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New sesquiterpenes from the red alga *Laurencia microcladia*

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Abstract—Three new aromatic sesquiterpenes (**1**, **2**, and **4**), one new dimeric sesquiterpene of the cyclolaurane-type (**3**), one sesquiterpene alcohol of bisabolene type (**8**) along with three previously reported metabolites (**5**–**7**), were isolated from the organic extracts of *Laurencia microcladia*, collected from the Chios island in the North Aegean Sea. The structures of the new natural products, as well as their relative stereochemistries, were established by means of spectral data analyses, including 2D experiments. The cytotoxicity of the isolated metabolites was evaluated against five human tumor cell lines.

1. Introduction

Red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae) are unique for their ability to biosynthesize a wide variety of secondary metabolites with diverse structural features depending on the species and localities.[1] The chemistry of halogenated compounds from *Laurencia* is a very interesting area of research and it never fails to offer the possibility of discovering new compounds with novel structures and properties.[2] The vast majority of *Laurencia* metabolites, so far, includes sesquiterpenes,[3,4] diterpenes,[5] triterpenes,[6] and C_{15}-acetogenins.[7] Most species of *Laurencia* biosynthesize a characteristic major metabolite or a class of compounds that are not commonly widely distributed within the genus.[8] Chemical studies based on cultured and field-collected materials of several species have revealed that the synthesis of halogenated secondary metabolites is not affected by environmental factors.[9] Thus, secondary metabolite chemistry can serve as an important tool for taxonomical studies in the genus *Laurencia* especially since many species appear morphologically very similar.[10] Moreover a number of halogenated metabolites have been shown to possess antibacterial, antifungal,[11] insecticidal[12] activities, as well as worth noting cytotoxicity against mammalian cells.[13]

During the course of our ongoing investigations toward the isolation and biological evaluation of compounds from marine organisms of the Greek seas,[14–16] we studied specimens of *Laurencia microcladia* Kützing, collected off the coasts of Chios island. In this report we describe the isolation and structure elucidation of five new metabolites (**1**–**4** and **8**) along with the known metabolites dibromophenol (**5**), (+)-α-isobromocuparene (**6**), and (−)-α-bromocuparene (**7**), all of which were obtained from the non-polar fractions of the organic extracts of *L. microcladia*.

An assessment of their cytotoxicity was performed on the following human tumor cell lines: HT29 (derived from colorectal adenocarcinoma), MCF7 (derived from a mammary adenocarcinoma), PC3 (derived from a prostate adenocarcinoma), HeLa (derived from cervix adenocarcinoma), and A431 (derived from epidermoid carcinoma).[17]

2. Results and discussion

L. microcladia was collected from the island of Chios and the CH_2Cl_2/MeOH extract of the freeze-dried alga was subjected to a series of vacuum column chromatography (VCC) on silica gel and normal phase high pressure liquid chromatography (HPLC), using mixtures of cyclohexane/EtOAc as the mobile phase, to yield compounds **1**–**8** in pure form.

Compound **1**, after HPLC purification, was isolated as colorless oil. The molecular formula $C_{15}H_{18}OBrI$ was deduced

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from HRFABMS data in combination with the NMR spectra (Tables 1 and 2). The LREIMS peaks at *m/z* 420/422 $[M]^+$, with relative intensities 1/0.9 $^{79}Br/^{81}Br$, indicated the presence of one bromine atom. The intense absorption at ν_{max} 3424 cm^{-1} showed the presence of a hydroxyl functionality in the molecule. The ^{13}C NMR and DEPT experiments allowed the determination of seven quaternary, two methine, three methylene, and three methyl carbon atoms. The ^1H and ^{13}C NMR spectra displayed resonances for one aromatic methyl ($\delta_{H/C}$ 2.61/29.8), two quaternary methyls ($\delta_{H/C}$ 1.28/18.7 and $\delta_{H/C}$ 1.37/22.4), one aromatic proton ($\delta_{H/C}$ 7.70/132.4), one methine ($\delta_{H/C}$ 1.09/24.2), cyclopropyl protons ($\delta_{H/C}$ 0.48, 0.52/16.2), and two methylenes ($\delta_{H/C}$ 1.61, 1.92/25.2 and $\delta_{H/C}$ 2.20, 1.19/35.1). All protonated carbons and their protons were assigned by the COSY and HMQC experiments. The structure elucidation was assisted by analyses of the HMBC experiments. The correlation in the HMBC experiments, between H-14 (δ_H 1.37) with C-1 (δ_C 49.1), C-5 (δ_C 35.1) and the aromatic carbon C-6 (δ_C 134.4) confirmed the position of H-14 on C-1. The correlation of signals at δ_H 1.28 (H-12) with 29.6 (C-2), 24.2 (C-3), and 16.2 (C-13) secured the position of H-12 on C-2. Comparison of the NMR data of **1** with reported values for laurinterol,[18,19] led to the assignment of the structure as the iodo-laurinterol. The shift of the aromatic methyl group (δ 2.61/29.8) in lower fields compared to laurinterol (δ 2.27/22.2) supported the position of the iodine on C-8. Furthermore, an HMBC correlation between H-11 with C-1 (δ_C 49.1) and C-6 (δ_C 134.4) confirmed the relative positions of the substituents on the aromatic ring. The strong NOE correlations between H-12/H-14 with H-5β and H-3 with H-4β determined the relative stereochemistry at C-1, C-2, and C-3. Consideration of the above data led to the determination of the structure as 8-iodo-laurinterol. As far as we know, this is only the third iodinated sesquiterpene from the genus *Laurencia*, besides 10-bromo-7-hydroxy-11-iodolaurene and a related iodo ether A that have been isolated from *Laurencia nana* Howe.[20]

