

Article

Cytotoxic Sesterterpenes from Thai Marine Sponge *Hyrtios erectus*

Wirongrong Kawetripob¹, Chulabhorn Mahidol^{1,2}, Pittaya Tuntiwachwuttikul³,
Somsak Ruchirawat^{1,2,4} and Hunsa Prawat^{1,*}

¹ Chulabhorn Research Institute, Kamphaeng Phet 6 Road, Bangkok 10210, Thailand; Kwirongrong@cri.or.th (W.K.); mahidol_natlab@cri.or.th (C.M.); Somsak@cri.or.th (S.R.)

² Chulabhorn Graduate Institute, Chemical Biology Program, Chulabhorn Royal Academy, Kamphaeng Phet 6 Road, Bangkok 10210, Thailand

³ Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket 83000, Thailand; pittaya_tun@yahoo.co.th

⁴ Center of Excellence on Environmental Health and Toxicology (EHT), CHE, Ministry of Education, Bangkok 10210, Thailand

* Correspondence: hunsa@cri.or.th; Tel.: +66-2-553-8982

Received: 30 October 2018; Accepted: 24 November 2018; Published: 28 November 2018



Abstract: Four sesterterpenes, erectusolides B, C, D, and seco-manoalide-25-methyl ether, two 2-furanone derivatives, erectusfuranones A and B, together with thirteen known sesterterpenes, (6Z)-neomanoalide-24-acetate, two diastereomers of 24-O-methylmanoalide, luffariolide B, manoalide, (6E)- and (6Z)-neomanoalide, seco-manoalide, scalarafuran, 12-acetylscalarolide, 12-epi-O-deacetyl-19-deoxy-scalarin, 12-epi-scalarin, and 12-O-deacetyl-12-epi-scalarin, three indole alkaloids, 5-hydroxy-1H-indole-3-carbaldehyde, hyrtiosine A, and variabine B, and one norterpene, cavernosine were isolated from the marine sponge *Hyrtios erectus*. Their structures were determined by means of spectroscopic methods and the absolute configurations of the asymmetric centers were determined using the modified Mosher's method. The cytotoxic activities for the isolated compounds have been reported.

Keywords: *Hyrtios erectus* (CRI 572 and CRI 588); Thorectidae; manoalides; scalarane; 2-furanone derivatives; cytotoxic activities

1. Introduction

Marine organisms have always been an attractive source of natural products with novel and exotic structures and useful biological activities [1–11]. Marine sponges of the genus *Hyrtios* (order Dictyoceratida family Thorectidae) have yielded scalarane [6,8,12–14] and manoalide [12] types sesterterpenes, which are important groups of active secondary metabolites. They have been reported to possess many biological activities, such as cytotoxic [3,6,15,16], antibacterial [9,11], antibiotic [17] activities, inhibition of the DNA-relaxing activity of mouse DNA topoisomerase I [12], and enhancement nerve growth factor synthesis in cultured astroglial cells [13]. As a part of our ongoing research program focused on the discovery of cytotoxic compounds from Thai marine sponges [18–20], drawing from the previous report, one sesterterpene, erectusolide A, six phenolic alkenes, erectuseneols A–F, together with nine known compounds, were isolated from the EtOAc soluble extract of *Hyrtios erectus* (Chulabhorn Research Institute (CRI) 588) [3]. Of these, some sesterterpenes exhibited significant cytotoxic activities against the MOLT-3 cell line with an IC₅₀ values of 3.79–5.82 μM [3]. Accordingly, further investigation of the EtOAc soluble extract was carried out, leading to the isolation of four new sesterterpenes, erectusolides B (1), C (2), D (4), and seco-manoalide-25-methyl ether (3), two new 2-furanone derivatives, erectusfuranones A and B (5 and 6), together with

seventeen known sesterterpenes, which were identified as (6*Z*)-neomanoalide-24-acetate (**7**) [9], two diastereomers of 24-*O*-methylmanoalide (**8** and **9**) [10], luffariolide B (**10**) [15], manoalide (**11**) [10,12,17], (6*E*)- and (6*Z*)-neomanoalides (**12** and **13**) [12,15,17], seco-manoalide (**14**) [12,17] scalarafuran (**15**) [8,16], 12-acetylscalarolide (**17**) [21], 12-*epi-O*-deacetyl-19-deoxyscalarin (**18**) [8,16], 12-*epi*-scalarin (**19**) [13,16], and 12-*O*-deacetyl-12-*epi*-scalarin (**20**) [13,14,16], three indole alkaloids, 5-hydroxy-1*H*-indole-3-carbaldehyde (**21**) [14,22], hyrtiosine A (**22**) [14,22], and variabine B (**23**) [7], and one norterpene, cavernosine (**16**) [3,23]. All of the known compounds (Figure 1) were readily identified by extensive study of their spectral data, including high resolution atmospheric pressure chemical ionization mass spectrometry (HRAPCIMS) or high resolution electrospray ionization mass spectrometry (HRESIMS), 1D and 2D nuclear magnetic resonance (NMR) data, as well as by comparison with those reported in the literatures.

2. Results

Two separate collections of *H. erectus* were studied; and they were collected in February 2011 from different locations of the Similan Island, Phangnga province, Thailand. The MeOH extract of the Thai marine sponge *H. erectus* from collection CRI 588 was dissolved in H₂O and partitioned with EtOAc. The EtOAc soluble extract was fractionated by column chromatography over silica gel, Sephadex LH-20, and reversed-phase high performance liquid chromatography (HPLC) to afford three new sesterterpenes, erectusolides B (**1**), C (**2**), and seco-manoalide-25-methyl ether (**3**), two new 2-furanone derivatives, erectusufuranones A (**5**) and B (**6**) together with eight known sesterterpenes, **7–14**. One additional new sesterterpene, erectusolide D (**4**), and ten known compounds (**13**, **15–23**) were obtained from the *H. erectus* collection CRI 572. All new compounds showed considerable structural similarity with the co-occurring known sesterterpenes.

Compound **1** was obtained as a weak optical rotation value ($[\alpha]_D^{26} -1.3$), and its molecular formula was determined to be C₂₅H₃₆O₃ (eight degrees of unsaturation) by HRAPCIMS. Infrared (IR) absorption bands of compound **1** suggested the presence of β -substituted α,β -unsaturated γ -lactone (β -substituted butenolide) at 1779 and 1746 cm⁻¹ and hydroxyl group at 3443 cm⁻¹. Four of the eight degrees of unsaturation implied by the molecular formula of **1** were taken up in one carbon–oxygen double bonds and three carbon–carbon double bonds, thus indicating the tetracyclic nature of the molecule. The ¹³C and (distortionless enhancement by polarization transfer (DEPT) NMR spectra of **1** showed 25 carbons including, 4 tertiary methyls, 7 methylenes, 2 oxymethylenes, 3 methines, 2 olefinic methines, and 7 quaternary carbons. The ¹H and ¹³C NMR spectral data of **1** (Table 1, Figures S1 and S2) were comparable to those of luffariolides A (Figure 1) and B (**10**) and implied that all compounds possessed identical two terminal units, which included a polyalkylated-cyclohexene (C₁₂–C₂₂) and β -substituted butenolide moieties. The major differences were found in the C₄–C₁₁, and C-23. In the ¹H–¹H COSY correlation of **1** (Figure 2), H-6 (δ_H 5.67) showed allylic coupling to H_{ax}-8 (1H, δ_H 1.90–2.00) and H₂-24 (2H, δ_H 4.03/4.06) and H-5 was coupled to H-4, H-6, and H-10. From its ¹H–¹H correlation spectroscopy (COSY) spectrum of **1** (Figure 2), typical allylic coupling of the olefinic methines at δ_H 5.86 (H-2) and 5.67 (H-6) with the respective methine signal at δ_H 2.74 (H-4) and oxymethylene at δ_H 4.03/4.06 (H₂-24) were discernable. Both the COSY and heteronuclear multiple bond correlation (HMBC) data indicated that the double bonds were not conjugated to each other. The ¹H, ¹³C, and COSY NMR data confirmed the presence of a 3-hydroxymethylbicyclo [4.2.0] oct-2-ene (C₄–C₁₁ and C-24) in the molecule. The HMBC correlations of **1** (Figures 2 and S4), the proton signal at δ_H 5.86 (H-2) correlated to methine carbon at δ_C 51.0 (C-4), H-4 at δ_H 2.74 correlated to quaternary carbon at δ_C 169.7 (C-3), methine carbon at δ_C 115.3 (C-2), and methylene carbon at δ_C 73.2 (C-25) allowed the connectivity of the butenolide with the bicyclic ring through the linkage of C-3 and C-4. The linkage of the polyalkylated-cyclohexene and CH₃-23 at quaternary C-11 on the molecule were deduced from the HMBC correlations of H₃-23 (δ_H 1.13) with C-4, C-10 (δ 37.0), C-11 (δ 43.0), and C-12 (δ 36.6) and the correlations of H₂-12 (δ_H 1.28–1.36 and 1.58–1.66) with C-4, C-10, C-14 (δ 136.3), and C-23 (δ 21.5). The large trans-diaxial coupling constant between H_{ax}-9 and H_{ax}-10 ($J = 12.2$

Hz) on the cyclohexene ring suggested the β -configuration of H_{ax}-10. The relative stereochemistry of **1** was established by analysis of the (nuclear overhauser effect spectroscopy (NOESY) spectrum (in CDCl₃ and C₆D₆; Figure 2). NOESY cross-peaks resonance of H-4, H_α-9, and H₃-23 and H-5, H-10, H-12, and H-2 implied a *cis*-junction for rings B/C and H-5, H_{ax}-10, β -substituted butenolide moiety, and polyalkylated-cyclohexene (C₁₂–C₂₂) are on the same face while the methyl group (CH₃-23) and H-4 on the opposite face of the molecule. Structure **1** with relative stereochemistry as shown was thus assigned to erectusolide B, which contained fused cyclohexene and cyclobutane rings. It could derive from luffariolide A (Figure 1) [11], through a biogenetic pathway involving a 2 + 2 cycloaddition of double bonds at C-4/C-5 and C-10/C-11 as suggested by Lin, H.W. and co-workers [4]. Furthermore, compounds **1** and **2** represent two important compounds which lend further support for the proposed biosynthetic pathway of Lin et. al. for the cyclobutane formation [4]. This substance has an optical rotation near zero ($[\alpha]_D^{26} -1.3$); attempting to separate the substance using various chiral columns was found to be inseparable.

