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Diversolides A–G, guaianolides from the roots of *Ferula diversivittata*

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ABSTRACT

Seven sesquiterpene lactone derivatives, diversolides A–G (**1**–**7**), together with two known compounds, diversin (**8**) and stigmasterol, were isolated from the roots of *Ferula diversivittata*. The structures of these compounds were elucidated by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, and NOESY) as well as high-resolution EIMS. Compounds **1**, **4** and **6**–**8** were tested for their in vitro antifungal and antibacterial activities. Some of the tested compounds showed moderate antifungal and antibacterial activities with minimum inhibitory concentration (MIC) values from 40 to 80 μg/ml.

1. Introduction

The exclusively old world genus *Ferula* belongs to the family Umbelliferae with about 130 species distributed throughout the Mediterranean area and central Asia, specially in the former USSR and neighboring countries such as Iran. This genus is well documented as a good source of biologically active compounds such as sesquiterpene derivatives (Ahmed, 1999,2001; Abd El-Razek et al., 2003; Iranshahi et al.,2004,2007,2008) and sulphur-containing compounds (Al-said et al., 1996; Iranshahi et al., 2003a).

The roots of *Ferula diversivittata* Regel & Schmalh.-Rech. have been used in folk medicine to prevent convulsion and hysteria (Zargari, 1996). The chemistry of the plant has previously been studied and only a few terpenoid coumarins have been identified to date (Abd El-Razek et al., 2003). In general, *Ferula* species are rich sources of sesquiterpenoids and sesquiterpenoid lactones, including humulanes, himachalanes, guaianes, germacranes, and especially carotanes (Valle et al., 1987; Garg and Agarwal, 1987; Miski et al., 1987; Casinovi et al., 1989; Garg et al., 1990,1998; Miski and Jakupovic, 1990; Appendino et al., 1990; Ahmed, 1990, 1991; Serkerov et al.,1992; Babekov et al., 2000; Iranshahi et al., 2003b; Shikishima et al., 2002; Suzuki et al., 2007). For example, 17 new sesquiterpenoid lactones, including 14 guaianolides with various esters attached to C-8 and C-11, were characterized from *Ferula penninervis* (Shikishima et al., 2002). In the present work, we wish to report seven new esterified guaianolides of this type from the roots of *F. diversivittata* as well as the in vitro antifungal and antibacterial activities of some of them.

2. Results and discussion

Normal-phase column chromatography of the dichloromethane extract of roots, followed by preparative TLC, afforded seven new natural products (Fig. 1), namely diversolides A–G (**1–7**), and two known compounds, diversin (**8**) and stigmasterol.

Compound **1** was obtained as white crystals, and its molecular formula, $C_{25}H_{30}O_7$, was established by HREIMS (*m*/*z* 442.1951, calcd 442.1991). The IR spectrum of compound **1** indicated the presence of γ-lactone (1790) and ester (1709) groups. It was clear from the ^{1}H and ^{13}C NMR data (Tables 1 and 2) that **1** consisted of an 8,11-dioxygenated guaianolide nucleus (Shikishima et al., 2002) bearing two ester groups. The main guaianolide proton resonances appeared at δ_H 6.19 (1H, *s*, H-3), 3.57 (1H, *d*, J = 10.1 Hz, H-5), 3.60 (1H, *dd*, J = 11.1 and 10.2 Hz, H-7), 4.63 (1H, *dd*, J = 11.1 and 10.1 Hz, H-6) and 5.54 (1H, *ddd*, J = 10.5, 10.2 and 3.2 Hz, H-8), 1.62 (3H, *s*, Me-13) and 2.24 and 2.25 (both 3H, *s*, Me-14 and Me-15). The oxygenated carbon resonances appeared at δ_C 194.9 (C-2), 78.6 (C-6), 67.1 (C-8), 77.8 (C-11) and 173.4 (C-12). Structure **1** was further supported by ^{1}H–^{1}H COSY data while NOESY cross-peaks between H-6 and H-7, between H-8 and H-5 and H-9β and between H-7 and H-9α supported the relative configuration. The

