The Anticancer Activities Phenolic Amides from the Stem of *Lycium barbarum*

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Received: 7 April 2017 / Accepted: 15 May 2017 / Published online: 6 June 2017
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**Abstract** Four new phenolic amides, 4-O-methylgrosamidine (1), (E)-2-(4,5-dihydroxy-2-{3-[(4-hydroxyphenethyl)amino]-3-oxopropyl}-phenyl)-3-(4-hydroxy-3-methoxyphenyl)-N-(4-hydroxyphenethyl)acryl-amide (2), (Z)-lyciumamide C (3), (Z)-thoreliamide B (4), together with thirteen known phenolic amides were identified from the stem of *Lycium barbarum*. The structures of the new compounds were determined by spectroscopic methods. All compounds were evaluated for their anti-cancer activities against human glioma stem cell lines.

**Graphical Abstract**

*Keywords* Lycium barbarum · Phenolic amides · Anticancer activities · Glioma stem cell

**Electronic supplementary material** The online version of this article (doi:10.1007/s13659-017-0134-x) contains supplementary material, which is available to authorized users.

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1 Introduction

*Lycium barbarum* had a long history of usage as a traditional herbal medicine and functional food in Asian countries [1]. Besides, its fruits known as goji or wolf berries, were beneficial to human health and very important agricultural products [2,3]. Modern pharmacological studies indicated *L. barbarum* possessed widely health-promoting and medical effects, including antioxidant [4], lipotropic [5], hepatic function protecting effects [5], immunomodulatory properties [6], antiaging [7], anticancer activities [8–11] and so on. Phytotoxicological studies showed that phenolic amides were not only characteristic compounds but also abundant ones in *L. Barbarum* [2]. Phenolic amides, originating from the condensation of cinnamic acid derivatives and tyramines, octopamines or aliphatic amines [2,12], had been reported a range of biological activities, like antioxidant [4], antiobesity [13], cytotoxicity [14], anti-inflammatory activity [15] and potent inhibitors of de novo nucleotide biosynthesis [16], and they also seemed to play an important role in plant defense against pathogens [12]. Continuation of our study on the phenolic amides had led to the isolation of four new phenolic amides, 4-O-methylgrossamide (1), (E)-2-(4,5-dihydroxy-2-([4-(4-hydroxyphenethyl)amino]-3-oxo-propyl)-phenyl)-3-(4-hydroxy-3-methoxyphenyl)-N-(4-hydroxyphenethyl)acryl-amide (2), (Z)-lyciumamide C (3), (Z)-thoreliamide B (4), together with thirteen known phenolic amides (Fig. 1) from *L. barbarum*. The known compounds were identified as grossamide (5) [17], lyciumamide C (6) [4], (Z)-3-([2,3-trans]-2-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-2,3-dihydrobenzo[1,4]-dioxin-6-yl]-N-(4-hydroxyphenethyl)acrylamide (7), [18] (E)-3-([2,3-trans]-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-methyl-2,3-dihydrobenzo[1,4]-dioxin-6-yl]-N-(4-hydroxyphenethyl)acryl-amide (8), [18] (E)-thoreliamide B (9) [19], canabisin E (10) [20], canabisin D (11) [21], 1,2-dihydro-6,8-dimethoxy-7-hydroxy-(3,5-dimethoxy-4-hydroxyphenyl)-N,N-2-bis(2-(4-hydroxyphenethyl)ethyl)-2,3-naphthalenedi-carboxamide (12) [22], cannabigin G (13) [20], N-E-p-coumaroyl tyramine (14) [23], N-E-caffeoyl tyramine (15) [24], N-E-feruloyl tyramine (16) [25], N-E-feruloyl octopamine (17) [25], by comparison with the data in the literature values. All of the compounds were evaluated for anti-cancer activity against human glioma stem cell lines, and compounds 1 and 5 exhibited moderate anti-cancer activities. Herein the isolation, structural elucidation and the bioactivity of the phenolic compounds were reported.

