Cytotoxic bibenzyl dimers from the stems of *Dendrobium fimbriatum* Hook

Feng-Qing Xu\(^a, b, ^\dagger\), Fang-Cheng Xu\(^d, ^\dagger\), Bo Hou\(^b\), Wei-Wei Fan\(^b\), Cheng-Ting Zi\(^b\), Yan Li\(^b\), Fa-Wu Dong\(^b\), Yu-Qing Liu\(^b\), Jun Sheng\(^c, ^*\), Zhi-Li Zuo\(^b, ^*\), Jiang-Miao Hu\(^b, ^*\)

\(^a\)Anhui University of Chinese Medicine, Hefei 230031, China
\(^b\)State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
\(^c\)Yunnan Agricultural University, Kunming 650201, China
\(^d\)The Department of Neurology of the First Affiliated Hospital of Medical University of Anhui, China

**ABSTRACT**

The bioassay-guided chemical investigation of the stems of *Dendrobium fimbriatum* Hook led to the isolation of seven first reported bibenzyl dimers with a linkage of a methylene moiety, fimbriadimerbibenzyls A–G (1–7), together with a new dihydrophenanthrene derivative (5)-2,4,5,9-tetrahydroxy-9,10-dihydrophenanthrene (8) and thirteen known compounds (9–21). The structure of the new compound was established by spectroscopic analysis. Biological evaluation of bibenzyl derivatives against five human cell lines indicated that seven of those compounds exhibited broad-spectrum and cytotoxic activities with \(IC_{50}\) values ranging from 2.2 to 21.2 \(\mu\)M. Those rare bibenzyl dimers exhibited cytotoxic activities in vitro and the cytotoxicity decreased as the number of oxygen-containing groups in the structure decreases.

© 2014 Elsevier Ltd. All rights reserved.

The stems of *Dendrobium fimbriatum* Hook is one of the common Orchidaceae species and has been used as Shi-Hu for nourishing the stomach, promoting secretion of saliva and reducing fever for the Chinese since thousands of years ago.\(^*\) It is distributed mainly in the southwest of China and some others Asian countries, such as Myanmar, Vietnam, Thailand, Nepal, and India.\(^\dagger\) Previous chemical investigations of its stems led to the isolation of phenanthrenes,\(^*\) bibenzyls,\(^\dagger\) diosgenin derivatives,\(^\dagger\) anthraquinones,\(^\dagger\) coumarins\(^\dagger\) and so on. In addition, bioactivity studies on pure compounds have been reported to possess multiple positive effects in vitro experiments, including anti-tumor, anti-mutagenic, anti-inflammatory and antioxidant effects.\(^\dagger\)–\(^\ddagger\) In our further investigation on herb medicine of Shi-Hu,\(^\dagger\)–\(^\ddagger\) *D. fimbriatum* was selected as material and led to the isolation of seven acyclic bis[bibenzyls] with a C–CH\(_2\)–C linkage of the benzene nucleus in a bibenzyl monomer (1–7),\(^\dagger\) a new compound named (5)-2,4,5,9-tetrahydroxy-9,10-dihydrophenanthrene (8), thirteen known compounds (Fig. 1) named moscatin (9),\(^\dagger\) 4-[3-hydroxy-4-methoxyphenethyl]-2,6-dimethoxyphenol (10),\(^\ddagger\) 3,4-dihydroxy-3′,4,5-trimethoxybibenzyl (11),\(^\dagger\) gigantol (12),\(^\dagger\) batatasin-III (13),\(^\dagger\) tristin (14),\(^\dagger\) 4,4′-dihydroxy-3,3′,5,α-tetramethoxybibenzyl (15),\(^\ddagger\) 4-methoxy-9,10-dihydro-phenanthrene-2,5-diol (16),\(^\ddagger\) 2,4-dimethoxy-9,10-dihydrophenanthren-7-ol (17),\(^\ddagger\) 2-methoxy-9,10-dihydrophenanthrene-4,7-diol (18),\(^\ddagger\) 9,10-dihydrophenanthrene-2,4,7-triol (19),\(^\dagger\) moscatin (20),\(^\ddagger\) botrydil (21),\(^\ddagger\) Departure from its traditional applications mentioned above, the bibenzyl compounds isolated from the species were tested for their cytotoxic activity against five human cancer cell lines, using the MTT method.

