Synthesis and antitumor activity in vitro of glioperazine C and its derivatives

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ABSTRACT

Glioperazine C, a naturally occurring diketopiperazine, together with its thirteen derivatives, were synthesized in 35-47% overall yields. The first total synthesis of glioperazine C was achieved by intermolecular condensation and intramolecular cyclization. Their structures were characterized on the basis of 1H, 13C NMR, and those compounds were evaluated for their anticancer activities against K562, HT-29 and HepG2 cell lines. Results showed that glioperazine C containing D-Trp and D-Thr demonstrated potent anticancer activity against HepG2 cell line (IC50 = 6.51 µM).

Keywords: Glioperazine C, Dioxopiperazine, Cyclic dipeptides, Anticancer

INTRODUCTION

2,5-Diketopiperazines are cyclic dipeptides from two amino acids with or without further structural modifications. These 2,5-diketopiperazines possessed attractive bioactivities including anti-tumor, neuroprotective, immune and metabolic regulatory, oxytocin inhibitory and anti-inflammatory effects, antibiotic activity, etc.[1] Glioperazine C (Fig. 1), a natural 2,5-diketopiperazine containing an indole fragment, was first isolated from the mycelia of liquid fermentation culture of fungus (Bionectra byssicola F120) in 2007.[2] The development of convenient and efficient synthetic approaches for the preparation of large quantities of glioperazine C and their analogues would be highly desirable for their biological studies. Herein, we would like to report the synthesis and the antitumor evaluation in vitro of glioperazine C and its cyclic dipeptides derivatives.

![Glioperazine C](image_url)

Fig. 1 The structure of glioperazine C
All reagents and solvents were purchased from commercially suppliers and used without further purification. \(^1\)H NMR spectra were recorded with a Bruker AM-400 NMR spectrometer with CDCl\(_3\)-d\(_6\) or DMSO-d\(_6\) as the solvent. \(^13\)C NMR spectra were recorded at 100 MHz. All chemical shifts (\(\delta_{\text{H}}\) and \(\delta_{\text{C}}\)) were reported in parts per million (ppm) and the coupling constants were measured in hertz (Hz). Thin layer chromatography was performed using silica gel 60 F\(_{254}\) plates (Merck) with observation under UV when necessary. Chromatography was performed on 230-400 mesh silica gel.

### 2.2.1 Synthesis route of glioperazine C and its derivatives

\[
\begin{align*}
\text{NH}_2 & \quad \text{COOMe} \quad R \quad \text{COOH} \\
& \quad \xrightarrow{a} \quad \text{NHBOc} \\
& \quad \xrightarrow{b} \quad \text{NHBOc} \\
& \quad \xrightarrow{c} \quad \text{NHBOc} \\
\end{align*}
\]

**Condition and reagents.** a) EDC, HOBt, Et\(_2\)N, DCM, r.t. 72%-90%; b) 1,4-dioxane/H\(_2\)O, 150 °C, 60%-73%.

![Scheme 1. Synthesis route of glioperazine C and its derivatives](image)

### 2.2.1.1 General procedure for the synthesis of N-Boc-dipeptide methyl esters (1a-II) [3] [4]

To a solution of D/L-tryptophan methyl esters (5.0 mmol) in CH\(_2\)Cl\(_2\), EDC (6.0 mmol), Et\(_2\)N (6 mmol), HOBT (6.0 mmol) and N-Boc-amino acids (6.0 mmol) was added at room temperature. The solution was stirred at room temperature for 3-5 h. After completion of the reaction, the solution was washed with brine (20 mL\(\times\)3). The organic layer was dried over anhydrous Na\(_2\)SO\(_4\) and the coupling constants were measured in hertz (Hz). Thin layer chromatography was performed using silica gel column chromatography (DCM:MeOH=100:1) in 72-90 % of yields.

