

Article

Briareolate Esters from the Gorgonian *Briareum asbestinum*

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Abstract: Two new briarane diterpenoids briareolate esters J (**1**) and K (**2**) were isolated from the methanolic extract of the octocoral *Briareum asbestinum* collected off the coast of Boca Raton, Florida. The structures of briaranes **1** and **2** were elucidated by interpretation of spectroscopic data. Briareolate ester K (**2**) showed weak growth inhibition activity against human embryonic stem cells (BG02).

Keywords: *Briareum asbestinum*; octocoral; briarane; briareolate ester; human embryonic stem cell

1. Introduction

Gorgonian corals have provided an abundance of novel structures with many of these demonstrating potentially useful biological activities. The gorgonian *Briareum asbestinum* has shown to be a plentiful source of diterpenoids belonging to the eunicellin, asbestinane, cembrane, and briarane classes and display numerous biological activities (e.g., cytotoxicity, antimicrobial, anti-inflammatory, antiviral, immunomodulatory, antifouling, and ichthotoxicity) [1]. Briarane-type diterpenoids contain a highly oxidized bicyclo[8.4.0] system of which most contain a γ -lactone ring. The briareolate esters are a

small group of briarane diterpenoids isolated from *Briareum asbestinum* that contain a C-19 methyl ester instead of the typical γ -lactone ring [2–4].

As part of an ongoing study to discover compounds that impact human embryonic stem cell (hESC) growth we have been screening pre-fractionated and semi-purified marine natural product extract libraries generated using a solid phase extraction (SPE) procedure followed by semi-preparative high pressure liquid chromatography (HPLC) using evaporative light scattering detection (ELSD) directed fractionation (Figure 1). The cell growth inhibitory activities of the pre-fractionated extract libraries are then evaluated against human embryonic stem cells (BG02) using a 96-well plate real-time cell electronic sensing (RT-CES) system to identify compounds that impact self-renewal, differentiation or apoptosis. A plot of cell index (impedance) *versus* time is used to indicate relative proliferation, differentiation, or death in real-time (Figure 2).

Our previous work using this approach on *B. asbestinum* resulted in the isolation of three new briareolate esters L–N (**3–5**) [5]. This included the biologically active compound briareolate ester L (**3**) that was found to possess a 10-membered macrocyclic ring with a (*E,Z*)-dieneone and was shown to contain a “spring loaded” Michael acceptor that is capable of forming a reversible covalent bond to model sulfur-based nucleophiles. In the present study we report the isolation and structural elucidation of two additional new briarane diterpenoids briareolate esters J (**1**) and K (**2**), along with three known compounds from the methanolic extract of *B. asbestinum* that was found to exhibit growth inhibition against BG02 cells (Figure 3).

Figure 1. HPLC chromatogram of the pre-fractionated extract of *B. asbestinum* showing the fractions separated using ELSD-directed collection to generate a semi-purified extract library for biological screening.