Compound 1\(^\dagger\) was obtained as a white powder, and its HRESIMS gave a pseudo-molecular ion at \(m/z 561.2464 [M+H]^+\), corresponding to the molecular formula C\(_{33}\)H\(_{30}\)O\(_9\) (calcd 561.2483). The IR spectrum showed the presence of hydroxyl (3422 cm\(^{-1}\)) and aromatic ring (1616 and 1514 cm\(^{-1}\)) functionalities. The assignment of \(^1\)H and \(^13\)C NMR spectroscopic data of 1 was based on analysis of HSQC and HMBC (Fig. 2). The \(^1\)H NMR spectrum (Table 1) reveals signals of two 1,3,4-trisubstituted aromatic rings \(\delta_H 6.79 (1H, d, J = 8.0 Hz), 6.63 (1H, dd, J = 8.0, 1.5 Hz), 6.54 (1H, d, J = 1.5 Hz); 6.83 (1H, dd, J = 8.0 Hz), 6.68 (1H, dd, J = 8.0, 1.5 Hz), 6.61 (1H, d, J = 1.5 Hz)) for A\(_1\) and B\(_2\); two 1,3,4,5-tetra-substituted aromatic rings \(\delta_H 6.26 (1H, br s), 6.22 (1H, br s), 6.33 (1H, d, J = 2.5 Hz), 6.28 (1H, d, J = 2.5 Hz)) for A\(_2\) and B\(_2\); four methylene groups \(\delta_H 2.73 (2H, m), 2.71 (2H, m), 3.08 (2H, m), 2.69 (2H, m),\) and a special aliphatic methylene group \(\delta_H 3.85 (2H, s)\). Four aromatic methoxy groups \(\delta_H 3.66 (s), 3.83 (s), 3.67 (s) and 3.76 (s)\). The \(^13\)C NMR and DEPT spectra (Table 1) revealed 33 carbons reso-
Figure 1. Structures of compounds 1–21.

Figure 2. Key correlations of 1–8.
The HMBC spectrum of 1 demonstrated the expected key correlations: from H-7a to C-1-a, C-2a and 7a, from H-8a to C-1-a, C-2a and 1-a, from H-10-a to C-9-a, C-12-a and 1-a, from H-14-a to C-13-a; from H-7b to C-2-b, C-6-b, C-9-b and 9-b, from H-12-b and H-6-b to C-7-b, from H-8-b to C-1-b, C-7-b, C-9-b and 14-b, from H-10-b and H-12-b to C-11-b. In addition, the correlations were observed from δH (OCH3) 3.83, 3.76, 3.67, 3.66 to C-3-a, C-3-b, C-11-b and C-11-a in the HMBC spectrum, respectively. The HSQC spectrum permitted the assignment of aliphatic methane protons δH 3.85→ΔC 18.9, which suggested that 1 may be composed of two gigantic monomer connected with an aliphatic methane carbon. Furthermore, from the HMBC correlations of the protons from δH 3.85 (2H, s) to C-11-a, C-12-a, C-3-a, C-9-b, and C-14-b, it could be predicted that the CH2 bridge should be linked with C-12-a and C-14-b. Therefore, the structure of 1 was finally identified as 12a,14b-methylene-didigantol, and named as fimbriadimerbibenzyl A.