Compound **2**, a bromo sesquiterpene ether, was purified by means of HPLC and was also isolated as colorless oil. Combination of its ^{13}C NMR data and HRFABMS measurements suggested a molecular formula of $C_{15}H_{17}OBr_3$. The LREIMS peaks at *m/z* 450/452/454/456 $[M]^+$, with relative intensities 1.0/3.0/2.9/0.9, indicated the presence of three bromine atoms. The IR absorption at 1240 cm^{-1} and the absence of an absorption band for hydroxyl or carbonyl groups indicated that the oxygen atom was involved in an ether linkage. The ^{13}C NMR spectrum of **2** (Table 2) exhibited signals for 15 carbons with the multiplicities of the carbon signals determined from the DEPT spectrum as: seven quaternary, two methine, three methylene and three methyl carbon atoms. The ^1H and ^{13}C NMR spectra displayed resonances for one aromatic methyl ($\delta_{H/C}$ 2.50/23.2), one quaternary methyl ($\delta_{H/C}$ 1.35/20.1), one secondary methyl ($\delta_{H/C}$ 0.72/6.7), one aromatic proton ($\delta_{H/C}$ 7.20/126.9), and two protons on carbon bearing bromine at $\delta_{H/C}$ 3.52, 3.71/34.8. With six degrees of unsaturation, the structure was suggested to contain besides the aromatic ring, two other rings, one of which incorporates the ether linkage and the other a five-membered carbocyclic ring. The ^1H and ^{13}C NMR data of the compound **2** were similar to the reported values for the known metabolite bromoether A, isolated previously from

Table 1. ^1H NMR data (400 MHz) of compounds **1–3** and **5–8**

No	1	2	3	5	6	7	8
				^1H (δ)			
1	—	—	—	—	—	—	1.67 (s)
2	—	1.86 (m)	—	—	—	—	—
3	1.09 (ddd, 7.8, 4.9, 3.9)	—	1.09 (ddd, 7.7, 4.7, 4.0)	1.08 (ddd, 7.8, 4.9, 3.0)	4.44 (dd, 9.2, 9.2)	4.04 (dd, 9.9, 8.9)	5.31 (m)
4	1.61 (−α) (dd, 12.2, 8.3);	2.02 (−a) (m);	1.61 (−α) (m);	1.61 (−α) (dd, 12.0, 7.9); 1.92	2.18 (−a) (m); 2.50	2.18 (−a) (m);	2.03 (−a) (m);
	1.92 (−β) (dddd, 12.2, 11.7, 8.2, 4.4)	2.17 (−b) (m)	1.92 (−β) (m)	(−β) (dddd, 12.0, 12.0, 7.5, 4.1)	(−b) (m)	2.49 (−b) (m)	2.31 (−b) (m)
5	1.19 (−α) (m); 2.20 (−β) (dd, 13.7, 8.2)	1.69 (−a) (m)	1.23 (−α) (m); 2.17 (−β)	1.22 (−α) (m); 2.21 (−β)	1.58 (−a) (m); 2.69	1.94 (−a) (m)	—
		1.89 (−b) (m)	(dd, 13.5, 8.0)	(dd, 13.4, 7.5)	(−b) (m)	2.26 (−b) (m)	
6	—	—	—	—	—	—	1.72 (−a) (m); 1.82 (−b) (m)
7	—	—	—	—	7.25 (d, 7.2)	7.07 (s)	2.16 (m)
8	—	—	—	—	7.11 (d, 8.2)	7.07 (s)	—
9	—	—	—	—	—	—	4.86 (−a) (br s)
							5.13 (−b) (br s)
10	—	—	—	—	7.11 (d, 8.2)	7.07 (s)	2.12 (m)
11	7.70 (s)	7.20 (s)	7.80 (s)	7.68 (s)	7.25 (d, 7.2)	7.07 (s)	2.13 (m)
12	1.28 (s)	0.72 (d, 7.1)	1.31 (s)	1.28 (s)	0.63 (s)	0.59 (s)	5.13 (br s)
13	0.48 (−a) (dd, 7.8, 4.9);	3.52 (−a) (d, 10.4);	0.54 (m)	0.47 (dd, 7.8, 4.9); 0.51	1.08 (s)	1.06 (s)	—
	0.52 (−b) (dd, 4.9, 3.9)	3.71 (−b) (d, 10.4)		(dd, 4.9, 3.0)			
14	1.37 (s)	1.35 (s)	1.39 (s)	1.38 (s)	1.27 (s)	1.40 (s)	1.67 (s)
15	2.61 (s)	2.50 (s)	1.95 (s)	2.51 (s)	2.32 (s)	2.29 (s)	1.60 (s)
–OH	5.59 (s)		4.81 (s)	5.76 (s)			

