FULL PAPER

Trichodermates A – F, New Cytotoxic Trichothecenes from the Plant Pathogenic Fungus *Trichoderma* sp.

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Trichodermates A – F (**1** – **6**, resp.), six new trichothecene polyunsaturated octadioic acid esters, and (−)-harzianum B (**7**) were isolated from the fermentation extract of *Trichoderma* sp., a plant pathogenic fungus isolated from stem rot of an unidentified tree in Thailand. The structures of **1** – **7** were elucidated by NMR experiments. The absolute configuration at C(12) in **3** was assigned by *in situ* dimolybdenum circular dichroism method, whereas that in **7** was deduced after hydrolysis of **7** to **8** *via* modified *Mosher*'s method. Compounds **1** and **2** showed modest cytotoxic activities against the K562 (human myelogenous leukemia) cell line with IC_{50} values of 12.12 and 13.08 μM, respectively.

Introduction. – Mycotoxins are toxic secondary metabolites produced by different types of fungi, belonging mainly to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Alternaria* [1]. Currently, more than 400 mycotoxins are known. The six major classes of frequently occuring mycotoxins are aflatoxins, trichothecenes, fumonisins, zearalenones, ochratoxins, and ergot alkaloids [1]. Trichothecenes, produced by several species of *Fusarium*, *Myrothecium*, *Stachybotrys*, *Trichoderma*, and *Trichothecium*, are the largest group of mycotoxins known to date, consisting of more than 180 structurally-related toxic compounds, but only a few are significant to human health [2]. Trichothecenes show various biological activities, such as cytotoxic and antitumor activities [3 – 6], and are extremely potent inhibitors of protein and/or DNA synthesis [7]. The 12,13-epoxide group of trichothecenes is essential for the inhibition of protein synthesis, and reduction of the C(9)=C(10) bond reduces toxicity [8]. *Trichoderma* is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. Many species of this genus can be characterized as opportunistic avirulent plant symbionts [9]. Some *Trichoderma* sp. produce enzymes and antibiotics and were used as biocontrol agents against plant diseases [10].

During an ongoing search for biologically active natural products from plant endophytic or pathogenic fungi [11], we initiated chemical investigation on *Trichoderma* sp. which was isolated from stem rot of an unidentified tree in Thailand. Bioassay-directed fractionation of the AcOEt extract prepared from the solid-substrate fermentation products resulted in the isolation of six new trichothecene polyunsaturated octadioic acid esters, which we named trichodermates A – F (**1** – **6**, resp.), and (−)-harzianum B (**7**; *Fig. 1*). Details of the isolation, structure elucidation, and cytotoxic activities of these metabolites are reported herein.

Results and Discussion. – The AcOEt extract of solid-substrate fermentation of *Trichoderma* sp. was subjected to chromatographic separation using silica gel, *ODS*, and *Sephadex LH-20*, and finally purified by preparative HPLC to yield six new trichothecene derivatives, trichodermates A – F (**1** – **6**, resp.), and (−)-harzianum B (**7**). Compound **7** was assigned the same constitutional formula and relative configuration as harzianum B by comparison of its spectroscopic data with the literature data [6]. However, the negative value of the optical rotation of **7** ($[\alpha]_D^{25} = -29.0$ ($c = 0.10$, $CHCl_3$) as opposed to $[\alpha]_D^{25} = +27.94$ ($c = 0.3$, $CHCl_3$) in [6]), indicated that **7** was the enantiomer of (+)-harzianum B. The absolute configuration of **7** was determined after hydrolysis of **7** to **8** by modified *Mosher*'s method [12]. Compound **8** was treated with (−)-(*R*)- and (+)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl), respectively, to give the corresponding (*S*)- and (*R*)-*Mosher* monoesters **8s** and **8r**. Interpretation of the ^{1}H-NMR chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) between **8s** and **8r**, established the absolute configuration at C(4) as (*R*) (*Fig. 2*). Related to the absolute configuration at C(4), the absolute configuration of **7** was assigned as (2*R*,4*R*,5*S*,6*R*,11*R*,12*S*).

Trichodermate A (**1**) was assigned the molecular formula of $C_{24}H_{32}O_6$ (nine degrees of unsaturation) on the basis of HR-ESI-MS. Analysis of the ^{1}H- and ^{13}C-NMR data (*Table 1*) revealed the presence of four Me (including one MeO), six CH_2, and three CH–O groups, three sp^3 C_q-atoms (one O-bearing), six olefinic C-atoms (five H-bearing), and two carboxylate C-atoms (δ(C) 173.3 and 166.4, resp.). These data accounted for all NMR resonances of **1** and five of the nine degrees of unsaturation, suggesting that **1** was a tetracyclic compound. Interpretation of the ^{1}H,^{1}H-COSY data of **1** revealed the presence of three isolated spin systems, which were CH(2)–

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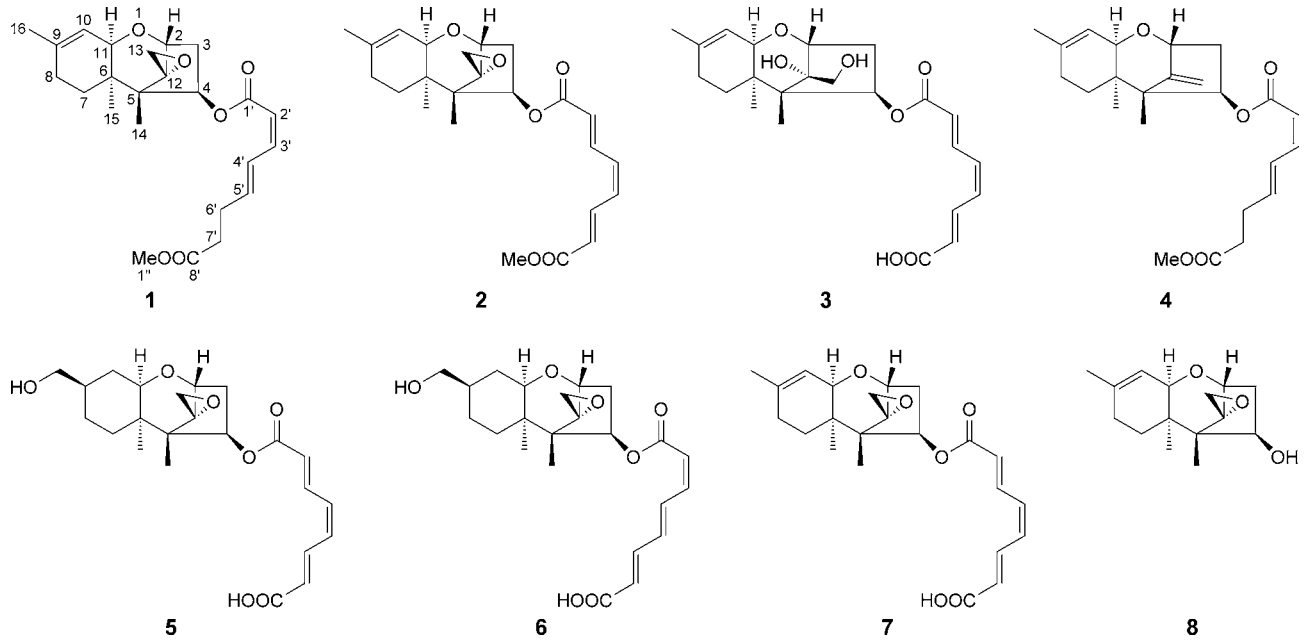

