Secondary Metabolites and Biological Activities of *Talaromyces* sp. LGT-2, an Endophytic Fungus from *Tripterygium Wilfordii*

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**Abstract**

In the present study, eleven compounds (1-11) including nine alkaloids (1-9), one triterpenoid saponin (10) and one formamide (11) were isolated from *Talaromyces* sp. LGT-2, an endophytic fungus from *Tripterygium wilfordii*. Their structures were determined based on NMR and ESI-MS spectral data, as well as comparing with previous literature data. This is the first report of the isolation of alkaloids (1-9) from *Talaromyces* genus. In the next step, all compounds were screened for their anti-monoamine oxidase, anti-acetylcholinesterase, antibacterial and antitumor activities. Compound 11 showed moderate anti-monoamine oxidase activity with IC$_{50}$ value of 61 μM; compounds 3, 4, 8 showed weaker anti-acetylcholinesterase activity; compounds 1, 3, 4, 7, 8, 9 showed moderate antibacterial activities; compounds 7, 8, 9 showed cytotoxicity against B16 cancer cell line with inhibitory rate of 86%, 82%, 78%, respectively, at the concentration of 500 μg/mL.

**Keywords:** endophytic fungus; secondary metabolites; monoamine oxidase inhibition; *Talaromyces; Tripterygium wilfordii.*

**Introduction**

Endophytic fungi have been proved to be a new source for natural compounds, literature reports that we can get secondary metabolites which have unique structure and wide range of biological activities, such as antitumor, antimicrobial and antituberculosis. Indeed, structural diversity of these metabolites make endophytic fungi a potential new lead for drug discovery and development (1, 2).

During our ongoing screening for new bioactive natural products from endophytes, we found the fermentation broth of *Talaromyces* sp. LGT-2 (GenBank Accession No. KF934203), an endophytic fungus inhabited in *Tripterygium wilfordii*, showed moderate monoamine oxidase (MAO) inhibitory activity with IC$_{50}$ value of 85 μg/mL. Further chemical investigation resulted in the isolation of compounds 1-11 (Figure 1.). Anti-MAO activity, anti-acetylcholinesterase (anti-AChE), antitumor and antibacterial activities of compounds 1-11 were also evaluated in this study (Table 1.).

**Experimental**

Chemicals and Instrumentation: Column Chromatography (CC): was performed on silica gel (200–300 mesh) and Sephadex LH-20 gel. HPLC was performed on JASCO liquid chromatograph with C$_{18}$ column. TLC: was carried out on silica gel GF254 by using various solvent systems. The structures of the compounds were determined based on their NMR and ESI-MS spectroscopy.

Fungus Material: Chinese medicine
Table 1. Anti-bacterial activity of monomer compounds (MIC, mg/mL).

| Sample | Escherichia coli | Pseudomonas Aeruginosa | Staphylococcus aureus | Bnfillus licheniformis | Streptococcus pneumoniae |
|--------|-----------------|------------------------|----------------------|------------------------|-------------------------|
| 1      | 0.5             | 0.8                    | 0.25                 | 0.25                   | 0.125                   |
| 3      | 0.5             | 0.5                    | 0.5                  | 0.125                  | 1                       |
| 4      | 0.5             | 0.5                    | 0.5                  | 0.25                   | 0.125                   |
| 7      | 0.25            | 0.25                   | 0.125                | 0.125                  | 0.125                   |
| 8      | 0.5             | 0.8                    | 0.25                 | 0.25                   | 0.125                   |
| 9      | 0.5             | 0.5                    | 0.5                  | 0.25                   | 1                       |

This study was focused on compounds isolated from second metabolites of Talaromyces sp. LGT-2, and evaluated biological activities. The methods of Column Chromatography and HPLC Chromatograph were simple and rapid for separation and purification of natural compounds. In the present study, eleven compounds (1-11) including nine alkaloids (1-9), one triterpenoid saponin (10) and one formamide (11) were isolated from Talaromyces sp. LGT-2. This is the first report of the isolation of alkaloids (1-9) from Talaromyces genus. Compound 11 showed moderate anti-monoamine oxidase activity with IC₅₀ value of 61μM, therefore, it was proved to be the responsible compound of anti-MAO activity; compounds 3, 4, 8 showed weaker anti-acetylcholinesterase activity; compounds 1, 3, 4, 7, 8, 9 showed moderate antibacterial activity (Table 1); compounds 7, 8, 9 showed weak cytotoxicity against B16 cancer cell line with inhibitory rate of 86%, 82%, 78%, respectively, at the concentration of 500 μg/mL.