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in parts per million. *J* values in parentheses are in hertz.

Table 2. ^{13}C NMR (50.3 MHz) and HMBC data of compounds **1**–**3** and **5**–**8**

No	**1**		**2**		**3**		**5**	**6**	**7**	**8**	
	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC	^{13}C (δ)	^{13}C (δ)	^{13}C (δ)	^{13}C (δ)	HMBC
1	49.1	H-4α, H-12, H-14	45.3	H-2, H-11, H-12, H-14	48.1	H-11, H-14	49.0	48.4	48.4	23.3	
2	29.6	H-5β, H-12, H-14	43.3	H-12, H-14	29.3	H-4a, H-12, H-14	29.5	47.7	47.4	133.8	H-1
3	24.2	H-5β, H-12	87.1	H-12	23.3	H-5β, H-12	24.2	63.6	62.1	118.4	H-1
4	25.2		36.8	H-14	24.3		25.2	31.2	33.1	37.7	H-1
5	35.1	H-14	41.1	H-14	34.6	H-14	35.0	33.5	36.5	72.4	H-9
6	134.4	H-14	129.9	H-14	135.0	H-11, H-14	135.1	143.0	143.9	32.3	
7	159.6	OH-C-7	148.6	H-11	151.8	H-11	150.1	126.5	127.4	27.3	
8	96.5	H-15	114.9	H-11, H-15	121.5	H-15	116.0	128.6	128.2	154.0	H-9
9	137.5	H-11, H-15	136.0	H-11, H-15	134.5	H-11, H-15, –OH	134.3	135.4	135.5	108.3	H-10
10	113.3	H-15	112.4	H-15	115.4	H-11, H-15	113.4	128.6	128.2	30.9	
11	132.4		126.9		132.7		131.1	126.5	127.4	27.5	
12	18.7		6.7		17.8		18.6	22.2	22.4	124.3	H-14, H-15
13	16.2	H-12	34.8		15.6	H-12	16.1	20.8	20.9	131.0	H-14, H-15
14	22.4		20.1		21.8		22.3	25.3	25.1	25.7	
15	29.8		23.2		19.3		24.1	20.6	20.8	17.9	H-14

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in parts per million.

Laurencia glandulifera.[20,21] On the basis of the absence of a second aromatic proton and the molecular formula, compound **2** was suggested to be the bromo analogue of bromoether A. The chemical shift of the aromatic methyl group resonating at δ 2.50/23.2, at lower field compared to bromoether A (δ 2.27/22.5) and the HMBC correlation between H-11 (δ 7.20) and C-1 (δ 45.3) supported the position of the bromine atom on C-8. The correlation of signals at δ 0.72 (H-12) with 45.3 (C-1), 43.3 (C-2), and 87.1 (C-3) as well as that at δ 1.35 (H-14) with 45.3 (C-1), 43.3 (C-2), 36.8 (C-4), 41.1 (C-5), and 129.9 (C-6) confirmed the position of the methyl groups H-12 and H-14 on C-2 and C-1, respectively. The relative stereochemistry of **2** was assigned on the basis of NOESY experiments. In view of the above-mentioned data, the proposed structure for metabolite **2** is shown in Figure 1.