Fig. 1. *Structures of* **1**–**8**

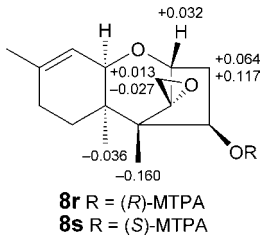

Fig. 2. $\Delta\delta$ $(=\delta_S - \delta_R)$ *Values obtained from MTPA esters* **8r** *and* **8s**

CH_2(3)–CH(4), CH_2(7)–CH_2(8)–C(9)=CH(10)–CH(11), and CH(2′)=CH(3′)–CH(4′)=CH(5′)–CH_2(6′)–CH_2(7′). In the HMBC spectrum of **1**, the correlations Me(14)/C(5,6,12), Me(15)/C(5,6,7), C(11) (δ(C) 70.7), H–C(11) (δ(H) 3.63)/C(2) (δ(C) 79.3), C(5,6,7), C(9) (δ(C) 140.4), C(10) (δ(C) 118.8), C(15), and H–C(2) (δ(H) 3.83)/C(5,11,12) established the C(6)/C(11)-fused hexahydro-2*H*-1-benzopyran moiety, with Me(14) and Me(15) attached to C(5) and C(6), respectively. The additional HMB correlations Me(16)/C(8,9,10) and CH_2(8), H–C(10)/C(16) indicated that the third Me group was attached to C(9). The HMB correlations CH_2(3)/C(2,5,12), H–C(4)/C(2,5,6,12), and Me(14)/C(4), indicated that the fragment C(3)–C(4) was connected to C(2) and C(5), respectively, establishing the third ring fused *via* C(2)–C(12)–C(5). The HMBC cross peaks H–C(4,3′)/C(1′) and CH_2(6′,7′)/C(8′) revealed that the 1′-*O*-1′,8′-dicarboxylate unit was attached to C(4). In addition, the MeO group was located at C(8′) which was supported by the correlation Me(1″)/C(8′). The HMB correlations CH_2(13)/C(2,5,12) confirmed that C(13) was connected to C(12). Based on the chemical shifts of C(12) (δ(C) 65.7) and C(13) (δ(C) 48.0), the degrees of unsaturation, and the number of O-atoms in the molecular formula of **1**, the remaining one degree of unsaturation was due to an epoxide ring at C(12) and C(13). Therefore, the constitutional formula of trichodermate A (**1**) was proposed.

The relative configuration of **1** was deduced by ^{1}H,^{1}H coupling constants and NOESY data. The C(2′)=C(3′) bond was assigned (*Z*) geometry on the basis of the coupling constant ($J = 11.4$) observed between H–C(2′) and H–C(3′), whereas the C(4′)=C(5′) bond was assigned (*E*) geometry due to the large coupling constant ($J = 15.2$) observed between H–C(4′) and H–C(5′). The NOESY correlations H–C(4)/H–C(11), Me(15) and H–C(11)/H_a–C(3), Me(15) indicated that these H-atoms were located at the same side of the 2-oxabicyclo[3.2.1]octane ring. In turn, the correlations H–C(2)/H_a–C(13) and H_b–C(13)/H_b–C(7,8), Me(14) showed that these H-atoms were located at the opposite side, thereby establishing the relative configuration of trichodermate A (**1**). Considering the identical relative configurations and similar optical rotation values of **1** and **7**, the absolute configuration of **1** was assigned as (2*R*,4*R*,5*S*,6*R*,11*R*,12*S*).

The elemental composition of trichodermate B (**2**) was determined to be $C_{24}H_{30}O_6$ (ten degrees of unsaturation) by HR-ESI-MS, which means that M_r is two mass units lower than for **1**. Analysis of the NMR data (*Table 1*) revealed that **2** possesses a similar structure to **1**, except that two CH_2 groups (δ(H) 2.53 – 2.52, δ(C) 28.1 and δ(H) 2.48 – 2.47, δ(C) 33.2, resp.) in **1** were replaced by an olefin unit (δ(H) 7.32, δ(C) 143.2 and δ(H) 6.07, δ(C) 124.7, resp.) in **2**. These observations were supported by ^{1}H,^{1}H-COSY and HMB correlations. By comparison of the ^{1}H,^{1}H coupling constants and NOESY data of **2** with those of **1**, the relative configuration of **2** was deduced to be the same as that of **1**, except for the configurations of the 1′,8′-dicarboxylate side chain. The large coupling constants ($J = 15.4$) observed between H–C(2′) and H–C(3′), H–C(6′) and H–C(7′)

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *for* **1** – **3**. δ in ppm, J in Hz.