Compound 2 was obtained as a white power, with the molecular formula C33H41O5 as established by position HR-ESIMS (m/z 555.2009 [M+Na]+, calcd for 555.2009 [M+Na]+), indicating sixteen degrees of unsaturation. The 1H NMR spectrum (Table 2) showed 13 resonances, including five aliphatic carbons at δC 38.1 (t), 37.2 (t), 35.3 (t), 37.4 (t), 18.9 (t), 24 aromatic carbons as well as four methoxy carbons. This peak data and its biological sources suggested that this compound should be a bibenzyl dimers derivative. The small differences were the correlations of the ring B2 in the HMBC spectrum (Fig. 2): from H-14-b to C-8-b, C-10-b, C-12-b and C-13-b, from H-12-b to C-10-b, C-11-b, C-13-b and C-14-b, from δH 3.90 (s, OCH3) to C-11-b. All assignments listed were based on HSQC and HMBC correlations. Thus, the structure of 2 was determined to be 12a,10b-methylene-didigantol, and named as fimbriadimerbibenzyl B.

Compound 3 was obtained as a white power, having a molecular formula C32H29O7, as determined by its negative HR-ESIMS (m/z 529.2227 [M−H]−, calcd for 529.2232). The NMR character (Table 3) was similar to 1 except that 3 exhibited a 1,3-disubstituted aromatic ring [δH: 7.07 (1H, J = 7.5 Hz), 6.61 (1H, br d, J = 7.5 Hz), 6.60 (1H, br d, J = 7.5 Hz)] instead of a 1,3,4-trisubstituted aromatic ring B1 in 1H NMR spectrum. Furthermore, the correlations (Fig. 2) from H-7-b to C-2-b and C-6-b, from H-6-b to C-2-b, C-4-b and C-5-b, from H-5-b to C-3-b in HMBC spectrum were observed. Therefore, compound 3 was elucidated as shown in Figure 1 and named as fimbriadimerbibenzyl C.

Compound 4 was obtained as a white power. The molecular formula was determined as C32H28O10 by HR-ESIMS (m/z 555.2009 [M+Na]+, calcd for 555.1989), indicating sixteen degrees of unsaturation. The 1H NMR spectrum (Table 2) showed 13 resonances, including a 1,3,4-trisubstituted aromatic ring [δH: 6.19 (1H, d, J = 2.4 Hz), 6.15 (1H, d, J = 2.4 Hz)], an aromatic methoxy group [δH 3.79 (s)], three methylene groups [δH 2.66 (2H, m), 2.29 (2H, m), 3.79 (1H, s)], the 13C NMR and DEPT spectrum (Table 2) exhibited sixteen resonances, containing three methylenes (δC: 36.6, 38.3, 21.4), five olefinic methines (δC: 121.7, 115.7, 113.2, 109.6, 101.4), seven olefinic quaternary carbons (δC: 156.8, 156.6, 148.4, 145.1, 144.9, 135.4, 119.5), and one methoxy (δC: 56.3). Detailed analysis of the NMR spectra, molecular formula and the aliphatic methylene

<table>
<thead>
<tr>
<th>No</th>
<th>1H</th>
<th>2H</th>
<th>3H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C1</td>
<td>C1</td>
<td>C1</td>
</tr>
<tr>
<td>2</td>
<td>C1</td>
<td>C1</td>
<td>C1</td>
</tr>
<tr>
<td>3</td>
<td>C1</td>
<td>C1</td>
<td>C1</td>
</tr>
</tbody>
</table>

*a* Recorded at 500 and 125 MHz for 1H and 13C NMR in chloroform-d6, respectively.

*b* Recorded at 600 and 150 MHz for 1H and 13C NMR in methanol-d4, respectively.

*c* Recorded at 600 and 150 MHz for 1H and 13C NMR in methanol-d4, respectively.
signal (δc 21.4, δh 3.94), it was supposed to be symmetrical molecular with the geometrical axis (center) of the –CH2. The correlations of 1H–1H COSY and HSQC spectra revealed the linkages of H-2a/H-3a, and H-7a/H-8a. In the HMBC spectra correlations of the protons δh 3.85 (2H, s) to C-9a, C-10a, and C-11a, and from H-8a to C-1a, C-10a, and C-14a. Therefore, 4 was identified as 10a,10b-methylene-digendentin, and named as fimbriadimerbibenzyl D.