Compound **3** was purified by means of HPLC separation and was isolated as colorless oil. Both ^{13}C NMR data and HRFABMS measurements supported the molecular formula of $C_{30}H_{36}O_2Br_2$. The LREIMS showed $[M]^+$ peaks at *m*/*z* 586/588/590 with intensities 1.0/2.0/1.0, indicating the presence of two bromine atoms. The presence of a hydroxyl group was evident from the IR band at 3538 and 3409 cm^{-1}. The ^{13}C NMR spectrum of **3** (Table 2) exhibited signals for 15 carbons, with the multiplicities of the carbon signals determined from the DEPT spectrum as: seven quaternary, two methine, three methylene, and three methyl carbon atoms. The ^{1}H and ^{13}C NMR spectra displayed resonances for one aromatic proton ($\delta_{H/C}$ 7.80/132.7), one aromatic methyl group ($\delta_{H/C}$ 1.95/19.3), two tertiary methyl groups ($\delta_{H/C}$ 1.31/17.8 and 1.39/21.8), cyclopropyl protons ($\delta_{H/C}$ 0.54/15.6), one methine ($\delta_{H/C}$ 1.09/23.3), two methylenes ($\delta_{H/C}$ 1.92, 1.61/24.3 and 1.23, 2.17/34.6), one exchangeable proton (δ_H 4.81), and six aromatic carbons (δ_C 115.4, 121.5, 132.7, 134.5, 135.0, 151.8). The spectral data of **3** were similar to the known dimeric sesquiterpene of the cyclolaurane type, isolated previously from *L. microcladia*.[17] The correlations observed in the HMBC spectrum between H-11 (δ_H 7.80) with C-1 (δ_C 48.1), C-6 (δ_C 135.0), C-10 (δ_C 115.4), and C-7 (δ_C 151.8), supported the fusion of the phenolic rings of the two laurinterol molecules, which is *ortho* to the methyl. The absence of a NOE effect between the methyls H-12 and H-14 (in contrast to the known dimeric sesquiterpene), along with the NOE effect between H-12 and H-5β, suggested an inversion of configuration at C-1. In view of the above-mentioned data, the proposed structure for metabolite **3** is shown in Figure 1.

Compound **4** was also purified by means of HPLC separation and was isolated as colorless oil. The compound was shown to be a hydrocarbon with the molecular formula $C_{15}H_{20}$ by LREIMS at *m*/*z* 200 $[M]^+$. The ^{1}H NMR spectrum exhibited two 2H doublets at δ 7.06 (d, J=7.9 Hz, 2H) and δ 7.25 (d, J=7.9 Hz, 2H), indicating the presence of a 1,4-disubstituted benzene ring, together with an aromatic methyl resonance at δ 2.29 (s, 3H). The remaining observable resonances consisted of two exocyclic methylene protons at δ 4.80 and 5.06 (s, 1H each), a quaternary methyl group at δ 1.42 (s, 3H), and a secondary methyl group at δ 1.12 (d, J=7.0 Hz, 3H) coupled to a deshielded allylic proton at δ 2.65 (dtt, J=8.5, 7.0, 2.9 Hz, 1H). With an unsaturation degree of six, the structure was suggested to contain one aromatic

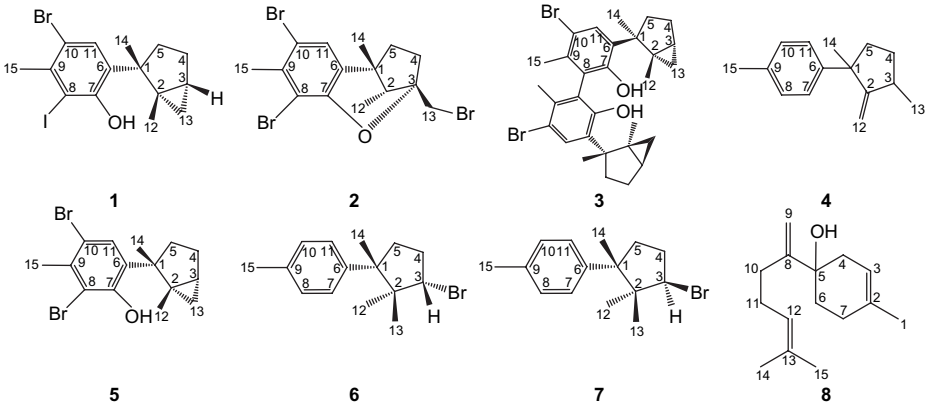

Figure 1. Metabolites isolated from *L. microcladia*.