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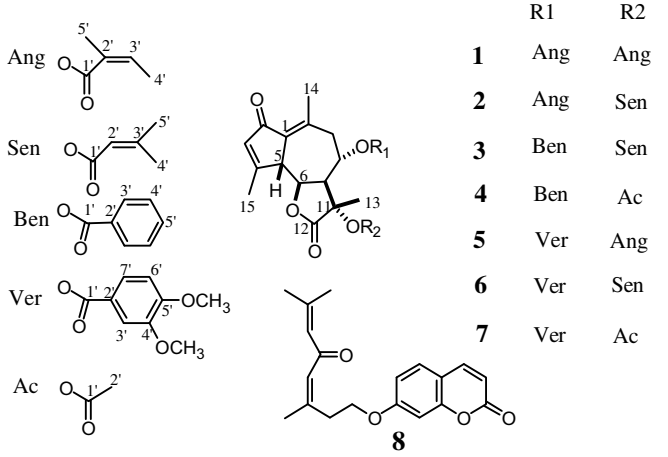

	R1	R2
1	Ang	Ang
2	Ang	Sen
3	Ben	Sen
4	Ben	Ac
5	Ver	Ang
6	Ver	Sen
7	Ver	Ac

Fig. 1. Compounds **1**–**8** isolated from the roots of *Ferula diversivittata*. Ac = Acetyl, Ang = Angeloyl, Ben = Benzoyl, Sen = Senecioyl, Ver = Veratroyl.

esters were identified as angelates on the basis of their spectroscopic properties [δ_H 1.86 ($2CH_3$), 1.96 (6H, *d*, J = 7.2 Hz), 6.17 (2H, *q*, J = 7.2 Hz), δ_C 166.4 and 166.6 (C-1′ and C-1″)]. In the HMBC spectrum a correlation from H-8 to C-1′ confirmed the attachment of one angelate to C-8. The second ester must be attached to C-11. The configuration of the angelate double bonds were confirmed as *Z* on the basis of a NOESY cross-peak from the H-3′ to the Me-5′ pairs.

The ^1H and ^{13}C NMR spectral data of diversolides B (**2**; $C_{25}H_{30}O_7$), C (**3**; $C_{27}H_{28}O_7$), D (**4**; $C_{24}H_{24}O_7$), E (**5**; $C_{29}H_{32}O_9$), F (**6**; $C_{29}H_{32}O_9$) and G (**7**; $C_{26}H_{28}O_9$) were very similar to those of **1**, except for the ester units. Compound **2** showed the presence of angelate and senecioyl [δ_H 5.64 (1H, *s*), 1.98 (3H, *s*), 2.13 (3H, *s*), δ_C 165.2 (C-1″), 114.3 (C-2″), 161.1 (C-3″), 27.7 (C-4″) and 20.3 (C-5″)] ester moieties in its ^1H and ^{13}C NMR spectra. The other ^1H and ^{13}C NMR spectral data of **2** were closely comparable to those of **1**. The location of angeloyl group on C-8 group was confirmed by an HMBC experiment and hence the senecioyl group must be attached to C-11.

Compounds **3** and **4** showed the presence of a benzoyl [**3**: δ_H 7.42 (2H, *t*), 7.58 (1H, *t*), 8.00 (2H, *d*), δ_C 165.3 (C-1′), 129.7 (C-2′), 129.8 (C-3′ and C-7′), 128.5 (C-4′ and C-6′) and 133.6 (C-5′); **4**: δ_H 7.44 (2H, *t*), 7.59 (1H, *t*), 8.00 (2H, *d*), δ_C 165.3 (C-1′), 129.3 (C-2′), 129.6 (C-3′ and C-7′), 128.6 (C-4′ and C-6′) and 133.7 (C-5′)] ester

Table 2
^{13}C NMR data for compounds **1**–**7** ($CDCl_3$, 125.7 MHz)