Position	**1**[a])			**2**[a])		**3**[b])	
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)	δ(H)	δ(C)
2	3.83 (*d*, *J* = 5.3)	79.3	4, 5, 11, 12	3.84 (*d*, *J* = 5.2)	79.3	4.10 (*dd*, *J* = 11.5, 6.3)	77.9
3	2.55 (*dd*, *J* = 15.5, 7.8), 2.02 (*ddd*, *J* = 15.5, 5.3, 3.6)	37.0	2, 4, 5, 12	2.57 (*dd*, *J* = 15.5, 7.8), 2.03 (*ddd*, *J* = 15.5, 5.3, 3.6)	36.8	2.34 (*dd*, *J* = 11.4, 5.6), 1.75 (*dd*, *J* = 11.4, 11.4)	37.9
4	5.63 (*dd*, *J* = 7.8, 3.6)	74.8	2, 5, 6, 12, 1′	5.65 (*dd*, *J* = 7.8, 3.6)	75.5	5.13 (*dd*, *J* = 11.6, 5.6)	72.9
5		49.2			49.4		57.8
6		40.6			40.6		44.1
7	1.96 – 1.92 (*m*), 1.43 – 1.40 (*m*)	24.6	6, 8, 9, 11	1.96 – 1.92 (*m*), 1.43 – 1.40 (*m*)	24.6	1.67 – 1.63 (*m*), 1.28 – 1.26 (*m*)	26.1
8	1.99 – 1.97 (*m*)	28.1	6, 7, 9, 10, 16	1.99 – 1.97 (*m*)	28.1	2.01 – 1.99 (*m*), 1.90 – 1.86 (*m*)	28.4
9		140.4			140.4		139.9
10	5.41 (*d*, *J* = 5.4)	118.8	6, 8, 11, 16	5.41 (*d*, *J* = 5.8)	118.7	5.48 (*d*, *J* = 5.5)	119.5
11	3.63 (*d*, *J* = 5.4)	70.7	2, 5 – 7, 9, 10, 15	3.63 (*d*, *J* = 5.8)	70.7	3.75 (*d*, *J* = 5.5)	77.4
12		65.7			65.7		90.9
13	3.12 (*d*, *J* = 4.1), 2.83 (*d*, *J* = 4.1)	48.0	2, 5, 12	3.13 (*d*, *J* = 4.0), 2.83 (*d*, *J* = 4.0)	48.0	3.79 (*d*, *J* = 11.7), 3.74 (*d*, *J* = 11.7)	65.2
14	0.72 (*s*)	6.1	4 – 6, 12	0.72 (*s*)	6.1	1.20 (*s*)	10.7
15	0.95 (*s*)	16.2	5 – 7, 11	0.95 (*s*)	16.2	0.70 (*s*)	13.6
16	1.71 (*s*)	23.4	8 – 10	1.71 (*s*)	23.4	1.68 (*s*)	23.4
1′		166.4			166.4		165.9
2′	5.62 (*d*, *J* = 11.4)	116.6	3′, 4′, 6′	6.03 (*d*, *J* = 15.4)	124.4	6.13 (*d*, *J* = 15.4)	125.2
3′	6.54 (*t*, *J* = 11.4)	145.2	1′, 4′, 5′	7.32 (*dd*, *J* = 15.4, 10.2)	143.1	7.35 (*dd*, *J* = 15.4, 10.3)	143.9
4′	7.43 (*dd*, *J* = 15.2, 11.4)	128.0	2′, 3′, 6′	6.61 (*dd*, *J* = 10.2, 10.2)	137.1	6.89 (*dd*, *J* = 10.3, 10.3)	137.8
5′	6.06 (*dt*, *J* = 15.2, 6.6)	142.9	3′, 4′, 6′, 7′	6.64 (*dd*, *J* = 10.2, 10.2)	137.3	6.89 (*dd*, *J* = 10.3, 10.3)	138.5
6′	2.53 – 2.52 (*m*)	28.1	4′, 5′, 7′, 8′	7.32 (*dd*, *J* = 15.4, 10.2)	143.2	7.39 (*dd*, *J* = 15.4, 10.3)	144.0
7′	2.48 – 2.47 (*m*)	33.2	5′, 6′, 8′	6.07 (*d*, *J* = 15.4)	124.7	6.14 (*d*, *J* = 15.4)	125.2
8′		173.3			167.0		165.9
1″	3.68 (*s*)	51.8	8′	3.77 (*s*)	51.9		

[a]) Recorded in $CDCl_3$. [b]) Recorded in (D_6)acetone.

indicated that the C(2′)=C(3′) and C(6′)=C(7′) bonds possess (*E*) geometry, whereas the C(4′)=C(5′) bond was assigned (*Z*) geometry due to the coupling constant (*J* = 10.2) observed between H–C(4′) and H–C(5′). The absolute configuration of the tetracyclic structure skeleton in **2** was determined to be the same as in **7** based on the identical relative configurations and similar optical rotation values.

The molecular formula of trichodermate C (**3**) was established as $C_{23}H_{30}O_7$ (nine degrees of unsaturation) on the basis of HR-ESI-MS data, which means that M_r is 18 mass units higher than for **7**. Analysis of the NMR data (*Table 1*) revealed that **3** possesses a similar structure to **7**, except that the chemical shifts of C(12) (δ(C) 65.5) and CH_2(13) (δ(H) 3.14 and 2.85, δ(C) 47.8) in **7** were obviously downfield shifted in **3** (δ(C) 90.9 and δ(H) 3.79 and 3.74, δ(C) 65.2, resp.). Considering the additional 18 mass units compared to **7**, the epoxide ring at C(12) and C(13) was opened to a 1,2-diol moiety in **3**. These observations were supported by the HMBC cross-peaks H–C(2)/C(12,13), CH_2(3)/C(12), CH_2(13)/C(2,5,12), and Me(14)/C(12). By comparison of the ^{1}H,^{1}H coupling constants and NOESY data of **3** with those of **2**, the relative configuration of **3** was deduced to be the same as that of **2**.

The absolute configuration at C(12) of the acyclic 12,13-diol unit in **3** was assigned using the *in situ* dimolybdenum circular dichroism (CD) method developed by *Frelek* [13 – 15]. Upon addition of $(AcO)_4Mo_2$ to a solution of **3** in DMSO, a metal complex was generated as an auxiliary chromophore. Since the contribution from the inherent CD resulting from the C(1′)=O group was subtracted to give the induced CD of the complex, the observed sign of the *Cotton* effect in the induced spectrum originates solely from the chirality of the *vic*-diol moiety expressed by the sign of the O–C–C–O torsion angle. The positive *Cotton* effect observed at 310 nm in the induced CD spectrum (*Fig. 3*) permitted assignment of the configuration (12*S*) on the basis of the empirical rule proposed by *Snatzke* [15]. In agreement with the relative configuration established by NOESY data, the configuration (2*R*,4*R*,5*S*,6*R*,11*R*,12*S*) was assigned for **3**.