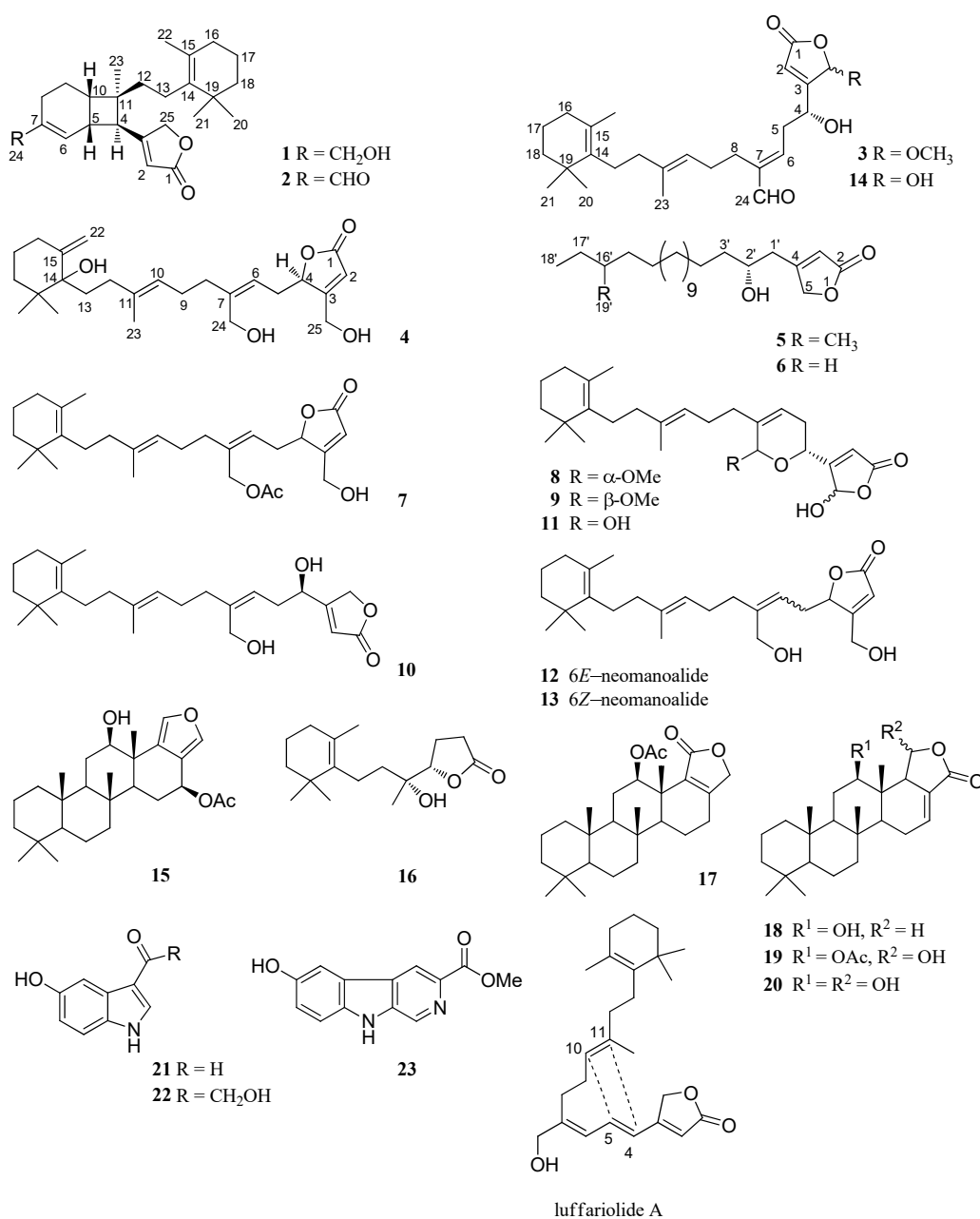


Figure 1. Isolated compounds 1–23 from sponge *Hyrtios erectus* and luffariolide A.

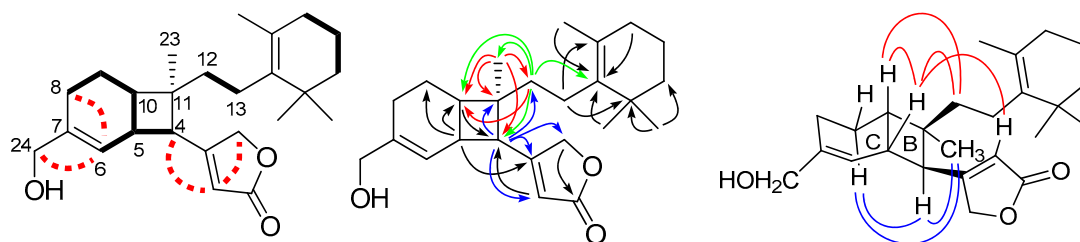


Figure 2. Key COSY (— and - - -), HMBC (↷), and NOESY (↷) correlations of **1**.

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data (^a in CDCl_3 ^b in C_6D_6) of compounds **1** and **2**.

Position	1			2		
	^a δ_{C} (Type)	^a δ_{H} , Mult. (J in Hz)	^b δ_{H} , Mult. (J in Hz)	^a δ_{C} (Type)	^a δ_{H} , Mult. (J in Hz)	^b δ_{H} , Mult. (J in Hz)
1	174.0 (C)	-	-	173.3 (C)	-	-
2	115.3 (CH)	5.86, brd (1.5)	5.66, q (1.6)	116.0 (CH)	5.93, brd (1.6)	5.46, brs
3	169.7 (C)	-	-	168.1 (C)	-	-
4	51.0 (CH)	2.74, d (10.0)	2.24, d (10.1)	50.8 (CH)	2.85, d (9.9)	1.93, d (10.5)
5	33.3 (CH)	2.85, brt (9.0)	2.60–2.66, m	34.5 (CH)	3.14, brt (8.8)	2.40, brt (10.5)
6	122.1 (CH)	5.67, brd (0.9)	5.48, brd (1.4)	149.1 (CH)	6.74, t (3.0)	5.85, brt (3.1)
7	140.9 (C)	-	-	143.7 (C)	-	-
8	24.0 (CH ₂)	1.90–2.00, o 2.09, dt (16.4, 3.4)	1.76, brt (13.0) 1.90–1.98, o	19.4 (CH ₂)	1.86–1.93, o 2.65, dt (16.8, 3.6)	1.51–1.62, o 2.60, dt (13.3, 3.1)
9	22.8 (CH ₂)	1.65, qd (12.2, 4.2) 1.82–2.00, o	1.42, qd (12.3, 4.1) 1.57–1.67, m	22.3 (CH ₂)	1.50–1.58, o 1.88–1.99, o	0.88–0.96, m 1.27–1.37, o
10	37.0 (CH)	2.25, dt (12.2, 6.9)	2.12, ddd (12.8, 7.9, 6.8)	38.0 (CH)	2.37, dt (11.7, 6.7)	1.82–1.88, o
11	43.0 (C)	-	-	43.7 (C)	-	-
12	36.6 (CH ₂)	1.08–1.20, m 1.49–1.61, o	1.28–1.36, m 1.58–1.66, o	36.6 (CH ₂)	1.15–1.18, o 1.55–1.58, o	1.10–1.17, m 1.35–1.40, o
13	22.4 (CH ₂)	1.90–2.00, o	1.94–2.06, o	22.2 (CH ₂)	1.88–1.99, o	1.23–1.40, o 1.78–1.84, o
14	136.3 (C)	-	-	136.0 (C)	-	-
15	127.5 (C)	-	-	127.7 (C)	-	-
16	32.7 (CH ₂)	1.89–1.98, o	1.92–2.06, o	32.7 (CH ₂)	1.85–1.99, o	1.78–1.89, o
17	19.4 (CH ₂)	1.53–1.62, o	1.60–1.68, o	19.4 (CH ₂)	1.52–1.62, o	1.49–1.58, o
18	39.7 (CH ₂)	1.40–1.44, m	1.49–1.52, m	39.7 (CH ₂)	1.40–1.44, m	1.37–1.42, o
19	35.1 (C)	-	-	35.1 (C)	-	-
20	28.71 (CH ₃)	0.96 *, s	1.14, s	28.74 (CH ₃)	0.96, s	0.99, s
21	28.72 (CH ₃)	0.97 *, s	1.11, s	28.7 (CH ₃)	0.98, s	1.02, s
22	19.8 (CH ₃)	1.55, s	1.66, s	19.8 (CH ₃)	1.56, s	1.52, s
23	21.5 (CH ₃)	1.13, s	0.88, s	21.5 (CH ₃)	1.16, s	0.63, s
24	66.8 (CH ₂)	4.03, d (13.2) 4.06, d (13.2)	3.88, s	192.9 (CH)	9.45, s	9.37, s
25	73.2 (CH ₂)	4.62, dd (17.4, 1.6) 4.65, dd (17.4, 1.0)	4.02, dd (17.1, 0.8) 4.16, dd (17.1, 1.7)	72.9 (CH ₂)	4.64, dd (17.3, 1.8) 4.68, dd (17.3, 1.1)	3.85, dd (17.2) 4.00, dd (17.2)

* Interchangeable; o = overlapped signals; Assignments are based on COSY, HSQC (Figures S3, S9 and S10), and HMBC experiments.

