New Biphenyl Compounds with DNA Strand-Scission Activity from *Clusia paralicola*

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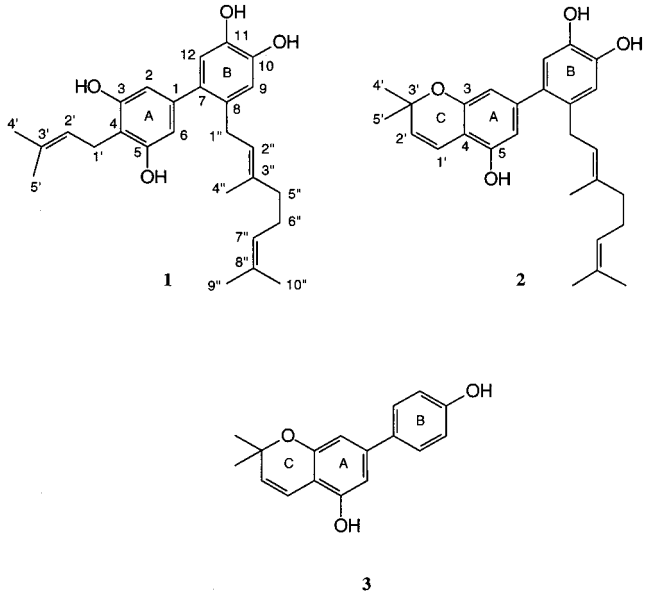
Three new biphenyl derivatives, clusiparalicolines A (**1**), B (**2**), and C (**3**), were isolated from the roots of *Clusia paralicola* by bioassay-directed fractionation using the DNA strand-scission and the KB human cancer cell line cytotoxicity assays. Compounds **1** and **2** were found to be active in the DNA strand-scission assay, whereas all three compounds exhibited modest cytotoxicity against the KB cell line. The structures of **1**−**3** were elucidated by spectroscopic methods including 1D and 2D NMR techniques.

In an ongoing collaborative program aimed at the discovery of novel, potential antineoplastic agents from plants, it was found that a chloroform extract of the roots of *Clusia paralicola* G. Mariz (Guttiferae) exhibited DNA strand-scission activity and significant cytotoxicity against the KB cell line. About 145 *Clusia* species are distributed throughout parts of Central and South America.[1] Extracts and decoctions of various *Clusia* species have been used for a variety of ailments by indigenous people of these regions.[2] Previous studies have shown that *C. paralicola* exhibits a broad range of antibacterial activity and has also shown anti-yeast activity.[3] Isoprenylated benzophenones[4−12] and triterpenes[11,13−15] have been found as the major compound classes in *Clusia* species. An unexpected prenylated dihydrophenanthrene, paralycoline A, has been isolated from *C. paralicola*.[16]

In the present investigation, the bioassay-guided fractionation using the DNA strand-scission and the KB cell-line cytotoxicity assays, led to the isolation of three new biphenyl compounds, **1**−**3**. Compounds **1** and **2** were found to be active in a DNA strand-scission assay, whereas all three compounds exhibited moderate cytotoxicity against the KB cell line. We report herein the isolation and structure elucidation of **1**−**3** using spectral methods such as HMQC, HMBC, and ROESY NMR techniques. In addition, DNA strand-scission activity and cytotoxicity in the KB cell line of compounds **1**−**3** are presented. Although a wide variety of compounds, both aromatic and non-aromatic, have been isolated from *Clusia* species, to our knowledge this is the first report therein of prenylated biphenyl compounds.

Results and Discussion

Compound **1** was deduced as having an elemental formula of $C_{27}H_{34}O_4$ by HREIMS, which showed a molecular ion peak at *m/z* 422.2455. The IR spectrum of **1** showed absorption bands at 3670 cm^{-1} for free hydroxyl groups, 3015 cm^{-1} for aromatic CH stretch, and 1220 cm^{-1} for C−O stretch.[17]