Compound 5 was determined to 10a,10b-methylene-digendentin, named as fimbriadimerbibenzyl E. The cytotoxic activity of these fourteen bibenzyl derivatives (1-7) were evaluated against human HL-60, SMMC-7721, A549, MCF-7 and SW480 cell lines. Among them, those new types of bibenzyl dimer, compounds 2, 4, 5, 6, 7 exhibited broad-spectrum and moderate cytotoxicity with IC50 values ranging from 2.2 to 12.3 μM. Furthermore, compounds 1 and 3 exhibited broad-spectrum and good cytotoxicity with IC50 values range from 5.85 to 21.23 μM. The bioassy results indicated that rich bifendyl in D. limbriatum exert the basic chemicals for the traditional usage of this herbal medicine. Compound 3 exhibited HL-60 and MCF-7 cell lines, having IC50 values of 22.33 and 24.84 μM, respectively. Compound 11 showed moderate cytotoxicities against HL-60 cell line.
and weak cytotoxicities against MCF-7 cell line, having \(IC_{50}\) values of 24.32 and 37.12 \(\mu M\), respectively. Others showed no cytotoxic activities up to a highest concentration of 40 \(\mu M\) in the cell lines tested (Table 5).

To the best of our knowledge, an acyclic bis[bibenzyl] with an aliphatic methylene bridge is rarely reported ever and this is the first example of compounds fimbriabibenzyl A–C (1–7) from the plant of Orchidaceae. This type of compound has only provided in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.052.

### Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.052.

### References and notes

19. The aerial stems of D. fimbriatum Hook were collected at Zhenyuan County, Yunnan Province, People’s Republic of China, in October 2011, and identified by Dr. En-De Liu, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (zsh 11) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.
29. Fimbriabibenzyl A (1) white amorphous powder; \(\delta_{H}^{1} 3.44 \text{ (0.14, MeOCH)}; \text{UV (MeOH)} \delta_{max}^{1} (log e): 204.496, 281.399 \text{ nm} \text{ IR (KBr)}: 3422, 3083, 2934, 2838, 1616, 1586, 1514, 1462, 1452, 1287, 1231, 1197, 1190, 1093, 1032 \text{ cm}^{-1} \); ESIMS (positive ion mode) m/z 561 \text{ [M+H]}^{+} \); HR-ESIMS (positive ion mode) m/z 561.2464 \text{ [M+H]}^{+} \) (calcd for C_{33}H_{36}O_{5} Na_{2}, 561.2483).
30. Fimbriabibenzyl B (2) white amorphous powder; \(\delta_{H}^{1} 3.37 \text{ (0.14, MeOCH)}; \text{UV (MeOH)} \delta_{max}^{1} (log e): 204.492, 281.389 \text{ nm} \text{ IR (KBr)}: 3439, 3422, 2933, 1609, 1581, 1514, 1461, 1453, 1431, 1271, 1220, 1194, 1176, 1138, 1125, 1098, 1033 \text{ cm}^{-1} \); ESIMS (positive ion mode) m/z 583 \text{ [M+Na]}^{+} \); HR-ESIMS (positive ion mode) m/z 583.2300 \text{ [M+Na]}^{+} \) (calcd for C_{35}H_{38}O_{5} Na, 583.2302).

"Table 4

<table>
<thead>
<tr>
<th>(\times 10^{4} \text{ values (log } e))</th>
<th>(\times 10^{4} \text{ values (log } e))</th>
<th>(\times 10^{4} \text{ values (log } e))</th>
<th>(\times 10^{4} \text{ values (log } e))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110.0 (d)</td>
<td>6.38 (1H, d, 2.4)</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>154.9 (s)</td>
<td>6.35 (1H, d, 2.4)</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>104.1 (d)</td>
<td>6.35 (1H, d, 2.4)</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>158.8 (s)</td>
<td>6.35 (1H, d, 2.4)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>152.9 (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>118.1 (d)</td>
<td>6.88 (1H, dd, 7.8, 1.3)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>128.3 (d)</td>
<td>7.14 (1H, dd, 7.8, 7.0)</td>
<td></td>
</tr>
</tbody>
</table>

a \(IC_{50}\) is defined as the concentration that resulted in a 50% decrease in cell number.

b Cisplatin was used as a position control.