ring, one five-membered ring, and an exocyclic methylene. The protons of **4** were assigned by ^{1}H–^{1}H COSY experiments and the position of H-13 was determined from correlations between H-13/H-3, H-13/H-4a, and H-13/H-4b. The isolation of metabolite **4** in minute amounts as well as its instability, made impossible ^{13}C NMR measurements. Considering the above-mentioned data, the proposed structure for metabolite **4** is shown in Figure 1. In the past, compound **4** has been mentioned as an intermediate for the synthesis of the known metabolite laurene.[22]

Compound **5** after purification by HPLC was isolated as colorless oil and identified by comparison of its ^{1}H NMR and MS spectra with previously reported data as being dibromophenol.[23] Extensive analyses of the ^{13}C NMR, HMQC, and HMBC spectra allowed ^{13}C NMR assignments for the dibromophenol, and this is the first report of metabolite **5** from *L. microcladia*.

Compound **6** was purified following chromatographic separations and was isolated as colorless oil. It was identified on the basis of its ^{1}H NMR and MS spectra and comparison with previously reported data as being (+)-α-isobromocuparene.[24] Extensive analyses of the ^{13}C NMR, HMQC, and HMBC spectra allowed the ^{13}C NMR assignments for compound **6**, reported for the first time as *L. microcladia* metabolite.

Compound **7** was isolated as colorless oil following HPLC purification and was identified by comparison of its ^{1}H NMR and MS spectra and with literature values as being (−)-α-bromocuparene.[24] Extensive analyses of the ^{13}C NMR, HMQC, and HMBC spectra allowed the ^{13}C NMR assignments for compound **7**, reported for the first time as *L. microcladia* metabolite.

Compound **8** was purified by HPLC and was isolated as colorless oil. The molecular formula for **8** was established as $C_{15}H_{24}O$ by combinations of HRFABMS and the NMR experiments. The IR spectrum displayed absorptions for a tertiary hydroxyl group (3409 cm^{-1}, broad) and an *exo*-methylene group (3010 and 1654 cm^{-1}). The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of four quaternary, two methine, six methylene, and three methyl carbon atoms. Among the carbons, one was bonded to oxygen resonating at δ 72.4 ppm and six were olefinic resonating at δ 154.0, 133.8, 131.0, 124.3, 118.4, and 108.3 ppm, indicating the presence of three double bonds. In addition, the ^{1}H NMR spectrum displayed a pair of singlets characteristic for an exocyclic methylene at δ_H 4.86 (br s, H-9a) and 5.13 (br s, H-9b), two olefinic protons at δ_H 5.31 (m, H-3) and 5.13 (br s, H-12), as well as three vinyl methyl groups at δ_H 1.60 (3H, s) and 1.67 (6H, s). With an unsaturation degree of four, the structure was suggested to contain, besides the three double bonds, one ring. All protonated carbons and their protons were matched precisely by COSY and HMQC experiments. Comparison of the spectral data (Tables 1 and 2) for **8** with literature values,[25,26] suggested that metabolite **8** possessed the skeleton of β-bisabolene. The main difference was the presence of an hydroxyl group attached to a quaternary carbon. The position of the hydroxyl group at C-5 was confirmed by the HMBC correlation of the protons of the exocyclic methylene H-9 (δ_H 4.86, 5.13) and C-5 (δ_C 72.4). Moreover the correlations between H-1/C-2, H-1/C-3, H-1/C-4 and H-14,H-15/C12,C-13 confirmed the positions of the methyl groups. In view of the above mentioned data, metabolite **8** is the hydroxyl derivative of β-bisabolene.

Metabolites **1**–**3** and **5**–**7** were evaluated for their cytotoxicity against five human tumor cell lines. The results of the cytotoxic activity of the tested compounds after 48 h of incubation are given in Table 3. All compounds were found to be deprived of significant cytotoxic activity. Compounds **1** and **5** exhibited relatively higher cytotoxicity against all tested cell lines. In combination with results obtained in the past with similar metabolites it seems that the presence of the aromatic hydroxyl group is increasing the cytotoxicity

Table 3. In vitro cytotoxic activities of metabolites **1**–**3** and **5**–**7**

Compound	Cell line				
	HT29	MCF7	PC3	HeLa	A431
	IC_{50} (μM)				
1	78.4	86.3	88.5	81.4	92.7
2	170.5	167.9	183.9	174.4	178.8
3	>300	>300	>300	>300	>300
5	98.7	104.1	75.2	114.6	93.4
6	130.4	177.6	191.2	204.3	198.4
7	287.3	265.4	271.3	240.8	277.4

For clarity, SEM values are not included in the table, but they were less than 5% of the mean in all the cases.