In the ^1H and ^{13}C NMR spectra of compound **1**, signals due to a prenyl group attached to an aromatic carbon were found at δ_H 5.28/δ_C 121.6 (C-2′), 3.43/22.4 (C-1′), 1.82/17.9 (C-5′), 1.75/25.8 (C-4′), and 135.4 (C-3′).[18] Signals for a geranyl group appeared at δ_H 5.15/δ_C 124.0 (C-2″), 5.09/124.4 (C-7″), 3.13/31.2 (C-1″), 2.06/26.5 (C-6″), 1.98/39.6 (C-5″), 1.67/25.6 (C-9″), 1.59/17.7 (C-10″), 1.52/15.9 (C-4″), 135.5 (C-3″), and 131.6 (C-8″).[19] The probability that the prenyl and geranyl groups were attached to an aromatic biphenyl structure composed of 12 aromatic carbon atoms was supported by the observations of signals for four aromatic methines as well as eight aromatic quaternary carbons in the NMR spectra of **1** (Table 1). The ^{13}C NMR signals for four aromatic quaternary carbons at δ_C 154.3 (C-3 and C-5), 142.9 (C-10), and 140.7 (C-11) suggested the existence of four hydroxyl groups as substituents on the two phenyl rings. In compound **1**, the fact that ring A is symmetrically substituted is supported by the presence of a signal at δ_H 6.26 (2H, s) due to H-2(6) and signals at δ_C 109.7 and 154.3 due to C-2(6) and C-3(5), respectively. The $^1H-^{13}C$ HMBC NMR technique was employed to position a prenyl, a geranyl, and four hydroxyl groups in the

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Table 1. NMR Data of Compound **1**

position	δ_H *a*	δ_C *b*	HMBC[a]	ROESY[c]
1		140.7 (s)		
2(6)	6.26 (2H, s)	109.7 (d)	C-2 (6), C-4, C-7	H-1″, H-4″
3(5)		154.3 (s)		
4		112.0 (s)		
7		133.9 (s)		
8		132.2 (s)		
9	6.73 (1H, s)	116.1 (d)	C-7, C-10, C-11, C-1″	H-1″, H-2″
10		142.9 (s)		
11		140.7 (s)		
12	6.55 (1H, s)	116.7 (d)	C-8, C-10, C-11	
1′	3.43 (2H, d, 6.3)	22.4 (t)	C-3 (5), C-4, C-2′, C-3′	H-5′
2′	5.28 (1H, t, 6.3)	121.6 (d)	C-3′, C-4′, C-5′	H-4′
3′		135.4 (s)		
4′	1.75 (3H, s)	25.8 (q)	C-2′, C-3′, C-5′	H-2′
5′	1.82 (3H, s)	17.9 (q)	C-2′, C-3′, C-4′	H-1′
1″	3.13 (2H, d, 6.8)	31.2 (t)	C-8, C-9, C-2″, C-3″	H-2(6), H-9, H-2″, H-4″
2″	5.15 (1H, t, 6.8)	124.0 (d)	C-4″, C-5″	H-9, H-1″, H-5″
3″		135.5 (s)		
4″	1.52 (3H, s)	15.9 (q)	C-2″, C-3″, C-5″	H-2(6), H-1″
5″	1.98 (2H, dd, 14.4, 6.7)	39.6 (t)	C-4″	H-2″
6″	2.06 (2H, dd, 13.6, 6.7)	26.5 (t)	C-5″	H-7″
7″	5.09 (1H, t, 6.4)	124.4 (d)	C-8″, C-9″, C-10″	H-6″, H-10″
8″		131.6 (s)		
9″	1.67 (3H, s)	25.6 (q)	C-7″, C-8″, C-10″	H-7″
10″	1.59 (3H, s)	17.7 (q)	C-7″, C-8″, C-9″	
OH-3(5)	5.41 (2H, s)		C-4, C-6	

[a] Operated at 500.1 MHz. [b] Operated at 62.9 MHz. [c] Operated at 300.1 MHz.

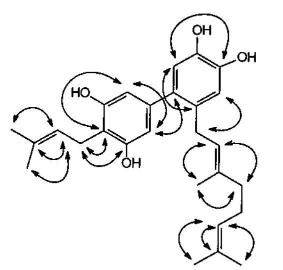

Figure 1. Important HMBC correlations of compound **1**.

