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# INDUCED PRODUCTION OF FURAN DERIVATIVES IN A FUNGAL ENDOPHYTE *Ceriporia lacerate* HS-ZJUT-C13A BY THE OSMAC METHOD

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*The One Strain Many Compounds (OSMAC) method was employed to explore the chemical diversities of secondary metabolites produced by* Ceriporia lacerate *HS-ZJUT-C13A, a fungal endophyte of* Huperzia serrata*. Four new furan derivatives **1–4**, together with a known compound, were obtained from the rice culture of the fungus. The structures of the new compounds were established by detailed analysis of the spectroscopic data, especially 1D and 2D NMR, and HR-ESI-MS. All compounds were evaluated for their acetylcholinesterase inhibitory activity and antiproliferative activities against HeLa, HepG2, and SGC7901 cell lines.*

**Keywords**: fungal endophyte, *Huperzia serrata*, secondary metabolites, OSMAC, *Ceriporia.*

Filamentous fungi are prolific producers of bioactive natural products [1], as exemplified by the antibiotic penicillin [2] and the anti-hypercholesterolemia drug lovastatin [3]. However, recent genome sequencing of many fungal species has revealed that fungi harbor a significant number of biosynthetic gene clusters (BGCs) encoding secondary metabolites far more than previously discovered [4]. This demonstrates that many BGCs are kept silent or cryptic under the present culturing conditions. Hence, the activation of silent or cryptic BGCs has become a hot spot in the field of fungal natural product research. The approach termed OSMAC (One Strain Many Compounds) by Bode and co-workers [5] refers to the fact that a single microorganism is capable of producing a diverse array of structurally different compounds and has been successfully used to induce or optimize the production of secondary metabolites in fungi [6–8]. Unlike genetic manipulations, the OSMAC approach is not targeted to the activation of a specific cryptic gene cluster, but to the systematic alteration of readily accessible culturing parameters including medium components (salts, amino acids, and carbon source), pH, culture aeration (including the type of culture vessel used), and temperature of growth [9]. This makes the OSMAC approach an accessible, versatile, inexpensive, and relatively simple tool for regulating the metabolism of fungi [9]. As part of our ongoing studies on the secondary metabolites of fungi [10–17], a fungal endophyte *Ceriporia lacerate* HS-ZJUT-C13A was isolated from the medicinal plant *Huperzia serrata.* Previous chemical investigations on the fungus cultivated in liquid potato-dextrose medium have led to the discovery of a series of triterpenoids [14] and sesquiterpenoids [15]. In the subsequent study, the OSMAC approach was employed to explore the structural diversities of secondary metabolites produced by this fungus. In the preliminary screening, the metabolites of the fungus cultivated on rice medium exhibited different HPLC and TLC profiles as compared with previous studies. Herein, we report the isolation, structure elucidation, and bioassay of four new furan derivatives **1–4** from the rice cultures of this fungus.

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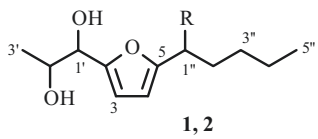
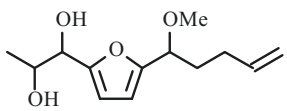
TABLE 1. $^{1}H$ (500 MHz) and $^{13}C$ NMR (125 MHz) Data for Compounds **1–4** ($CD_3OD$, δ, ppm, J/Hz)