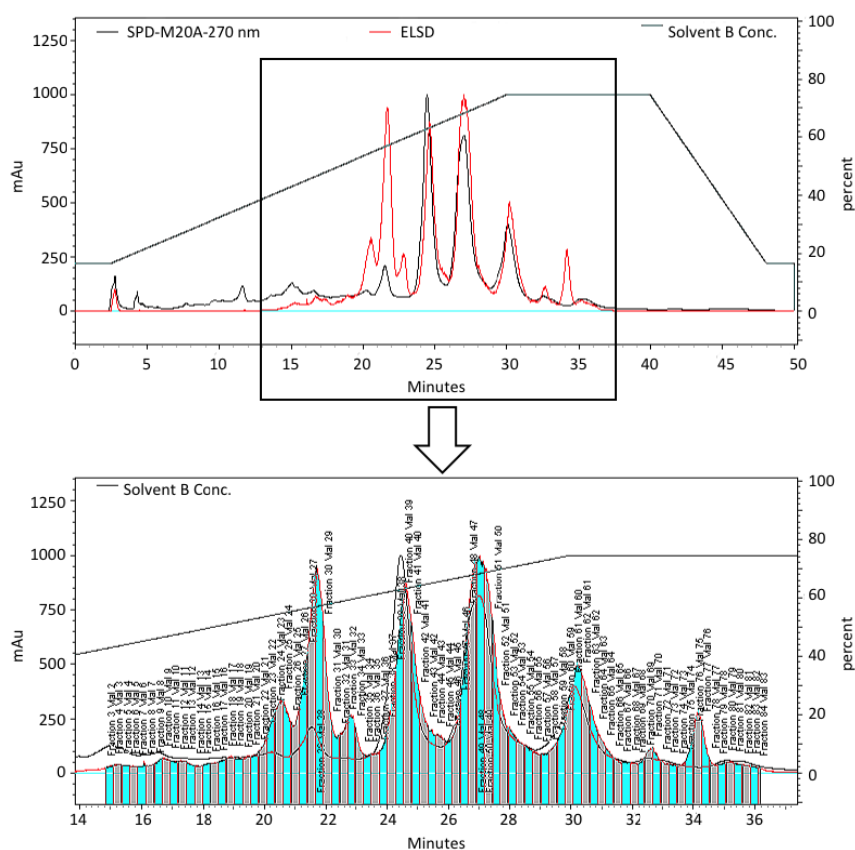


Figure 2. A cell index vs. time plot for a pre-fractionated extract library of *B. asbestinum* against the BG02 cell line. A drop in cell index within the first 24–48 h after addition of the compounds is interpreted as toxicity or induction of apoptosis.

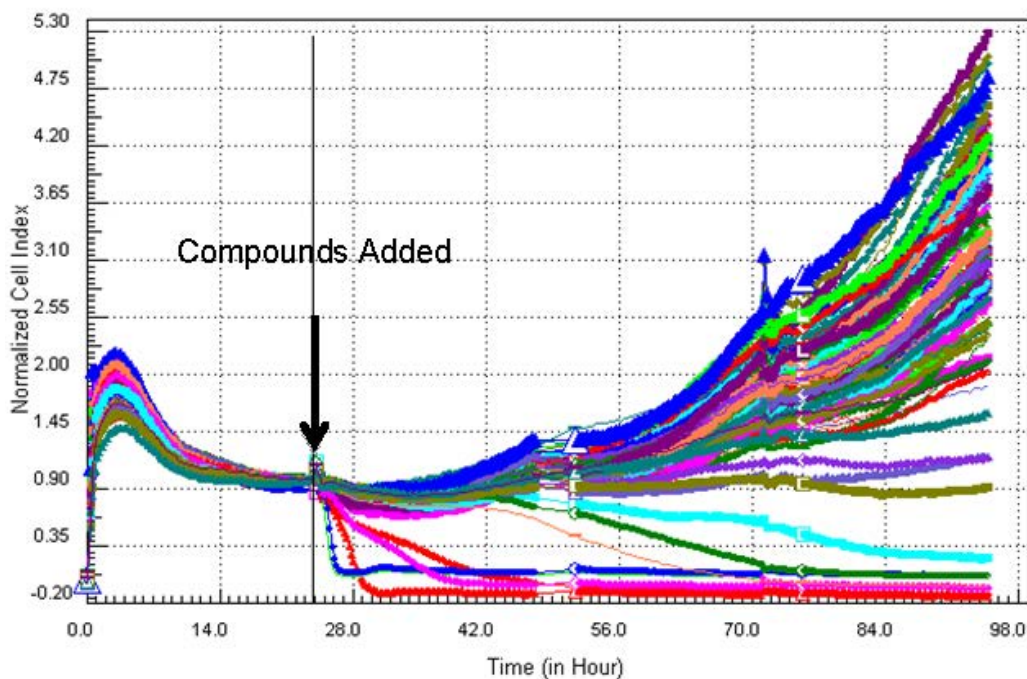
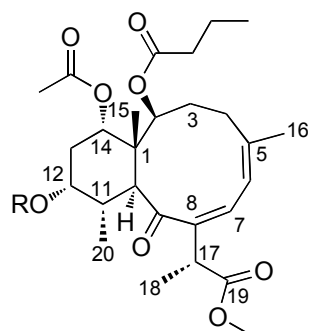
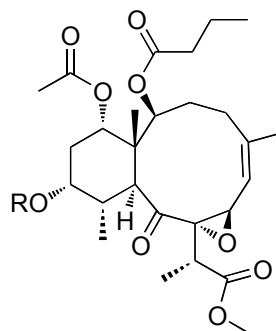


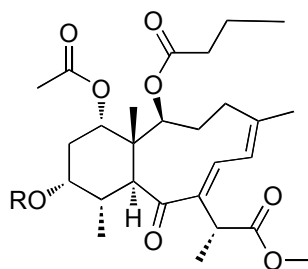
Figure 3. Structures of compounds 1–7.



