FULL PAPER

Aurovertin-Type Polyketides from *Calcarisporium arbuscula* with Potent Cytotoxic Activities against Triple-Negative Breast Cancer

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Two new polyene polyketides, namely aurovertins T and U (**1** and **2**), were isolated from *Calcarisporium arbuscula*, together with aurovertins B (**3**), D and E (**4** and **5**), and M (**6**). The structures were elucidated by extensive spectroscopic methods (especially 2D-NMR techniques). The cytotoxic activities of all isolates against human triple-negative breast cancer cell line (MDA-MB-231) were evaluated. As a result, compounds **3**, **4**, and **6** exhibited more potent cytotoxic activities against MDA-MB-231 cell line than the positive control taxol. Also, discussion about the relationships between structure and activity of these aurovertins was presented.

Keywords: Aurovertin, *Calcarisporium arbuscula*, Triple-negative breast cancer, Polyketides, Fungi.

Introduction

Breast cancer is the most frequently diagnosed cancer in women and is associated with substantial morbidity and mortality. Triple-negative breast cancer (TNBC) refers to breast tumor characterized by lack of expression of estrogen, progesterone, and HER2/neureceptors, and comprises 15% of all breast cancers. TNBC is the most aggressive of breast cancer subtypes, with a high propensity for metastasis and poor prognosis [1][2]. Current treatment modalities for TNBC are limited to surgery, radiation, and systemic chemotherapy, given the absence of more specific therapeutic targets. Although systematic therapeutic approaches have reduced cancer-specific mortality, TNBC remains associated with high rates of cancer recurrence and metastasis. It is urgent to find lead compounds for potential treatment of TNBC [3][4].

During our preliminary screening, the $CHCl_3$ extract of the culture of *Calcarisporium arbuscula* exhibited significant cytotoxic against MDA-MB-231 cell line. A chemical investigation of the extract led to the isolation of two new and four previously known aurovertin derivatives (**1** – **6**; *Fig. 1*). The details regarding the isolation, structural characterization, and cytotoxic assay of compounds **1** – **6** are presented here.

Results and Discussion

Aurovertin T (**1**) was obtained as a yellow amorphous powder. The molecular formula was deduced as $C_{21}H_{28}O_6$ on the basis of $[M + Na]^+$ at m/z 399.1780 ($C_{21}H_{28}NaO_6^+$; calc. 399.1784), indicating the existence of eight degrees of unsaturation. The IR spectrum exhibited the absorption bands due to OH (3440 cm^{-1}) and C=C bonds (1632 cm^{-1}). The UV spectrum showed the typical absorption of conjugated triene pyrone moiety in aurovertins [5 – 7]. The ^{1}H-NMR data (*Table 1*) of **1** revealed the presence of five Me group (δ(H) 1.06 (*t*, *J* = 7.5), 1.22 (*s*), 1.28 (*s*), 1.93 (*s*), 3.86 (*s*)), seven olefinic H-atoms, and two O-bearing CH atoms (δ(H) 3.68 (*dd*, *J* = 7.5, 4.5), 3.80 (*d*, *J* = 3.0)). The ^{13}C-NMR and DEPT spectra showed 21 C-atom signals that attributed to five Me groups, one CH_2, nine CH, and six quaternary C-atoms. The data mentioned above were similar to those of **7**, a biosynthetic intermediate of aurovertins [8]. An in-depth comparison of the NMR data between compounds **1** and **7** suggested that the major difference lied in the α-pyrone and polyene moieties. The ^{1}H-NMR spectrum of **1** only displayed three pairs of C=C H-atom signals, which indicated that **1** had one less C=O than that in **7**. The HMBCs Me(20)/C(15), Me(20)/C(16), and Me(20)/C(17) showed that the Me group (C(20)) was located at C (16) but not C(14) (*Fig. 2*). The planar structure of **1** was