on the tested cell lines, whereas the significantly more bulky dimer appears deprived of any cytotoxicity. Based on these preliminary results a structure–activity study might help in the future synthesis of related compounds possessing selectivity to tumor cells.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a Perkin–Elmer model 341 polarimeter and a 10-cm cell. UV spectra were determined in spectroscopic grade CH_2Cl_2 and $CHCl_3$ on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained using a Paragon 500 Perkin–Elmer spectrophotometer. NMR spectra were recorded using a Bruker AC 200 and Bruker DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as an internal standard. The 2D experiments (^{1}H–^{1}H COSY, HMQC, HMBC, NOESY) were performed using standard Bruker microprograms. High-resolution mass spectral data were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana, USA. EIMS data were recorded on a Hewlett Packard 5973 Mass Selective Detector. VCC separation was performed with Kieselgel 60H (Merck), and TLC was performed with Kieselgel 60 F_{254} aluminum support plates (Merck) and spots were detected with 15% H_2SO_4 in MeOH reagent. HPLC separation was conducted using a Pharmacia LKB 2248 model equipped with a refractive index detector RI GBC LC-1240 and a Spherisorb HPLC normal phase column, 25 cm×10 mm, S10W, 64340 plates/meter.

3.2. Plant material

L. microcladia was collected by hand at Vroulidia bay, Chios island in the North Aegean Sea, Greece, at a depth of 0.5–1 m in May of 2002. A specimen is kept at the Herbarium of the Laboratory of Pharmacognosy and Chemistry of Natural Products, University of Athens (ATPH/MO/151).

3.3. Extraction and isolation

L. microcladia was initially freeze-dried (291.4 g dry weight) and then exhaustively extracted with mixtures of CH_2Cl_2/MeOH (3/1) at room temperature. The extract was concentrated to give a dark green residue (12.0 g), which later was subjected to vacuum column chromatography (VCC) on silica gel, using cyclohexane with increasing amounts (10%) of EtOAc as mobile phase and finally MeOH. Fraction A (100% cyclohexane) (307.8 mg) was subjected to normal phase HPLC chromatography, using 100% *n*-hexane as mobile phase to yield pure compounds **1** (1.2 mg), **4** (0.4 mg), and **5** (1.5 mg). Fraction C (20% EtOAc in cyclohexane) (27.3 mg) was subjected to normal phase HPLC chromatography, using 100% *n*-hexane as mobile phase to yield pure compounds **2** (0.9 mg), **6** (9.8 mg), and **7** (4.8 mg). Fraction D (30% EtOAc in cyclohexane) (2.8 g) was further purified by gravity column on silica gel using cyclohexane with increasing amounts (1%) of EtOAc as mobile phase. Fraction V (4% EtOAc) (4.4 mg) was subjected to normal phase HPLC chromatography, using 100% *n*-hexane as mobile phase to yield pure compound **3** (0.7 mg). Fraction VI (5% EtOAc) (53.6 mg) was subjected to normal phase HPLC chromatography, using *n*-hexane/EtOAc (96/4) as mobile phase to yield pure compound **8** (0.8 mg).

3.3.1. Compound 1. Colorless oil; $[\alpha]_D^{20}$ −20 (*c* 0.04, CH_2Cl_2); UV $\lambda_{max}^{CH_2Cl_2}$ (log ε): 242.6 (3.1), 263.0 (2.6), 292.0 (2.8), 303.4 (2.6) (nm); IR (CH_2Cl_2) ν_{max} 3424, 3020, 1030, 1630, 1470 cm^{-1}; HRFABMS (*m/z*): 419.9611 $[M]^+$ (calcd for $C_{15}H_{18}O^{79}BrI$ 419.9586), $\Delta m/m$=−59 (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel int. %): 420/422 $[M]^+$ (20/19), 405/407 $[M-CH_3]^+$ (44/40), 352/354 (65/60), 325/327 (14/15), 300 (31), 173 (88), 128 (77), 115 (100), 77 (76), 41 (66).

3.3.2. Compound 2. Colorless oil; $[\alpha]_D^{20}$ +33 (*c* 0.09, CH_2Cl_2); UV $\lambda_{max}^{CH_2Cl_2}$ (log ε): 234 (2.9), 270 (2.1), 295.4 (2.3) (nm); IR (CH_2Cl_2) ν_{max} 1634, 1565, 1381, 1240, 1169, 1041 cm^{-1}; HRFABMS (*m/z*): 449.8835 $[M-H]^+$ (calcd for $C_{15}H_{17}O^{79}Br_3$ 449.8829), $\Delta m/m$=−13 (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel int. %): 450/452/454/456 $[M]^+$ (33/100/97/30), 371/373/375 $[M-Br]^+$ (42/77/40), 315/317/319 (40/75/40), 292/294 (64/61), 279 (70), 251 (21), 238 (20), 197 (17), 128 (25), 107 (77), 55 (60).

