DNA strand-scission activity and were evaluated for cytotoxicity against the KB cancer cell line as described in Section 3. Vismiaguianone B (**2**) exhibited moderate DNA strand-scission activity ($43 \pm 12\%$ nicked at 2.5 μg ml^{-1}). Compounds **4**–**6** were found to be moderately cytotoxic when tested against the KB cell line, with EC_{50} values of 2.4, 3.3, and 1.3 μg ml^{-1}, respectively (Table 4).

Compounds **1**–**3** have similar structure, except for variations in the substitution pattern and functionality of ring C. Only compound **2**, which has ring C attached at C-4 and C-5, showed moderate DNA strand-scission activity, whereas **1** and **3**, which have ring C affixed to C-5 and C-6, did not show any activity. None of the remaining compounds (**4**–**7**) was deemed active in the DNA strand-scission assay.

Compounds **4** and **5** are regioisomers varying in the position of ring D. Both compounds showed moderate cytotoxic activity for KB cells, indicating that their differential substitution of ring D did not affect such activity. The benzocoumarins, **6** and **7**, have similar structures except for their respective ring D. Compound **6** with a dimethylpyran ring as ring D, exhibited cytotoxicity against the KB cell line, whereas **7**, containing a dihydrofuran ring with a hydroxyisopropyl group was devoid of activity. Accordingly, a dihydropyran ring in the structure of **6** appears to be important for the mediation of cytotoxic activity.

3. Experimental

Mps: uncorrelated IR: $CHCl_3$. ^{1}H and ^{13}C NMR: 300 or 500 MHz instruments with TMS as int. standard. EIMS: direct probe. Molecular modeling software: HyperChem™ 4.0 (Hypercube, Waterloo, ON, Canada)

3.1. Plant material

The roots of *V. guianensis* (Aubl.) Choisy were collected in August 1993, in Recife, Pernambuco, Brazil, and identified by one of us (R. Mukherjee). A voucher specimen (A3090) has been deposited in the Field Museum of Natural History, Chicago, IL, USA.

3.2. Extraction and isolation

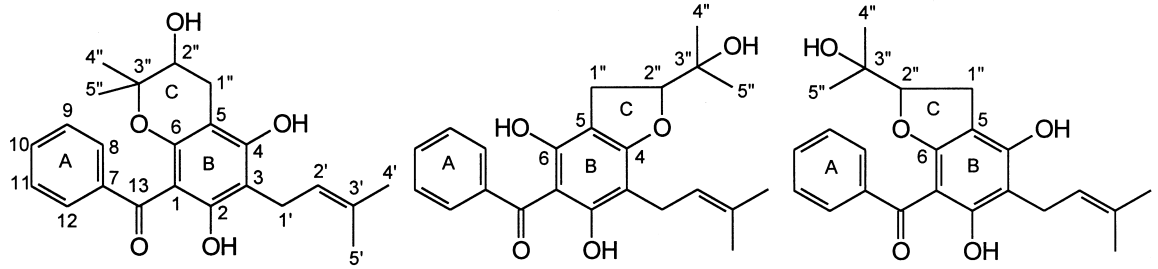
Dried roots of *V. guianensis* (875 g) were ground and extracted with MeOH (2×2 l) for 24 h by percolation. The MeOH extract was concentrated, redissolved in MeOH–H_2O (9:1) and subsequently washed with hexane (300 ml). The aq. MeOH solution (300 ml) was further partitioned with $CHCl_3$ (2×300 ml). The $CHCl_3$ extract was washed with 1% saline solution, and then evaporated, affording 17 g of a residue which showed cytotoxic activity against the KB cell line.