2 Results and Discussion

Compound 1 was obtained as a white powder. Its molecular formula was determined to be C37H39N2O8, from its 13C NMR and HRESIMS (m/z 639.2721 [M+H]+, calcd. for C37H39N2O8, 639.2701), suggesting 20 degrees of unsaturation. The UV maxima (249, 289 and 320 nm) showed the existence of aromatic rings [19]. The IR spectrum indicated the presence of OH (3387 cm−1), aromatic rings (1651, 1605, 1517 cm−1), and C–N bond (1265 cm−1) groups [14]. In the 1H NMR spectrum, four pairs symmetrical aromatic protons signals at δH 7.04 (2H, d, J = 8.4 Hz), 6.71 (2H, d, J = 8.4 Hz), 7.01 (2H, d, J = 8.5 Hz), and 6.69 (2H, J = 8.5 Hz) were readily assigned to two AA′BB′ system of 1 [26]. Four pairs of vicinal methylene protons signals at δH 2.67 (4H, t, J = 5.1 Hz), 3.36 (4H, t, J = 5.1 Hz), along with four methylenes carbons signals at δC 169.4 (s), 165.2 (s) were readily assigned to two amide moieties, which indicated by HMBC correlations from δH 5.93 (1H, d, J = 8.1 Hz, H-7) and 3.36 (2H, t, J = 5.1 Hz, H-8′) to δC 169.4 (s, C-9), from δH 7.38 (1H, d, J = 15.7 Hz, H-7′) and 3.36 (2H, t, J = 5.1 Hz, H-8″) to δC 165.2 (s, C-9′). According to these 1D NMR data (Table 1), compound 1 was readily identified as a phenolic amide with two tyramine moieties [18]. Detailed analysis of 1D NMR spectra of 1 displayed similarities to those of 5 [27], except for a methoxy at C-4 in 1 instead of a hydrogen at C-4 in 5, which indicated by HMBC correlations from δH 3.76 (3H, s, 4-OCH3), 6.85 (1H, dd, J = 8.4, 2.0 Hz, H-6) and 6.94 (1H, d, J = 2.0 Hz, H-2) to δC 149.0 (s, C-4), from δH 3.78 (3H, s, 3-OCH3) and 6.98 (1H, d, J = 8.4 Hz, H-5) to δC 148.9 (s, C-3).

The large coupling constants of H-7′ with H-8′ (J = 15.7 Hz) suggested the configuration of the double bond at C-7′/C-8′ was E [17]. The configuration of H-7 with H-8 was three, which was assigned by the ROESY correlations of H-8 and H-6 (Fig. 3), while there was no ROESY correlation between H-7 and H-8 [28]. The absolute configuration at C-7 and C-8 were 7R, 8S, respectively, which determined by the negative cotton effect at 257 nm (Δε = −0.45) observed in circular dichroic spectrum [28–31]. Detailed analysis of 2D NMR data (HSQC, HMBC, ROESY) established the structure of 1 to be as shown, named 4-O-methylgrossamide.

Compound 2 was obtained as a yellow powder. Its molecular formula C35H36N2O8 was established by 13C NMR and positive HR–ESI–MS data (M+Na)+ 635.2368, calcd. for C35H36N2O8Na, 635.2364. The IR spectrum absorptions showed the existence of OH (3422 cm−1), conjugated C=O (1636 cm−1) and Ph (1614 and 1514 cm−1) groups [19]. In the 1D-NMR spectrum, four pairs symmetrical aromatic protons at δH 6.94 (2H, d, J = 8.4 Hz), 6.68 (2H, d, J = 8.4 Hz), 6.91 (2H, d,
Table 1 $^1$H (600 MHz) and $^{13}$C NMR (150 MHz) Data for compounds 1, 2 in DMSO-$d_6$ and 4 in CD$_3$OD ($\delta$ in ppm, $J$ in Hz)