3.3.3. Compound 3. Colorless oil; $[\alpha]_D^{20}$ −10.0 (*c* 0.04, CH_2Cl_2); UV $\lambda_{max}^{CH_2Cl_2}$ (log ε): 240.0 (3.5), 280 (3.2) (nm); IR (CH_2Cl_2) ν_{max} 3538, 3409, 2955, 2866, 1706, 1605, 1493, 1448, 1392, 1380, 1268, 1066, 1042, 848 cm^{-1}; HRFABMS (*m/z*): 586.1087 $[M]^+$ (calcd for $C_{30}H_{36}O_2{}^{79}Br_2$ 586.1084), $\Delta m/m$=−5 (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel int. %): 586/588/590 $[M]^+$ (29/62/31), 571/573/575 (31/64/33), 518/520/522 (53/100/51), 503/505/507 (29/60/35), 450/452/454 (14/27/15), 294/296 (17/16), 279 (28), 109 (41), 67 (32).

3.3.4. Compound 4. Colorless oil; $[\alpha]_D^{20}$ +40.0 (*c* 0.04, $CHCl_3$); UV $\lambda_{max}^{CHCl_3}$ (log ε): 292.6 (1.6) (nm); IR ($CHCl_3$) ν_{max} 2960, 2927, 2869, 1653, 1512, 1451, 1370, 1143 cm^{-1}; ^{1}H NMR data ($CDCl_3$): 7.06 (d, *J*=7.9 Hz, 2H), 7.25 (d, *J*=7.9 Hz, 2H), 2.29 (s, 3H, H-15), 4.80 and 5.06 (s, 1H each, H-12a and H-12b), 1.42 (s, 3H, H-14), 1.12 (d, *J*=7.0 Hz, 3H, H-13), 2.65 (dtt, *J*=8.5, 7.0, 2.9 Hz, 1H, H-3), 2.11 (m, 2H, H-4a, H-5a), 1.80 (m, 1H, H-4b), 1.66 (m, 1H, H-5b); EIMS 70 eV, *m/z* (rel int. %): 200 $[M]^+$ (63), 185 (75), 171 (13), 158 (41), 143 (100), 129 (28), 115 (22), 105 (31), 91 (19), 77 (13).

3.3.5. Compound 5. Colorless oil; $[\alpha]_D^{20}$ +10.0 (*c* 0.04, $CHCl_3$); UV $\lambda_{max}^{CHCl_3}$ (log ε): 292.4 (2.3) (nm); IR ($CHCl_3$) ν_{max} 3485, 3393, 1651, 1616, 1558, 1385, 1288, 1155, 1022 cm^{-1}; NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel int. %): 372/374/376 $[M]^+$ (31/62/32), 357/359/361 (51/100/55), 315/317/319 (15/32/18), 304/306/308 (45/85/45), 252/254 (26/23), 212/214 (12/12), 199 (15), 173 (31), 158 (16), 115 (25), 91 (17), 77 (18).

3.3.6. Compound 8. Colorless oil; $[\alpha]_D^{20}$ −2.5 (*c* 0.04, $CHCl_3$); UV $\lambda_{max}^{CHCl_3}$ (log ε): 285 (2.29) (nm); IR ($CHCl_3$) ν_{max} 3409, 3010, 1654, 1629, 1275 cm^{-1}; HRFABMS (*m/z*): 220.1844 $[M]^+$ (calcd for $C_{15}H_{24}O$ 220.1828), $\Delta m/m$=−72 (ppm); NMR data ($CDCl_3$), see Tables 1 and

2; EIMS 70 eV, *m/z* (rel int. %): 220 (1), 202 (13), 187 (10), 159 (100), 109 (47), 69 (44).

3.4. Conditions of cell cultures

Cells were grown as monolayer cell cultures in Dulbecco's minimal essential medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in an incubator with humidified atmosphere and 5% CO_2. Cells were passaged by trypsinization two times a week to keep them in log phase.