C	1	2	3	4	5	6	7
1	129.1	129.0	129.1	129.1	130.2	130.2	129.1
2	194.9	195.1	195.0	194.9	195.3	195.4	194.9
3	136.1	136.1	136.1	136.1	136.2	136.1	136.1
4	169.2	169.3	169.2	169.0	170.7	170.7	169.1
5	48.1	48.0	48.1	48.0	48.3	48.3	48.1
6	78.6	78.5	78.4	78.3	79.4	79.3	78.5
7	47.6	47.5	47.2	47.8	48.3	48.4	47.6
8	67.1	67.0	68.3	68.2	69.0	69.1	68.0
9	44.3	44.2	43.9	43.8	44.2	44.2	43.9
10	144.9	145.1	144.9	144.9	145.4	145.3	145.0
11	77.8	77.8	77.8	78.0	78.9	78.3	78.1
12	173.4	173.8	173.1	173.1	174.0	174.1	173.1
13	20.5	20.6	20.7	20.6	20.7	20.7	20.6
14	19.9	19.9	20.0	20.0	19.7	19.7	20.0
15	20.0	19.9	20.2	20.2	20.1	20.1	20.2
1′	166.4	166.4	165.3	165.3	165.6	165.6	165.1
2′	126.4	126.8	129.7	129.3	122.7	122.7	121.7
3′	140.4	141.7	129.8	129.6	113.2	113.2	112.4
4′	15.8	15.9	128.5	128.6	150.0	150.0	149.0
5′	20.2	20.3	133.6	133.7	155.0	155.0	153.9
6′	–	–	–	–	111.6	111.7	110.4
7′	–	–	–	–	124.6	124.7	123.7
4′-OCH_3	–	–	–	–	56.2	56.2	56.1
5′-OCH_3	–	–	–	–	56.2	56.2	56.1
1″	166.6	165.2	165.1	169.8	166.9	165.6	169.8
2″	126.9	114.3	114.4	20.8	127.6	115.4	20.8
3″	141.4	161.1	161.2	–	141.2	161.4	–
4″	15.9	27.7	27.7	–	16.0	27.4	–
5″	20.2	20.3	20.5	–	20.4	20.4	–

The numbers 1′–7′ and 1″–5″ are related to R_1 and R_2, respectively.

Table 1
^1H NMR data for compounds **1**–**7** ($CDCl_3$, 500 MHz)[a]