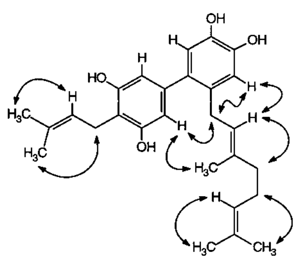

Figure 2. Important ROESY correlations of compound **1**.

molecule. The signal due to H-1′ at δ_H 3.43 showed two-bond connectivity with C-4 (δ_C 112.0), and three-bond connectivity with C-3 and C-5 (δ_C 154.3) in the HMBC spectrum of **1**. Hence, the prenyl group was assigned to C-4. The HMBC correlations of H-1″/C-8 and H-1″/C-9, which are two-bond and three-bond connectivities, respectively, suggested that the geranyl group is attached at C-8 in the molecule. Further evidence for the position of this geranyl group was provided by the ROESY experiment, which displayed cross-peaks between H-1″/H-2(6) and H-1″/H-9. Therefore, **1** was determined to be a new biphenyl compound, namely, clusiparalicoline A. All ^1H and ^{13}C NMR signals of compound **1** were unambiguously assigned by detailed analysis of the $^1H-^{13}C$ HMQC, $^1H-^{13}C$ HMBC (Figure 1), and $^1H-^1H$ ROESY (Figure 2) NMR spectra as shown in Table 1.

Compound **2** gave a molecular ion peak at *m/z* 420.2306 from HREIMS, corresponding to an elemental formula of $C_{27}H_{32}O_4$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of compound **1** except for signals for the prenyl functionality at C-4 in **1**. Signals for a dimethylpyran ring appeared at δ_H 6.69/δ_C 116.5 (C-1′), 5.59/129.0 (C-2′), 1.44/27.8 (C-4′ and C-5′), and 76.2 (C-3′) in the ^1H and ^{13}C NMR spectra of **2**. These results suggested that compound **2** was formed from the cyclization of the prenyl group in **1** with an adjacent hydroxyl group. Accordingly, **2** is a new compound, clusiparalicoline B. Assignments of ^1H and ^{13}C NMR data of **2** were made by comparison with the NMR data of compounds **1** and **3** see below as shown in Table 2. The APT experiment was also used to support the ^{13}C NMR assignments.

Compound **3** was shown to have the elemental formula $C_{17}H_{16}O_3$ from HREIMS, which exhibited a molecular ion peak at *m/z* 268.1095. Comparison of ^1H and ^{13}C NMR spectra with those of compounds **1** and **2** suggested that **3** was also a biphenyl compound but with different substituents. The ^1H and ^{13}C NMR spectra of **3** displayed signals for a dimethylpyran ring at δ_H 6.64/δ_C 116.2 (C-1′), 5.60/129.0 (C-2′), 1.45/27.8 (C-4′ and C-5′), and 76.1 (C-3′), which also appeared in the spectra of compound **2**, indicating the presence of a similarly substituted ring A in **3**. The ^1H NMR spectrum of **3** showed a set of *ortho*-coupled doublets at δ_H 7.41 ($J = 7.5$) and 6.85 ($J = 7.5$) integrated as two protons for each, which were correlated to the ^{13}C NMR signals at δ_C 128.0 and 115.6, respectively, in the HMQC spectrum. These results indicated the existence of a *para*-substituted phenyl group in the molecule. Further support for the presence of the *para*-substituted phenyl ring was provided by the $^1H-^{13}C$ HMBC spectrum of **3** as shown in Figure 3. Signals of the ^1H and ^{13}C NMR spectra of **3** were assigned unambiguously by detailed analysis of $^1H-^1H$ COSY, $^1H-^{13}C$ HMQC, and $^1H-^{13}C$ HMBC spectra as shown in Table 2. The data indicated that compound **3** was a new biphenyl compound, clusiparalicoline C.

Compounds **1** and **2** exhibited significant DNA strand-scission activity (77% and 65% relaxation, respectively, at 2.5 μg/mL), whereas **3** was found to be inactive (cf. Experimental Section and Table 3). In parallel assays, 0.025 μg/mL bleomycin nicked approximately 50% of the supercoiled DNA. Compounds **1** and **2** have identical rings