| No. | 1 |  | 2 |  | 4 |  |  |
|-----|---|---|---|---|---|---|---|
| 1   | $\delta_H$ | 132.1 | $\delta_C$ | 126.5 | $\delta_H$ | 112.4 | $\delta_C$ | 7.21 d (2.1) | 119.0 |
| 2   | 6.94 d (2.0) | 109.6 | 6.39 d (2.0) | 112.4 | 7.21 d (2.1) | 119.0 |
| 3   | 148.9 | 147.4 |
| 4   | 149.0 | 146.9 |
| 5   | 6.98 d (8.4) | 111.7 | 6.66 d (8.3) | 115.2 | 6.92 d (8.4) | 117.8 |
| 6   | 6.85 dd (8.4, 2.0) | 118.4 | 6.73 dd (8.3, 2.0) | 124.7 | 6.98 dd (8.4, 2.1) | 124.5 |
| 7   | 5.93 d (8.1) | 87.5 | 7.57 s | 135.0 | 6.63 d (12.6) | 137.2 |
| 8   | 4.23 d (8.1) | 56.0 |  | 125.6 | 5.88 d (12.6) | 123.3 |
| 9   | 169.4 | 166.8 | 170.4 |
| 3-OMe | 3.78 s | 55.7 | 3.37 s | 54.6 |
| 4-OMe | 3.76 s | 55.5 |
| 1'   | 128.6 | 130.7 |
| 2'   | 7.16 d (1.6) | 111.7 | 6.52 s | 116.7 | 6.70 br. s | 106.0 |
| 3'   | 144.1 | 144.4 |
| 4'   | 148.7 | 145.5 |
| 5'   | 128.4 | 116.6 | 149.5 |
| 6'   | 6.91 s | 115.9 | 131.4 | 6.70 br. s | 106.0 |
| 7'   | 7.38 d (15.7) | 138.7 | 2.51 m | 27.9 | 4.88 d (8.1) | 78.0 |
| 8'   | 6.49 d (15.7) | 119.7 | 2.13 td (9.4, 6.5) | 36.0 | 4.07 ddd (8.1, 4.3, 2.5) | 80.3 |
| 9'   | 165.2 | 171.4 | 3.71 dd (12.5, 2.5) | 62.2 |
| 3'-OMe | 3.85 s | 55.7 | 3.75 s | 56.9 |
| 1''  | 129.7 | 129.6 | 131.3 |
| 2''  | 7.04 d (8.4) | 129.6 | 6.94 d (8.4) | 129.5 | 6.98 d (8.4) | 130.8 |
| 3''  | 6.71 d (8.4) | 115.2 | 6.68 d (8.4) | 115.1 | 6.67 d (8.4) | 116.4 |
| 4''  | 155.7 | 155.7 | 157.1 |
| 5''  | 6.71 d (8.4) | 115.2 | 6.68 d (8.4) | 115.1 | 6.67 d (8.4) | 116.4 |
| 6''  | 7.04 d (8.4) | 129.6 | 6.94 d (8.4) | 129.5 | 6.98 d (8.4) | 130.8 |
| 7''  | 2.67 t (5.1) | 34.4 | 2.66 t (7.5) | 34.6 | 2.68 t (7.6) | 35.5 |
| 8''  | 3.36 t (5.1) | 40.9 | 3.41 t (7.5) | 41.5 | 3.41 m | 42.5 |
| 1''' | 129.3 | 129.6 |
| 2''' | 7.01 d (8.5) | 129.5 | 6.91 d (8.4) | 129.4 |
| 3''' | 6.69 d (8.5) | 115.1 | 6.65 d (8.4) | 115.1 |
| 4''' | 155.7 | 155.6 |
| 5''' | 6.69 d (8.5) | 115.1 | 6.65 d (8.4) | 115.1 |
| 6''' | 7.01 d (8.5) | 129.5 | 6.91 d (8.4) | 129.4 |
| 7''' | 2.67 t (5.1) | 34.2 | 2.58 t (7.4) | 34.5 |
| 8''' | 3.36 t (5.1) | 40.8 | 3.20 t (7.4) | 40.7 |

$J = 8.4$ Hz), and 6.65 (2H, d, $J = 8.4$ Hz) suggested the presence of two AA'BB' systems [26]. Four pairs of vicinal methylenes protons signals at $\delta_H$ 2.66 (2H, t, $J = 7.5$ Hz), 3.41 (2H, t, $J = 7.5$ Hz), 2.58 (2H, t, $J = 7.4$ Hz), and 3.20 (2H, t, $J = 7.4$ Hz), along with two carbonyl carbons at $\delta_C$ 171.4 (s), and 166.8 (s) were the characteristic resonances for two –CONHCH$_2$CH$_2$– moieties, which were supported by HMBC correlations of $\delta_H$ 7.57 (1H, s, H-7), 3.41 (2H, t, $J = 7.5$ Hz, H-8") with $\delta_C$ 166.8 (s, C-9), of $\delta_H$ 2.43 (1H, m, H-7), 3.20 (2H, t, $J = 7.4$ Hz, H-8"”) with $\delta_C$ 171.4 (s, C-9”) [19]. Analysis of the $^1$H, $^{13}$C NMR data (Table 1) indicated that 2 was similar to those of (E)-2-(4,5-dihydroxy-2-[(4-hydroxyphenethyl)amino]-3-oxo-propyl)-phenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-N-
Fig. 1 Structures of compounds 1–17
The only methoxyl was substituted at C-3, which was supported by the HMBC correlations of \( \delta_{H} 3.37 \) (3H, s), 6.66 (1H, d, \( J = 8.3 \) Hz, H-5) with \( \delta_{C} 147.4 \) (s, C-3). The HMBC correlations of \( \delta_{H} 2.43 \) (1H, m, H-7) with \( \delta_{C} 171.4 \) (s, C-9), 131.4 (s, C-6'), and 116.7 (d, C-2'), of \( \delta_{H} 2.13 \) (2H, td, \( J = 9.4 \), 6.5 Hz, H-8') with \( \delta_{C} 130.7 \) (s, C-1'), suggested the \(-\text{CH}_2\text{CH}_2-\) fragment was connected between C-1' and C-9' (Fig. 2). ROESY correlation of H-6 with H-5' (Fig. 3) suggested two benzene rings, connected to C-7 and C-8 respectively, were on the same side, further confirmed the double bond of C-7/C-8 was E [18].