H	1	2	3	4	5	6	7
3	6.19 *s*[b]	6.18 *s*[b]	6.20 *s*	6.20 *s*	6.15 *s*[b]	6.15 *s*[b]	6.20 *s*
5	3.57 *d* (10.1)	3.55 *d* (10.1)	3.60 *d* (11.2)	3.59 *d* (11.3)	4.05 *d* (11.1)	4.03 *d* (11.1)	3.59 *d* (11.3)
6	4.63 *dd* like *t* (11.1, 10.1)	4.65 *dd* like *t* (10.3, 10.1)	4.69 *dd* like *t* (11.2, 10.0)	4.66 *dd* like *t* (11.3, 10.4)	4.79 *dd* like *t* (11.1, 10.0)	4.76 *dd* like *t* (11.1, 10.0)	4.66 *dd* like *t* (11.3, 10.5)
7	3.60 *dd* like *t* (11.1, 10.2)	3.60 *dd* like *t* (11.2, 10.3)	3.69 *dd* like *t* (10.8, 10.0)	3.66 *dd* like *t* (10.4, 10.4)	3.82 *dd*[b]	3.87 *dd*[b]	3.64 *dd* like *t* (10.5, 10.5)
8	5.54 *ddd* (10.5, 10.2, 3.2)	5.57 *ddd* (11.2, 11.2, 3.2)	5.69 *ddd* (11.1, 10.8, 3.2)	5.69 *ddd* (10.4, 10.4, 3.2)	5.82 *ddd* (11.5, 11.0, 3.2)	5.76 *ddd* (11.5, 11.0, 3.2)	5.67 *ddd* (10.5, 10.5, 3.2)
9	2.44 *dd* (18.9, 10.5) α 2.84 *dd* (18.9, 3.2) β	2.46 *dd* (18.9, 11.2) α 2.86 *dd* (18.9, 3.2) β	2.51 *dd* (18.9, 11.1) α 2.98 *dd* (18.9, 3.2) β	2.53 *dd* (18.9, 10.4) α 2.98 *dd* (18.9, 3.2) β	2.78 *dd* (18.9, 11.0) α 2.94 *dd* (18.9, 3.2) β	2.82 *dd* (18.9, 11.0) α 2.91 *dd* (18.9, 3.2) β	2.52 *dd* (18.9, 10.5) α 2.97 *dd* (18.9, 3.2) β
13	1.62 *s*	1.57 *s*	1.59 *s*	1.60 *s*	1.70 *s*	1.63 *s*	1.60 *s*
14	2.24 *s*	2.23 *s*	2.25 *s*	2.25 *s*	2.24 *s*	2.24 *s*	2.26 *s*
15	2.25 *s*	2.24 *s*	2.26 *s*	2.26 *s*	2.25 *s*	2.25 *s*	2.26 *s*
3′	6.17 *q* (7.2)[b]	6.17 *q* (7.2)[b]	8.00 *d* (7.5)	8.00 *d* (7.5)	7.53 *d* (1.9)	7.53 *d* (1.9)	7.51 *d* (1.5)
4′	1.96 *d* (7.2)	1.96 *d* (7.2)	7.42 t (7.5)	7.44 t (7.5)	–	–	–
5′	1.86 *s*	1.86 *s*	7.58 t (7.5)	7.59 t (7.5)	–	–	–
6′	–	–	–	–	7.04 *d* (8.3)	7.06 *d* (8.3)	6.87 *d* (8.4)
7′	–	–	–	–	7.66 *dd* (8.3, 1.9)	7.67 *dd* (8.3, 1.9)	7.62 *dd* (8.4, 1.5)
4′-OCH_3	–	–	–	–	3.83	3.85	3.92
5′-OCH_3	–	–	–	–	3.89	3.90	3.94
2″	–	5.64 *s*	5.71 *s*	2.14 *s*	–	5.75 *s*	2.13 *s*
3″	6.17 *q* (7.2)[b]	–	–	–	6.25 *q* (7.0)	–	–
4″	1.96 *d* (7.2)	1.98 *s*	1.95 *s*	–	1.90 *d* (7.0)	1.97 *s*	–
5″	1.86 *s*	2.13 *s*	2.16 *s*	–	1.84 *s*	2.14 *s*	–

The numbers 1′–7′ and 1″–5″ are related to R_1 and R_2, respectively.

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by ^1H–^1H COSY, HMQC, HMBC and NOESY experiments.

[b] Resonance partially obscured.

Table 3
Antimicrobial activity of compounds **1**, **4** and **6–8**

Compounds	*Staphylococcus aureus* (ATCC 29737)	*Escherichia coli* (ATCC 8739)	*Aspergillus niger* (ATCC 1624)	*Candida albicans* (ATCC 14053)
Minimum inhibitory concentrations (μg/ml)				
1	>160	80	>160	>160
4	40	>160	80	>160
6	80	80	80	>160
7	>160	>160	40	>160
8	>160	>160	80	>160
Gentamycin	16	8	ND	ND
Fluconazole	ND	ND	8	16

ND: Not determined.

moiety in their ^{1}H and ^{13}C NMR spectra. In the HMBC spectra of **3** and **4**, the correlations of δ_H 5.69 (H-8) with δ_C 165.3 (C-1′), confirmed the presence of benzoyl linkage at C-8. The ester moieties located on C-11 were determined as senecioyl and acetyl, respectively (Tables 1 and 2). The IR spectrum of compound **4** indicated the additional carbonyl group (1739), confirming acetyl functional group.