Compound **2** was obtained as optically active ($[\alpha]_{\text{D}}^{26} -18.5$), and its molecular formula was determined to be $\text{C}_{25}\text{H}_{34}\text{O}_3$ (nine degrees of unsaturation) by HRAPCIMS. Infrared (IR) absorption bands of compound **2** suggested the presence of β -substituted α,β -unsaturated γ -lactone at 1779 and 1746 cm^{-1} and α,β -unsaturated carbonyl group at 1682 cm^{-1} . The ^1H and ^{13}C NMR spectroscopic data of **2** (Table 1, Figures S4–S8) indicated that it was essentially identical to compound **1**, except for the presence of an aldehyde group (δ_{H} 9.45, s; δ_{C} 192.9) for **2** in place of the hydroxymethyl group (δ_{H} 4.03/4.06, each d, $J = 13.2$ Hz, δ_{C} 66.8) for **1**. This was further confirmed by the HMBC correlations (Figures S11 and S12) between olefinic H-6 (δ_{H} 6.74) and aldehydic carbon (δ_{C} 192.9) and between proton aldehyde (δ_{H} 9.45) and C-7 (δ_{C} 143.7) and C-8 (δ_{C} 19.4). The NOESY spectrum of **2** was similar to that observed of **1** indicating the same relative stereochemistry. Thus, compound **2** was suggested to be the formaldehyde analog of **1**, and named erectusolide C. This compound has low optical rotation ($[\alpha]_{\text{D}}^{26} -18.5$); attempting to separate the compound using various chiral columns was unsuccessful.

Compound **3** was obtained as a pale yellow solid, exhibiting similar ultraviolet (UV), infrared (IR), ^1H , and ^{13}C NMR spectra (Table 2, Figures S13 and S14) as (6*E*) seco-manoalide (**14**) [17]. Accurate mass measurement by HRAPCIMS of **3** indicated a pseudo molecular ion peak at m/z 465.2414 $[\text{M} + \text{Cl}]^-$ (calcd for $\text{C}_{26}\text{H}_{38}\text{ClO}_5$, 465.2413), consistent with the molecular formula $\text{C}_{26}\text{H}_{38}\text{O}_5$. This difference of 14 amu compared to the molecular formula of **14**, and the appearance of a signal of methoxy group in

^1H , and ^{13}C NMR spectra of **3** at δ_{H} 3.65 and δ_{C} 57.9. It is suggested that the hydroxyl group at C-25 was replaced by a methoxyl group. This was further confirmed by the HMBC correlations (Figures S16) between the methoxyl protons (δ_{H} 3.65) and hemiacetal carbon (δ_{C} 103.3) and between H-25 (δ_{H} 5.84) and methoxyl carbon (δ_{C} 57.9). The absolute stereochemistry at C-4 of **3** was determined by the modified Mosher's method [24]. The hydroxyl group of **3** was converted into both the *S*- and *R*-MTPA esters **3a** and **3b**, respectively. The ^1H NMR chemical shifts were assigned by the analysis of the ^1H - ^1H COSY NMR data for each MTPA ester (experimental section). The calculated $\Delta\delta_{\text{S-R}}$ values were positive for the H₂-5 (+0.03 and +0.03), H-6 (+0.15), H₂-8 (+0.04), and H-24 (+0.11) and negative for the H-2 (−0.13), 25-OMe (−0.004), and H-25 (−0.02) (Figure 3), implying that the absolute configuration of C-4 was *R*. Thus, compound **3** was characterized as (4*R*,6*E*) seco-manoalide-25-methyl ether.

Table 2. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data (CDCl_3) of compounds **3** and **4**.

Position	3		4	
	δ_{C} (Type)	δ_{H} , Mult. (J in Hz)	δ_{C} (Type)	δ_{H} , Mult. (J in Hz)
1	169.2 (C)	-	172.7 (C)	-
2	119.3 (CH)	6.09, brt (1.3)	116.0 (CH)	6.04, brd (1.4)
3	166.3 (C)	-	171.7 (C)	-
4	66.9 (CH)	4.73, brdd (6.7, 4.1)	81.8 (CH)	5.08, m
5	34.7 (CH ₂)	2.78, dt (15.7, 7.5) 2.92, ddd (15.7, 7.1, 4.1)	30.2 (CH ₂)	2.57, ddd (15.0, 7.3, 5.5) 2.78, dt (15.0, 7.0)
6	147.0 (CH)	6.54, t (7.1)	119.9 (CH)	5.19, t (7.5)
7	146.1 (C)	-	143.0 (C)	-
8	24.6 (CH ₂)	2.32, t (7.4)	35.4 (CH ₂)	2.10–2.22, m
9	26.7 (CH ₂)	2.09, q (7.4)	26.3 (CH ₂)	2.07–2.17, m
10	122.4 (CH)	5.13, td (7.3, 1.0)	123.6 (CH)	5.07, m
11	137.5 (C)	-	136.6 (C)	-
12	40.2 (CH ₂)	1.95–2.07, m	33.7 (CH ₂)	1.68, td (13.5, 3.5) 1.86–1.96, m
13	27.8 (CH ₂)	1.97–2.07, m	31.0 (CH ₂)	1.49–1.60, m 1.88, td (11.7, 4.3)
14	137.0 (C)	-	80.4 (C)	-
15	127.0 (C)	-	150.5 (C)	-
16	32.8 (CH ₂)	1.90, t (6.2)	34.0 (CH ₂)	1.97, td (13.0, 5.7) 2.32, brd (13.0)
17	19.5 (CH ₂)	1.54–1.59, m	22.7 (CH ₂)	1.43–1.65, m
18	39.9 (CH ₂)	1.39–1.43, m	38.0 (CH ₂)	1.37, brd (13.7) 1.63, td (13.7, 4.5)
19	35.0 (C)	-	39.7 (C)	-
20	28.6 (CH ₃)	0.99, s	24.1 (CH ₃)	0.96, s
21	28.6 (CH ₃)	0.99, s	22.1 (CH ₃)	0.88, s
22	19.8 (CH ₃)	1.59, s	108.3 (CH ₂)	4.83, brs 4.91, brs
23	16.0 (CH ₃)	1.61, s	16.3 (CH ₃)	1.60, s
24	194.2 (CH)	9.43, s	60.3 (CH ₂)	4.10, d (12.1) 4.13, d (12.1)
25	103.3 (CH)	5.84, s	58.6 (CH ₂)	4.44, d (16.9) 4.52, d (16.9)
OMe-25	57.9 (CH ₃)	3.65, s		

Assignments are based on COSY, HSQC (Figures S15) or HMQC (Figures S19), and HMBC experiments.

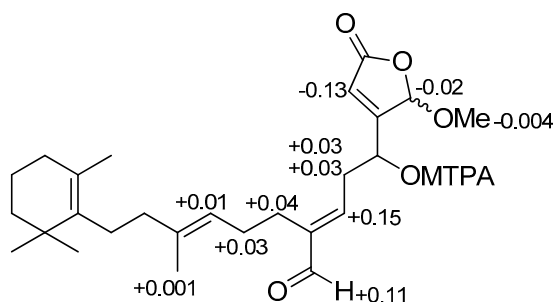


Figure 3. $\Delta\delta_{S-R}$ values in ppm for *S*- and *R*-MTPA esters of compound **3** in CDCl_3 .

Compound **4** was obtained as an optically active ($[\alpha]_D^{26} -20.9$), and its molecular formula was determined to be $\text{C}_{25}\text{H}_{38}\text{O}_5$ (seven degrees of unsaturation) by HRAPCIMS. Five of the seven degrees of unsaturation implied by the molecular formula of **4** were taken up in one carbon–oxygen double bonds and four carbon–carbon double bonds, thus indicating the bicyclic nature of the molecule. The IR spectrum exhibited absorption bands corresponding to a hydroxyl group (3424 cm^{-1}), an ester carbonyl (1739 cm^{-1}), and an exomethylene substituent (898 cm^{-1}). Similarities in the NMR spectra between compounds **4** (Table 2, Figures S17 and S18) and 6*Z*-neomanoalide (**13**) suggested that compound **4** was also a neomanoalide-type sesterterpene [17].