Results and Discussion

This study was focused on compounds isolated from second metabolites of Talaromyces sp. LGT-2, and evaluated biological activities. The methods of Column Chromatography and HPLC Chromatograph were simple and rapid for separation and purification of natural compounds. In the present study, eleven compounds (1-11) including nine alkaloids (1-9), one triterpenoid saponin (10) and one formamide (11) were isolated from Talaromyces sp. LGT-2. This is the first report of the isolation of alkaloids (1-9) from Talaromyces genus. Compound 11 showed moderate anti-monoamine oxidase activity with IC₅₀ value of 61μM, therefore, it was proved to be the responsible compound of anti-MAO activity; compounds 3, 4, 8 showed weaker anti-acetylcholinesterase activity; compounds 1, 3, 4, 7, 8, 9 showed moderate antibacterial activity (Table 1); compounds 7, 8, 9 showed weak cytotoxicity against B16 cancer cell line with inhibitory rate of 86%, 82%, 78%, respectively, at the concentration of 500 μg/mL.

Structure elucidation of the isolated compounds:

Fumitremorgin C (1). Colorless amorphous powder. EI-MS m/z (%): 379 (80) [M]+, 364 (14) [M-CH₃]+, 324 (32), 281 (100), 212 (67). ¹H-NMR (400, CDCl₃, δ, ppm, J/Hz): 7.81 (1H, s, H-1), 7.43 (1H, d, J = 8.0, H-16), 6.86 (1H, s, H-19), 6.80 (1H, d, J = 8.0, H-17), 5.98 (1H, d, J = 9.2, H-3), 4.90 (1H, d, J = 9.2, H-21), 4.18 (1H, dd, J = 11.6, 4.8, H-12), 4.11 (1H, t, J = 8.0, H-6), 3.83
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(3H, s, OMe), 3.64 (2H, m, H-9), 3.50 (1H, dd, J = 16.0, 4.8, H-13a), 3.09 (1H, dd, J = 16.0, 11.6, H-13b), 2.38 (1H, m, H-7a), 2.25 (1H, m, H-7b), 2.06 (1H, m, H-8a), 1.99 (3H, s, H-24), 1.89 (1H, m, H-8b), 1.68 (3H, s, H-23); 13C-NMR (100 MHz, CDCl3, δ, ppm): 169.5 (C-5), 165.7 (C-11), 156.6 (C-18), 137.0 (C-20), 133.9 (C-22), 132.2 (C-2), 124.2 (C-21), 120.7 (C-15), 118.8 (C-16), 109.5 (C-17), 106.3 (C-14), 95.3 (C-19), 59.2 (C-6), 56.8 (C-12), 55.7 (OMe), 51.0 (C-3), 45.4 (C-9), 28.6 (C-7), 25.7 (C-23), 23.0 (C-8), 21.9 (C-13), 18.1 (C-24)[7].

Brevianamide F (2). White amorphous powder. EI-MS m/z (%): 283 (10) [M]+, 185 (8), 130 (100), 84 (20), 43 (17). 1H-NMR (400 MHz, CDCl3, δ, ppm, J/Hz): 8.61 (1H, s, -NH), 7.58 (1H, d, J = 8.0, H-7), 7.38 (1H, d, J = 8.0, H-4), 7.24 (1H, t, J = 8.0, H-6), 7.14 (1H, t, J = 8.0, H-5), 7.05 (1H, s, H-2), 5.86 (1H, s, -NH), 4.36 (1H, d, J = 9.2, H-9), 4.06 (1H, t, J = 8.0, H-12), 3.76 (1H, dd, J = 1.2, 14.4, H-8b), 3.63 (2H, m, H-15), 2.97 (1H, dd, J = 9.2, 14.4, H-8a), 1.86-2.34 (4H, m, H-16, 17); 13C-NMR (100 MHz, CDCl3, δ, ppm): 169.4 (C-11), 165.5 (C-14), 136.6 (C-7a), 126.6 (C-3a), 123.4 (C-2), 122.7 (C-5), 119.7 (C-6), 118.5 (C-4), 111.5 (C-7), 109.5 (C-3), 59.1 (C-12), 54.5 (C-9), 45.3 (C-15), 28.2 (C-17), 26.8 (C-8), 22.5 (C-16)[8].