Compounds **5**, **6** and **7** showed the presence of a veratroyl ester [**5**: δ_H 3.83 (3H, *s*), 3.89 (3H, *s*), 7.04 (1H, *d*), 7.53 (1H, *d*), 7.66 (1H, *dd*), δ_C 165.6 (C-1′), 122.7 (C-2′), 113.2 (C-3′), 150.0 (C-4′), 155.0 (C-5′), 111.6 (C-6′), 124.6 (C-7′) and 56.2 (4′- and 5′-OCH_3); See the Tables 1 and 2 for ^{1}H and ^{13}C NMR spectral data of **6** and **7**]. In the HMBC spectra of **5**, **6** and **7**, correlation of H-8 with C-1′, indicated that the veratroyl group is located on C-8. The esters on C-11 were identified as angeloyl, senecioyl and acetyl respectively for **5**, **6** and **7** (Tables 1 and 2). The acetate carbonyl absorption was observed at 1741 cm^{-1} in the IR spectrum of **7**. Other NMR spectral data were also in agreement with the structures **5**, **6** and **7**. NOESY spectra indicated that the relative configurations of compounds **2**-**7** were the same as that of **1**.

In conclusion, most of sesquiterpenes reported from *Ferula* species belong to humulanes, himachalanes, guaianes, germacranes, and especially carotanes (Valle et al., 1987; Garg and Agarwal 1987; Miski et al., 1987; Garg et al., 1990, 1998; Miski and Jakupovic 1990; Appendino et al., 1990; Ahmed, 1990, 1991; Serkerov et al., 1992; Babekov et al., 2000; Iranshahi et al., 2003). There are a few reports on sesquiterpene lactones from the genus *Ferula* (Serkerov et al., 1992; Shikishima et al., 2002; Suzuki et al., 2007). For an example, 17 new sesquiterpene lactones including 14 guaianolides were characterized from *F. penninervis* in a previous study (Shikishima et al., 2002). The structures of the mentioned guaianolides were very similar to those of the present study, except for the ester groups.

Compounds **1**, **4** and **6–8** were tested for their in vitro antifungal and antibacterial activities against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* using gentamycin and fluconazole as positive controls. The tested compounds did not show any antifungal activity against *C. albicans* at a range of 1.25–160 μg/ml. However, other fungi and bacteria strains had different susceptibility to the compounds at concentrations lower than 160 μg/ml (Table 3). The best antimicrobial activity was observed against the filamentous fungus *A. niger*. This fungus was susceptible to all tested compounds, except to compound **1** (The higher test concentrations of 160 μg/ml were considered inactive). The complete results are exhibited in Table 3.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected. The optical rotation was measured on a Polax-2L ATAGO Polarimeter. UV spectra were obtained in CH_2Cl_2 on a Shimadzu UV-1650 PC spectrophotometer, and IR spectra were recorded on a Perkin–Elmer Paragin 1000 FT-IR spectrometer. ^{1}H and ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC spectra were recorded on Bruker Avance 500 and 600 MHz (with Cryo Probe) for both ^{1}H NMR, ^{13}C NMR and 2D spectra.

The HREIMS spectra were obtained on a JEOL JMS HX-110 mass spectrometer. Column chromatography was conducted with silica gel 230–400 mesh, Merck. Preparative TLC was performed on silica gel GF_{254s} plates (20 × 20 cm, glass plates) and observation of plates was carried out under UV CAMAG spectrometer (254 nm).

3.2. Plant material

The plant material (*F. diversivittata* Regel & Schmalh.-Rech.) was collected from Hezarmasjed mountains, Khorasan Razavi province, Iran, in May 2006. The plant material was identified by Mohammad Reza Joharchi, Ferdowsi University of Mashhad Herbarium (FUMH).

A voucher specimen (No. 1006) has been deposited at the herbarium of Faculty of Pharmacy, Mashhad University of Medical Sciences.

3.3. Extraction and isolation

The air-dried roots (350 g) were ground into a powder and extracted exhaustively by maceration at room temperature with CH_2Cl_2. After filtration, the extract was concentrated under vacuum to yield 15 g of a brown residue.