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **2** and **3**

	2[a]		**3**[b]	
position	δ_H	δ_C	δ_H	δ_C
1		142.4 (s)		141.7 (s)
2	6.18 (1H, s)	109.3 (d)	6.49 (1H, d, 1.6)	106.1 (d)
3		150.7 (s)		151.4 (s)
4		108.0 (s)		108.2 (s)
5		153.3 (s)		154.2 (s)
6	6.33 (1H, s)	110.6 (d)	6.63 (1H, dd, 1.6, 0.8)	107.7 (d)
7		133.9 (s)		133.2 (s)
8		132.1 (s)	7.41 (1H, d, 7.5)	128.0 (d)
9	6.74 (1H, s)	116.1 (d)	6.85 (1H, d, 7.5)	115.6 (d)
10		142.9 (s)		155.2 (s)
11		140.8 (s)	6.85 (1H, d, 7.5)	115.6 (d)
12	6.62 (1H, s)	116.5 (d)	7.41 (1H, d, 7.5)	128.0 (d)
1′	6.69 (1H, d, 10.0)	116.3 (d)	6.64 (1H, dd, 10.0, 0.8)	116.2 (d)
2′	5.59 (1H, d, 10.0)	129.0 (d)	5.60 (1H, d, 10.0)	129.0 (d)
3′		76.2 (s)		76.1 (s)
4′ (5′)	1.44 (6H, s)	27.8 (q)	1.45 (6H, s)	27.8 (q)
1″	3.17 (2H, d, 6.9)	31.3 (t)		
2″	5.17 (1H, t, 6.9)	124.0 (d)		
3″		135.4 (s)		
4″	1.53 (3H, s)	16.0 (q)		
5″	1.98 (2H, d-like, 6.6)	39.6 (t)		
6″	2.05 (2H, m)	26.5 (t)		
7″	5.09 (1H, t, 6.6)	124.4 (t)		
8″		131.6 (s)		
9″	1.68 (3H, s)	25.7 (q)		
10″	1.59 (3H, s)	17.8 (q)		

[a] Operated at 300.1and 75.5 MHz for ^{1}H and ^{13}C NMR experiments, respectively. [b] Operated at 250.1 and 62.9 MHz for ^{1}H and ^{13}C NMR experiments, respectively.

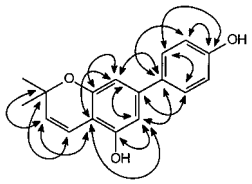

Figure 3. HMBC correlations of compound **3**.

Table 3. DNA Strand-Scission and KB Cytotoxicity of Compounds **1**−**3**

compound	% relaxation of supercoiled DNA	KB[a]
1	77 ± 0.7[b,c]	3.8 ± 0.4[c]
2	65 ± 2.0[b,c]	4.5 ± 2.2[c]
3	inactive	5.3 ± 1.6

[a] Data expressed as EC_{50} values (μg/mL). [b] Assayed at 2.5 μg/mL. [c] Mean ± SEM from at least three separate determinations.

A and B, with a 10,11-dihydroxy catechol moiety. They differ only in that the ring C in compound **2** is formed by cyclization involving the prenyl group at C-4 and the hydroxyl group at C-3. Therefore, the prenyl as well as the 3-hydroxyl group in ring A of **1** are not required for the observed DNA strand-scission activity. As stated above, compound **3** is inactive as a DNA strand-scission agent. This biphenyl analogue is identical to compound **2** in regard to rings A and C, but lacks the geranyl moiety attached to C-8 in ring B and more importantly lacks the catechol moiety present in ring B of **1** and **2**. In several of our previous reports the presence of a catechol moiety has been associated with DNA strand-scission activity.[20−22] Singh et al. have made a comprehensive study of the DNA cleavage by di- and tri-hydroxyalkylbenzenes.[23] It was reported that the 6-alkyl-1,2,4-trihydroxyalkylbenzenes were 50−100 times more potent in the relaxation of supercoiled DNA than the 5-alkyl-1,3-dihydroxybenzenes. It has been postulated that this is due to the chelation of Cu^{2+} by the catechol moiety. Compounds **1**, **2**, and **3** showed modest KB inhibitory activity (Table 3). Additional in vivo biological studies are required to assess the antineoplastic potential of compounds **1** and **2**.

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR experiments were performed on either a Bruker AMX 500, a Bruker AMX 250, or a Bruker DPX 300 spectrometer. For COSY, HMQC, and HMBC experiments, a Bruker AMX 500 spectrometer was used. ROESY and APT NMR spectra were run using the Bruker DPX 300 instrument. $CDCl_3$ was used as a solvent and TMS as internal standard. UV and IR spectra were recorded on a Varian Cary 3G UV−vis spectrophotometer and a Shimadzu IR-460 spectrometer, respectively. EIMS were recorded on HP 5989A instrument, and HREIMS were obtained using VGZAB-E magnetic sector instrument. Column chromatography was carried out either on Si gel 60 (70−230 mesh, Merck, Darmstadt, Germany), on pre-packed Si gel columns utilizing equipment for flash chromatography (Biotage, Charlottesville, VA), or on Sephadex LH-20 (Sigma, St. Louis, MO). Fractions were monitored by TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) with visualization under UV light (254 and 365 nm) and with 5% phosphomolybdic acid in EtOH.