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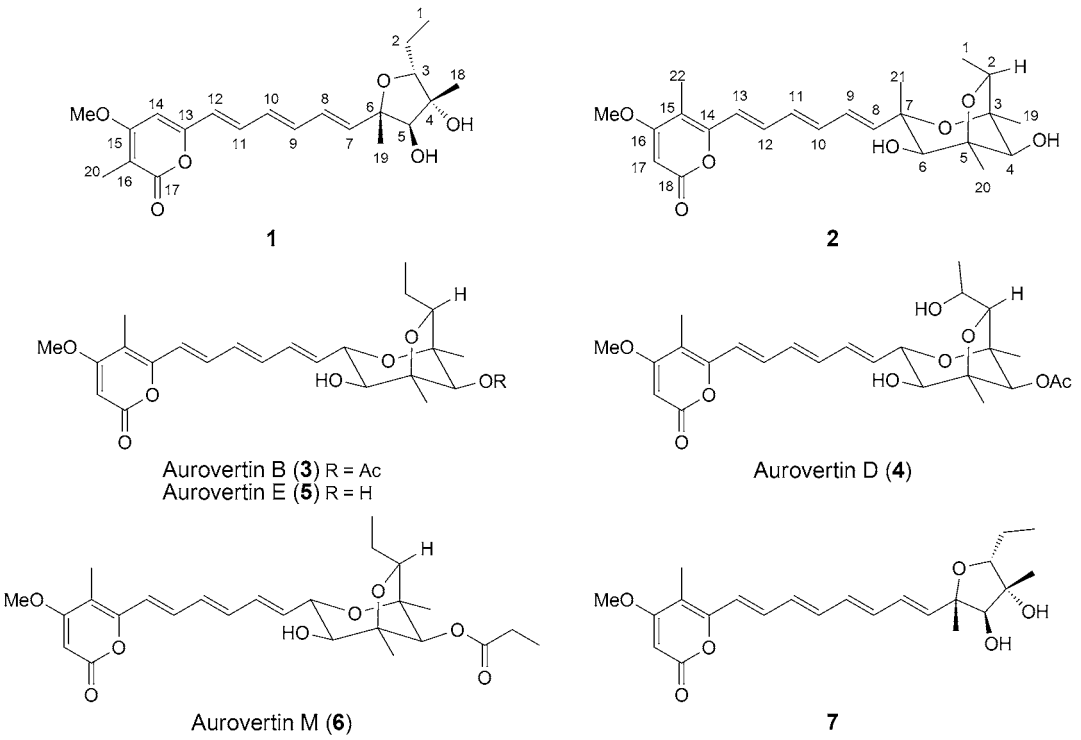

Fig. 1. Structures of compounds **1** – **6** isolated from *Calcarisporium arbuscula*.

thus formulated. The assignments of the ^{1}H- and ^{13}C-NMR data of **1** were achieved unambiguously from the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra (*Table 1*, *Fig. 2*).

The configuration of **1** was deduced by coupling constant and NOESY analyses. The large vicinal coupling constants clearly indicated that the C(7)=C(8), C(9)=C(10), and C(11)=C(12) bonds all had (*E*)-configuration.

Table 1. ^{1}H- and ^{13}C-NMR data for **1** and **2** in $CDCl_3$. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.06 (*t*, *J* = 7.5)	11.6	1.18 (*d*, *J* = 6.5)	13.4
2	1.54 – 1.59 (*m*)	21.4	4.07 (*q*, *J* = 6.5)	80.5
3	3.68 (*dd*, *J* = 7.5, 4.5)	85.0	–	83.2
4	–	81.6	4.24 (*s*)	75.5
5	3.80 (*d*, *J* = 3.0)	85.1	–	82.8
6	–	83.3	3.56 (*s*)	79.8
7	6.04 (*d*, *J* = 15.5)	143.4	–	78.4
8	6.48 (*dd*, *J* = 14.5, 11.5)	127.2	5.94 (*d*, *J* = 15.5)	146.7
9	6.44 (*dd*, *J* = 14.0, 11.5)	137.5	6.30 – 6.35 (*m*)	128.4
10	6.30 (*dd*, *J* = 14.0, 11.5)	131.6	6.42 (*dd*, *J* = 14.5, 10.5)	137.2
11	7.15 (*dd*, *J* = 15.5, 11.5)	135.5	6.30 – 6.35 (*m*)	131.8
12	6.02 (*d*, *J* = 15.5)	122.5	7.16 (*dd*, *J* = 15.0, 11.0)	135.7
13	–	157.5	6.34 (*d*, *J* = 15.0)	119.7
14	6.02 (*s*)	96.0	–	154.5
15	–	165.8	–	108.3
16	–	103.1	–	170.8
17	–	165.0	5.48 (*s*)	89.1
18	1.22 (*s*)	18.4	–	163.9
19	1.28 (*s*)	21.4	1.31 (*s*)	17.5
20	1.93 (*s*)	9.1	1.35 (*s*)	18.8
21			1.25 (*s*)	26.8
22			1.95 (*s*)	9.1
MeO	3.86 (*s*)	56.4	3.81 (*s*)	56.4