The extract (15 g) was subjected to column chromatography on silica gel (5 × 60 cm) using petrol with increasing volumes of EtOAc [petrol:EtOAc (20:1, 2.1 L), (15:1, 3.2 L), (10:1, 3.3 L), (9:1, 3 L), (8:1, 2.7 L), (7:1, 2.7 L), (6:1, 4.2 L), (5.5:1, 1.3 L), (5:1, 3.6 L), (4.5:1, 1.65 L), (4:1, 1.5 L), (3:1, 3.6 L), (2:1, 1.8 L), (1:1, 2.5 L) and (0:1, 3 L)]. The fractions (200 ml each) were compared by TLC (silica gel using petrol:EtOAc, different ratios), and those giving similar spots were combined. Fifteen fractions were finally obtained. Fraction 2, 6, 9, 11, 12 and 15 afforded stigmasterol (79.7 mg), **8** (568.9 mg), **1** (283.5 mg), **3** (3.2 mg), **4** (222.7 mg) and **7** (93.4 mg) as white crystals, respectively. Fraction 10 needed more purification with PTLC (silica gel using petrol:EtOAc, 3:1, 20 × 20 cm, glass plates, each plate was run four times) to give 99.8 mg of **2**. Fraction 14 was also further purified by PTLC (silica gel using petrol:EtOAc, 3:1.2, 20 × 20 cm, glass plates, each plate was run six times) to give **5** (548.4 mg) and **6** (622.3 mg).

3.4. Diversolide A (**1**); 2-methyl-but-2-enoic 3,6,9-trimethyl-3-(2-methyl-but-2-enoyloxy)-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 193–197 °C; $[\alpha]_D^{22}$ − 44.8 (*c* 0.223, CH_2Cl_2); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 229 (4.32); $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2969, 2826, 1790 (lactone), 1709 (ester), 1688, 1639, 1381, 1349, 1225, 1149, 1090, 1036; EIMS (70 eV) *m/z* 442 (2.8) $[M]^+$, 243 (7.1), 242 (7.7), 84 (5.3), 83 (100), 55 (64.3); HREIMS *m/z* 442.1951, calcd 442.1991.

3.5. Diversolide B (**2**); 2-methyl-but-2-enoic 3,6,9-trimethyl-3-(3-methyl-but-2-enoyloxy)-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 190–191.2 °C; $[\alpha]_D^{22}$ − 45.2 (*c* 0.221, CH_2Cl_2); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 230 (4.28); $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2909, 2840, 1790 (lactone), 1709 (ester), 1688, 1639, 1375, 1225, 1144,

1096; EIMS (70 eV) *m/z* 442 (59.4) $[M]^+$, 243 (62.3), 242 (86.0), 84 (20.8), 83 (100), 55 (82.3); HREIMS *m/z* 442.1970, calcd 442.1991.

3.6. Diversolide C (**3**) ; benzoic acid 3,6,9-trimethyl-3-(3-methyl-but-2-enoyloxy)-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 255–258 °C; $[\alpha]_D^{22}$ − 21 (*c* 0.028, MeOH); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 225; $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2924, 2854, 1791, 1715, 1634, 1437, 1384, 1272, 1113; EIMS (70 eV) *m/z* 464 (5.5) $[M]^+$, 242 (18.7), 199 (8.7), 186 (8.3), 105 (69.0), 83 (100), 77 (21.0); HREIMS *m/z* 464.1806, calcd 464.1835.

3.7. Diversolide D (**4**); benzoic acid 3-acetoxy-3,6,9-trimethyl-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 235–237 °C; $[\alpha]_D^{22}$ + 44 (*c* 0.113, CH_2Cl_2); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 228 (4.48), 258 (4.39), 293 (3.89); $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2940, 2852, 1788 (lactone), 1739 (acetyl), 1709 (benzoyl), 1690, 1641, 1616, 1376, 1373, 1091, 714; EIMS (70 eV) *m/z* 424 (36.4) $[M]^+$, 242 (73.6), 105 (100), 77 (41.5); HREIMS *m/z* 424.1521, calcd 424.1522.