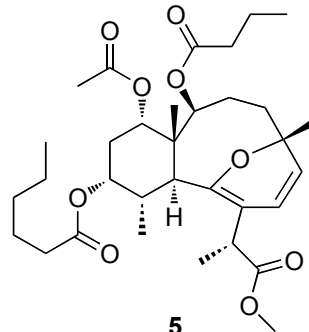
1 R = $-\text{CO}(\text{CH}_2)_4\text{CH}_3$
7 R = H



2 R = $-\text{CO}(\text{CH}_2)_4\text{CH}_3$
6 R = H



3 R = H
4 R = $-\text{CO}(\text{CH}_2)_4\text{CH}_3$



5

2. Results and Discussion

Specimens of *Briareum asbestinum* (Figure 4) were collected at Hillsboro Ledge, Boca Raton Florida and kept frozen until extraction. The methanolic extract was first fractionated on polymeric HP-20 resin using cyclic loading [6]. The HP-20 column was eluted with 800 mL fractions of (1) H₂O, (2) 40% Me₂CO/H₂O, (3) 75% Me₂CO/H₂O and (4) Me₂CO. The Me₂CO fraction was then subjected to column chromatography on HP-20SS and normal phase HPLC to yield two new briareolate esters J (1) and K (2) and three known briareolate esters D (6), G (7), and M (4).

Figure 4. *Briareum asbestinum*.



Briareolate ester J (1) was isolated as a colorless oil. The molecular formula of briareolate ester J (1), C₃₃H₅₀O₉, was determined from the HRESIMS of the [M + Na]⁺ ion at *m/z* 613.3346, 98 mass units higher than that of briareolate ester G (7). The presence of a ketone conjugated with two double bonds ($\alpha,\beta,\gamma,\delta$ -unsaturated ketone) was indicated from a carbonyl carbon with a chemical shift of δ_C 207 (C-9), and the C-C double bond carbons [δ_C 146.3 (C-5), δ_C 125.3 (C-6), δ_C 140.9 (C-7), δ_C 146.3 (C-8)]. The observation of absorption maxima at $\lambda_{max} = 287$ and 225 nm in the UV spectrum was consistent with this assignment. NOE correlations observed from the olefinic proton H-6 to H-7 and H₃-16, together with correlations from H-7 to both H-17 and H₃-18, established the (*Z,Z*)-configuration of the dieneone and the *s-cis* conformation of the diene (Figure 5). Additionally, the upfield chemical shift of the olefinic proton H-7 in 1 at δ_H 6.74 compared to δ_H 7.64 in the (*E,Z*)-dieneone briareolate ester L (3), with the *s-trans* diene conformation, confirmed this assignment [5].