Pseurotin A1 (3). Light yellow oil. [α]D25 –4.8 (c 0.1, MeOH). ESI-MS m/z 454.1 [M + Na]+. 1H-NMR (400 MHz, DMSO-d6, δ, ppm, J/Hz): 9.94 (1H, s, NH), 8.10 (2H, d, J = 8.0 Hz, H-19, 23), 7.63 (1H, t, J = 7.3, H-21), 7.52 (2H, t, J = 8.0, H-20, 22), 6.07 (1H, d, J = 6.2, 9-OH), 5.75 (1H, d, J = 5.5, 10-OH), 5.42 (1H, dd, J = 7.0, 11.0, H-13), 5.39 (1H, dd, J = 8.1, 11.0, H-12), 4.96 (1H, d, J = 5.1, 11-OH), 4.62 (1H, d, J = 6.2, H-9), 4.45 (1H, dd, J = 5.2, 11.0, H-11), 4.36 (1H, t, J = 5.2, H-10), 3.12 (3H, s, OMe-8), 2.01 (1H, m, H-14), 1.95 (1H, m, H-14), 1.66 (3H, s, H-16), 0.86 (3H, t, J = 7.2, H-15); 13C-NMR (100 MHz, DMSO-d6, δ, ppm): 201.4 (C-4), 194.6 (C-17), 187.2 (C-2), 168.1 (C-6), 135.8 (C-13), 134.6 (C-18), 133.9 (C-21), 130.5 (C-19), 130.5 (C-23), 129.4 (C-12), 128.1 (C-20), 128.1 (C-22), 113.1 (C-3), 97.6 (C-8), 89.5 (C-5), 77.0 (C-9), 72.7 (C-10), 69.9 (C-11), 52.1 (OMe-8), 22.3 (C-14), 14.4 (C-15), 5.8 (C-16)[9].

Pseurotin A2 (4). Light yellow oil. [α]D25 –30.0 (c 0.1, MeOH). ESI-MS m/z 454.1 [M + Na]+. 1H-NMR (400 MHz, DMSO-d6, δ, ppm, J/Hz): 9.98 (1H, s, NH), 8.28 (2H, d, J = 8.0, H-19, 23), 7.69 (1H, t, J = 7.3, H-21), 7.55 (2H, t, J = 8.0, H-20, 22), 6.36 (1H, d, J = 10.0, 9-OH), 5.88 (1H, d, J = 5.5, 10-OH), 5.47 (1H, dt, J = 7.0, 11.0, H-13), 5.41 (1H, dd, J = 8.1, 11.0, H-12), 5.10 (1H, d, J = 5.1, 11-OH), 4.18 (1H, d, J = 10.0, H-9), 4.57 (1H, dd, J = 5.2, 8.1, H-11), 4.51 (1H, t, J = 5.2, H-10), 3.21 (3H, s, OMe-8), 2.07 (2H, m, H-14), 1.66 (3H, s, H-16), 0.93 (3H, t, J = 7.2, H-15); 13C-NMR (100 MHz, DMSO-d6, δ, ppm): 199.8 (C-4), 196.1 (C-17), 188.8 (C-2), 169.2 (C-6), 135.7 (C-13), 134.6 (C-18), 133.9 (C-21), 130.5 (C-19), 130.5 (C-23), 129.4 (C-14), 14.4 (C-15), 5.8 (C-16)[9].
12), 128.1 (C-20), 128.1 (C-22), 114.0 (C-3), 95.4 (C-8), 88.0 (C-5), 75.8 (C-9), 71.9 (C-10), 69.6 (C-11), 51.7 (O Me-8), 21.7 (C-14), 14.4 (C-15), 5.8 (C-16) [3].

Spirotryprostatin A (5). Colorless acicular crystals. ESI-MS m/z 396.0 [M+H]+ [1]. 1H-NMR (400 MHz, CDCl3, δ, ppm, J/Hz): 7.51 (1H, s, H-1), 6.93 (1H, d, J = 8.4, H-4), 6.50 (1H, d, J = 8.4, H-5), 6.43 (1H, s, H-7), 5.00 (2H, m, H-18, 9), 4.77 (1H, J = 9.0, H-19), 4.29 (1H, t, J = 8.4, H-12), 3.80 (3H, s, OMe), 3.68 (2H, m, H-15), 2.60 (1H, dd, J = 10.8, 13.2, H-13b), 1.95-2.41 (7H, m, H-13a, 14, 15, 8), 1.59 (3H, s, H-21), 1.25 (3H, s, H-22) [10].

6-Methoxyspirotryprostatin B (6). Colorless acicular crystals. ESI-MS m/z 392.2 [M+H]+ [1]. 1H-NMR (400 MHz, CDCl3, δ, ppm, J/Hz): 7.64 (1H, s, H-1), 6.95 (1H, d, J = 8.4, H-4), 6.51 (1H, d, J = 8.4, H-5), 6.44 (1H, s, H-7), 5.76 (1H, s, H-8), 5.38 (1H, d, J = 8.8, H-18), 5.19 (1H, d, J = 8.8, H-19), 4.34 (1H, dd, J = 10.0, H-12), 3.80 (3H, s, OMe), 3.83 (1H, m, H-15b), 3.55 (1H, m, H-15a), 2.48 (1H, m, H-13b), 2.12 (1H, m, H-14b), 1.98 (2H, m, H-13a, 14a), 1.59 (3H, s, H-21), 1.25 (3H, s, H-22) [11].