Detailed analysis of 1D, 2D NMR spectral data (Table 2) suggested that the planar structure of 3 was the same as 6. The visible difference was that the configuration of the double bond at C-7/C-8 in 3 was Z, which was suggested by coupling constants (\( J = 12.6 \) Hz) between H-7 and H-8 [4, 25]. The NOESY correlations of H-7' and H-8' suggested the configuration of H-7'/H-8' was erythro (Fig. 3) [4], which also supported by the specific optical rotation of 3 \( [\alpha]_{D}^{25} + 5.0^\circ \) (c 0.11, MeOH) was the same side as that of 6 \( [\alpha]_{D}^{22} - 7.9^\circ \) (c 0.34, MeOH) [4]. Other parts of the structure were identical to those of 6 by detailed analysis of its 2D NMR spectra. Thus, the structure of 3 was established and named Z-lyciumamide C.

The aromatic rings of the phenylpropanoid units exhibited various oxygenation patterns, and it had been summarized that the deoxygenation patterns might involve the 3,4-, 2,4-, 2,5- and 2,6-positions [4, 17, 19–21], but seldom the 3,5-positions [18]. Some compounds which had 3,5-positions substituents
always been proven to be 3,4- 2,4- or 2,5-positions substituents, owing to the existence of “deceptively simple” protons signals when changed the deuterated solvent or temperature [32]. Interestingly, in our experiment, the “deceptively simple” protons signals that exhibited two broad singlets with approximate integrations of 1:2 for H-2° and for H-5°, H-6° were found when using DMSO-d6, and they would be a set of proton signals with a m-coupling constant for H-2° (d, J = 2.0 Hz), an o-coupling constant for H-5° (d, J = 8.1 Hz), and o-m-coupling constants for H-6° (dd, J = 8.1, 2.0 Hz) in 3, and with a m-coupling constant for H-2° (d, J = 2.0 Hz), an o-coupling constant for H-5° (d, J = 8.2 Hz), and o-m-coupling constants for H-6° (dd, J = 8.2, 2.0 Hz) in 6 when using CD3OD (SI, Figs. S37, S38).

Compound 4 was obtained as faint yellow powder. Its molecular formula C28H29NO8 was established by 13C NMR and positive ESIMS m/z 508 [M+H]+. The UV maxima (207, 240 and 278 nm) showed the existence of aromatic rings and IR bands (3421, 1648, 1614, 1512, and 1274 cm−1) displayed aromatic rings, hydroxyl functions and a C–N bond [18]. Analysis of the 1H, 13C NMR data (Table 1) revealed that compounds 4 and 9 are structurally similar except for the configuration of the double bone at C-7/C-8. The coupling constants (J = 12.6 Hz) between H-7 and H-8 suggested the configuration of the double bone at C-7/C-8 in 4 was Z [21]. The 1H–1H COSY correlations of δH 4.88 (1H, d, J = 8.1 Hz, H-7°) 4.07 (1H, ddd, J = 8.1, 4.3, 2.5 Hz, H-8°)/3.71 (1H, dd, J = 12.5, 2.5 Hz, H-9°) 3.48 (1H, dd, J = 12.5, 4.3 Hz, H-9′) revealed the presence of –CH(7°)–CH(8°)–CH2(9°)– fragment, also supported by HMBC correlations of δH 4.88 (1H, d, J = 8.1 Hz, H-7°), 4.07 (1H, ddd, J = 8.1, 4.3,
0.25 Hz, H-8') to δC 106.0 (d, C-2', 6') (Fig. 2). The relative configuration of H-7'/H-8' was threo orientation, for the large coupling constant (J = 8.1 Hz) between H-7' and H-8', along with ROESY correlation of H-2' and H-6' with H-8' and there was no NOE correlation between H-7' and H-8' [33–35] (Fig. 3). Other parts of the structure were identical to those of 9 by detailed analysis of its 2D NMR spectra. Thus, compound 4 was assigned as (Z)-thoreliamide B.