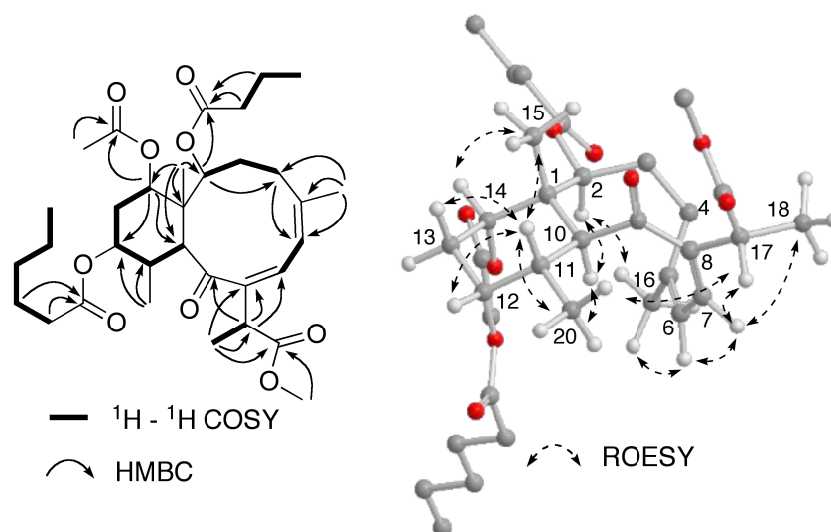
A close inspection of the ¹H and ¹³C NMR data (Table 1) revealed the similarity of 1 to that of briareolate ester G (7), except that H-12 [δ_H 4.89, br s] was shifted downfield by 1.24 ppm as compared with that of 1. In addition, in the ¹³C NMR spectrum the resonance of C-12 (δ_C 73.7) was shifted downfield by 2.7 ppm and those of C-11 (δ_C 38.4) and C-13 (δ_C 30.2) were shifted upfield by 0.5 and 1.3 ppm, respectively, in comparison with those of 1 [4]. This suggested that the 12-hydroxy group of 7, was replaced by a hexanoate group at C-12 in 1, as observed in briareolate esters M (4) and N (5). The presence of the hexanoate group was confirmed by the NMR data [δ_H 0.92 (3H, t, *J* = 8.0 Hz), *ca.* 1.31 (4H, overlapped), 1.63 (2H, overlapped), 2.32 (2H, m), δ_C 17.6 (q), 24.0, 26.3, 33.0, and 36.1 (each t), 175.1 (CO)]. These assignments were confirmed by COSY, HSQC, and HMBC correlations similar to those observed for 4, 5 and 7 (Figure 5). The relative configuration of briareolate ester J (1)

was determined to be the same as that of **7** from the similarity of proton-proton coupling constants and ^1H and ^{13}C chemical shifts together with NOE correlations observed in a ROESY experiment (Figure 5).

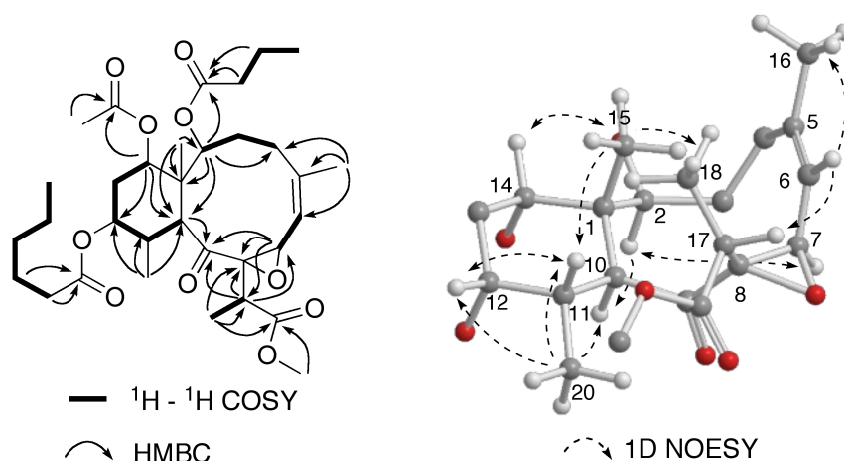
Table 1. NMR Spectroscopic Data for Briareolate Esters J (**1**) and K (**2**)^a.