Acknowledgments

This work was financially supported by Pu’er Tea Research Institute commissioned project and Yunnan province (Nos. 2012CG001 and 2013IB021). The authors also thank Ji-Jun Chen team and the staff of analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measurements of all spectra. We also thank National Supercomputing center in Jinan for providing the computational resource.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.052.
ESIMS (negative ion mode) m/z 529 [M−H]−; HR-ESIMS (negative ion mode) m/z 529.2227 [M−H]− (calcd for C32H33O7, 529.2232).

33. Fimbriadimerbibenzyl D (4) white amorphous power; [α]D -3.50 (c 0.115, MeOH); UV (MeOH) λmax (log ε): 204 (4.96), 281 (3.99) nm; IR (KBr): νmax 3406, 2934, 2857, 1611, 1514, 1451, 1363, 1346, 1327, 1271, 1231, 1208, 1130, 1030 cm−1; ESIMS (positive ion mode) m/z 555 [M+Na]+; HR-ESIMS (positive ion mode) m/z 555.2009 [M+Na]+ (calcd for C31H32O8Na, 555.1989).

34. Fimbriadimerbibenzyl E (5) white amorphous power; [α]D -13.93 (c 0.144, MeOH); UV (MeOH): λmax (log ε) 204 (5.02), 282 (4.02) nm; IR (KBr): νmax 3417, 2934, 2837, 1604, 1514, 1462, 1430, 1350, 1231, 1192, 1141, 1124, 1086, 1033, 817 cm−1; ESIMS (positive ion mode) m/z 561 [M+H]+; HR-ESIMS (positive ion mode) m/z 561.2456 [M+H]+ (calcd for C33H37O8, 561.2478).

35. Fimbriadimerbibenzyl F (6) white amorphous power; [α]D -10.54 (c 0.117, MeOH); UV (MeOH): λmax (log ε): 204 (4.84), 282 (3.91) nm; IR (KBr): νmax 3440, 3431, 2933, 1616, 1586, 1514, 1463, 1451, 1431, 1382, 1271, 1232, 1198, 1140, 1053, 1033 cm−1; ESIMS (negative ion mode) m/z 559 [M−H]−; HR-ESIMS (negative ion mode) m/z 559.2329 [M−H]− (calcd for C33H35O8, 559.2317).

36. Fimbriadimerbibenzyl G (7) white amorphous power; [α]D -7.12 (c 0.22, MeOH); UV (MeOH) λmax (log ε): 205 (5.10), 282 (4.11) nm; IR (KBr): νmax 3424, 3061, 2999, 2834, 1610, 1514, 1462, 1430, 1345, 1271, 1232, 1195, 1079, 1052, 1032, 839, 818, 795 cm−1; ESIMS (positive ion mode) m/z 583 [M+Na]+; HR-ESIMS (positive ion mode) m/z 583.2399 [M+Na]+ (calcd for C33H36O8Na, 583.2302).

37. 2,4,5,9-Tetrahydroxy-9,10-dihydrophenanthrene (8) yellow amorphous power; [α]D 32.51 (c 0.149, MeOH). UV (MeOH) λmax (log ε): 215 (4.42), 272 (3.96), 306 (3.86) nm; IR (KBr): νmax 3424, 2923, 1621, 1514, 1462, 1430, 1345, 1284, 1254, 1209, 1155, 1052, 1005 cm−1; EIMS m/z: 244 [M]+; HR-EIMS m/z 244.0728 [M]+ (calcd for C14H12O4, 244.0736).