Plant Material. The roots of *C. paralicola* were collected in August 1993, in Recife, Pernambuco, Brazil, and identified by one of authors (R. M.). A voucher specimen (voucher no. B1050) has been deposited in the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. Dried roots of *C. paralicola* (575 g) were ground and extracted with MeOH (1.5 L × 2) for 24 h by percolation. The MeOH extracts were concentrated, mixed with H_2O (MeOH−H_2O, 9:1), and partitioned with hexane (300 mL). The aqueous MeOH fraction (300 mL) was further partitioned with $CHCl_3$ (300 mL × 2). The $CHCl_3$ fraction was washed with 1% saline solution, then evaporated, affording 30.8 g of a dried $CHCl_3$ extract that showed cytotoxic activity against the KB cell line.

The $CHCl_3$ extract (30 g) mixed with Celite (100 g) homogeneously and subjected to Si gel column chromatography (200

g) using $CHCl_3$−MeOH (gradient, 100:0 → 10:1) as an elution system provided eight fractions. Fraction 7 (5.5 g) eluted with $CHCl_3$−MeOH (49:1 → 19:1) from the initial separation, was further separated by flash column chromatography using $CHCl_3$−MeOH (98.5:1.5 → 97.5: 2.5) as a solvent, affording six fractions. Fractions 2 and 3, which were combined and subsequently subjected to Sephadex LH-20 (MeOH), RP_{18} column chromatography (MeOH−H_2O, 7:3), and Si gel column chromatography (hexanes−EtOAc, 3:1) for final purification, produced compound **3** (33 mg, 0.0057% w/w). Fractions 4 and 5 from the flash column chromatography were also combined and further subjected to Si gel column chromatography (hexanes−EtOAc, 3:1), RP_{18} preparative HPLC (MeOH−H_2O, 7:3), and finally RP_{18} column chromatography (MeOH−H_2O, 7:3) to afford two pure compounds **1**, (20 mg, 0.0035% w/w) and **2** (8 mg, 0.0014% w/w).

Clusiparalicoline A (1): brown oil; UV (MeOH) λ_{max} (log ϵ) 321 (1.2), 284 (3.8), 252 (3.9), 212 (4.6) nm; IR ($CHCl_3$) ν_{max} 3670, 3015, 2395, 1520, 1475, 1425, 1220, 927, 743, 667, 623 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 62.9 MHz) NMR data, see Table 1; EIMS *m/z* $[M]^+$ 422 (41), 405 (7), 366 (7), 335 (13), 311 (78), 297 (45), 281 (19), 255 (100), 243 (74), 187 (11), 152 (9), 105 (7), 69 (65); HREIMS *m/z* $[M]^+$ 422.2455 (calcd for $C_{27}H_{34}O_4$ 422.2457).

Clusiparalicoline B (2): brown oil; UV (MeOH) λ_{max} (log ϵ) 292 (4.1), 220 (4.5) nm; IR ($CHCl_3$) ν_{max} 3670, 3015, 2390, 1519, 1475, 1421, 1222, 927, 743, 664, 622 cm^{-1}; ^1H ($CDCl_3$, 300.1 MHz) and ^{13}C ($CDCl_3$, 75.5 MHz) NMR data, see Table 2; EIMS *m/z* $[M]^+$ 420 (27), 405 (100), 335 (13), 319 (13), 309 (98), 293 (23), 281 (20), 279 (19), 267 (9), 253 (11), 69 (52); HREIMS *m/z* $[M]^+$ 420.2306 (calcd for $C_{27}H_{32}O_4$ 420.2301).

Clusiparalicoline C (3): white brown powder, mp 75−78 °C; UV (MeOH) λ_{max} (log ϵ) 306 (4.3), 233 (4.2), 208 (4.3) nm; IR (KBr) ν_{max} 3410, 1607, 1247, 1207, 1113, 1054, 825 cm^{-1}; ^1H ($CDCl_3$, 250.1 MHz) and ^{13}C ($CDCl_3$, 62.9 MHz) NMR data, see Table 2; EIMS *m/z* $[M]^+$ 268 (15), 253 (100), 224 (3), 215 (4), 181 (2), 165 (1), 152 (2), 134 (2), 126 (17), 112 (3), 77 (2), 69 (2); HREIMS *m/z* $[M]^+$ 268.1095 (calcd for $C_{17}H_{16}O_3$ 268.1099).