The above $CHCl_3$ extract, mixed with Celite (17 g), was subjected to a silica gel CC (flash, 500 g, 230–400 mesh) using $CHCl_3$–MeOH (gradient, 100:0 → 10:1) solvent mixtures, affording 11 fractions (I–XI). Fraction II (571 mg), eluted with $CHCl_3$–MeOH (99.5:0.5 → 98.8:1.2) from the initial CC, was further separated by silica gel CC (flash, 70 g, 230–400 mesh) using hexane–acetone (3:2) as eluent, giving 15 fractions (II-1–II-15). Fraction II-13 from this CC, was triturated with MeOH, affording compound **4** (78 mg, 0.0089% w/w). Preparative HPLC of fraction II-14 (133 mg) (column: Dynamax-60A 8 μm, C_8, 250×21.4 mm I.D., flow rate: 10 ml min^{-1}) using MeOH–H_2O (9:1), gave compound **5** (102 mg, 0.0117% w/w; t_R 21 min). Fractions III–V, eluted with $CHCl_3$–MeOH (98.7:1.3 → 98.5:1.5) from the initial CC, were combined due to their overlapping TLC pattern. This combined fraction (5.6 g) was subjected to a silica gel (flash, 200 g, 230–400 mesh) CC using hexane–acetone (gradient, 99:1 → 1:1) mixtures for elution, giving 11 fractions (III-1–III-11). Fraction III-4 from this column, that eluted with hexane–acetone (23:2), was further separated by Sephadex LH-20 using $CHCl_3$–MeOH (1:1) as eluent, affording compound **6** (65 mg, 0.0074% w/w) as needles. Fraction III-8 (1.0 g) eluted with hexane–acetone (7:3) from the CC of combined fractions of III–V, was

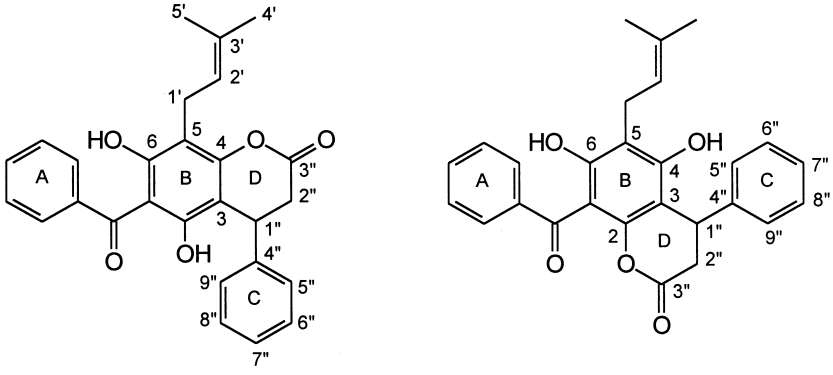
Table 4
Cytotoxic activity of isolates from *V. guianensis* with the KB cell line

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**
KB[a]	> 20	> 20	> 20	2.4 ± 0.9[b]	3.3 ± 1.5[b]	1.3 ± 0.8[b]	> 20

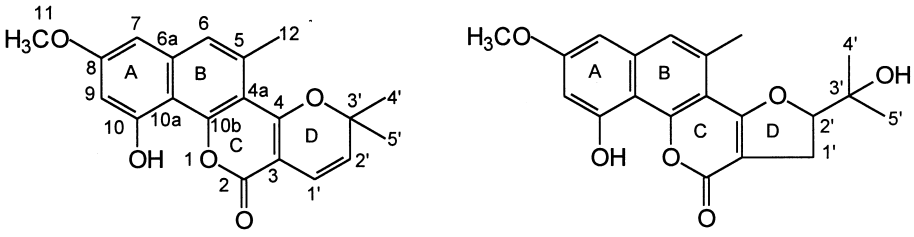
[a] Oral epidermoid carcinoma. Results are expressed as EC_{50} values (μg ml^{-1}).
[b] Mean ± SEM determined from three separate experiments.