A literature survey shows that the E-isomers of this type of compounds are widespread in some genera and small amount of Z-isomers [12, 18]. In this paper, three pairs of Z-E-isomers (3 and 6; 4 and 9; 7 and 8) were reported. Due to their different retention times on the Rp-C18 column, every pair of isomers was separated by HPLC.

All the compounds were evaluated for their bioactivity against two human glioma stem cell lines (GSC-3# and GSC-12#), by the cell viability assay and phenotypic screening. The results showed that compound 5 exhibited the moderate cytotoxicity against GSC-3# and GSC-12# at the concentration of 10 μg/mL (Fig. 4a), and the IC50 values were 6.40 and 5.85 μg/mL respectively (Fig. 5).

Compound 1 showed the moderate cytotoxicity against GSC-3# and GSC-12# at the concentration of 25 μg/mL (Fig. 4b), and the IC50 values were 28.51 and 19.67 μg/mL respectively (Fig. 5).

### 3 Experimental Section

#### 3.1 General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were detected on a SHMDZU.

| Table 2 | 1H (500 MHz) and 13C NMR (125 MHz) Data for compounds 3 and 6 in DMSO-d6 and CD3OD (δ in ppm, J in Hz) |
|---------|---------------------------------------------------------------------------------|
| No.    | 3 (in DMSO-d6) | 3 (in CD3OD) | 6 (in DMSO-d6) | 6 (in CD3OD) |
|        | δH         | δC         | δH         | δC         | δH         | δC         | δH         | δC         |
| 1      | 128.7      | 130.5      | 128.5      | 130.8      |
| 2      | 7.60 s     | 114.4      | 7.22 d (1.7) | 115.4      | 7.10 s     | 111.9      | 7.07 d (1.5) | 113.2      |
| 3      | 142.9      | 145.2      | 143.9      | 145.7      |
| 4      | 148.1      | 150.2      | 149.0      | 151.3      |
| 5      | 128.7      | 129.9      | 130.1      | 130.2      |
| 6      | 7.23 s     | 119.7      | 7.05 s     | 120.2      | 7.16 s     | 116.6      | 7.12 s     | 116.2      |
| 7      | 6.55 d (12.9) | 136.7      | 6.66 d (12.6) | 138.5      | 7.38 d (15.7) | 139.1      | 7.46 d (15.7) | 141.9      |
| 8      | 5.81 d (12.9) | 121.5      | 5.85 d (12.6) | 122.3      | 6.50 d (15.7) | 119.4      | 6.42 d (15.7) | 119.7      |
| 9      | 166.2      | 170.3      | 165.4      | 169.0      |
| 5'-OMe | 3.73 s     | 55.6       | 3.78 s     | 56.5       | 3.73 s     | 55.6       | 3.80 s     | 56.4       |
| 1'      | 132.2      | 134.5      | 132.0      | 134.1      |
| 2'      | 6.90 s     | 110.3      | 6.93 d (2.0) | 110.6      | 6.94 s     | 110.4      | 6.93 d (2.0) | 110.5      |
| 3'      | 147.6      | 149.2      | 147.7      | 149.1      |
| 4'      | 146.4      | 147.7      | 146.6      | 147.7      |
| 5'      | 6.75 s     | 115.3      | 6.75 d (8.1) | 116.3      | 6.79 s     | 115.4      | 6.77 d (8.2) | 116.2      |
| 6'      | 6.75 s     | 118.5      | 6.81 d (8.1, 2.0) | 119.8      | 6.79 s     | 118.8      | 6.81 dd (8.2,2.0) | 118.5      |
| 7'      | 5.50 d (6.5) | 87.6      | 5.56 d (6.2) | 89.7      | 5.52 d (6.9) | 87.7      | 5.55 d (6.4) | 89.7      |
| 8'      | 3.46 d (6.5) | 52.8      | 3.41 d (5.8) | 55.2      | 3.49 d (6.9) | 52.7      | 3.45 dd (8.0,6.7) | 54.8      |
| 9'      | 3.61 dd (10.6,7.2) | 63.1      | 3.82 d (4.8) | 64.9      | 3.71 dd (10.6,6.0) | 62.8      | 3.83 d (5.2) | 64.6      |
| 3'-OMe | 3.76 s     | 55.6       | 3.85 s     | 56.8       | 3.77 s     | 55.7       | 3.87 s     | 56.7       |
| 1''     | 129.5      | 131.3      | 129.5      | 131.2      |
| 2''     | 6.98 d (8.5) | 129.4      | 6.96 d (8.4) | 130.8      | 7.04 d (8.6) | 129.6      | 7.04 d (8.4) | 130.7      |
| 3''     | 6.67 d (8.4) | 115.1      | 6.68 d (8.5) | 116.4      | 6.71 d (8.4) | 115.2      | 6.71 d (8.5) | 116.3      |
| 4''     | 155.6      | 157.1      | 155.7      | 156.9      |
| 5''     | 6.67 d (8.4) | 115.1      | 6.68 d (8.5) | 116.4      | 6.71 d (8.4) | 115.2      | 6.71 d (8.5) | 116.3      |
| 6''     | 6.98 d (8.5) | 129.4      | 6.96 d (8.4) | 130.8      | 7.04 d (8.6) | 129.6      | 7.04 d (8.4) | 130.7      |
| 7''     | 2.61 t (7.5) | 34.2      | 2.65 t (7.5) | 35.9      | 2.67 t (7.4) | 34.4      | 2.74 t (7.3) | 35.8      |
| 8''     | 3.26 t (7.5) | 40.6      | 3.31 t (7.5) | 42.5      | 3.35 t (7.4) | 40.8      | 3.45 t (7.3) | 42.5      |
Fig. 4 Compounds 1–5 against human glioma stem cells by phenotypic screening; a Compound 5 against GSC-3# and GSC-12# at 10 μg/mL; b Compounds 1–4 against GSC-12# at 25 μg/mL.