The molecular formula of trichodermate D (**4**) was assigned as $C_{24}H_{32}O_5$ (nine degrees of unsaturation) on the basis of HR-ESI-MS data. Analysis of the NMR data (*Table 2*) revealed that **4** possesses a similar structure to **1**, except that the epoxide ring at C(12) (δ(C) 65.7) and C(13) (δ(H) 3.12 and 2.83, δ(C) 48.0) in **1** was replaced by a C=C bond in **4** (δ(C) 152.8 and δ(H) 5.12 and 4.70, δ(C) 105.1, resp.). These observations were supported by the HMBC cross-peaks H–C(2)/C(12,13), CH_2(3)/C(12), H–C(4)/C(12), CH_2(13)/C(2,5,12), and Me(14)/C(12). The relative configuration of **4** was deduced to be the same as that of **1** by comparing their ^{1}H,^{1}H coupling constants and NOESY data. The absolute configurations at C(2), C(4) – C(6), and C(11) in **4** were the same as in **1**, in view of their identical relative configurations and similar optical rotation values.

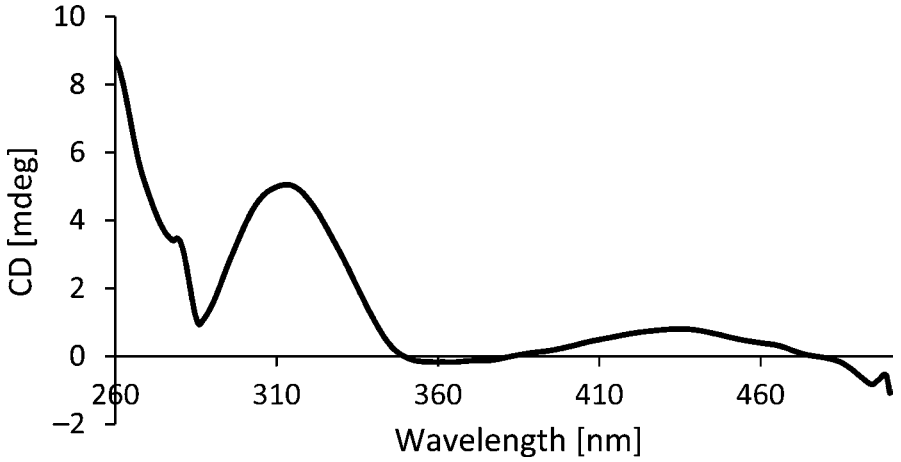

Fig. 3. *CD Spectrum of* **3** *in DMSO containing* $(AcO)_4Mo_2$ *with inherent CD spectrum subtracted*

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; in $CDCl_3$) *for* **4** – **6**. δ in ppm, J in Hz.

Position	**4**		**5**		**6**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
2	4.43 (*d*, *J* = 5.2)	78.9	3.84 (*d*, *J* = 5.1)	79.5	3.87 (*d*, *J* = 5.0)	79.5
3	2.59 (*dd*, *J* = 15.4, 7.7), 1.74 (*ddd*, *J* = 15.4, 5.2, 3.5)	38.1	2.51 (*dd*, *J* = 15.4, 7.8), 2.01 (*ddd*, *J* = 15.4, 5.0, 3.6)	36.9	2.53 (*dd*, *J* = 15.4, 7.7), 2.02 (*ddd*, *J* = 15.4, 5.0, 3.6)	37.0
4	5.61 (*dd*, *J* = 7.7, 3.5)	74.9	5.55 (*dd*, *J* = 7.8, 3.6)	75.4	5.57 (*dd*, *J* = 7.7, 3.6)	75.2
5		52.2		49.8		49.7
6		40.4		41.5		41.4
7	1.86 – 1.80 (*m*), 1.30 – 1.26 (*m*)	23.7	1.88 (*dt*, *J* = 13.0, 3.4), 1.41 (br. *d*, *J* = 13.0)	28.1	1.89 (*dt*, *J* = 13.3, 3.7), 1.43 (br. *d*, *J* = 13.3)	28.1
8	1.98 – 1.95 (*m*)	28.2	1.65 (br. *d*, *J* = 13.2), 1.17 (*dd*, *J* = 13.0, 2.7)	24.7	1.65 (br. *d*, *J* = 13.0), 1.18, (*dd*, *J* = 13.0, 2.8)	24.7
9		140.0	1.81 – 1.77 (*m*)	34.7	1.81 – 1.77 (*m*)	34.7
10	5.37 (*d*, *J* = 5.2)	119.0	1.72 (br. *d*, *J* = 15.2), 1.48 (*dt*, *J* = 15.4, 2.9)	30.3	1.73 (br. *d*, *J* = 14.5), 1.49 (*dt*, *J* = 14.1, 2.8)	30.3
11	3.66 (*d*, *J* = 5.2)	70.6	3.56 – 3.54 (*m*)	72.4	3.57 – 3.55 (*m*)	72.4
12		152.8		65.8		65.8
13	5.12 (*s*), 4.70 (*s*)	105.1	3.17 (*d*, *J* = 4.0), 2.87 (*d*, *J* = 4.0)	48.7	3.19 (*d*, *J* = 4.0), 2.89 (*d*, *J* = 4.0)	48.7
14	0.95 (*s*)	10.4	0.69 (*s*)	5.8	0.70 (*s*)	6.0
15	0.95 (*s*)	16.4	1.03 (*s*)	17.8	1.04 (*s*)	17.9
16	1.68 (*s*)	23.4	3.45 – 3.43 (*m*), 3.48 – 3.46 (*m*)	68.2	3.45 – 3.43 (*m*), 3.50 – 3.48 (*m*)	68.2
1′		166.2		166.3		166.8
2′	5.57 (*d*, *J* = 11.4)	116.5	6.09 (*d*, *J* = 15.3)	125.2	5.86 (*d*, *J* = 11.4)	121.6
3′	6.53 (*t*, *J* = 11.4)	145.2	7.33 (*dd*, *J* = 15.3, 10.2)	143.0	6.65 (*dd*, *J* = 11.4, 11.4)	142.8
4′	7.38 (*dd*, *J* = 15.2, 11.4)	127.9	6.65 (*dd*, *J* = 10.2, 10.2)	137.9	7.93 (*dd*, *J* = 14.8, 11.4)	136.7
5′	6.06 (*dt*, *J* = 15.2, 6.6)	142.8	6.66 (*dd*, *J* = 10.2, 10.2)	136.9	6.56 (*dd*, *J* = 14.8, 11.4)	137.5
6′	2.53 – 2.51 (*m*)	28.2	7.38 (*dd*, *J* = 15.3, 10.2)	144.9	7.50 (*dd*, *J* = 15.3, 11.4)	145.8
7′	2.47 – 2.46 (*m*)	33.2	6.02 (*d*, *J* = 15.3)	123.8	6.02 (*d*, *J* = 15.3)	123.6
8′		173.2		170.7		171.1
1″	3.69 (*s*)	51.9				