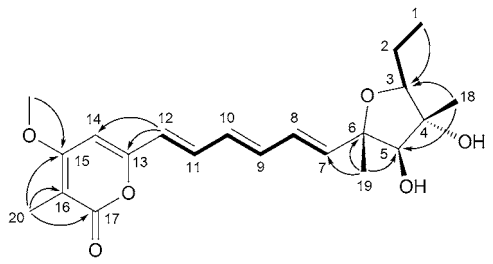

Fig. 2. Key ^{1}H,^{1}H-COSY (▬) and HMBC (H → C) correlations of **1**.

NOE correlations H–C(3)/Me(19) and Me(19)/Me(18) indicated that the H–C(3), Me(18), and Me(19) were on the same side of the molecular plane, tentatively assumed as *β*. As a consequence, the orientation of the Et group at C(3) was α. A strong NOE between CH_2(2) and H–C(5) supported the α-configuration of H–C(5). Thus, the structure of aurovertin T (**1**) was established as depicted.

Aurovertin U (**2**) was also obtained as a yellow amorphous powder, which had the molecular formula $C_{23}H_{30}O_7$ based on HR-ESI-MS analysis. The IR, UV, and NMR spectra were similar to those of aurovetin K, except for an additional Me group signal and loss a low-field signal in **2** [9]. The location of the Me(22) group at C(15) was deduced by HMBCs Me(22)/C(14), Me(22)/C(15), and Me(22)/C(16). Further analysis of other spectroscopic data (HMQC, HMBC, ^{1}H,^{1}H-COSY, NOESY) established the structure of **2** as shown in *Fig. 1*.

The known compounds were identified as aurovertins B (**3**) [10], D and E (**4** and **5**) [5][11], and M (**6**) [9] by comparing their spectroscopic data with literature data. Aurovertins are a class of toxic polyketide with the basic structure of 2,6-dioxabicyclo[3.2.1]octane (DBO) ring system and a conjugated α-pyrone moiety. To date, these metabolites had been reported from fungal species like *C. arbuscula* [10][11], *Albatrellus confluens* [5][9], *Pochonia chlamydosporia* [7], and *Metarhizium anisopliae* [6]. Among these isolates, aurovertin T (**1**) is structurally like a biosynthetic intermediate [8], and has different substituted pattern in its α-pyrone moiety from the other aurovertins previously reported [5 – 11].

All compounds (**1** – **6**) were evaluated for their cytotoxic activities against MDA-MB-231 with taxol as a positive control. As shown in *Table 2*, most of isolates showed cytotoxicity against MDA-MB-231, especially **3**, **4**, and **6** exhibiting more potent cytotoxicity than taxol. Compound **1** bearing no DBO moiety, a typical function in the aurovertins, showed no cytotoxictiy against MDA-MB-231. The result indicated that the DBO moiety in aurovetins was an important factor for the retention of bioactivity. Moreover, compounds **2** and **5**, lacking the Ac group at C(4), showed reduced cytotoxic activity compared to the similar, but Ac-bearing compounds **4** and **3**, respectively, which indicated that the Ac group at C(4) was a key group for the improvement of the cytotoxic activity.

Table 2. 50% Inhibitory concentration of **1** – **6** against MDA-MB-231

Compound	**1**	**2**	**3**	**4**	**5**	**6**	Taxol[a])
IC_{50} [μM]	>50	5.43	0.09	0.08	8.79	0.25	2.85

[a]) Taxol was used as positive control.

Aurovertins were initially reported as a group of toxic substances from *C. arbuscula* [12]. Aurovertin B (**3**) was found to be a specific and uncompetitive inhibitor of F1 ATPase [13][14]. Recent research showed that aurovetins displayed nematodetoxic [7] and cytotoxic activities [15]. In this study, several aurovetins were found to show potent cytotoxicity against TNBC cell line. Herein, compounds **3**, **4**, and **6** can be used as lead compounds for further optimization for the potential use of treatment of TNBC. Further investigations are undergoing and the details will be reported in due course.