The main differences in the ^1H NMR spectra of **4** and compound **13** were the absence of one olefinic methyl group resonance in compound **4** and the appearance of a resonance attributable to an exomethylene moiety (δ_{H} 4.83 and 4.91, H_2 -22). Observation of a sp^2 methylene carbon resonance (δ_{C} 108.3) and a corresponding quaternary carbon (δ_{C} 150.5) further supported the presence of a exocyclic methylene functionality in the structure of **4**. An oxygenated quaternary carbon (δ_{C} 80.4, C-14) was observed by ^{13}C NMR and DEPT experiments. The assignment and placement of the hydroxyl substituent at C-14 and the exocyclic methylene at C-15 were deduced by observation of long range ^1H - ^{13}C HMBC correlations (Figures 4 and S20) (H_2 -13, H_2 -16, H_2 -18, H_3 -21/22 to C-14, H_2 -17 to C-15, and H_2 -16 to C-22). The relative stereochemistry of the methylenecyclohexane unit was elucidated mainly on the basis of NOESY correlations (Figure 4). The correlations among axial H_{α} -18 (δ_{H} 1.63, td, $J = 13.0, 5.7\text{ Hz}$), axial methylene H_2 -13, and equatorial CH_3 -20 (δ_{H} 0.96) and correlations among axial H_3 -21 (δ_{H} 0.88), exocyclic methylene H_2 -22, and equatorial H_{β} -18 (δ_{H} 1.37, brd, $J = 13.7\text{ Hz}$) that were observed in **4** indicated that the equatorial hydroxyl group at C-14 occupied the β -face. The geometry of the olefinic bonds was assigned as 6*Z*,10*E* on the basis of NOESY correlations between H_2 -5 and H_2 -24 and between H_2 -9 and H_3 -23, respectively. Compound **4** exhibited a negative optical rotation ($[\alpha]_D^{25} -20.9$ (c 1.64, CHCl_3)), similar to that of 6*Z*-neomanoalide (**13**) ($[\alpha]_D^{25} -28$ (c 0.8, CH_2Cl_2)) [12] the relative stereochemistry at C-4 of **4** was then assigned to be *R*. Thus, the structure **4** was concluded as shown in Figure 1 and was named erectusolide D.

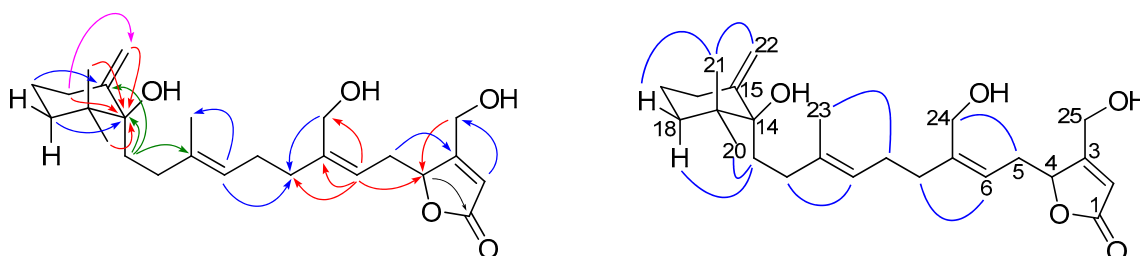


Figure 4. Key HMBC (↔), and NOESY (↔) correlations of **4**.

Compound **5** was obtained as optically active ($[\alpha]_D^{25} +1.3$), and its molecular formula was determined to be $\text{C}_{23}\text{H}_{42}\text{O}_3$ (three degrees of unsaturation) by HRESIMS. The IR absorption bands data of compound **5** suggested the presence of β -substituted α, β -unsaturated γ -lactone (β -substituted butenolide for three degrees of unsaturation in the structure at 1777 and 1739 cm^{-1} in addition

to the hydroxyl group at 3442 cm^{-1} . The ^1H and ^{13}C NMR spectra of **5** as shown in Table 3 (Figures S11 and S22) suggests that compound **5** possess β -substituted butenolide moiety to which a saturated long chain hydrocarbon group is attached. The ^1H NMR signals at δ_{H} 5.93 (1H) and 4.85 (2H) assignable to an olefinic proton and methylene protons at the α - and γ -positions of the α,β -unsaturated γ -lactone, respectively, reveals that the long chain hydrocarbon group is attached at the β -position (C-4). The 2-hydroxy-16-methyloctadecane group (long chain hydrocarbon) was assigned by a combination of HRESIMS, ^1H and ^{13}C NMR, DEPT, COSY, HSQC (Figure S23), and HMBC analyses. The HMBC spectrum of **5** (Figures 5 and S24) showed the correlations from two of the methyl protons at δ_{H} 0.85 ($\text{H}_3\text{-18}'$, t, $J = 7.2$ Hz) and δ_{H} 0.84 ($\text{H}_3\text{-19}'$, d, $J = 6.0$ Hz) to the methine carbon at δ_{C} 34.4 (C-16') and methylene carbon at δ_{C} 29.42 (C-17') suggesting that the position of the second methyl group was attached at C-16'. In the HMBC spectrum of **5** also showed the correlations between methylene $\text{H}_2\text{-1}'$ (δ_{H} 2.52/2.64) and C-3 (δ_{C} 117.3), C-2' (δ_{C} 70.2), C-3' (δ_{C} 37.7), and C-5 (δ_{C} 73.8) and between $\text{H}_2\text{-2}'$ (δ_{H} 3.84–3.90) and C-4 (δ_{C} 167.3), C-4' (δ_{C} 25.5) indicating clearly that the methylene carbon (C-1') of 2-hydroxy-16-methyloctadecyl attached to the C-4 of the α,β -unsaturated γ -lactone. The absolute stereochemistry at C-2' of **5** was determined by the modified Mosher's method [24]. The hydroxyl group of **5** was converted into both the *S*- and *R*-MTPA esters **5a** and **5b**, respectively, each of which was a single diastereoisomer by ^1H and COSY NMR experiments. The calculated $\Delta\delta_{\text{S-R}}$ values were positive for the $\text{H}_2\text{-3}'$ (+0.04) and $\text{H}_2\text{-4}'$ (+0.06), while $\Delta\delta_{\text{S-R}}$ values were negative for $\text{H}_2\text{-1}'$ (−0.07 and −0.06), H-3 (−0.09), and $\text{H}_2\text{-5}$ (−0.13 and −0.27) (Figure 6), which implied the absolute configuration of C-2' was *R*. Thus, compound **5** was characterized as 4-((*2R*)-2-hydroxy-16-methyloctadecyl)furan-2(5*H*)-one and was named erectusfuranone A.

Table 3. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data (CDCl_3) of compounds **5** and **6**.

Position	5		6	
	δ_{C} (Type)	δ_{H} , Mult. (J in Hz)	δ_{C} (Type)	δ_{H} , Mult. (J in Hz)
2	173.8 (C)	-	173.9 (C)	-
3	117.3 (CH)	5.93, s	117.2 (CH)	5.93, s
4	167.3 (C)	-	167.4 (C)	-
5	73.8 (CH ₂)	4.85, s	73.8 (CH ₂)	4.85, s
1'	36.3 (CH ₂)	2.52, dd (14.8, 8.2) 2.64, dd (14.8, 3.2)	36.5 (CH ₂)	2.52, dd (15.2, 8.2) 2.64, dd (15.2, 3.4)
2'	70.2 (CH)	3.84–3.90, m	70.2 (CH)	3.84–3.89, m
3'	37.7 (CH ₂)	1.49–1.54, m	37.7 (CH ₂)	1.49–1.55, m
4'	25.5 (CH ₂)	1.28–1.38, m 1.39–1.46, m	25.5 (CH ₂)	1.30–1.47, m
	(CH ₂)-5'– (CH ₂)-14' 30.0, 29.68, 29.64, 29.63, 29.61, 29.57, 29.49, 29.49, 29.48, 27.1 (C-14')	1.22–1.50, m	(CH ₂)-5'– (CH ₂)-14' 29.65, 29.65, 29.65, 29.63, 29.61, 29.61, 29.58, 29.50, 29.50, 29.42	1.22–1.38, m
15'	36.6 (CH ₂)	1.07–1.17, m 1.23–1.37, m	29.3 (CH ₂)	1.22–1.38, m
16'	34.4 (CH)	1.23–1.37, m	31.9 (CH ₂)	1.22–1.31, m
17'	29.42 (CH ₂)	1.22–1.50, m	22.6 (CH ₂)	1.22–1.38, m
18'	11.3 (CH ₃)	0.85, t (7.2)	14.0 (CH ₃)	0.88, t (7.0)
19'	19.2 (CH ₃)	0.84, d (6.0)	-	-

Assignments are based on COSY, HSQC (Figures S23 and S27), and HMBC (Figures S24 and S28) experiments.

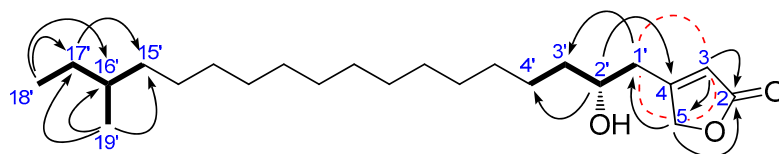


Figure 5. Key COSY (—), long range COSY (---), and HMBC (↷) correlations of 5.