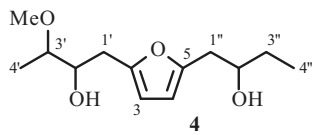
| C atom | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | – | 156.0 | – | 156.5 |
| 3 | 6.24 (1H, d, J = 3.0) | 108.9 | 6.28 (1H, d, J = 3.0) | 109.1 |
| 4 | 6.19 (1H, d, J = 3.0) | 107.7 | 6.27 (1H, d, J = 3.0) | 110.4 |
| 5 | – | 158.0 | – | 155.1 |
| 1′ | 4.32 (1H, d, J = 6.0) | 73.5 | 4.32 (1H, d, J = 7.0) | 74.1 |
| 2′ | 3.98 (1H, m) | 70.8 | 3.95 (1H, m) | 71.3 |
| 3′ | 1.19 (3H, d, J = 6.5) | 19.13 | 1.02 (3H, d, J = 6.5) | 19.6 |
| 4′ | – | – | – | – |
| 1″ | 4.55 (1H, t, J = 7.0) | 68.7 | 4.15 (1H, t, J = 7.0) | 78.3 |
| 2″ | 1.81 (2H, m) | 36.7 | 1.84 (1H, m); 1.78 (1H, m) | 35.0 |
| 3″ | 1.38 (1H, m); 1.31 (1H, m) | 29.3 | 1.20 (2H, m) | 29.2 |
| 4″ | 1.37 (2H, m) | 23.8 | 1.33 (2H, m) | 23.8 |
| 5″ | 0.89 (3H, t, J = 7.0) | 14.6 | 0.89 (3H, t, J = 7.0) | 14.6 |
| OMe | – | – | 3.21 (3H, s) | 56.8 |
| C atom | **3** | | **4** | |
| 2 | – | 156.5 | – | 151.9 |
| 3 | 6.28 (1H, d, J = 3.0) | 109.0 | 6.27 (1H, d, J = 3.0) | 111.8 |
| 4 | 6.28 (1H, d, J = 3.0) | 110.4 | 6.08 (1H, d, J = 3.0) | 108.5 |
| 5 | – | 154.9 | – | 155.2 |
| 1′ | 4.32 (1H, d, J = 7.5) | 74.1 | 1.21 (2H, d, J = 3.5) | 20.1 |
| 2′ | 3.96 (1H, m) | 71.2 | 3.98 (1H, m) | 70.2 |
| 3′ | 1.02 (3H, d, J = 6.5) | 19.6 | 3.87 (1H, m) | 83.9 |
| 4′ | – | – | 0.97 (3H, d, J = 6.5) | 19.6 |
| 1″ | 4.18 (1H, t, J = 7.0) | 77.5 | 2.74 (2H, t, J = 5.5) | 31.0 |
| 2″ | 1.84 (1H, m); 1.93 (1H, m) | 34.6 | 3.83 (1H, m) | 71.3 |
| 3″ | 2.06 (2H, m) | 31.1 | 1.41 (2H, m) | 40.3 |
| 4″ | 5.81 (1H, m) | 139.4 | 0.92 (3H, t, J = 6.0) | 14.7 |
| 5″ | 4.95 (1H, dd, J = 10.0, 1.0)<br>4.99 (1H, dd, J = 17.0, 1.0) | 115.7 | – | – |
| OMe | 3.21 (3H, s) | 56.8 | 3.24 (3H, s) | 57.4 |



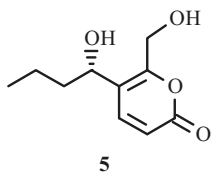

**1, 2**




**3**




**4**




**5**

**1:** R = OH; **2:** R = $OCH_3$

Extensive column chromatography of the ethanol extract of the rice culture of *C. lacerate* HS-ZJUT-C13A afforded four new furan derivatives **1**–**4** and a known one **5**. The known compound was identified as taiwapyrone (**5**) by comparing its spectroscopic data with that reported in the literature [18].

Compound **1** was obtained as a colorless oil. The molecular formula was determined to be $C_{12}H_{20}O_4$ on the basis of HR-ESI-MS molecular-ion peak at *m/z* 251.1246 $[M + Na]^+$ (calcd for $C_{12}H_{20}O_4Na$, 251.1254), indicating three degrees of unsaturation. The IR spectrum of **1** showed absorption bands for hydroxyl groups (3339 $cm^{-1}$). The $^{1}H$ NMR spectrum of **1** (Table 1) displayed signals of one methyl triplet at δ 0.89 (t, J = 7.0 Hz), one methyl doublet at δ 1.19 (d, J = 6.5 Hz), three oxygenated methine protons at δ 3.98 (m), 4.32 (d, J = 6.0 Hz), and 4.55 (t, J = 7.0 Hz), and two coupled olefinic [δ 6.19 (d, J = 3.0 Hz) and 6.24 (d, J = 3.0 Hz)]. The $^{13}C$ NMR and DEPT NMR spectra contained 12 resonances, including two methyl, three methylene, five methine (three oxygenated at δ 68.7, 70.8 and 73.5, and two olefinic at δ 107.7 and 108.9), and two olefinic quaternary carbons at δ 156.0 and 158.0. These data revealed the presence of a 2,5-disubstituted furan ring in compound **1**.