The molecular formula of trichodermate E (**5**) was assigned as $C_{23}H_{30}O_7$ (nine degrees of unsaturation) on the basis of HR-ESI-MS data. Analysis of the NMR data (*Table 2*) revealed that **5** possesses a similar structure to **7**, except that the C(9)=C(10) bond in **7** was hydrogenated to one CH (δ(H) 1.81 – 1.77, δ(C) 34.7) and one CH_2 group (δ(H) 1.72 and 1.48, δ(C) 30.3). These observations were supported by the HMBC cross-peaks CH_2(8)/C(9,10), H–C(9)/C(7,8), CH_2(10)/C(6,8,9,11), and H–C(11)/C(9,10). Furthermore, Me(16) in **7** was oxidized to a $HOCH_2$ group in **5** (δ(H) 3.48 – 3.46 and 3.45 – 3.43, δ(C) 68.2), which was confirmed by the HMB correlations H–C(9)/C(16) and CH_2(16)/C(8,9,10). H–C(9) in **5** possesses the same orientation as H–C(4), H–C(11), and Me(15) as supported by the NOESY correlations H–C(4)/H–C(11), Me(15), and H–C(11)/H–C(9), Me(15), and CH_2(16)/H_a–C(13). The relative configurations at the other C-atoms in **5** were deduced to be the same as in **7**

by comparing their ^{1}H,^{1}H coupling constants and NOESY data. Considering the similar optical rotation values, and the biosynthesis homology of **5** and **7**, and in agreement with the relative configuration determined by NOESY data, the absolute configuration of **5** was assigned as (2*R*,4*R*,5*S*,6*R*,9*R*,11*R*,12*S*).

The molecular formula of trichodermate F (**6**) was assigned as $C_{23}H_{30}O_7$ (nine degrees of unsaturation) on the basis of HR-ESI-MS data, which is same as of **5**. Comparison of the NMR data (*Table 2*) with those of **5** suggested **6** to possess a similar structure to **5**; they were only differing in the chemical shifts and coupling constants of the C(1′) – C(8′) side chain. The (2′*Z*,4′*E*,6′*E*) geometry in **6**, instead of (2′*E*,4′*Z*,6′*E*) geometry occurring in **5**, was deduced by the coupling constants observed between H–C(2′) and H–C(3′) ($J = 11.4$), H–C(4′) and H–C(5′) ($J = 14.8$), and H–C(6′) and H–C(7′) ($J = 15.3$). The absolute configuration of **6** was assigned to be (2*R*,4*R*,5*S*,6*R*,9*R*,11*R*,12*S*) as for **5**, based on the same relative configuration and the obvious biogenetic homology.

Compounds **1** – **7** and hydroxylation product **8** were tested for their cytotoxic activities against K562 (human myelogenous leukemia), HCT116 (human colon carcinoma), and HepG2 (human hepatocellular liver carcinoma) cell lines by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay. Compounds **1**, **2**, and **7** showed modest cytotoxic effects against K562 cells with IC_{50} values of 12.12 ± 1.03, 13.08 ± 1.12, and 25.17 ± 3.09 μM, respectively (the positive control taxol showed an IC_{50} value of 0.34 ± 0.08 μM). In contrast, **1** – **8** did not show detectable cytotoxic activities against HCT116 and HepG2 cell lines at 100 μM.

Trichodermates A – F (**1** – **6**, resp.) represent further examples of trichothecene sesquiterpenoid polyunsaturated octadioic acid esters [6]. Compounds **1** and **2** are structurally closely related to harzianum B (**7**) [6], except that the terminal COOH group is methyl-esterified and the geometry of the C(2′)=C(3′) and C(4′)=C(5′) bonds differs. In addition, the C(6′)=C(7′) bond is hydrogenated in **1**. Compounds **3** and **4** are new trichothecene sesquiterpenes with the 12,13-expoxide moiety opened to form a *vic*-diol and an olefin unit, respectively. Compounds **5** and **6** differ from harzianum B (**7**) in having a $HOCH_2$ group instead of a Me group linked to C(9), and in the hydrogenation of the C(9)=C(10) bond to sp^3 CH and CH_2 C-atoms.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 300 – 400 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), *ODS* (12 nm; *S-50* μm, *YMC Co., Ltd.*), and *Sephadex LH-20* (*GE Healthcare Bio-Sciences AB*). HPLC: *Waters 1525* instrument (*Waters*, USA); *Waters Symmetry Prep® C_{18}* column (7.8 × 300 mm, 7 μm); variable wavelength UV detector. Optical rotations: *Anton Paar MCP 200* automatic polarimeter. UV Spectra: *Thermo Genesys-10S* UV/VIS spectrophotometer; λ_{max} ($\log \varepsilon$) in nm. CD Spectra: *Applied Photophysics Chirascan* spectropolarimeter; λ_{max} in nm. IR Spectra: *Nicolet IS5* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance-500* spectrometer (500 and 125 MHz, resp.; *Bruker*, Rheinstetten, Germany); δ in ppm rel. to residual solvent peaks ($CDCl_3$: δ(H) 7.26, δ(C) 77.7; (D_6)acetone: δ(H) 2.05, δ(C) 29.8 and 206.1), *J* in Hz. HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, resp. ESI-MS and HR-ESI-MS: *Agilent Accurate-Mass-Q-TOF LC/MS 6520* instrument; in *m*/*z*. The fragmentor and capillary voltages were kept at 125 and 3500 V, resp. N_2 was supplied as nebulizing and drying gas. The temp. of the drying gas was set at 300°. The flow rate of the drying gas and the pressure of the nebulizer were 10 l min^{-1} and 10 psi, resp. All MS experiments were performed in positive-ion mode. Full-scan spectra were acquired at 1.03 spectra/s.