Compound 6 was obtained as optically active ($[\alpha]_D^{26} -4.9$), and its molecular formula was determined to be $C_{22}H_{40}O_3$ (three degrees of unsaturation) by HRESIMS. The UV, IR, 1H and ^{13}C NMR (Figures S25 and S26) spectroscopic data of 6 indicated that it was essentially identical to compound 5. The signals of a methine moiety (C-16'; δ_C 34.3, δ_H 1.23–1.37) and a methyl group (C-19'; δ_C 19.2, δ_H 0.84) were not evident in 6, instead a methylene group (C-16'; δ_C 31.9, δ_H 1.22–1.31) was observed. The absolute stereochemistry at C-2' of 6 was determined by the modified Mosher's method [24]. The hydroxyl group of 6 was converted into both the *S*- and *R*-MTPA esters 6a and 6b, respectively, each of which was a single diastereoisomer judged by 1H and COSY NMR experiments. The $\Delta\delta_{S-R}$ (Figure 6) values observed in the 1H NMR spectra were calculated and the resulting positive $\Delta\delta$ values for H₂-6 (+0.04 and +0.04) and H₂-7 (+0.06), and negative $\Delta\delta$ values for H₂-1' (−0.06 and −0.06), H-3 (−0.09), and H₂-5 (−0.12 and −0.26) were consistent with the 2'*R* configuration. Thus, compound 6 was characterized as (*R*)-4-(2-hydroxyoctadecyl) furan-2(5*H*)-one and was named erectusfuranone B.

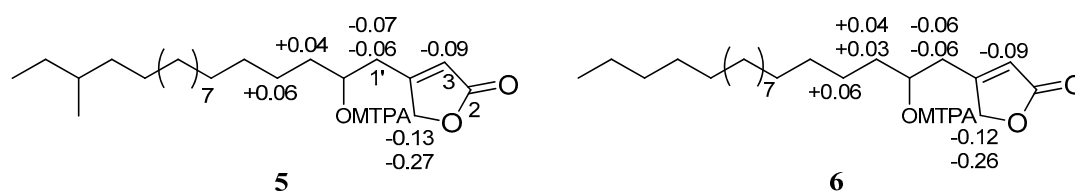


Figure 6. $\Delta\delta_{S-R}$ values in ppm for *S*- and *R*-MTPA esters of compounds 5 and 6 in $CDCl_3$.

Cytotoxicity of the isolated compounds 3, 5, 7–20 and 23 were evaluated against several cancer cell lines such as (Table 4), MOLT-3 (acute lymphoblastic leukemia), HepG2 (hepatocarcinoma), HeLa (human cervical carcinoma), HuCCA-1 (human cholangiocarcinoma), A549 (non-small-cell lung cancer), H69AR (multidrug resistance small-cell lung cancer), KB (human epidermoid carcinoma in the mouth), T47D (hormone dependent breast cancer), MDA-MB-231 (hormone independent breast cancer), and MRC-5 (normal embryonic lung cell). Compounds 8, 9, 14 and 20 showed good cytotoxic activity against MOLT-3 cell line with IC_{50} values of 1.77, 1.30, 6.68, and 7.64 μM , respectively, compounds 8 and 9 also showed cytotoxic activity against HeLa, HuCCA-1, and A549 cell lines with IC_{50} values of 1.51–16.77 μM . In addition, compounds 8 and 20 selectively exhibited cytotoxic activity toward the MOLT-3 (IC_{50} 1.77 and 7.64 μM , respectively) cancer cell line with the selectivity index (SI) value of 7 (IC_{50} 13.00 μM for normal cell line, MRC-5) and 9 (IC_{50} 70.97 μM for MRC-5), respectively. The SI value is the ratio of IC_{50} of normal cell (MRC-5) and IC_{50} of cancer cell line. Compound 9 selectively exhibited cytotoxic activity against MOLT-3 (IC_{50} 1.30 μM) and HeLa (IC_{50} 1.51 μM) cell lines with respective SI values of 8 and 7 (IC_{50} 10.84 μM for MRC-5). Manoalides 8 and 9, the acetal derivatives of the hemiacetal 11, showed higher activity than 11, suggesting that the presence of 24-*O*-methyl of manoalides 8 and 9 were important for cytotoxic activity (Table 4). Manoalide 11 was previously reported to possess good cytotoxicity against L1210 (mouse lymphocytic leukemia) and KB (mouth epidermal carcinoma) cell lines with the IC_{50} value of 0.053 and 0.63 μM , respectively [12]. Compound 20 (12-*O*-deacetyl-12-*epi*-scalarin) exhibited cytotoxic activity against A549 cells with an IC_{50} of 73.38 μM (Table 4), while its IC_{50} have been reported in the literature to be 36.82 μM [16]. Luffariolide B (10), (6*E*)- and (6*Z*)-neomanoalides (12 and 13) showed weak cytotoxicity against the MOLT-3 cell line with IC_{50} of 35.45, 37.81, and 34.10 μM , respectively, but they exhibited cytotoxicity against the L1210 cell line with IC_{50} of 3.23, 24.38, and 13.93 μM , respectively [15].

Table 4. Cytotoxicity of compounds 3, 5, 7–20 and 23 (IC₅₀, μM).

Compound	MOLT-3	HepG2	HeLa	HuCCA-1	A549	H69AR	KB	T47D	MDA-MB-231	MRC-5
3	15.84	27.91	20.02	24.72	41.12	- ^a	- ^a	- ^a	40.09	- ^a
5	31.75	107.46	28.79	65.57	57.24	- ^a	- ^a	- ^a	89.26	- ^a
7	40.11	I	87.95	I	I	- ^a	- ^a	- ^a	I	- ^a
8	1.77	53.49	8.42	11.02	16.77	- ^a	- ^a	- ^a	39.53	13.00
9	1.30	27.91	1.51	8.60	12.00	- ^a	- ^a	- ^a	18.60	10.84
10	35.45	I	78.71	I	I	- ^a	- ^a	- ^a	I	- ^a
11	17.38	I	87.04	84.52	I	- ^a	- ^a	- ^a	I	- ^a
12	37.81	I	79.20	119.35	I	- ^a	- ^a	- ^a	I	- ^a
13	34.10	88.73	59.30	94.65	114.30	- ^a	- ^a	- ^a	92.04	- ^a
14	6.68	101.75	21.80	47.38	58.13	- ^a	- ^a	- ^a	84.13	17.48
15	- ^a	- ^a	63.08	49.07	- ^a	51.40	58.41	28.04	14.02	- ^a
16	124.32	I	77.54	I	I	- ^a	- ^a	- ^a	I	- ^a
17	- ^a	- ^a	63.08	60.75	- ^a	107.48	63.08	42.06	60.75	- ^a
18	- ^a	- ^a	23.32	41.45	- ^a	56.99	6.99	5.18	5.96	- ^a
19	13.83	75.45	65.32	60.81	60.81	- ^a	- ^a	- ^a	- ^a	- ^a
20	7.64	27.36	- ^a	87.06	73.38	- ^a	- ^a	- ^a	- ^a	70.97
23	77.98	I	I	I	I	- ^a	- ^a	- ^a	- ^a	- ^a
Doxorubicin	- ^a	0.36 ± 0.05	0.19 ± 0.00	0.40 ± 0.03	0.35 ± 0.02		1.64 ± 0.15		1.64 ± 0.17	3.16 ± 0.10
Etoposide	0.04 ± 0.01	- ^a	- ^a	- ^a	- ^a		- ^a		- ^a	- ^a

I = inactive at IC₅₀ > 50 μg/mL; ^a not determined; etoposide and doxorubicin were used as the reference compounds.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were recorded on a JASCO DIP 1020 polarimeter using cylindrical glass cell (10 mm inner diameter (I.D.) × 10 mm). UV spectra were measured with a UV-1700 Pharma Spec (Shimadzu, Kyoto, Japan) spectrophotometer. Fourier transform infrared (FTIR) spectra were obtained using a universal attenuated total reflectance attached on Perkin Elmer Spectrum One spectrometer (PerkinElmer, Waltham, MA, USA). Nuclear magnetic resonance (NMR) spectra were recorded in a CDCl₃ or C₆D₆ solution containing Me₄Si as internal standard on Bruker AM400 or AVANCE600 spectrometer (Bruker Corporation, Billerica, MA, USA). HR-MS was performed on a Bruker (Micro ToF, Bruker Corporation, Billerica, MA, USA) spectrometer. HPLC was carried out on a Waters 600 system (Waters Corporation, Milford, MA, USA) equipped with a Waters Delta 600 pump, a Waters 600 Controller, a Waters 2998 photodiode array detector, and Waters Empower 2 software. Sephadex™ LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was used for a column gel filtration. All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

3.2. Sponge Material

Sponges (*Hyrtios erectus*) CRI 572 and CRI 588 were collected by hand using scuba at a depth of 30–40 feet in the Similan Island at the Andaman Sea (Phangnga province, Thailand) on 22 and 23 February 2011, respectively. The sponges were identified by Dr. Sumaitt Putchakarn, Head of Marine Biodiversity Research, Unit Curator of Porifera and Echinodermata, Institute of Marine Science, Burapha University, Bangsaen, Chonburi, Thailand. The voucher specimens (CRI 572 and CRI 588) were presently deposited at the Laboratory of Natural Products, Chulabhorn Research Institute, Bangkok, Thailand.