### 2.2.1.2 General procedure for the synthesis of cyclic dipeptides (2a-2l) [5]

N-Boc-dipeptide methyl esters (1a-II, 4.0 mmol) was dissolved in 20 mL of 1,4-dioxane/H\(_2\)O (1:3, v/v) in sealed tube. The solution was stirred at 150 °C for 10-16 h. After completion of the reaction, the crude products were obtained by evaporation under reduced pressure. The crude product was washed with water, ethanol and dichloromethane to give 2a-2l in 63-82 % of yields.

**Glioperazine C (2a).** White solid, 63 %. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 10.86 (s, 1H), 8.03 (d, \(J = 2.0\) Hz, 1H), 7.78 (d, \(J = 2.1\) Hz, 1H), 7.54 (d, \(J = 8.0\) Hz, 1H), 7.32 (d, \(J = 8.0\) Hz, 1H), 7.15 (d, \(J = 0.2\) Hz, 1H), 7.04 (t, \(J = 7.2\) Hz, 1H), 6.96 (t, \(J = 7.2\) Hz, 1H), 4.99 (d, \(J = 5.6\) Hz, 1H), 3.92 (s, 1H), 3.87-3.63 (m, 1H), 3.49 (d, \(J = 2.8\) Hz, 1H), 3.20 (t, \(J = 16.1\) Hz, 2H), 0.94 (d, \(J = 6.5\) Hz, 3H); \(^13\)C NMR (100 MHz, DMSO-d\(_6\)) 20.1, 31.6, 56.2, 60.9, 67.7, 110.2, 111.7, 118.7, 119.0, 121.3, 124.5, 128.1, 136.6, 166.9, 168.4.

(3R,6S)-3-((1H-indol-3-yl)methyl)-6-((R)-1-hydroxyethyl)piperazine-2,5-dione (2b). White solid, 65 %. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 10.84 (s, 1H), 7.89 (s, 1H), 7.81 (s, 1H), 7.62 (d, \(J = 8.0\) Hz, 1H), 7.32 (d, \(J = 8.0\) Hz, 1H), 7.13 (s, 1H), 7.04 (t, \(J = 7.6\) Hz, 1H), 6.96 (t, \(J = 7.6\) Hz, 1H), 4.96 (d, \(J = 4.8\) Hz, 1H), 4.17 (d, \(J = 4.0\) Hz, 1H), 3.95 (m, 1H), 3.26 (m, 4H), 1.03 (d, \(J = 6.4\) Hz, 3H); \(^13\)C NMR (100 MHz, DMSO-d\(_6\)) 20.2, 27.7, 54.6, 60.8, 68.4, 109.3, 111.6, 118.8, 119.0, 121.3, 124.9, 128.2, 136.3, 166.5, 169.1.

(3S,6S)-3,6-bis((1H-indol-3-yl)methyl)piperazine-2,5-dione (2c). White solid, 60 %. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 10.83 (s, 2H), 7.69 (s, 2H), 7.34 (d, \(J = 8.0\) Hz, 2H), 7.28 (d, \(J = 8.0\) Hz, 2H), 7.03 (t, \(J = 7.2\) Hz, 2H), 6.95 (t, \(J = 7.2\) Hz, 2H), 6.60 (d, \(J = 0.2\) Hz, 2H), 3.87 (s, 2H), 2.71 (dd, 2H), 2.19 (dd, 2H); \(^13\)C NMR (100 MHz, DMSO-d\(_6\)) 30.5, 30.5, 55.8, 55.8, 109.3, 109.3, 111.7, 111.7, 118.8, 118.8, 119.0, 119.0, 121.3, 121.3, 124.9, 124.9, 127.8, 127.8, 136.5, 136.5, 167.2, 167.2.