Fig. 5 The IC_{50} value for compounds 1 and 5 against human glioma stem cell lines.
UV-2401PC spectrometer. IR spectra were determined on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr disks. 1D and 2D NMR spectra were recorded on Bruker DRX-400, DRX-500, and DRX-600 spectrometers using TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESI–MS and EI–MS (HR–EI–MS) analysis were carried out on Waters Xevo TQS and Waters AutoSpec Premier P776 mass spectrometers, respectively. Semi-preparative HPLC was performed on a Waters 600 with a COSMOSIL C18 (10 × 250 mm) column. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd., People’s Republic of China), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Fractions were visualized by 10% sulfuric acid ethanol solution and spots were monitored by thin-layer chromatography (TLC) (GF254, Qingdao Marine Chemical Co., Ltd.), and spots were further purified on a silica gel column, eluting with CHCl3–MeOH (20:1–9:1) to give nine fractions. Fraction VI (22 g) was chromatographed on silica gel, eluted with CHCl3–MeOH (20:1–5:1) to give 12 (568.9 mg) and the residue, then the latter was further purified over HPLC to afford 2 (4.86 mg, tR 18 min; CH3CN/H2O 20:80, 3 mL/min), and 11 (15.5 mg, tR 23.5 min; CH3CN/H2O 20:80, 3 mL/min). Fraction VII (92 g) was separated on a MCI column eluted successively with CHCl3/MeOH (20:1–9:1) to obtain subfractions VII-5-2a and VII-5-2b. Purified of subfraction VII-5-2a by HPLC afforded 6 (54.3 mg, tR 18 min; CH3CN/H2O 38:62, 3 mL/min), 3 (43.2 mg, tR 23 min; CH3CN/H2O 38:62, 3 mL/min). Subfraction VII-5-2b was separated by Sephadex LH-20 column (elution with MeOH), and then chromatographed on silica gel column (CHCl3/MeOH, 10:1), further purified by HPLC to afford 7 (12.5 mg, tR 20 min; CH3CN/H2O 38:62, 3 mL/min) and 8 (7.8 mg, tR 25 min; CH3CN/H2O 38:62, 3 mL/min).