3.3. Extraction and Isolation

A frozen sample (2.6 kg) of *H. erectus* collection CRI 572 was cut into small pieces and extracted exhaustively with MeOH. The extract was filtered through cotton, and then evaporated under reduced pressure to give an aqueous residue, which was partitioned with EtOAc. The organic layer was concentrated to give a dark brown solid (7.52 g). The EtOAc-soluble fraction was subjected to vacuum liquid chromatography on silica gel and eluted with an EtOAc-hexane gradient (0→100% of EtOAc). Nine fractions (F1–F9) were obtained. F2 (340.5 mg) was subjected to column chromatography on Sephadex LH-20, using CH₂Cl₂-MeOH (1:1) to give compound **15** (16 mg). F4 (293.3 mg) was repeatedly fractionated by Sephadex LH-20, using CH₂Cl₂-MeOH (1:1) to give a mixture compounds (96.8 mg) that was separated using on a HPLC column (Hichrome C18, 5 μm, 21.2 mm × 250 mm with MeOH-H₂O (gradient, 77→97% MeOH for 40 min), flowrate 12 mL/min, λ 220, 265 nm) to afford compounds **16** (28 mg, at 12.7 min), **17** (4.9 mg, at 32.5 min). F5 (574.4 mg) was subjected to column chromatography on Sephadex LH-20, using 100% MeOH to obtain a mixture compound (9.3 mg) that was separated using on a HPLC column (Sunfire C18, 5 μm, 10 mm × 250 mm, with 80% CH₃CN-H₂O, flowrate 3 mL/min, λ 220 nm) to afford **18** (4.0 mg, at 25.7 min). F6 (976.4 mg) was subjected to column chromatography on Sephadex LH-20, using CH₂Cl₂-MeOH (1:1) to give three fractions (f1–f3). Fraction f1 was subjected to repeated chromatography on Sephadex LH-20, using 100% MeOH to give a mixture compound (73.5 mg) that was separated using a HPLC column (Hichrome C18, 5 μm, 21.2 mm × 250 mm, with MeOH-H₂O (gradient, 75→100% MeOH for 50 min), flowrate 12 mL/min, λ 220 nm) to afford compound **19** (2.3 mg, at 35.2 min). Fraction f2 was subjected to column chromatography on Sephadex LH-20, using 100% MeOH to give a mixture compound (45.6 mg) that was separated using on a HPLC column (Sunfire C18, 5 μm, 19 mm × 250 mm, with 88% MeOH-H₂O, flowrate 12 mL/min, λ 220 nm) to afford **20** (20 mg, at 16.7 min). Fraction f3 was subjected to repeated chromatography on Sephadex LH-20, using 100% MeOH to give a mixture

compound (6.9 mg) that was separated using on a preparative thin layer chromatography (PTLC) [hexane–CH₂Cl₂–acetone (2:2:1) as eluent] to afford **21** (1.4 mg). F7 (508.1 mg) was subjected to column chromatography on Sephadex LH-20, using 100% MeOH to give a mixtures compound (12.1 mg) that was separated using on a HPLC column (Sunfire C18, 5 μm, 19 mm × 250 mm, with 20% MeOH–H₂O, flowrate 12 mL/min, λ 254 nm) to afford compound **22** (7.5 mg, at 12.8 min). F8 (298.1 mg) was repeatedly fractionated by Sephadex LH-20, using CH₂Cl₂–MeOH (1:1) to give a mixture compound (127.6 mg) that was separated using a HPLC column (Hichrome C18, 5 μm, 21.2 mm × 250 mm with 75% MeOH–H₂O, flowrate 12 mL/min, λ 222 nm) to afford compounds **4** (16.4 mg, at 11.3 min), and **13** (16.1 mg, at 33.5 min). F9 (249.2 mg) was subjected to repeated chromatography on Sephadex LH-20, using CH₂Cl₂–MeOH (1:1) to give a mixtures compound (20.3 mg) that was separated using a HPLC column (Hichrome C18, 5 μm, 21.2 mm × 250 mm, with MeOH–H₂O (45–55% MeOH for 20 min), flowrate 12 mL/min, λ 234 nm) to afford compound **23** (5.4 mg, at 14.7 min). The flesh sponge *Hyrtios erectus* collection CRI 588 (14.0 kg) was cut into small pieces and extracted repeatedly with MeOH (20 L) three times (3 × 20 L). After the evaporation of the solvent, the concentrated MeOH extracts was partitioned between EtOAc and water and the EtOAc fraction was chromatographed on a silica gel column with CH₂Cl₂–hexane (1:1) containing increasing proportions of acetone as eluent, to give 7 fractions (A–G). Fraction C (4.0 g) was fractionated by Sephadex LH-20 with MeOH–CH₂Cl₂ (1:1) as eluent, to give 2 fractions (C1–C2). C2 (230.3 mg) was repeatedly chromatography on Sephadex LH-20, using CH₂Cl₂–MeOH (4:1) to give a mixtures compound (200 mg) that was separated using a HPLC column (Sunfire Prep C18, 5 μm, 10 mm × 250 mm) with MeOH–H₂O (93:7), flowrate 2.5 mL/min, λ 224 nm, to afford compounds **5** (4.1 mg at 45.5 min), and **6** (7.2 mg at 48.2 min). Fraction D (9.0 g) was further fractionated on a Sephadex LH-20 column chromatography with MeOH–CH₂Cl₂ (1:1) to yield 4 fractions (D1–D4). D4 (400 mg) was further purified by HPLC column (Sunfire C18, 5 μm, 19 mm × 250 mm) with CH₃CN–H₂O (80:20), flowrate 8 mL/min, λ 224, 236 nm, to afford compounds **1** (5.9 mg, at 23.0 min), **2** (1.6 mg, at 29.6 min), and **3** (7.2 mg, at 35.6 min). Fraction E (25.0 g) was further fractionated by vacuum liquid chromatography eluted with CH₂Cl₂–hexane (1:1) containing increasing proportions of acetone as eluent, to give 4 fractions (E1–E4). Fraction E1 (2 g) was further purified by a HPLC column (Sunfire C18, 5 μm, 19 mm × 250 mm) with CH₃CN–H₂O (85:15), flowrate 8 mL/min, λ 224, 236 nm, to afford compounds **7** (74 mg, at 32.3 min), **8** (52 mg, at 47.9 min), and **9** (229 mg, 52.8 min). Fraction E3 (3 g) was further purified by a HPLC column (Sunfire C18, 5 μm, 19 mm × 250 mm) with CH₃CN–H₂O (80:20), flowrate 8 mL/min, λ 224, 236 nm, to afford compounds **10** (27 mg, at 19.9 min), and **11** (56 mg, at 32.5 min). Fraction E4 (1.4 g) was further purified by HPLC column (Sunfire C18, 5 μm, 19 mm × 250 mm) with CH₃CN–H₂O (82:18), flowrate 8 mL/min, λ 224, 236 nm, to afford compounds **13** (72 mg, at 27.35 min) and **14** (33 mg, at 29.3 min). Fraction G (4.0 g) was further filtrated on a Sephadex LH-20 column chromatography with MeOH to give a residue (655 mg), which was further purified by HPLC column (Cosmosil, 5 μ C18-MS-II, 20 mm × 250 mm) with CH₃CN–H₂O (60:40), flowrate 8 mL/min, λ 224, 236 nm, to afford compound **12** (70.0 mg, at 64.2 min).

Preparation of (S)- and (R)-MTPA Esters of compound 3: To a solution of **3** (3.1 mg) in pyridine (0.9 mL) was added (R)-MTPA-chloride (17 μL). The mixture was stirred at room temperature for 3 h, checked with thin layer chromatography (TLC) to make sure that the reaction was complete, quenched by the addition of 2 mL of H₂O, and the mixture was subsequently extracted with CH₂Cl₂ (2 mL) three times (3 × 2 mL). The CH₂Cl₂ soluble layers were combined, dried over anhydrous MgSO₄, and evaporated. The residue was subjected to short silica gel column chromatography using hexane–EtOAc (4:1) to give the (S)-MTPA ester **3a** (1.6 mg).

Selected ¹H NMR (CDCl₃ 600 MHz) of **3a**: δ_H 9.32, s, H-24; 6.28, t (*J* = 7.1 Hz), H-6; 5.86, brs, H-2; 5.79, s, H-25; 5.74, dd (*J* = 7.2, 4.2 Hz), H-4; 5.09, t (*J* = 7.1 Hz), H-10; 3.03, ddd (*J* = 16.1, 6.6, 4.2 Hz), Ha-5; 2.90, dt (*J* = 16.1, 8.0 Hz), Hb-5; 2.24, t (*J* = 7.6 Hz), H₂-8; 2.02, m, H₂-9.

The same procedure was used to prepare the (*R*)-MTPA ester **3b** (2.5 mg from 3.9 mg of **3**) with (*S*)-MTPA chloride.