(3S)-3-((1H-indol-3-yl)methyl)piperazine-2,5-dione (2d). White solid, 70%. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 10.91
(3S,8aR)-3-(((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (2e). White solid, 60 %. 1H NMR (400 MHz, DMSO-d6) δ 10.73 (s, 1H), 8.02 (s, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.43 (d, J = 7.8 Hz, 1H), 6.98 (s, 1H), 6.89 (t, J = 7.5 Hz, 1H), 6.76 (t, J = 7.5 Hz, 1H), 4.60 (d, J = 4.7 Hz, 1H), 3.94-4.20 (m, 1H), 3.36 (m, 1H), 3.20 (m, 2H), 3.00 (dd, 1H), 1.9 (m, 1H), 1.52-1.74 (m, 2H), 1.23-1.45 (m, 1H); 13C NMR (100 MHz, DMSO-d6) 26.3, 28.2, 55.8, 58.8, 108.8, 112.3, 117.6, 118.6, 121.8, 123.9, 127.8, 135.4, 164.9, 168.3, 169.3.

(3R,6S)-3-(((1H-indol-3-yl)methyl)-6-((S)-1-hydroxyethyl)piperazine-2,5-dione (2f). White solid, 72 %. 1H NMR (400 MHz, DMSO-d6) δ 10.84 (s, 1H), 7.90 (d, J = 2.4 Hz, 1H), 7.80 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 7.03 (t, J = 7.2 Hz, 1H), 6.95 (t, J = 7.2 Hz, 1H), 4.92 (d, J = 5.6 Hz, 1H), 4.18 (t, J = 4.4 Hz, 1H), 3.04 (dd, 1H), 1.9 (m, 1H), 1.52-1.74 (m, 2H), 1.23-1.45 (m, 1H); 13C NMR (100 MHz, DMSO-d6) 20.2, 27.7, 54.6, 60.9, 68.4, 109.4, 111.6, 118.7, 119.4, 121.2, 124.9, 128.2, 136.5, 136.5, 167.2, 167.2.

(3R,6R)-3-(((1H-indol-3-yl)methyl)-6-(((R)-1-hydroxyethyl)piperazine-2,5-dione (2g). White solid, 60 %. 1H NMR (400 MHz, DMSO-d6) δ 10.86 (s, 1H), 8.02 (s, 1H), 7.78 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.15 (s, 1H), 7.05 (t, J = 7.5 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H), 4.98 (d, J = 5.8 Hz, 1H), 3.92 (s, 1H), 3.76 (s, 1H), 3.52 (s, 1H), 3.22 (d, J = 6.0 Hz, 2H), 0.94 (d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, DMSO-d6) 20.1, 31.6, 56.2, 60.9, 67.7, 110.2, 111.7, 118.7, 119.0, 121.3, 124.9, 124.9, 127.8, 127.8, 136.5, 136.5, 167.2, 167.2.

(R)-3-(((1H-indol-3-yl)methyl)piperazine-2,5-dione (2h). White solid, 70 %. 1H NMR (400 MHz, DMSO-d6) δ 10.91 (s, 1H), 8.08 (s, 1H), 7.75 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.06 (s, 1H), 7.04 (d, J = 7.2 Hz, 1H), 6.94 (t, J = 7.2 Hz, 1H), 4.01 (d, J = 2.6 Hz, 1H), 3.57 (s, 1H), 3.33 (d, J = 2.6 Hz, 1H), 3.28 (d, J = 2.6 Hz, 1H), 3.24 (dd, 1H), 3.02 (dd, 1H), 2.82 (s, 1H), 2.77 (s, 1H); 13C NMR (100 MHz, DMSO-d6) 29.7, 44.3, 55.9, 108.9, 111.7, 118.9, 119.2, 121.4, 125.1, 127.9, 136.4, 165.9, 169.5.

(3R,8aR)-3-(((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (2j). White solid, 65 %. 1H NMR (400 MHz, DMSO-d6) δ 10.85 (s, 1H), 7.73 (s, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.18 (s, 1H), 7.06 (t, J = 7.5 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H), 4.30 (d, J = 4.7 Hz, 1H), 4.0 (m, 1H), 3.4 (m, 1H), 3.2 (m, 2H), 3.07 (dd, 1H), 1.9 (m, 1H), 1.6 (m, 2H), 1.3 (m, 1H); 13C NMR (100 MHz, DMSO-d6) 22.3, 26.2, 28.1, 55.7, 58.9, 109.8, 111.7, 118.7, 119.1, 121.3, 124.9, 127.8, 136.4, 165.9, 169.5.