All protonated carbons were assigned by HMQC correlations. In the $^1H$–$^1H$ COSY plots of compound **1**, correlations between $H_3$-3′/H-2′ and H-2′/H-1′, as well as $H_3$-5″/$H_2$-4″, $H_2$-4″/$H_2$-3″, $H_2$-3″/$H_2$-2″, and $H_2$-2″/H-1″, revealed the presence of two isolated spin systems –CH(1′)–CH(2′)– $CH_3$(3′) and –CH(1″)–$CH_2$(2″)–$CH_2$(3″)–$CH_2$(4″)–$CH_3$(5″). The connection between C-1′ and C-2 was determined by the HMBC correlations from H-1′ to C-2 and C-3, and from H-2′ to C-2, while HMBC correlations from H-1″ to C-4 and from $H_2$-2″ to C-5″ supported the connection between C-1″ and C-5. All the three oxygenated methines were replaced by hydroxyl groups as indicated by the molecular formula of compound **1** and confirmed by the NMR data. Thus, compound **1** was determined to possess the structure of 1-(5-(1-hydroxypentyl)furan-2-yl)propane-1,2-diol.

Compound **2** was also afforded as a colorless oil with a molecular formula of $C_{13}H_{22}O_4$ as indicated by the HR-ESI-MS molecular-ion peak at *m/z* 265.1416 $[M + Na]^+$ (calcd for $C_{13}H_{22}O_4Na$, 265.1410). The NMR data of compound **2** (Table 1) resembled those of **1**, except for the signals assignable to a methoxyl group ($\delta_H$ 3.21 (s) and $\delta_C$ 56.8), which was elucidated to anchor at C-1″ by the HMBC correlation from the oxygenated methyl singlet at $\delta_H$ 3.21 (s) to the oxygenated methine C-1″. Compound **2** was thus assigned the structure of 1-(5-(1-methoxypentyl)furan-2-yl)propane-1,2-diol.

Compound **3** was obtained as a pale yellow oil with a molecular formula of $C_{13}H_{20}O_4$ as indicated by the HR-ESI-MS molecular-ion peak at *m/z* 263.1234 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1236). The NMR data of compound **3** (Table 1) exhibited similarities with those of **2**. Key differences include the replacement of one methyl [$\delta_H$ 0.89 (t, J = 7.0 Hz) and $\delta_C$ 14.6] and one methylene [$\delta_H$ 1.33 (m) and $\delta_C$ 23.8] in **2** with an $sp^2$ methine [$\delta_H$ 5.81 (m) and $\delta_C$ 139.4] and an $sp^2$ methylene [$\delta_H$ 4.95 (dd, J = 10.0, 1.0 Hz); 4.99 (dd, J = 17.0, 1.0 Hz) and $\delta_C$ 115.7] in **3**, suggesting the oxidation of **2** to form a terminal double bond in **3**. The terminal double bond was deduced to be located between C-4″ and C-5″ according to the $^1H$–$^1H$ COSY plots between $H_2$-5″/$H_2$-4″, H-4″/$H_2$-3″, $H_2$-3″/$H_2$-2″, and $H_2$-2″/H-1″. Compound **3** was determined to have the structure of 1-(5-(1-methoxypent-4-en-1-yl)furan-2-yl)propane-1,2-diol.