DNA Strand-Scission Assay. The basic DNA strand-scission assay was first reported from Hecht's laboratory.[24] As described previously, we have developed a modified Hecht's procedure.[20] The original $CHCl_3$ extract, chromatographic fractions, and isolates **1**−**3** were tested in duplicate at a concentration of 25 μg/mL. This concentration was then diluted 10 times and tested until the isolates exhibited less than 80% relaxation. The final diluted concentration for compounds **1** and **2** was 2.5 μg/mL. These results were compared with a positive control, bleomycin sulfate at 0.025 μg/mL (Table 3).

KB Cytotoxicity Assay. Fractions and compounds **1**−**3** were tested in a human oral epidermoid carcinoma (KB) cell line using established protocols.[25]

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References and Notes

(1) Mabberley, D. J. *The Plant Book*; Cambridge University Press: New York, 1993.
(2) Schultes, R. E. *Bot. Mus. Leafl. Harv. Univ.* **1983**, *29*, 49−57.
(3) Chiappeta, A. D. A.; Francisco de Mello, J.; Maciel, G. M. *Rev. Inst. Antibiot. Univ. Fed. Pernambuco Recife* **1983**, *21*, 43−50.
(4) Dreyer, D. L. *Phytochemistry* **1974**, *13*, 2883−2884.
(5) delle Monache, F.; delle Monache, G.; Pinheiro, R. M.; Radics, L. *Phytochemistry* **1988**, *27*, 2305−2308.
(6) delle Monache, F.; delle Monache, G.; Gács-Baitz, E. *Phytochemistry* **1991**, *30*, 703−705.
(7) delle Monache, F.; delle Monache, G.; Gács-Baitz, E. *Phytochemistry* **1991**, *30*, 2003−2005.
(8) Gustafson, K. R.; Blunt, J. W.; Munro, M. H. G.; Fuller, R. W.; McKee, T. C.; Cardellina, J. H., II; McMahon, J. B.; Cragg, G. M.; Boyd, M. R. *Tetrahedron* **1992**, *48*, 10093−10102.
(9) Cerrini, S.; Lamba, D.; delle Monache, F.; Pinherio, R. M. *Phytochemistry* **1993**, *32*, 1023−1028.
(10) Olivares, E. M.; Gonzalez, J. G.; delle Monache, F. *Phytochemistry* **1994**, *36*, 473−475.
(11) Gonzalez, J. G.; Olivares, E. M.; delle Monache, F. *Phytochemistry* **1995**, *38*, 485−489.
(12) Henry, G. E.; Jacobs, H.; McLean, S.; Reynolds, W. F.; Yang, J.-P. *Tetrahedron Lett.* **1995**, *36*, 4575−4578.
(13) Nagem, T. J.; Mesquita, A. A. L.; Silva, R. *Fitoterapia* **1993**, *64*, 380.
(14) Salama, A. M. *Rev. Colomb. Cienc. Quim. Farm.* **1986**, *15*, 99−104.
(15) Barrios, M.; Calvo, M.; Arguedas, E.; Castro, O. *Fitoterapia* **1990**, *61*, 479.
(16) delle Monache, F.; delle Monache, G.; Cavalcanti, J. F.; Pinheiro, R. M. *Tetrahedron Lett.* **1989**, *28*, 563−566.
(17) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectroscopic Identification of Organic Compounds*, 5th ed.; John Wiley & Sons: New York, 1991.
(18) Seo, E.-K.; Silva, G. L.; Cahi, H.-B.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **1997**, *45*, 509−515.
(19) Dai, J.-D.; Decosterd, L. A.; Gustafson, K. R.; Cardellina, J. H., II; Gray, G. N.; Boyd, M. R. *J. Nat. Prod.* **1994**, *57*, 1511−1516.
(20) Chaudhuri, S. K.; Huang, L.; Fullas, F.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tucker, J. C.; Beecher, C. W. W.; Kinghorn, A. D. *J. Nat. Prod.* **1995**, *58*, 1966−1969.
(21) Huang, L.; Fullas, F.; McGivney, R. J.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tucker, J. C.; Beecher, C. W. W.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1996**, *59*, 290−292.
(22) Huang, L.; Wall, M. E.; Wani, M. C.; Navarro, H.; Santisuk, T.; Reutrakul, V.; Seo, E.-K.; Farnsworth, N. R.; Kinghorn, A. D. *J. Nat. Prod.* **1998**, *61*, 446−450.
(23) Singh, U. S.; Scannell, R. T.; An, H.; Carter, B. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 12691−12699.
(24) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kikuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869−877.
(25) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30−38.

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