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Experimental Part

General

All solvents were of anal. grade and obtained from commercially available sources. Thin-layer chromatography (TLC): precoated silica gel GF_{254} plates (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*, Shangdong, China); visualized by UV light (at 254 and/or 360 nm) and by spraying with 10% H_2SO_4 reagent followed by heating at 110° for 5 – 10 min. Column chromatography (CC): SiO_2 (200 – 300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), *RP-C18* SiO_2 (*ODS-A* 12 nm *S-150*; *YMC Co*., Japan), and *MCI CHP20P* gel (75 – 150 mm; *Mitsubishi Chemical Industries, Ltd*., Japan). Optical rotations: *Rudolph-AutoPolIV* polarimeter (USA). UV Spectra: Shimadzu UV-2450 spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Avatar-370* spectrometer (Thermo Electron Corporation, USA); $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AM-500* apparatus (USA); δ in ppm rel. to Me_4Si standard, *J* in Hz. ESI-MS: *Agilent-6210-Lc/Tof* mass spectrometer (USA); in *m*/*z*.

Fungus and Culture Conditions

The fungus was purchased from was purchased from the China Forestry Culture Collection Center (cfcc 89533). The cultivation was carried out on shakers at 28 °C and 185 rpm for 6 days in liquid PD medium (potato extracts, 200 g; glucose, 20 g, dist. H_2O, 1 l), followed by static cultivation for another 24 d.

Extraction and Isolation

The cultures (10 l) were filtered through cheesecloth to separate the broth and mycelia. The air-dried mycelia

(380 g) were powdered and extracted with acetone (3 times) at r.t. The solvent was evaporated under reduced pressure to give a crude extract (58 g). The acetone extract was suspended in 3.0 l of H_2O and partitioned with $CHCl_3$ (5 × 0.5 l). The $CHCl_3$ fraction (18.0 g) was then subjected to CC (SiO_2; petroleum ether (PE)/acetone, 10:1→4:1) to give three fractions, *Frs. 1 – 3*. *Fr. 1* was purified by CC (*MCI CHP20P*; MeCN/H_2O 5:5→7:3) to provide aurovertins D (**4**) (230 mg) and E (**5**) (78 mg). Compounds **1** (2.3 mg) and **2** (5.8 mg) were isolated from *Fr. 2* by CC (*RP-C18;* MeCN–H_2O 6:4→8:2). Aurovertin B (**3**) (287 mg) was purified by recrystallization of *Fr. 3* from MeCN/H_2O (9:1). The filtrate was evaporated to give a yellow residue, which was further purified by CC (SiO_2, PE/acetone 4:1 →2:1) to give aurovertins B (**3**) (55.0 mg) and M (**6**) (11.0 mg).

Aurovertin T (= **6-{(1*E*,3*E*,5*E*)-6-[(2*R**,3*S**,4*S**,5*S**)-5-Ethyltetrahydro-3,4-dihydroxy-2,4-dimethylfuran-2-yl]hexa-1,3,5-trien-1-yl}-4-methoxy-3-methyl-2*H*-pyran-2-one**; **1**). Yellow amorphous powder. $[\alpha]_D^{20} = -29.5$ ($c = 0.05$, $CHCl_3$). UV (MeOH): 368 (3.99), 276 (4.11), 265 (4.15), 230 (3.94). IR (KBr): 3440, 2938, 1632, 1450, 1378, 1094, 1067, 987, 758. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 399 ($[M + Na]^+$). HR-ESI-MS: 399.1780 ($[M + Na]^+$, $C_{21}H_{28}NaO_6^+$; calc. 399.1784).

Aurovertin U (= **6-{(1*E*,3*E*,5*E*)-6-[(1*R**,3*S**,4*S**,5*S**,7*R**,8*S**)-4,8-Dihydroxy-1,3,5,7-tetramethyl-2,6-dioxabicyclo[3.2.1]octan-3-yl]hexa-1,3,5-trien-1-yl}-4-methoxy-5-methyl-2*H*-pyran-2-one**; **2**). Yellow amorphous powder. $[\alpha]_D^{20} = -21.4$ ($c = 0.05$, $CHCl_3$). UV (MeOH): 365 (4.02), 278 (4.23), 266 (4.15), 230 (3.88). IR: 3438, 2961, 1685, 1621, 1550, 1460, 1375, 1095, 972, 752. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 441 ($[M + Na]^+$). HR-ESI-MS: 441.1894 ($[M + Na]^+$, $C_{23}H_{30}NaO_7^+$; calc. 441.1889).

Biological Assay

All isolates were tested for cytotoxic activities against MDA-MB-231 cell line according to standard protocols [15][16], and taxol was used as positive control. Five concentrations were used to calculate the IC_{50} values.

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