Compound **4**, colorless oil, was obtained as an isomer of compound **2** as proposed by the HR-ESI-MS molecular-ion peak at *m/z* 265.1233 $[M + Na]^+$, (calcd for $C_{13}H_{22}O_4Na$, 265.1236). The NMR data of compound **4** (Table 1) resambled those of **2**, exhibiting charicteristics of a 2,5-disubstituted furan derivative. Comprehensive interpretation of the 2D NMR spectra revealed that compound **4** differed from **2** in the length of the side chains at C-2 and C-5, respectively. Specifically, $^1H$–$^1H$ COSY plots of $H_3$-4″/$H_2$-3″, $H_2$-3″/H-2″, and H-2″/$H_2$-1″ established a side chain –$CH_2$(1″)–CH(2″)–$CH_2$(3″)–$CH_3$(4″), which was anchored at C-5 by the HMBC correlations from $H_2$-1″ to C-4 and from H-2″ to C-5. In a similar way, another side chain –$CH_2$(1′)–CH(2′)–CH(3′)–$CH_3$(4′) could be easily constructed by the $^1H$–$^1H$ COSY plots of $H_3$-4′/H-3′, H-3′/H-2′, and H-2′/$H_2$-1′, and connected to C-2 by the HMBC correlations from $H_2$-1′ to C-2 and C-3, and from H-2′ to C-2. The methoxyl group was determined to be located at C-3′ by the HMBC correlation from the oxygenated methyl at $\delta_H$ 3.24 (s) to C-3′, while C-2′ and C-2″ were both replaced by hydroxyl groups as indicated by the molecular formula. Compound **4** was elucidated as 1-(5-(2-hydroxybutyl)furan-2-yl)-3-methoxybutan-2-ol.

The relative and absolute configurations at the chiral centers of **1**–**4** remain undefined since we failed in preparing the chemical derivatives for chemical communication or suitable single crystals for X-ray detection.

Compounds **1**–**5** were evaluated for their acetylcholinesterase inhibitory activity and antiproliferative activities against HeLa, HepG2, and SGC7901 cell lines. However, none of them exhibited significant activities.

In our previous studies, the fungus *C. lacerate* HS-ZJUT-C13A was reported to produce a series of triterpenoids [14] and sesquiterpenoids [15] that were believed to originate from the mevalonate pathway. However, the metabolites **1**–**5** isolated in the present study belong to polyketides, a class of structurally complex natural products usually biosynthesized via the acetate-malonate pathway. Variations in the metabolic profiles of the fungus implied that different BGCs may be activated in two different culture media. It suggested that the OSMAC method, as exemplified by changing culture media in our case, represented one of the effective strategies for enhancing the chemical diversity of microbial secondary metabolites.

## EXPERIMENTAL

**General Experiment Procedures**. Optical rotations were determined on a Rudolph Autopol IV polarimeter (Rudolph Research Analytical, New Jersey, USA). IR spectra were recorded on a Thermo-Nicolet-6700 photometer (Thermo Fisher Scientific, Massachusetts, USA). NMR spectra were recorded on Bruker AM-500 NMR apparatus with TMS as internal standard (Bruker Corporation, Zurich, Switzerland). ESI-MS were recorded on an Agilent-6210-Lc/Tof mass spectrometer (Agilent Technologies, California, USA). All solvents used were of analytical grade (Hangzhou Gaojin Fine Chemical Plan Chemical Plant). Silica gel (300–400 mesh, Qingdao Marine Chemical Factory, Qingdao, P. R. China),

MCI CHP20P gel (75–150 μm; Mitsubishi Chemical Industries, Ltd., Japan), Toyopearl HW-40C gel (Tosoh Corporation, Japan), and YMC ODS C-18 gel (50 μ, YMC Co. Ltd., Kyoto, Japan) were used for column chromatography (CC), and a precoated silica gel $GF_{254}$ plate (Qingdao Marine Chemical Factory, Qingdao, P. R. China) was used for TLC.