1 2 3



4 5



6 7

further separated by silica gel CC (flash, 150 g, 230–400 mesh, solvent: $CHCl_3$–acetone gradient, 99:1 → 1:1) leading to 14 fractions (III-8-1–III-8-14). Fraction III-8-4 was purified by preparative HPLC (column: MetaChem, Intersil ODS-3 8 μm, C_{18}, 250 × 20 mm I.D., flow rate: 10 ml min^{-1}) using MeOH–H_2O. (4:1), affording compound **1** (110 mg, 0.0126% w/w, t_R 9.6 min). Compound **3** (27 mg, 0.0031% w/w, t_R 14.7 min) was purified by preparative HPLC (column: MetaChem, Intersil ODS-3 8 μm, C_{18}, 250 × 20 mm I.D., solvent: MeOH–H_2O, 4:1, flow rate: 10 ml min^{-1}) of fraction III-8-5. Preparative HPLC of fraction III-8-8 (column: MetaChem, Intersil ODS-3 8 μm, C_{18}, 250 × 20 mm I.D.) using MeOH–H_2O mixtures as eluents (gradient for 30 min, 70:30 → 90:10) afforded compound **2** (12 mg, 0.0014% w/w, t_R 23.9 min). Fraction III-9 (470 mg), eluted with hexane–acetone (3:2) from the CC of combined fractions III–V, was further separated by silica gel CC (flash, 100 g, 230–400 mesh, solvent: $CHCl_3$–acetone, gradient, 19:1 → 7:3), affording compound **7** (4.2 mg, 0.0005% w/w, eluted with $CHCl_3$–acetone, 3:1).

3.3. Vismiaguianone A (1)

Yellow powder (MeOH), mp 140–142°C. $[\alpha]_D^{22}$ + 10.9° ($CHCl_3$; *c* 0.11). UV λ_{max}^{MeOH} nm (log ε): 308 (4.02), 210 (4.35). IR ν_{max} ($CHCl_3$) cm^{-1}: 3355, 3015, 1610, 1564, 1440, 1368, 1320, 1259, 1122, 1059. ^1H NMR (300.1 MHz; $CDCl_3$) and ^{13}C NMR (75.5 MHz; $CDCl_3$) data: see Table 1. ^1H–^{13}C HMBC correlations (500.1 MHz; $CDCl_3$): see Fig. 1. EIMS *m/z*: $[M]^+$ 382

(54), 349 (30), 309 (20), 295 (55), 255 (40), 177 (52), 149 (100), 129 (37), 111 (37), 105 (77). HR-EIMS *m/z*: 382.1784 (calculated for $C_{23}H_{26}O_5$, 382.1780).

3.4. Vismiaguianone B (**2**)

Yellow powder (MeOH), mp 58–60°C. $[\alpha]_D^{22}$ + 5.0° ($CHCl_3$; *c* 0.14). UV λ_{max}^{MeOH} nm (log ε): 318 (4.11), 213 (4.42). IR ν_{max} ($CHCl_3$) cm^{-1}: 3495, 2965, 1640, 1440, 1363, 1324, 1207, 1116. ^1H NMR (300 MHz; $CDCl_3$) and ^{13}C NMR (75 MHz; $CDCl_3$) data: see Table 1. ^1H–^{13}C HMBC correlations (500.1 MHz; $CDCl_3$): H-8(12)/C-8(12), C-10, C-13; H-9(11)/C-9(11); H-10/C-8(12); H-1′/C-2, C-3, C-4, C-2′; H-2′/C-1′, C-4′, C-5′; H-4′/C-2′, C-5′; H-5′/C-2′, C-4′; H-1″/C-4, C-5, C-6, C-2″; H-2″/C-4, C-5, C-1″, C-5″; H-4″/C-2″, C-3″, C-5″; H-5″/C-2″, C-3″, C-4″; OH-2/C-2, C-3; OH-6/C-5, C-6. EIMS *m/z*: $[M]^+$ 382 (100), 367 (33), 349 (22), 339 (15), 327 (66), 309 (20), 295 (17), 281 (15), 267 (47), 255 (37), 231 (13), 217 (10), 203 (9), 189 (21), 177 (25), 129 (9), 115 (7), 105 (78). HR-EIMS *m/z*: 382.1784 (calculated for $C_{23}H_{26}O_5$, 382.1780).