3.8. Diversolide E (**5**); 3,4-dimethoxy-benzoic acid 3,6,9-trimethyl-3-(2-methyl-but-2-enoyloxy)-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 189–191 °C; $[\alpha]_D^{22}$ + 129 (*c* 0.07, CH_2Cl_2); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 227 (4.48), 258 (4.39), 293 (3.89); $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2937, 2829, 1790 (lactone), 1709 (ester), 1693, 1639, 1511, 1268, 1219, 1149, 1096; EIMS (70 eV) *m/z* 524 (27.1) $[M]^+$, 242 (19.0), 182 (76.4), 165 (100), 83 (56.3), 55 (10.5); HREIMS *m/z* 524.2072, calcd 524.2046.

3.9. Diversolide F (**6**); 3,4-dimethoxy-benzoic acid 3,6,9-trimethyl-3-(3-methyl-but-2-enoyloxy)-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 198–200 °C; $[\alpha]_D^{22}$ − 129 (*c* 0.038, CH_2Cl_2); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 228 (4.33), 258 (4.22), 293 (3.73); $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2940, 2832, 1793 (lactone), 1709 (ester), 1695, 1641, 1513, 1268, 1219, 1149, 1096; EIMS (70 eV) *m/z* 524 (27.1) $[M]^+$, 242 (19.0), 182 (76.4), 165 (100), 83 (56.3), 55 (10.5); HREIMS *m/z* 524.2072, calcd 524.2046.

3.10. Diversolide G (**7**); 3,4-dimethoxy-benzoic acid 3-acetoxy-3,6,9-trimethyl-2,7-dioxo-2,3,3a,4,5,7,9a,9b-octahydro-azuleno[4,5-b]furan-4-yl ester

White crystalline solid; mp 212–215 °C; $[\alpha]_D^{22}$ − 80 (*c* 0.062, CH_2Cl_2); $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 227 (4.33), 258 (4.29), 293 (3.83); $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2937, 2840, 1790 (lactone), 1741 (acetyl), 1709 (veratroyl), 1688, 1639, 1375, 1225, 1144, 1096; EIMS (70 eV) *m/z* 484 (13.5) $[M]^+$, 243 (8.1), 242 (18.3), 183 (11.7), 182 (97.5), 171 (12.4), 166 (10.4), 165 (100), 137 (10.7), 77 (11.1); HREIMS *m/z* 484.1754, calcd 484.1734.

3.11. Biological activity

The antibacterial and antifungal activities of compounds **1**, **4** and **6**–**8** were determined against *S. aureus* (ATCC 29737), *E. coli* (ATCC 8739), *C. albicans* (ATCC 14053) and *A. niger* (ATCC 16404) by the broth dilution method (National Committee for Clinical Laboratory Standards, 1993, 1997). The bacteria and *C. albicans* were maintained on Nutrient broth (Difco) and adjusted to 1×10^6 and 1×10^4 organism/ml, respectively. Inoculum for *A. niger* was prepared as a spore suspension from a five days old agar-surface culture and adjusted to a concentration of approximately 1×10^4 in a final volume of cultures.

The susceptibility of the strains to compounds **1**, **4** and **6**–**8** was performed by determination of the minimum inhibitory concentrations (MICs) of the isolates using the broth dilution method. First stock solution of the sample of concentration of 40 mg/ml was prepared in DMSO. The test sample was further diluted in sterile water to obtain serial dilution concentrations from 1.25 μg/ml to 160 μg/ml. Then 0.5 ml of each concentration was added to 0.5 ml of double strength medium broth for test strain. Inoculum which was prepared as described above was introduced to each test tube. The cultured tubes were incubated at 37 °C for bacteria for 24 h, at 30 °C for 24 h for *C. albicans* and at 25 °C for 3 days for *A. niger*. The lowest concentration at which no growth was observed, recorded as MICs. Culture media with different concentration of gentamycin and fluconazole were used as control and DMSO (4 μl) was used as negative control. All experiments were performed in triplicate.

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