3-Dehydroxymethylbisdethio-3, 10a-bis(methylthio)gliotoxin (7). Colorless acicular crystals. ESI-MS m/z 349.0 [M+Na]+ [1]. 1H-NMR (400 MHz, CD3COCD3, δ, ppm, J/Hz): 2.17 (3H, s, -SMe), 2.43 (3H, s, -SMe), 2.86 (2H, brs, H-10), 3.11 (3H, s, -NMe), 4.71 (1H, d, J = 13.2, H-5a), 4.81 (1H, m, H-6), 5.63 (1H, m, H-7), 5.89 (1H, m, H-8), 5.99 (1H, brs, H-9); 13C-NMR (100 MHz, CD3COCD3, δ, ppm): 168.7 (C-1), 165.0 (C-4), 133.9 (C-9a), 131.2 (C-8), 123.8 (C-7), 120.2 (C-9), 75.0 (C-6), (C-9a) 72.8 (C-11), 70.0 (C-5a), 68.1 (C-3), 38.8 (C-10), 31.9 (-NMe), 17.7 (-SMe), 14.6 (-SMe) (12).

Bisdethiobis(methylthio)gliotoxin (8). Light yellow oil. EI-MS m/z (%): 354 (9) [M]+, 307 (79) [M-SCH3]+, 259 (100), 243 (41), 229 (88), 160 (58). 1H-NMR (400 MHz, CDCl3, δ, ppm, J/Hz): 5.00 (1H, s, -OH), 7.16 (1H, t, J = 8.0, H-8), 6.89 (1H, d, J = 8.0, H-9), 6.81 (1H, d, J = 8.0, H-7), 4.52 (1H, d, J = 12.0, H-15b), 3.98 (1H, d, J = 12.0, H-15a), 3.60 (1H, d, J = 12.6, H-10b), 3.46 (1H, d, J = 12.6, H-10a), 3.21 (3H, s, -NMe), 2.33 (3H, s, -SMe), 2.25 (3H, s, -SMe) [14].

Cyclosieviersoside F (10). Colorless amorphous powder. 1H-NMR (400 MHz, CD3OD, δ, ppm, J/Hz): 4.90 (1H, d, J = 7.6, H-1 of D-glucose), 4.65 (1H, d, J = 7.6, H-1 of D-xylose), 4.29 (1H, m, H-16), 3.13-3.85 (11H, m, D-xylose + D-glucose), 1.00, 1.01, 1.12, 1.20, 1.25, 1.25, 1.27 (each 3H, s, Me-18, 21, 26, 27, 28, 29, 30), 0.27 and 0.58 (each 1H, d, J = 4.0, H-19); 13C-NMR (100 MHz, CD3OD, δ, ppm): 107.4 (C-1′, Xyl), 104.9 (C-1′′, Xyl), 90.0 (C-20), 88.4 (C-3), 82.5 (C-24), 80.0 (C-6), 78.6 (C-3′′), 77.7 (C-5′′, Xyl), 75.6 (C-2′′, Xyl), 75.5 (C-2′′, Xyl), 74.7 (C-3′′, Xyl), 74.7 (C-16), 71.8 (C-4′, Xyl), 71.3 (C-4′′, Xyl), 71.3 (C-25), 66.7 (C-5′′, Xyl), 62.9 (C-6′′, Xyl), 58.9 (C-17), 53.3 (C-5), 46.7 (C-13), 47.0 (C-14), 46.7 (C-8), 46.1 (C-15), 43.1 (C-4′, Xyl), 35.4 (C-7), 35.1 (C-22), 34.2 (C-12), 33.0 (C-1), 30.4 (C-2), 29.6 (C-19), 28.2 (C-26), 29.6 (C-10), 28.5 (C-27), 27.0 (C-29), 27.6 (C-21), 26.5 (C-11), 26.8 (C-23), 22.1 (C-9), 21.5 (C-28), 20.2 (C-18), 16.6 (C-30) [15].

(Z)-N-(4-hydroxystyrlyl)formamide [11]. Colorless acicular crystals. ESI-MS m/z 162.0[M-H]+. 1H-NMR (CD3OD, 400 MHz, δ, ppm, J/Hz): 8.08 (1H, s, -CHO), 7.17 (2H, d, J = 8.0, H-2, H-6), 6.75 (1H, d, J = 9.6, H-7), 6.73 (2H, d, J = 8.0, H-3, H-5), 5.73 (1H, d, J = 9.6, H-8); 13C-NMR (100 MHz, CD3OD, δ, ppm): 118.0 (C-1), 111.1 (C-2), 126.8 (C-1′), 130.2 (C-2′′/6′′), 116.2 (C-3′′/5′′), 157.0 (C-4′′), 160.7 (-CHO) (16).

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