position	1		2	
	δ_{C} , mult	δ_{H} (J in Hz)	δ_{C} , mult	δ_{H} (J in Hz)
1	46.4, C		48.8, C	
2	73.7, CH	5.17, br d (6.0)	80.5, CH	5.52, d (8.0)
3 α	31.3, CH ₂	1.89, m	31.1, CH ₂	2.31, m
3 β		1.89, m		1.58, m
4 α	30.2, CH ₂	2.20, m	34.1, CH ₂	2.53, dd (18.0, 8.0)
4 β		2.08, m		2.28, m
5	146.3, C		147.1, C	
6	125.3, CH	6.12, br s	116.2, CH	5.47, br d (4.0)
7	140.9, CH	6.74, br s	64.3, CH	4.42, br d (4.0)
8	146.3, C		71.3, C	
9	207.0, C		211.5, C	
10	48.3, CH	3.79, d (12.0)	44.7, CH	3.06, d (12.0)
11	38.4, CH	2.19, m	36.2, CH	2.34, m
12	73.7, CH	4.89, br s	72.6, CH	4.98, br s
13 α	30.2, CH ₂	2.05, m	29.9, CH ₂	2.05, br d (16.0)
13 β		1.86, m		1.99, m
14	75.6, CH	4.68, br s	74.6, CH	4.69, br s
15	14.9, CH ₃	1.19, s	12.8, CH ₃	0.99, s
16	26.3, CH ₃	2.19, s	26.8, CH ₃	1.75, s
17	46.4, CH	3.45, q (8.0)	40.8, CH	2.39, q (8.0)
18	19.8, CH ₃	1.32, d (8.0)	13.5, CH ₃	1.29, d (8.0)
19	176.7, C		175.3, C	
20	17.6, CH ₃	0.79, d (8.0)	16.8, CH ₃	0.75, d (8.0)
OMe	52.8, CH ₃	3.62, s	52.5, CH ₃	3.61, s
ester at C-2	175.1, C		174.3, C	
	37.7, CH ₂	2.19, m	37.9, CH ₂	2.25, m
	19.2, CH ₂	1.57, m	19.7, CH ₂	1.60, m
	14.5, CH ₃	0.92, t (8.0)	14.5, CH ₃	0.93, t (8.0)
ester at C-12	175.1, C		175.4, C	
	36.1, CH ₂	2.32, m	35.9, CH ₂	2.37, m
	26.3, CH ₂	1.63, m	26.4, CH ₂	1.64, m
	33.0, CH ₂	1.31, m	33.0, CH ₂	1.33, m
	24.0, CH ₂	1.31, m	24.0, CH ₂	1.33, m
	17.6, CH ₃	0.92, t (8.0)	14.8, CH ₃	0.95, t (8.0)
ester at C-14	172.1, C		172.6, C	
	22.1, CH ₃	1.98, s	22.3, CH ₃	1.99, s

^a In CD₃OD, 400 MHz for ^1H and 100 MHz for ^{13}C NMR.

Figure 5. Selected 2D NMR correlations for briareolate ester J (**1**).

Briareolate ester K (**2**) was isolated as a colorless oil. The molecular formula of briareolate ester K (**2**), $\text{C}_{33}\text{H}_{50}\text{O}_{10}$, was determined from the HRESIMS of the $[\text{M} + \text{Na}]^+$ ion at m/z 629.3291, one more oxygen atom than that of briareolate ester J (**1**). A comparison of the ^1H and ^{13}C NMR data (Table 1) revealed that **2** was similar to **1**, except that the NMR signals for the C-7–C-8 double bond were missing in the NMR spectra of **2**, and instead, resonances for an epoxide were observed in the ^1H NMR (δ 4.42, H-6) and ^{13}C NMR [δ_{C} 64.3 (s); 71.3 (d)] spectra indicating oxidation of the C-7–C-8 double bond to be an epoxide. HMBC correlations from the oxygenated methine signal at δ 4.42 (H-7) to C-5 (δ 147.1) and C-6 (δ 116.2) of the trisubstituted double bond, and to C-8 (δ 71.3) and C-9 (δ 211.5) are consistent with this assignment. Additional HMBC correlations observed from H-17 to C-7, C-8 and C-9 confirmed this assignment (Figure 6).

Figure 6. Selected NMR correlations for briareolate ester K (**2**).

The relative configuration of briareolate ester K (**2**) was determined to be identical to that of **1** on the basis of NOE enhancements revealed in a series of 1D NOESY experiments (Figure 6). The configuration of the C-7/8 epoxide was determined from NOE enhancements observed between H-7 and H-2 together with NOE enhancements observed from H-6 to H-17 which placed H-7 on the inside

of the 10-membered ring and indicated the *trans* orientation of the epoxide. This assignment is consistent with that of briareolate ester D (**6**) whose structure was assigned on the basis of X-ray crystallography [4].