Fungal Material. The culture of *Trichoderma* sp. was isolated from the stem rot of an unidentified tree collected in Thailand in August 2012. The isolate was identified by *X. L.* based on morphology and sequence (Genbank Accession No. KP317835) analysis of the ITS region of the rDNA. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25° for 5 d. Agar plugs were cut into small pieces (*ca.* 0.5 × 0.5 × 0.5 cm) under aseptic conditions, 15 pieces were used to inoculate three *Erlenmeyer* flasks (250 ml), each containing 50 ml of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract; the final pH of the media was adjusted to 6.5, and the medium was sterilized by autoclave). Three flasks of the inoculated media were incubated at 25° on a rotary shaker at 170 rpm for 5 d to prepare the seed culture. Fermentation was carried out in 60 *Fernbach* flasks (500 ml), each containing 80 g of rice. Dist. H_2O (120 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to r.t., each flask was inoculated with 5.0 ml of the spore inoculum and incubated at 25° for 40 d.

Extraction and Isolation. The fermented material was extracted repeatedly with AcOEt (4 × 9.0 l), and the org. solvent was evaporated to dryness *in vacuo* to afford the crude extract (35.0 g), which was fractionated by reversed-phase (RP) CC (*ODS*; H_2O/MeOH, gradient) to obtain 20 fractions. The fraction (1.5 g) eluted with 65% MeOH was separated by CC (SiO_2; CH_2Cl_2/MeOH, gradient) to give 15 subfractions, *Frs. 1 – 15*. *Fr. 2* (25 mg) was separated by semi-prep. RP-HPLC (MeOH/H_2O 45 : 55 over 45 min; 2 ml min^{-1}) to afford **4** (t_R 27.0 min; 2.3 mg). Using the same conditions as described above, *Fr. 4* (40 mg) was separated to afford **1** (t_R 18.3 min; 9.6 mg) and **2** (t_R 20.5 min; 3.5 mg), *Fr. 6* (50 mg) was separated to afford **7** (t_R 34.5 min; 18.0 mg), and *Fr. 13* (40 mg) was separated to afford **3** (t_R 25.0 min; 3.4 mg). *Fr. 2* (650 mg) was separated by CC (*Sephadex LH-20*; MeOH) and the resulting subfractions were further purified by semi-prep. RP-HPLC (MeOH/H_2O 55 : 45 over 50 min; 2 ml min^{-1}) to afford **5** (t_R 29.0 min; 6.5 mg) and **6** (t_R 40.0 min; 6.7 mg).

Trichodermate A (= *8-Methyl 1-[(2*R*,4*R*,5*S*,5a*R*,9a*R*)-2,3,4,5,5a,6,7,9a-Octahydro-5,5a,8-trimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxiran]-4-yl] (2*Z*,4*E*)-Octa-2,4-dienedioate*; **1**). Colorless oil. $[\alpha]_D^{25} = -20.0$ ($c = 0.10$, MeOH). UV (MeOH): 302 (3.88). IR (neat): 3673 (br.), 2972, 1654, 1032. ^{1}H- and ^{13}C-NMR, and HMBC: see *Table 1*. Key NOESY correlations (500 MHz, $CDCl_3$): H–C(2)/H_a–C(13); H_a–C(3)/H–C(11); H–C(4)/H–C(11), Me(15); H–C(11)/Me(15); H_b–C(13)/H_b–C(7,8), Me(14). HR-ESI-MS: 417.2272 ($[M+H]^+$, $C_{24}H_{33}O_6^+$; calc. 417.2272).

Trichodermate B (= *Methyl (2*R*,4*R*,5*S*,5a*R*,9a*R*)-2,3,4,5,5a,6,7,9a-Octahydro-5,5a,8-trimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxiran]-4-yl (2*E*,4*Z*,6*E*)-Octa-2,4,6-trienedioate*; **2**). Colorless oil. $[\alpha]_D^{25} = -25.0$ ($c = 0.14$, MeOH). UV (MeOH): 302 (4.44). IR (neat): 3689 (br.), 2889, 1653, 1037. ^{1}H- and ^{13}C-NMR: see *Table 1*. HMB Correlations (500 MHz, $CDCl_3$): H–C(2)/C(4,5,11,12); CH_2(3)/C(2,4,5,12); H–C(4)/C(1′,2,6,12); CH_2(7)/C(6,8,9,11); CH_2(8)/C(6,7,9,10); H–C(10)/C(6,8,11,16); H–C(11)/C(2,6,7,9,10,15); CH_2(13)/C(2,5,12); Me(14)/C(4,5,6,12); Me(15)/C(5,6,7,11); Me(16)/

C(8,9,10); H–C(2′)/C(1′,3′,4′); H–C(3′)/C(1′,2′,5′); H–C(4′)/C(2′,3′,6′); H–C(5′)/C(3′,4′,7′); H–C(6′)/C(4′,7′,8′); H–C(7′)/C(5′,6′,8′); Me(1″)/C(8′). Key NOESY correlations (500 MHz, $CDCl_3$): H–C(2)/H_a–C(13); H_a–C(3)/H–C(11); H–C(4)/H–C(11), Me(15); H–C(11)/Me(15); H_b–C(13)/H_b–C(7,8), Me(14). HR-ESI-MS: 415.2115 ([$M+H$]$^+$, $C_{24}H_{31}O_6^+$; calc. 415.2115).