Selected ^1H NMR (CDCl_3 , 600 MHz) of **3b**: δ_{H} 9.21, s, H-24; 6.13, t ($J = 7.1$ Hz), H-6; 6.00, brs, H-2; 5.81, s, H-25; 5.74, dd ($J = 7.0, 4.2$ Hz), H-4; 5.07, t ($J = 6.9$ Hz), H-10; 3.00, ddd ($J = 16.2, 6.8, 4.3$ Hz), Ha-5; 2.87, dt ($J = 16.2, 7.5$ Hz), Hb-5; 2.20, t ($J = 7.7$ Hz), H₂-8; 1.99, m, H₂-9

(*S*)-MTPA esters **5a** (1.3 mg from 1.4 mg of **5**) and **6a** (1.8 mg from 1.4 mg of **6**) and (*R*)-MTPA esters **5b** (1.5 mg from 1.4 mg of **5**), **6b** (1.7 mg from 1.2 mg of **6**) were prepared by the method mentioned above.

Selected ^1H NMR (CDCl_3 , 400 MHz) of **5a**: δ_{H} 5.77, brs, H-3; 2.72, dd ($J = 15.7, 6.9$ Hz), Ha-1'; 2.62, dd ($J = 15.7, 3.1$ Hz), Hb-1'; 5.28, m, H-2'; 1.67, m, H₂-3'; 4.56, dd ($J = 17.8, 1.8$ Hz), Ha-5; 4.25, brd ($J = 17.6$ Hz), Hb-5.

Selected ^1H NMR (CDCl_3 , 400 MHz) of **5b**: δ_{H} 5.86, s, H-3; 2.78, dd ($J = 15.7, 7.3$ Hz), Ha-1'; 2.69, dd ($J = 15.7, 3.1$ Hz), Hb-1'; 5.29, m, H-2'; 1.63, m, H₂-3'; 4.69, brd ($J = 17.4$ Hz), Ha-5; 4.52, brd ($J = 17.4$ Hz), Hb-5.

Selected ^1H NMR (CDCl_3 , 600 MHz) of **6a**: δ_{H} 5.77, brs, H-3; 2.72, dd ($J = 15.6, 7.1$ Hz), Ha-1'; 2.63, dd ($J = 15.6, 3.4$ Hz), Hb-1'; 5.28, m, H-2'; 1.71, m, Ha-3'; 1.59, m, Hb-3'; 1.29, m, H₂-4'; 4.57, dd ($J = 17.8, 1.6$ Hz), Ha-5; 4.26, brd ($J = 16.7$ Hz), Hb-5.

Selected ^1H NMR (CDCl_3 , 600 MHz) of **6b**: δ_{H} 5.86, brs, H-2; 2.78, dd ($J = 15.4, 6.58$ Hz), Ha-1'; 2.69, dd ($J = 15.4, 3.6$ Hz), Hb-1'; 5.29, m, H-2'; 1.67, m, Ha-3'; 1.55, m, Hb-3'; 1.23, m, H₂-4'; 4.69, d ($J = 17.5$ Hz), Ha-5; 4.52, d ($J = 17.5$ Hz), Hb-5.

Erectusolide B (**1**): Colorless powder; $[\alpha]_{\text{D}}^{26} -1.3$ (c 0.56, CHCl_3); UV λ_{max} (MeOH) nm ($\log \epsilon$) 202 (3.9), 224 (3.4), IR (ATR) ν_{max} 3443 (br), 2927, 2861, 1779, 1746, 1629, 1456, 1379, 1266, 1143, 1041, 888, 851, 735, 702 cm^{-1} ; ^1H and ^{13}C NMR data see Table 1; HRAPCIMS m/z 385.2727 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{25}\text{H}_{37}\text{O}_3$, 385.2737).

Erectusolide C (**2**): Colorless powder; $[\alpha]_{\text{D}}^{26} -18.5$ (c 0.16, CHCl_3); UV λ_{max} (MeOH) nm ($\log \epsilon$) 234 (4.3); IR (ATR) ν_{max} 2924, 2855, 1779, 1748, 1682, 1632, 1458, 1378, 1261, 1142, 1040, 887 cm^{-1} ; ^1H and ^{13}C NMR data see Table 1; HRESIMS m/z 405.2390 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{25}\text{H}_{34}\text{NaO}_3$, 405.2400).

seco-Manoalide-25-methyl ether (**3**): Pale yellow solid; $[\alpha]_{\text{D}}^{24} -22.2$ (c 1.86, CHCl_3); UV λ_{max} (MeOH) nm ($\log \epsilon$) 203.5 (4.3), 232 (sh); IR (ATR) ν_{max} 3446 (br), 2930, 2863, 1762, 1687, 1457, 1371, 1203, 1120, 1070, 960, 898, 870, 735, 702 cm^{-1} ; ^1H and ^{13}C NMR data see Table 2; HRAPCIMS m/z 465.2414 $[\text{M} + \text{Cl}]^-$ (calcd for $\text{C}_{26}\text{H}_{38}\text{ClO}_5$, 465.2413).

Erectusolide D (**4**): Pale yellow gum; $[\alpha]_{\text{D}}^{25} -20.9$ (c 1.64, CHCl_3); UV λ_{max} (MeOH) nm ($\log \epsilon$) 202 (4.3), IR (ATR) ν_{max} 3424 (br), 2928, 2857, 1739, 1642, 1456, 1382, 1258, 1172, 1063, 898 cm^{-1} ; ^1H and ^{13}C NMR data see Table 2; HRAPCIMS m/z 453.2418 $[\text{M} + \text{Cl}]^-$ (calcd for $\text{C}_{25}\text{H}_{38}\text{ClO}_5$, 453.2413).

erectusfuranone A (**5**): Colorless powder; $[\alpha]_{\text{D}}^{25} +1.3$ (c 0.32, CHCl_3); UV λ_{max} (MeOH) nm ($\log \epsilon$) 206 (3.9); IR (ATR) ν_{max} 3442 (br), 2923, 2853, 1777, 1739, 1637, 1457, 1378, 1175, 1027, 888, 720 cm^{-1} ; ^1H and ^{13}C NMR data see Table 3; HRAPCIMS m/z 367.3213 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{23}\text{H}_{43}\text{O}_3$, 367.3207).

erectusfuranone B (**6**): Colorless powder; $[\alpha]_{\text{D}}^{26} -4.9$ (c 0.61, CHCl_3); UV λ_{max} (MeOH) nm ($\log \epsilon$) 206 (3.9); IR (ATR) ν_{max} 3402 (br), 2916, 2851, 1779, 1756, 1736, 1634, 1469, 1025, 892, 720 cm^{-1} ; ^1H and ^{13}C NMR data see Table 3; HRESIMS m/z 353.3053 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{22}\text{H}_{41}\text{O}_3$, 353.3050).

3.4. Cytotoxicity Assay

Cytotoxic activity for adhesive cell lines including HepG2, HeLa, HuCCA-1, A549, H69AR, KB, T47D, MDA-MB-231, and MRC-5 cell lines were evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay [25,26]. For the non-adhesive MOLT-3 cell line, the

cytotoxicity was assessed using the 2,3-bis-(2-methoxy-4-nitro-5-sulphenyl-(2H)-tetrazolium-5-carboxanilide (XTT) assay [27]. Etoposide and doxorubicin were used as positive controls (Table 4)

4. Conclusions

Two separate collections of *H. erectus* were studied. Three new sesterterpenes, erectusolides B (1), C (2), and seco-manoalide-25-methyl ether (3), two new 2-furanone derivatives, erectusfuranones A (5) and B (6) together with eight known sesterterpenes, 7–14 were isolated from *H. erectus* collection CRI 588. One additional new sesterterpene, erectusolide D (4), and ten known compounds (13, 15–23) were also obtained from the *H. erectus* collection CRI 572. Three sesterterpenes 8, 9, and 20 showed good cytotoxic activity against the MOLT-3 cell line with IC₅₀ values of 1.30–7.64 μM and with SI (selectivity index) values 7–9.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1660-3397/16/12/474/s1>, Figure S1. ¹H NMR spectrum (600 MHz) of compound 1 in C₆D₆, Figure S2. ¹³C NMR spectrum (150 MHz) of compound 1 in C₆D₆, Figure S3. HSQC spectrum of compound 1 in C₆D₆, Figure S4. HMBC spectrum of compound 1 in C₆D₆, Figure S5. ¹H NMR spectrum (600 MHz) of compound 2 in CDCl₃, Figure S6. ¹H NMR spectrum (600 MHz) of compound 2 in C₆D₆, Figure S7. ¹³C NMR spectrum (150 MHz) of compound 2 in CDCl₃, Figure S8. ¹³C NMR spectrum (150 MHz) of compound 2 in C₆D₆, Figure S9. HSQC spectrum of compound 2 in CDCl₃, Figure S10. HSQC spectrum of compound 2 in C₆D₆, Figure S11. HMBC spectrum of compound 2 in CDCl₃, Figure S12. HMBC spectrum of compound 2 in C₆D₆, Figure S13. ¹H NMR spectrum (600 MHz) of compound 3 in CDCl₃, Figure S14. ¹³C NMR spectrum (150 MHz) of compound 3 in CDCl₃, Figure S15. HSQC spectrum of compound 3 in CDCl₃, Figure S16. HMBC spectrum of compound 3 in CDCl₃, Figure S17. ¹H NMR spectrum (600 MHz) of compound 4 in CDCl₃, Figure S18. ¹³C NMR spectrum (150 MHz) of compound 4 in CDCl₃, Figure S19. HMQC spectrum of compound 4 in CDCl₃, Figure S20. HMBC spectrum of compound 4 in CDCl₃, Figure S21. ¹H NMR spectrum (600 MHz) of compound 5 in CDCl₃, Figure S22. ¹³C NMR spectrum (150 MHz) of compound 5 in CDCl₃, Figure S23. HSQC spectrum of compound 5 in CDCl₃, Figure S24. HMBC spectrum of compound 5 in CDCl₃, Figure S25. ¹H NMR spectrum (600 MHz) of compound 6 in CDCl₃, Figure S26. ¹³C NMR spectrum (150 MHz) of compound 6 in CDCl₃, Figure S27. HSQC spectrum of compound 6 in CDCl₃, Figure S28. HMBC spectrum of compound 6 in CDCl₃.