Compounds **1** and **2** were evaluated for cell growth inhibitory activities against human embryonic stem cells (BG02) using a 96-well plate real-time cell electronic sensing (RT-CES) system [7]. Briareolate ester K (**2**) showed weak growth inhibition against BG02 cells with an EC₅₀ value of 40 μM. No inhibitory activity was detected for briareolate ester J (**1**) at 40 μM. Previously the (*E,Z*)-dienone containing briareolate esters L (**3**) and M (**4**) were found to have growth inhibition against both the BG02 and a pancreatic cancer cell line (BxPC-3) cells with EC₅₀ values of 2.4 and 9.3 μM, respectively for **3**, and 8.0 μM against BG02 cells and only cytostatic effects at 13.0 and 17.0 μM against the BxPC-3 cells for **4** [5]. No growth inhibition was found for the briareolate esters B, C, G (**7**), and N (**5**). The absence of significant growth inhibition activity for the (*Z,Z*)-dieneone compound **1** and the epoxide containing compound **2** further confirms the importance of the (*E,Z*)-dieneone for biological activity.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were measured on a Jasco P-2000 polarimeter (*c*: g/100 mL) at 589 nm. UV spectra were obtained on a Perkin-Elmer Lambda EZ 210 UV-vis spectrophotometer. IR spectra were recorded on a Thermo Electronic Corporation Nicolet IR-100 spectrophotometer. All NMR spectra were recorded on a Varian MercuryPlus 400 spectrometer. All chemical shifts (δ) were referenced internally to the residual solvent peak (CD₃OD: ¹H, δ 3.30; ¹³C, δ 49.0; CDCl₃: ¹H 7.26 ppm; ¹³C 77.0 ppm). Short- and long-range ¹H–¹³C correlations were determined with gradient-enhanced inverse-detected HSQC and HMBC experiments respectively. NOE correlations were detected with NOESY or ROESY experiments with a 0.5 s mixing time. The high-resolution ESI mass spectra were obtained using an Agilent 6210 LC-TOF mass spectrometer at the Mass Spectrometer Facility at the University of Florida, Gainesville, Florida. HPLC purifications were performed on Beckman System Gold HPLC system with a 168 UV detector and a SEDEX 85 (Sedere) evaporative light scattering detector. Thin layer chromatography (TLC) analyses were performed using Merck Kieselgel (Aufoilen) 60 F₂₅₄ plates. TLC plates were visualized by spraying with 1:1 MeOH:H₂SO₄.

3.2. Animal Material

The gorgonian *Briareum asbesinum* was collected by hand using SCUBA at a depth of 45–50 ft from Hillsboro Ledge, Boca Raton Florida. The specimen was immediately frozen and kept at –20 °C until extraction. A voucher specimen has been deposited in the Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton Florida (FAU02-010).

3.3. Extraction and Isolation

The sample of *Briareum asbestinum* (500 g wet wt.), were extracted with MeOH (3 × 800 mL) for 24 h. The third, second and then the first extracts were passed through a column of HP-20 resin

(2.5 × 25 cm) equilibrated with MeOH. The combined eluents was diluted with H₂O (2.5 L) and passed again through the column. The column was eluted with 800 mL fractions of: (1) H₂O, (2) 40% Me₂CO/H₂O, (3) 75% Me₂CO/H₂O and (4) Me₂CO. Fraction 4 was concentrated to dryness and was subjected to column chromatography on HP-20SS resin eluting with increasing concentrations of MeCN in H₂O (40%–100%). A late eluting fraction was further subjected to semi-preparative silica gel HPLC (Luna 5 μm; 10 × 250 mm; 4 mL/min; 5%–50% EtOAc/Hexane over 30 min) to give **1** (6.0 mg), **2** (10.0 mg), **4** (10.0 mg), **6** (10.0 mg) and **7** (5.0 mg).

Briareolate ester J (**1**): Colorless oil; $[\alpha]_D^{25} -19$ (*c* 0.05, MeOH); UV (MeOH) λ_{\max} 225 nm (ϵ 2711), 287 (ϵ 1713); IR (MeOH) ν_{\max} 2956, 2867, 1737, 1641, 1454, 1371 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 613.3346 [M + Na]⁺ (calcd. for C₃₃H₅₀O₉Na, 613.3353).

Briareolate ester K (**2**): Colorless oil; $[\alpha]_D^{25} -8$ (*c* 0.05, MeOH); IR (MeOH) ν_{\max} 2951, 2869, 1742, 1454, 1367 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 629.3291 [M + Na]⁺ (calcd. for C₃₃H₅₀O₁₀Na, 629.3302).