Trichodermate C (=(2E,4Z,6E)-8-{[(2R,4R,5S,5aR,9aR)-2,3,4,5,5a,6,7,9a-Octahydro-10-hydroxy-10-(hydroxymethyl)-5,5a,8-trimethyl-2,5-methano-1-benzoxepin-4-yl]oxy}-8-oxoocta-2,4,6-trienoic Acid; **3**). Colorless oil. $[\alpha]_D^{25} = -3.6$ ($c = 0.14$, MeOH). UV (MeOH): 302 (4.32). IR (neat): 3675 (br.), 2981, 1685, 1032. ^{1}H- and ^{13}C-NMR: see *Table 1*. HMB Correlations (500 MHz, (D_6)acetone): H–C(2)/C(3,12,13); CH_2(3)/C(2,4,5,12); H–C(4)/C(1′,3,5,6,14); CH_2(7)/C(6,8,9,11,15); CH_2(8)/C(6,7,9,10); H–C(10)/C(6,8,11,16); H–C(11)/C(2,6,7,9,10,15); CH_2(13)/C(2,5,12); Me(14)/C(4,5,6,12); Me(15)/C(5,6,7,11); Me(16)/C(8,9,10); H–C(2′)/C(1′,3′,4′); H–C(3′)/C(1′,2′,5′); H–C(4′)/C(2′,3′,6′); H–C(5′)/C(3′,4′,7′); H–C(6′)/C(4′,7′,8′); H–C(7′)/C(5′,6′,8′). Key NOESY correlations (500 MHz, (D_6)acetone): H–C(2)/H_a–C(13); H–C(4)/H–C(11), Me(15); H–C(11)/Me(15); H_b–C(13)/Me(14). HR-ESI-MS: 419.2064 ([$M+H$]$^+$, $C_{23}H_{31}O_7^+$; calc. 419.2064).

Trichodermate D (=*8-Methyl 1-[(2R,4R,5S,5aR,9aR)-2,3,4,5,tbcrules=bot5a,6,7,9a-Octahydro-5,5a,8-trimethyl-10-methylidene-2,5-methano-1-benzoxepin-4-yl] (2Z,4E)-Octa-2,4-dienedioate*; **4**). Colorless oil. $[\alpha]_D^{25} = -26.5$ ($c = 0.16$, MeOH). UV (MeOH): 302 (4.26). IR (neat): 3670 (br.), 2732, 1655, 1037. ^{1}H- and ^{13}C-NMR: see *Table 2*. HMB Correlations (500 MHz, $CDCl_3$): H–C(2)/C(4,5,11,13); CH_2(3)/C(2,5,12); H–C(4)/C(1′,2,6,12); CH_2(7)/C(6,8,9,11); CH_2(8)/C(6,7,9,10); H–C(10)/C(6,8,11,16); H–C(11)/C(7,9,10,15); CH_2(13)/C(2,5,12); Me(14)/C(4,5,6,12); Me(15)/C(5,6,7,11); Me(16)/C(8,9,10); H–C(2′)/C(1′,4′); H–C(3′)/C(1′,5′); H–C(4′)/C(2′,3′,6′); H–C(5′)/C(3′,6′,7′); H–C(6′)/C(3′,4′,7′,8′); H–C(7′)/C(5′,6′,8′); Me(1″)/C(8′). Key NOESY correlations (500 MHz, $CDCl_3$): H–C(2)/H_a–C(13); H_a–C(3)/H–C(11); H–C(4)/H–C(11), Me(15); H–C(11)/Me(15); H_b–C(13)/Me(14). HR-ESI-MS: 401.2325 ([$M+H$]$^+$, $C_{24}H_{33}O_5^+$; calc. 401.2323).

Trichodermate E (=(2E,4Z,6E)-8-{[(2R,4R,5S,5aR,8R,9aR)-Decahydro-8-(hydroxymethyl)-5,5a-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxiran]-4-yl]oxy}-8-oxoocta-2,4,6-trienoic Acid; **5**). Colorless oil. $[\alpha]_D^{25} = -27.0$ ($c = 0.10$, MeOH). UV (MeOH): 302 (4.80). IR (neat): 3687 (br.), 1699, 1081. ^{1}H- and ^{13}C-NMR: see *Table 2*. HMB Correlations (500 MHz, $CDCl_3$): H–C(2)/C(4,5,11,13); CH_2(3)/C(2,5,12); H–C(4)/C(1′,2,6,12); CH_2(7)/C(6,8,9,11,15); CH_2(8)/C(6,7,9,10); H–C(9)/C(7,8,16); CH_2(10)/C(6,8,9,11); H–C(11)/C(7,9,10,15); CH_2(13)/C(2,5,12); Me(14)/C(4,5,6,12); Me(15)/C(5,6,7,11); CH_2(16)/C(8,9,10); H–C(2′)/C(1′,3′,4′); H–C(3′)/C(1′,5′); H–C(4′)/C(2′,3′,6′); H–C(5′)/C(3′,6′,7′); H–C(6′)/C(4′,7′,8′); H–C(7′)/C(5′,6′,8′). Key NOESY correlations (500 MHz, $CDCl_3$): H–C(2)/H_a–C(13); H_a–C(3)/H–C(11); H–C(4)/H–C(11), Me(15); H–C(11)/H–C(9), Me(15); H_a–C(13)/CH_2(16); H_b–C(13)/Me(14). HR-ESI-MS: 419.2069 ([$M+H$]$^+$, $C_{23}H_{31}O_7^+$, calc. 419.2064).

Trichodermate F (=(2E,4E,6Z)-8-{[(2R,4R,5S,5aR,8R,9aR)-Decahydro-8-(hydroxymethyl)-5,5a-dimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxiran]-4-yl]oxy}-8-oxoocta-2,4,6-trienoic Acid; **6**). Colorless oil. $[\alpha]_D^{25} = -6.3$ ($c = 0.14$, MeOH). UV (MeOH): 302 (4.23). IR (neat): 3687 (br.), 2889, 1708, 1037. ^{1}H- and ^{13}C-NMR: see *Table 2*. HMB Correlations (500 MHz, $CDCl_3$): H–C(2)/C(4,5,11,12); CH_2(3)/C(2,5,12); H–C(4)/C(1′,2,5,6,12); CH_2(7)/C(5,6,8,9,15); CH_2(8)/C(6,7,9); H–C(9)/C(8,10,16); CH_2(10)/C(6,8,9,11,16); H–C(11)/C(2,7,9,10,15); CH_2(13)/C(2,5,12); Me(14)/C(4,5,6,12); Me(15)/C(5,6,7,11); CH_2(16)/C(8,9,10); H–C(2′)/C(1′,3′,4′); H–C(3′)/C(1′,5′); H–C(4′)/C(2′,3′,6′); H–C(5′)/C(3′,6′,7′); H–C(6′)/C(4′,7′,8′); H–C(7′)/C(5′,6′,8′). Key NOESY correlations (500 MHz, $CDCl_3$): H–C(2)/H_a–C(13); H_a–C(3)/H–C(11); H–C(4)/H–C(11), Me(15); H–C(11)/H–C(9), Me(15); H_a–C(13)/CH_2(16); H_b–C(13)/Me(14). HR-ESI-MS: 419.2069 ([$M+H$]$^+$, $C_{23}H_{31}O_7^+$; calc. 419.2064).