Author Contributions: Conceptualization, C.M. and S.R.; Funding acquisition, C.M.; Investigation, W.K. and H.P.; Project administration, S.R.; Supervision, S.R.; Validation, H.P.; Writing—original draft, H.P.; Writing—review & editing, P.T. and S.R.

Funding: This research received no external funding.

Acknowledgments: The authors thank P. Intachote, S. Sengsai and B. Saimanee for testing cytotoxic activity.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Bugni, S.T.; Ireland, C.M. Marine-derived fungi: A chemically and biologically diverse group of microorganisms. *Nat. Prod. Rep.* **2004**, *21*, 143–163. [[CrossRef](#)] [[PubMed](#)]
2. Blunt, J.W.; Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2018**, *35*, 8–53. [[CrossRef](#)] [[PubMed](#)]
3. Kawetripob, W.; Mahidol, C.; Wongbundit, S.; Tuntiwachwuttikul, P.; Ruchirawat, S.; Prawat, H. Sesterterpenes and phenolic alkenes from the Thai sponge *Hyrtios erectus*. *Tetrahedron* **2018**, *74*, 316–323. [[CrossRef](#)]
4. Jiao, W.H.; Hong, L.L.; Sun, J.B.; Piao, S.J.; Chen, G.D.; Deng, H.; Wang, S.P.; Yang, F.; Lin, H.W. (±)-Hippolide J—A Pair of Unusual Antifungal Enantiomeric Sesterterpenoids from the Marine Sponge *Hippospongia lachne*. *Eur. J. Org. Chem.* **2017**, *24*, 3421–3426. [[CrossRef](#)]
5. Hawas, U.W.; Abou El-Kassem, L.T.; Abdelfattah, M.S.; Elmallah, M.I.Y.; Eid, M.A.G.; Monier, M.; Nithiyanandam, M. Cytotoxic activity of alkyl benzoate and fatty acids from the red sea sponge *Hyrtios erectus*. *Nat. Prod. Res.* **2018**, *32*, 1369–1374. [[CrossRef](#)] [[PubMed](#)]

6. Elhady, S.S.; Al-Abd, A.M.; El-Halawany, A.M.; Alahdal, A.M.; Hassanean, H.A.; Ahmed, S.A. Antiproliferative Scalarane-Based Metabolites from the Red Sea Sponge *Hyrtios erectus*. *Mar. Drugs* **2016**, *14*, 130. [[CrossRef](#)] [[PubMed](#)]
7. Sakai, E.; Kato, H.; Rotinsulu, H.; Losung, F.; Mangindaan, R.E.P.; de Voogd, N.J.; Yokosawa, H.; Tsukamoto, S. Variabines A and B: New β -carboline alkaloids from the marine sponge *Luffariella variabilis*. *J. Nat. Med.* **2014**, *68*, 215–219. [[CrossRef](#)] [[PubMed](#)]
8. Mahidol, C.; Prawat, H.; Sangpetsiripan, S.; Ruchirawat, S. Bioactive Scalaranes from the Thai Sponge *Hyrtios gumminae*. *J. Nat. Prod.* **2009**, *72*, 1870–1874. [[CrossRef](#)] [[PubMed](#)]
9. Konig, G.; Wright, A.D.; Sticher, O. Four new antibacterial sesterterpenes from a marine sponge of the genus *Luffariella*. *J. Nat. Prod.* **1992**, *55*, 174–178. [[CrossRef](#)]
10. Namikoshi, M.; Suzuki, S.; Meguro, S.; Nagai, H.; Koike, Y.; Kitazawa, A.; Kobayashi, H.; Oda, T.; Yamada, J. Manoalide derivatives from a marine sponge *Luffariella* sp. collected in Palau. *Fish. Sci.* **2004**, *70*, 152–158. [[CrossRef](#)]
11. Pailee, P.; Mahidol, C.; Ruchirawat, S.; Prachyawarakorn, V. Sterols from Thai Marine Sponge *Petrosia* (Strongylophora) sp. and Their Cytotoxicity. *Mar. Drugs* **2017**, *15*, 54. [[CrossRef](#)] [[PubMed](#)]
12. Kobayashi, M.; Okamoto, T.; Hayashi, K.; Yokoyama, N.; Sasaki, T.; Kitagawa, I. Marine Natural Products. XXXII. Absolute Configurations of C-4 of the Manoalide Family, Biologically Active Sesterterpenes from the Marine Sponge *Hyrtios erecta*. *Chem. Pharm. Bull.* **1994**, *42*, 265–270. [[CrossRef](#)] [[PubMed](#)]
13. Doi, Y.; Shigemori, H.; Ishibashi, M.; Mizobe, F.; Kawashima, A.; Nakaie, S.; Kobayashi, J. New Sesterterpenes with Nerve Growth Factor Synthesis-Stimulating Activity from the Okinawan Marine Sponge *Hyrtios* sp. *Chem. Pharm. Bull.* **1993**, *41*, 2190–2191. [[CrossRef](#)] [[PubMed](#)]
14. Ashour, M.A.; Elkhayat, E.S.; Ebel, R.; Edrada, R.; Proksch, P. Indole alkaloid from the Red Sea sponge *Hyrtios erectus*. *Arkivoc* **2007**, *XV*, 225–231.
15. Tsuda, M.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. Luffariolides AE, new cytotoxic sesterterpenes from the Okinawan marine sponge *Luffariella* sp. *J. Org. Chem.* **1992**, *57*, 3503–3507. [[CrossRef](#)]
16. Tsukamoto, S.; Miura, S.; van Soest, R.W.M.; Ohta, T. Three New Cytotoxic Sesterterpenes from a Marine Sponge *Spongia* sp. *J. Nat. Prod.* **2003**, *66*, 438–440. [[CrossRef](#)] [[PubMed](#)]
17. De Silva, E.D.; Scheuer, P.J. Three new sesterterpenoid antibiotics from the marine sponge *Luffariella variabilis* (Polejaff). *Tetrahedron Lett.* **1981**, *22*, 3147–3150. [[CrossRef](#)]
18. Prawat, H.; Mahidol, C.; Kawetripob, W.; Prachyawarakorn, V.; Tuntiwachwuttikul, P.; Ruchirawat, S. Sesquiterpene isocyanides, isothiocyanates, thiocyanates, and formamides from the Thai sponge *Halichondria* sp. *Tetrahedron* **2016**, *72*, 4222–4229. [[CrossRef](#)]
19. Prawat, H.; Mahidol, C.; Kawetripob, W.; Wittayalai, S.; Ruchirawat, S. Iodo-sesquiterpene hydroquinone and brominated indole alkaloids from the Thai sponge *Smenospongia* sp. *Tetrahedron* **2012**, *68*, 6881–6886. [[CrossRef](#)]
20. Prawat, H.; Mahidol, C.; Wittayalai, S.; Intachote, P.; Kanchanapoom, T.; Ruchirawat, S. Nitrogenous sesquiterpenes from the Thai marine sponge *Halichondria* sp. *Tetrahedron* **2011**, *67*, 5651–5655. [[CrossRef](#)]
21. Bergquist, P.R.; Cambie, R.C.; Kernan, M.R. Scalarane sesterterpenes from *Collosporgia auris*, a new thorectid sponge. *Biochem. Syst. Ecol.* **1990**, *18*, 349–357. [[CrossRef](#)]
22. Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. Hyrtiosins A and B, new indole alkaloids from the Okinawan marine sponge *Hyrtios erecta*. *Tetrahedron* **1990**, *46*, 7699–7702. [[CrossRef](#)]
23. Jefford, C.W.; Jaggi, D.; Bernardinelli, G.; Boukouvalas, J. The synthesis of (\pm)-cavernosine. *Tetrahedron Lett.* **1987**, *28*, 4041–4044. [[CrossRef](#)]
24. Ohtani, I.; Kusumi, T.; Ishitsuka, M.O.; Kakisawa, H. Absolute configurations of marine diterpenes possessing a xenicane skeleton. An application of an advanced Mosher's method. *Tetrahedron Lett.* **1989**, *30*, 3147–3150. [[CrossRef](#)]
25. Carmichael, J.; DeGraff, W.G.; Gazdar, A.F.; Minna, J.D.; Mitchell, J.B. Evaluation of a tetrazolium-based semi-automated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936–942. [[PubMed](#)]

26. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [[CrossRef](#)]
27. Doyle, A.; Griffiths, J.B. *Mammalian Cell Culture: Essential Techniques*; John Wiley & Sons: Chichester, UK, 1997.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).