3.4. Cell Culture

BG02 hESCs were grown in defined medium, containing 10 ng/mL HRG1β (Peprotech), 10 ng/mL ActA (R&D Systems), 200 ng/mL LR³-IGF1 (JRH Biosciences), and 8 ng/mL FGF2 (Sigma or R&D Systems). Cultures were passaged with Accutase (Innovative Cell Technologies) and plated on tissue culture flasks coated with growth factor-reduced matrigel (BD Biosciences) diluted 1:200, as described by Robins and Schulz [8].

3.5. RT-CES Cytotoxicity Assays

The xCELLigence real-time impedance system (Roche) was used to monitor the effects of compounds on cells [7]. BG02 cells were plated at 1 × 10⁴ cells/well in matrigel coated plates. The culture was placed in the reader station under standard humidified conditions and incubated at 37 °C with 5% CO₂. The media was changed every 24 h and impedance was measured every 15 min for 3 days. Compounds were added to quadruplicate wells 24 h after plating. Vincristine was used as a positive control for cytotoxicity, and DMSO alone was used as a negative control. Cell index plots were normalized immediately prior to addition of compound, and EC₅₀ values were generated using the xCELLigence analysis software RTCA ver 1.1 after 2 days of treatment.

4. Conclusions

Two new briarane diterpenoids briareolate esters J (**1**) and K (**2**), along with three known compounds were isolated from the methanolic extract of *B. asbestinum* collected off the coast of Boca Raton, Florida. The compounds were identified from screening semi-purified and pre-fractioned extract libraries against hESCs and briareolate ester K (**2**) containing a *trans* C-7/8 epoxide was found to exhibit weak growth inhibition activity against human embryonic stem cells (BG02). The lack of biological activity for briaranes **1** and **2** further confirmed the requirement of the α,β,γ,δ-unsaturated ketone in conjunction with the double bond configuration for biological activity.

Acknowledgments

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References

1. Berrue, F.; Kerr, R.G. Diterpenes from gorgonian corals. *Nat. Prod. Rep.* **2009**, *26*, 681–710.
2. Maharaj, D.; Mootoo, B.S.; Lough, A.J.; McLean, S.; Reynolds, W.F.; Tinto, W.F. Methyl briareolate, the first briarein diterpene containing a C-19 methyl ester. *Tetrahedron Lett.* **1992**, *33*, 7761–7764.
3. Dookran, R.; Maharaj, D.; Mootoo, B.S.; Ramsewak, R.; McLean, S.; Reynolds, W.F.; Tinto, W.F. Briarane and asbestinane diterpenes from *Briareum asbestinum*. *Tetrahedron* **1994**, *50*, 1983–1992.
4. Mootoo, B.S.; Ramsewak, R.; Sharma, R.; Tinto, W.F.; Lough, A.J.; McLean, S.; Reynolds, W.F.; Yang, J.P.; Yu, M. Further briareolate esters and briareolides from the Caribbean gorgonian octocoral *Briareum asbestinum*. *Tetrahedron* **1996**, *52*, 9953–9962.
5. Gupta, P.; Sharma, U.; Schulz, T.C.; Sherrer, E.S.; McLean, A.B.; Robins, A.J.; West, L.M. Bioactive diterpenoid containing a reversible “spring-loaded” (*E,Z*)-dieneone michael acceptor. *Org. Lett.* **2011**, *13*, 3920–3923.
6. Housen, W.E.; Jaspars, M. Methods in Biotechnology. In *Natural Products Isolation*, 2nd ed.; Sarker, S.D., Latif, Z., Gray, A.I., Eds.; Humana Press: Totowa, NJ, USA, 2006; Volume 20, pp. 353–391.
7. Solly, K.; Wang, X.; Xu, X.; Strulovici, B.; Zheng, W. Application of real-time cell electronic sensing (RT-CES) technology to cell-based assays. *Assay Drug Dev. Technol.* **2004**, *2*, 363–372.
8. Robins, A.J.; Schulz, T.C. Media and Extra Cellular Matrix Requirements for Large-Scale ESC Growth. In *Emerging Technology Platforms for Stem Cells*; Lakshmipathy, U., Chesnut, J.D., Thyagarajan, B., Eds.; Wiley-Blackwell: Oxford, UK, 2009; pp. 251–274.

Samples Availability: Available from the authors.

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