3.5. Vismiaguianone C (**3**)

Yellow powder (MeOH), mp 162–164°C. $[\alpha]_D^{22}$ + 2.7° ($CHCl_3$; *c* 0.15). UV λ_{max}^{MeOH} nm (log ε): 308 (4.21), 212 (4.40). IR ν_{max} ($CHCl_3$) cm^{-1}: 3560, 3350, 2915, 1631, 1465, 1432, 1371, 1326, 1256, 1121, 1089. ^1H NMR (300.1 MHz; $CDCl_3$) and ^{13}C NMR (75.5 MHz; $CDCl_3$) data: see Table 1. ^1H–^{13}C HMBC correlations (500.1 MHz; $CDCl_3$): H-8(12)/C-8(12), C-13; H-9(11)/C-9(11), C-7; H-10/C-8(12); H-1′/C-2, C-3, C-4, C-2′, C-3′; H-2′/C-1′, C-4′, C-5′; H-4′/C-2′, C-3′, C-5′; H-5′/C-2′, C-3′, C-4′; H_2-1″/C-5, C-6, C-2″, C-3″; OH-2/C-1, C-2, C-3. EIMS *m/z*: $[M]^+$ 382 (100), 349 (35), 327 (30), 309 (21), 267 (43), 255 (22), 189 (21), 105 (76). HR-EIMS *m/z*: 382.1784 (calculated for $C_{23}H_{26}O_5$, 382.1780).

3.6. Vismiaguianone D (**4**)

Light yellow needles (MeOH), mp 171–172°C. $[\alpha]_D^{22}$ − 147.2° ($CHCl_3$; *c* 0.25). UV λ_{max}^{MeOH} nm (log ε): 298 (4.20), 263 (sh, 4.15). IR ν_{max} ($CHCl_3$) cm^{-1}: 3495, 3040, 2905, 1773, 1623, 1435, 1308, 1233, 1179, 1122, 983, 697. ^1H NMR (300.1 MHz; $CDCl_3$) and ^{13}C NMR (75.5 MHz; $CDCl_3$) data: see Table 2. ^1H–^{13}C HMBC correlations (500.1 MHz; $CDCl_3$): H- 8(12)/C-8(12), C-13; H-9(11)/C-9(11), C-8(12); H-10/C-8(12); H-1′/C-4, C-5, C-6, C-2′, C-3′; H-2′/C-5, C-1′, C-4′, C-5′; H-4′/C-2′, C-3′, C-5′; H-5′/C-2′, C-3′, C-4′; H-1″/C-2, C-3 C-4, C-3″, C-4″; H-5″(9″)/C-1″, C-5″(9″), C-7″; H-6″(8″)/C-6″(8″); H-7″/C-5″(9″); OH-2/C-1, C-2, C-3; OH-6/C-1, C-5, C-6. EIMS *m/z*: $[M]^+$ 428 (100), 413 (17), 385 (30), 373 (100), 359 (9), 343 (30), 331 (26), 309 (32), 293 (12), 265 (12), 253 (12), 231 (6), 215 (8), 129 (7), 115 (6), 105 (29). HR-EIMS *m/z*: 428.1618 (calculated for $C_{27}H_{24}O_5$, 428.1624).

3.7. Vismiaguianone E (**5**)

Light yellow powder (MeOH), mp 70–72°C. $[\alpha]_D^{22}$ + 198.9° ($CHCl_3$; *c* 0.37). UV λ_{max}^{MeOH} nm (log ε): 305 (4.07), 250 (sh, 4.18). IR ν_{max} ($CHCl_3$) cm^{-1}: 3340, 1774, 1615, 1440, 1320, 1255, 1230, 1122, 697, 663. ^1H NMR (300.1 MHz; $CDCl_3$) and ^{13}C NMR (75.5 MHz; $CDCl_3$) data: see Table 2. ^1H–^{13}C HMBC correlations (500.1 MHz; $CDCl_3$): H-8(12)/C-8(12), C-10, C-13; H-9(11)/C-9(11); H-10/C-8(12); H-1′/C-4, C-5, C-6, C-2′, C-3′; H-2′/C-1′, C-3′, C-5′; H-4′/C-5′; H-5′/C-4′; H-1″/C-2, C-3, C-2″, C-4″, C-5″(9″); H-2″/C-3, C-3″, C-4″; H-5″(9″)/C-1″, C-7″; H-6″(8″)/C-4″, C-6″(8″); H-7″/C-5″(9″). EIMS *m/z*: $[M]^+$ 428 (100), 400 (12), 385 (62), 373 (89), 343 (43), 279 (16), 331 (30), 309 (76), 293 (16), 265 (18), 253 (20), 231 (17), 215 (17), 129 (13), 115 (14), 105 (72). HR-EIMS *m/z*: 428.1619 (calculated for $C_{27}H_{24}O_5$, 428.1624).