3.5. Determination of cytotoxicity

Cells were seeded into 96-well plates (100 μl/well at a density of 1×10^5 cells ml^{-1}) and exposed to various concentrations of the compounds for 48 h. The cytotoxicity was determined with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay,[27] with minor modifications.[28] Briefly, after incubation with the test compounds, MTT solution (5 mg ml^{-1} in PBS) was added (20 μl/well). Plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved by adding 100 μl/well of 0.1 N HCl in 2-propanol. Absorption was measured by an enzyme-linked immunosorbant assay (ELISA) reader at 545 nm, with reference filter at 690 nm. For each concentration at least nine wells were used from three separate experiments. One-hundred microliters of culture medium supplemented with the same amount of MTT solution and solvent was used as blank solution. Data obtained were presented as IC_{50} (μM), which is the concentration of the compound, where $100\times(A_0-A)/A_0=50$. In this formula, A is the optical density of the wells after 48 h of exposure to test compound and A_0 is the optical density of the control wells.

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References and notes

1. Vairappan, C. S.; Suzuki, M.; Abe, T.; Masuda, M. *Phytochemistry* **2001**, *58*, 517–523.
2. Vairappan, C. S. *Biomol. Eng.* **2003**, *20*, 255–259.
3. Norte, M.; Fernández, J. J.; Padilla, A. *Phytochemistry* **1992**, *31*, 326–327.
4. Wright, A. D.; Goclik, E.; König, G. M. *J. Nat. Prod.* **2003**, *66*, 435–437.
5. Davyt, D.; Fernandez, R.; Suescun, L.; Mombrú, A. W.; Saldaña, J.; Dominguez, L.; Fujii, M. T.; Manta, E. *J. Nat. Prod.* **2006**, *69*, 1113–1116.
6. De Nys, R.; Wright, A. D.; König, G. M.; Sticher, O. *J. Nat. Prod.* **1993**, *56*, 883–887.
7. Howard, B. M.; Fenical, W. *Tetrahedron Lett.* **1976**, *1*, 41–44.
8. Fenical, W. *Phytochemistry* **1976**, *15*, 511–512.
9. Suzuki, M.; Matsuo, Y.; Masuda, M. *Tetrahedron* **1993**, *49*, 2033–2042.
10. Takahashi, Y.; Daitoh, M.; Suzuki, M.; Abe, T.; Masuda, M. *J. Nat. Prod.* **2002**, *65*, 395–398.
11. König, G. M.; Wright, A. D. *Planta Med.* **1997**, *63*, 186–187.
12. El Sayed, K. A.; Dunbar, D. C.; Perry, T. L.; Wilkins, S. P.; Hamann, M. T. *J. Agric. Food Chem.* **1997**, *45*, 2735–2739.
13. Monde, K.; Tanigughi, T.; Miura, N.; Vairappan, C. S.; Suzuki, M. *Chirality* **2006**, *18*, 335–339.
14. Kladi, M.; Vagias, C.; Furnari, G.; Moreau, D.; Roussakis, C.; Roussis, V. *Tetrahedron Lett.* **2005**, *46*, 5723–5726.
15. Iliopoulou, D.; Roussis, V.; Pannecouque, C.; De Clercq, E.; Vagias, C. *Tetrahedron* **2002**, *58*, 6749–6755.
16. Kontiza, I.; Vagias, C.; Jakupovic, J.; Moreau, D.; Roussakis, C.; Roussis, V. *Tetrahedron Lett.* **2005**, *46*, 2845–2847.
17. Kladi, M.; Xenaki, H.; Vagias, C.; Papazafiri, P.; Roussis, V. *Tetrahedron* **2006**, *62*, 182–189.
18. Irie, T.; Suzuki, M.; Kurosawa, E.; Masamune, T. *Tetrahedron Lett.* **1966**, *17*, 1837–1840.
19. Ooi, T.; Utsumi, K.; Kusumi, T. *Heterocycles* **2001**, *54*, 577–579.
20. Izac, R. R.; Sims, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 6136–6137.
21. Suzuki, M.; Kurosawa, E. *Tetrahedron Lett.* **1976**, *52*, 4817–4818.
22. Oh, C. H.; Han, J. W.; Kim, J. S.; Um, S. Y.; Jung, H. H.; Jang, W. H.; Won, H. S. *Tetrahedron Lett.* **2000**, *41*, 8365–8369.
23. Suzuki, M.; Kurosawa, E. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3352–3354.
24. Suzuki, T.; Suzuki, M.; Kurosawa, E. *Tetrahedron Lett.* **1975**, *35*, 3057–3058.
25. Manjarrez, A.; Guzmán, A. *J. Org. Chem.* **1966**, *31*, 348–349.
26. Miyazawa, M.; Kameoka, H. *Phytochemistry* **1983**, *22*, 1040–1042.
27. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
28. Alley, M. C.; Scudiero, A. D.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.