3.3.1 4-O-methylgossamine (1)

White powder; [α]D25° – 16.8° (c 0.11, DMSO); UV (MeOH) λmax (log ε) 249 (4.2), 289 (4.4), 320(4.4); IR (KBr) νmax 3387, 1651, 1605, 1517, 1265 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz) data, see Table 1; positive ESI-MS m/z 639 [M+H]+; positive HRESIMS m/z 639.2721 [M+H]+ (calcd. for C33H39N2O8, 639.2701).

3.3.2 (E)-2-(4,5-dihydroxy-2-{3-{[(4-hydroxyphenethyl)amino]-3-oxopropyl}phenyl}-3-(4-hydroxy-3-methoxyphenyl)-N-(4-hydroxyphenethyl)-acrylamide (2)

Yellow powder; [α]D19° + 2.3° (c 0.47, MeOH); UV (MeOH) λmax (log ε) 203 (4.7), 217 (4.6), 289 (4.1), 324 (4.1); IR (KBr) νmax 3422, 2927, 1636, 1614, 1449, 1262 cm−1; 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz) data, see Table 1; positive HRESIMS [M+Na]+ 635.2368 (calcd. for C33H36N2O8Na, 635.2364).

3.3.3 Z-lyciumamide C (3)

White powder; [α]D25° – 5.0° (c 0.11, MeOH); UV (MeOH) λmax (log ε) 203 (4.6), 225 (4.5), 286 (4.2), 304 (4.2); IR
Cell viability assay was performed by the MTS method as previously described. GSCs were digested with 20000 cells/well. The compounds were added with a serial gradient concentration (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 μg/mL) and cultured in cell incubator for 72 h. MTS reagent was diluted 1:5 with fresh medium and mixed well. The old medium was removed and subsequently the fresh medium was added with 100 μL/well. The cells were incubated for 1.5 h. Absorbance was measured by Hybrid Reader (BioTek synergy H1) at 490 nm. The cell viability was evaluated by percentage compared with DMSO control group. The half-maximal inhibitory concentration (IC50) was measured and calculated by Graph Pad Prism 5 software.

3.3.4 Z-thoreliamide B (4)

Faint yellow powder; [α]D21 — 11.1° (c 0.11, MeOH); UV (MeOH) λmax (log ε) 207 (4.7), 240 (4.3), 278 (4.0); IR (KBr) νmax 3408, 1647, 1608, 1516, 1273, 1219 cm−1; 1H NMR (CD3OD, 500 MHz) and 13C NMR (CD3OD, 125 MHz) data, see Table 1; positive ESIMS m/z 492 [M+H]+; negative HRESIMS m/z 492.2027 [M−H]− (calcd. for C28H30NO7, 492.2017).

3.4 Anticancer Activities

GSC-3# and GSC-12# were human glioma stem cell lines that were established by Kunming institute of zoology from two human glioblastoma multiforme samples. The glioma stem cell was cultured in serum-free medium DMEM F12 supplied with 1xB27 and 50 ng/mL EGF, BFGF and 1% penicillin/streptomycin. The glioma stem cell was cultured in serum-free medium DMEM F12 in the laminin pre-coating dishes and cultured in 37 °C, 5% CO2 incubator. Cell viability assay was performed by the MTS method as previously described. GSCs were digested and counted, seeded in laminin pre-coating 96-well-plate with 20000 cells/well. The compounds were added with a serial gradient concentration (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 μg/mL) and cultured in cell incubator for 72 h. MTS reagent was diluted 1:5 with fresh medium and mixed well. The old medium was removed and subsequently the fresh medium was added with 100 μL/well. The cells were incubated for 1.5 h. Absorbance was measured by Hybrid Reader (BioTek synergy H1) at 490 nm. The cell viability was evaluated by percentage compared with DMSO control group. The half-maximal inhibitory concentration (IC50) was measured and calculated by Graph Pad Prism 5 software.

4 Supporting Information

1D, 2D NMR spectra, ESIMS/MS, HRESIMS, UV and IR of compounds 1–4 and influence of deuterated solvent on the 1H NMR spectra of compounds 3 and 6 are available).

Acknowledgements

The authors are grateful to agricultural comprehensive development project of science and technology in Ningxia province (Research on Chinese wolfberry active substances and health products), and STS project of Chinese Academy of Sciences for the financial support.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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