3.8. Vismiaguianin A (**6**)

Light yellow needles (MeOH), mp 191–192°C. UV λ_{max}^{MeOH} nm (log ε): 388 (4.17), 263 (4.42), 238 (4.59). IR ν_{max} ($CHCl_3$) cm^{-1}: 3405, 2965, 1718, 1632, 1575, 1542, 1496, 1462, 1352, 1297, 1213, 1155, 1104, 1032, 997. ^1H NMR (300.1 MHz; $CDCl_3$) and ^{13}C NMR (75.5 MHz; $CDCl_3$) data: see Table 3. ^1H–^{13}C HMBC correlations (500.1 MHz; $CDCl_3$): see Fig. 2. EIMS *m/z*: $[M]^+$ 338 (63), 323 (41), 230 (100), 204 (19), 162 (13), 135 (22), 81 (27), 68 (50). EIMS *m/z*: $[M + Na]^+$ 361. HR-EIMS *m/z*: 338.1157 (calculated for $C_{29}H_{18}O_5$, 338.1154).

3.9. Vismiaguianin B (**7**)

Pale yellow powder (MeOH), mp 222–224°C. $[\alpha]_D^{22}$ + 18.0° ($CHCl_3$; *c* 0.05). UV λ_{max}^{MeOH} nm (log ε): 374 (4.07), 293 (4.41), 235 (4.80). IR ν_{max} ($CHCl_3$) cm^{-1}: 3475, 3015, 1716, 1622, 1496, 1467, 1379, 1151, 1032, 993, 929. ^1H NMR (300.1 MHz; pyridine-d_6) and ^{13}C NMR (75.5 MHz; pyridine-d_6) data: see Table 3. ^1H–^{13}C HMBC correlations (500.1 MHz; pyridine-d_6): H-11/C-8; H-12/C-5, C-6, C-4a; H_2-4/C-3, C-3′; H-4′/C-2′, C-3′; H-5′/C-2′, C-3′. EIMS *m/z*: $[M]^+$ 356 (100), 323 (9), 297 (16), 284 (30), 270 (11), 242 (7), 241 (10), 230 (80), 201 (16), 169 (9), 127 (12). HR-EIMS *m/z*: 356.1255 (calculated for $C_{20}H_{20}O_6$, 356.1260).

3.10. Biological assays

3.10.1. DNA strand-scission assay

The DNA strand-scission assay was carried out by a modified Hecht procedure (Sugiyama et al., 1985; Huang et al., 1998). The results were compared with a positive control, bleomycin sulfate at 0.025 μg ml^{-1}.

3.10.2. Cytotoxicity testing

Compounds **1**–**7** were evaluated for cytotoxic activity against the KB cell line as follows. The assays were run in 96-well plastic plates and each well contained 190 μl of a 9-KB cell suspension (5×10^4 cells ml^{-1}) and 10 μl of the test compound assayed in triplicate at 11 concentrations ranging from 0.0025 to 20 μg ml^{-1}. Each plate also contained triplicate samples of vehicle (10% DMSO) and an 11-point camptothecin curve that was used to monitor assay performance. The plates were incubated for 72 h (37°C; 95% CO_2 balanced with nitrogen) before the addition of 100 μl of 50% trichloroacetic acid to stop the assay and fix the cells to the well bottom. The relative amount of cell growth in each well was determined by a spectrophotometric assay essentially as previously described (Likhitwitayawuid et al., 1993). The absorbance values were converted to percent survival using the formula: $(Abs_{sample}/Abs_{vehicle}) \times 100$. The concentration of test compound that caused 50% cell death (EC_{50}) was determined from the semilog plot of percent survival versus log concentration that was fit to a sigmoid curve using GraphPad Prism (v. 3.00 for Windows, GraphPad Software, San Diego, CA, USA).

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