(−)-Harzianum B (=(2E,4Z,6E)-8-{[(2R,4R,5S,5aR,9aR)-2,3,4,5,5a,6,7,9a-Octahydro-5,5a,8-trimethylspiro[2,5-methano-1-benzoxepine-10,2′-oxiran]-4-yl]oxy}-8-oxoocta-2,4,6-trienoic Acid; **7**). Colorless oil. $[\alpha]_D^{25} = -29.0$ ($c = 0.10$, $CHCl_3$).

Hydrolysis of **7**. To a soln. (2.0 ml) of **7** (6.0 mg, 15 μmol) in CH_2Cl_2/MeOH 9:1, MeONa (3.3 mg, 60 μmol) was added, and the mixture was stirred for 1 h. The solvents were removed *in vacuo*, the residue was extracted repeatedly with CH_2Cl_2 (4 × 1.0 ml), and the org. solvent was evaporated to dryness *in vacuo*. The product was purified by semi-prep. RP-HPLC (MeOH/H_2O 45:55 over 45 min; 2 ml min^{-1}; detected at 195 nm [16]) to afford **8** (t_R 31.0 min; 2.6 mg). Colorless powder. $[\alpha]_D^{25} = -12.0$ ($c = 0.16$, MeOH). ^{1}H-NMR (500 MHz, $CDCl_3$): 5.39 (*d*, $J = 5.5$, H–C(10)); 4.33 (*dd*, $J = 7.5$, 2.9, H–C(4)); 3.82 (*d*, $J = 5.4$, H–C(2)); 3.51 (*d*, $J = 5.5$, H–C(11)); 3.10 (*d*, $J = 3.9$, H_a–C(13)); 2.81 (*d*, $J = 3.9$, H_b–C(13)); 2.61 (*dd*, $J = 15.7$, 7.5, H_a–C(3)); 1.99 – 1.97 (*m*, H_b–C(3), CH_2(8)); 1.92 – 1.90 (*m*, H_a–C(7)); 1.70 (*s*, Me(16)); 1.46 – 1.42 (*m*, H_b–C(7)); 0.85 (*s*, Me(15)); 0.80 (*s*, Me(14)).

Preparation of (R)- and (S)-MTPA Esters (= **8r** and **8s**). A sample of **8** (1.1 mg, 4.4 μmol), (*S*)-MTPACl (5.0 μl, 26 μmol), and (D_5)pyridine (0.5 ml) were quickly added to a clean NMR tube at ambient temp., and all compounds were mixed thoroughly by shaking the NMR tube carefully. The ^{1}H-NMR spectrum of the (*R*)-MTPA ester derivative was recorded directly every 4 h, and the reaction was found to be complete after 12 h. ^{1}H-NMR (500 MHz, (D_5)pyridine) for **8r**: 5.96 (*dd*, $J = 7.7$, 3.5, H–C(4)); 5.52 (*d*, $J = 5.5$, H–C(10)); 3.92 (*d*, $J = 5.2$, H–C(2)); 3.68 (*d*, $J = 5.5$, H–C(11)); 3.10 (*d*, $J = 4.2$, H_a–C(13)); 2.82 (*d*, $J = 4.2$, H_b–C(13)); 2.68 (*dd*, $J = 15.4$, 7.7, H_a–C(3)); 2.08 (*ddd*, $J = 15.4$, 5.2, 3.5, H_b–C(3)); 1.93 – 1.91 (*m*, H_a–C(8)); 1.90 – 1.88 (*m*, H_b–C(8)); 1.85 – 1.83 (*m*, H_a–C(7)); 1.63 (*s*, Me(16)); 1.30 – 1.27 (*m*, H_b–C(7)); 0.93 (*s*, Me(15)); 0.91 (*s*, Me(14)).

Similarly, a mixture of **8** (1.1 mg, 4.4 μmol), (*R*)-MTPACl (5.0 μl, 26 μmol), and (D_5)pyridine (0.5 ml) was processed as described above for **8r** to afford **8s**. ^{1}H-NMR (500 MHz, (D_5)pyridine) for **8s**: 5.89 (*dd*, $J = 7.6$, 3.3, H–C(4)); 5.52 (*d*, $J = 5.8$, H–C(10)); 3.96 (*d*, $J = 5.2$, H–C(2)); 3.68 (*d*, $J = 5.5$, H–C(11)); 3.11 (*d*, $J = 4.2$, H_a–C(13)); 2.79 (*d*, $J = 4.2$, H_b–C(13)); 2.75 (*dd*, $J = 15.4$, 7.7, H_a–C(3)); 2.20 (*ddd*, $J = 15.4$, 5.2, 3.5, H_b–C(3)); 1.93 – 1.91 (*m*, H_a–C(8)); 1.90 – 1.88 (*m*, H_b–C(8)); 1.85 – 1.83 (*m*, H_a–C(7)); 1.63 (*s*, Me(16)); 1.30 – 1.27 (*m*, H_b–C(7)); 0.90 (*s*, Me(15)); 0.75 (*s*, Me(14)).

Absolute Configuration at C(12) of the 12,13-Diol Moiety in **3** [13]. HPLC Grade DMSO was dried (4 Å molecular sieves). According to the published procedure, a 1:1.5 mixture of diol **3**/$(AcO)_4Mo_2$ was subjected to CD measurements at a concentration of 0.2 mg ml^{-1}. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (*ca.* 10 min after mixing). The inherent CD was subtracted. The observed sign of the diagnostic band at 310 nm in the induced CD spectrum correlated with the absolute configuration at C(12) in the 12,13-diol moiety.

MTS Assay [17]. In a 96-well plate, each well was plated with (2 – 5)·10^3 cells (depending on the cell multiplication rate). After cell attachment overnight, the medium was removed, and each well was treated with 100 μl of medium containing 0.1% DMSO, or appropriate concentrations of the test compounds and the positive control cisplatin (100 mM stock soln. in DMSO and serial dilutions). The plate was incubated for 48 h at 37° in a humidified 5%-CO_2 atmosphere. Proliferation was assessed by adding 20 μl of MTS (*Promega*) to each well in the dark, followed by 90 min incubation at 37°. The assay plate was read at 490 nm using a microplate reader. The assay was run in triplicate.

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