**Fungal Material and Culture Conditions**. The fungus was isolated from the medicinal plant *H. serrata* collected in Pan-An County, Zhejiang Province, China, in July 2010. It was identified as *C. lacerate* based on DNA sequence analysis conducted by Sangon Biotech (Shanghai) Co. Ltd. The original culture (voucher number HS-ZJUT-C13A) was deposited at the China Centre for Type Culture Collection with the deposit number CCTCC M 2012433. The fungus was first inoculated to three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract and 0.4% yeast extract). After inoculation, they were incubated at 28°C on a rotary shaker at 180 rpm for 5 days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled water to give a final spore/cell suspension of $1 \times 10^{-6}$/mL. The fermentation was carried out in 100 Fernbach flasks (500 mL) each containing 80 g of rice. Distilled water (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 121°C for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 28°C for 40 days under static conditions.

**Extraction and Isolation**. The cultures were soaked with ethanol to afford an extract, which was then suspended in water and partitioned with EtOAc. The organic phases were evaporated under reduced pressure to yield a residue (42.3 g), which was subjected to MCI CHP20P gel CC eluting with MeOH–$H_2O$ (20:80–100:0) to afford four fractions (Frs. 1–4). Fraction 2 (6.3 g) was subjected to silica gel CC using petroleum–EtOAc (2:1) as eluent to afford three subfractions (Frs. 2A–2C). Fraction 2B (432 mg) was chromatographed over an ODS C-18 column eluting with a gradient of MeOH–$H_2O$ (35:65–45:55) to yield **3** (5.3 mg) and **4** (10.2 mg). Fraction 2C (55.3 mg) was successively subjected to ODS C-18 CC eluting with MeOH–$H_2O$ (40:60) and Toyopearl HW-40C gel CC eluting with methanol to afford **1** (5.9 mg), **2** (7.6 mg), and **5** (11.0 mg).

**1-(5-(1-Hydroxypentyl)furan-2-yl)propane-1,2-diol (1)**. Colorless oil. $[\alpha]_D^{20}$+2.7° (*c* 0.01, MeOH). IR ($\nu_{max}$, $cm^{-1}$): 3339, 2931, 2869, 1654, 1377, 1242, 1037, 788. For $^1H$ and $^{13}C$ NMR, see Table 1. HR-ESI-MS *m/z* 251.1246 $[M + Na]^+$ (calcd for $C_{12}H_{20}O_4Na$, 251.1254).

**1-(5-(1-Methoxypentyl)furan-2-yl)propane-1,2-diol (2)**. Colorless oil. $[\alpha]_D^{20}$–7.2° (*c* 0.28, MeOH). IR ($\nu_{max}$, $cm^{-1}$): 3402, 2933, 2867, 1456, 1377, 1127, 1087, 793. For $^1H$ and $^{13}C$ NMR, see Table 1. HR-ESI-MS *m/z* 265.1416 $[M + Na]^+$ (calcd for $C_{13}H_{22}O_4Na$, 265.1410).

**1-(5-(1-Methoxypent-4-en-1-yl)furan-2-yl)propane-1,2-diol (3)**. Pale yellow oil. $[\alpha]_D^{20}$+5.3° (*c* 0.11, MeOH). IR ($\nu_{max}$, $cm^{-1}$): 3368, 2928, 2858, 1601, 1371, 1095, 1036, 798. For $^1H$ and $^{13}C$ NMR, see Table 1. HR-ESI-MS *m/z* 263.1234 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_4Na$, 263.1236).

**1-(5-(2-Hydroxybutyl)furan-2-yl)-3-methoxybutan-2-ol (4)**. Colorless oil. $[\alpha]_D^{20}$+10.0° (*c* 0.12, MeOH). IR ($\nu_{max}$, $cm^{-1}$): 3392, 2959, 1373, 1261, 1091, 1029, 797. For $^1H$ and $^{13}C$ NMR, see Table 1. HR-ESI-MS *m/z* 265.1233 $[M + Na]^+$ (calcd for $C_{13}H_{22}NaO_4$, 265.1236).

**Bioassays**. Antiproliferative [12] and acetylcholinesterase inhibitory [13] assays of compounds **1**–**5** were performed employing the method we previously reported.

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