(S)-3-((S)-1-hydroxyethyl)piperazine-2,5-dione (2k). White solid, 68 %. 1H NMR (400 MHz, DMSO-d6) δ 8.15 (s, 1H), 7.95 (s, 1H), 5.06 (d, J = 6.0 Hz 1H), 4.02 (d, J = 6.0 Hz 1H), 3.83 (d, J = 16.8Hz 1H), 3.46 (d, J = 16.8Hz, 1H), 3.42 (s, 1H), 1.08 (d, J=60.1Hz); 13C NMR (100 MHz, DMSO-d6) 20.4, 44.8, 61.2, 68.7, 167.4, 168.6.

(3S,6S)-3,6-bis((S)-1-hydroxyethyl)piperazine-2,5-dione (2l). White solid, 61 %. 1H NMR (400 MHz, DMSO-d6) δ 8.09 (s, 3H), 5.13 (d, J = 4.0Hz 2H), 3.99 (s, 2H), 3.33 (s, 2H), 3.10 (d, J = 4.0Hz 6H); 13C NMR (100 MHz, DMSO-d6) 20.8, 20.8, 60.8, 60.8, 67.8, 67.8, 166.9, 166.9.

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2.2.2 Synthesis route of benzoyl substituted glioperazine C

![Chemical structures](image)

Scheme 2. Synthesis route of benzoyl substituted glioperazine C

2.2.2.1 (S)-methyl-2-((tert-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanoate (3)

To a stirred solution of D-tryptophan methyl ester (1.1 g, 5.0 mmol) and Et₃N (6.0 mmol, 0.88 mL) in 10% Na₂CO₃ (15 mL) was slowly added (Boc)₂O (6.0 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h. After completion of the reaction, the solution was extracted with CH₂Cl₂ (15 mL×3). The organic layers were combined and dried over anhydrous Na₂SO₄ and evaporated. The crude product was used in the next step without further purification.

2.2.2.2 (S)-methyl-3-(1-benzoyl-1H-indol-3-yl)-2-((tert-butoxycarbonyl)amino)propanoate (4)

To a stirred solution of compound 3 (844 mg, 2.0 mmol) in anhydrous CH₂Cl₂ was slowly added Et₃N (2.4 mmol, 0.35 mL), DMAP (0.2 mmol, 24 mg), benzoyl chloride (2.4 mmol, 0.28 mL) at 0 °C. The mixture was stirred at room temperature for 3 h under Ar. The reaction was quenched with water. The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by silica gel column chromatography (PE:EA=3:1) to give product 4 in 89% yield.

2.2.2.3 (S)-methyl-2-amino-3-(1-benzoyl-1H-indol-3-yl)propanoate (5)

To a stirred solution of compound 4 (1.0 g, 0.24 mmol), in CH₂Cl₂ (5 mL) was dropwise added TFA (1.0 mL) at 0 °C. The solution was stirred at room temperature for 30 min. The reaction mixture was evaporated and the residue was used in the next step without further purification.

2.2.2.4 (S)-methyl-3-(1-benzoyl-1H-indol-3-yl)-2-((2R,3R)-2-((tert-butoxycarbonyl)amino)-3-hydroxybutanamido)propanoate (6)

To a solution of (S)-methyl-2-amino-3-(1-benzoyl-1H-indol-3-yl)propanoate TFA salt (500 mg, 1.1 mmol) in CH₂Cl₂ (10 mL), EDC (230 mg, 1.3 mmol), Et₃N (0.18 mL, 2.4 mmol), HOBT (178 mg, 1.3 mmol) and N-Boc-D-Thr (289 mg, 1.3 mmol) was added at room temperature. The solution was stirred at room temperature for 4 h. After completion of the reaction, the solution was washed with brine. The organic layer was dried over anhydrous Na₂SO₄ and evaporated. The crude product was purified by silica gel column chromatography (DCM:MeOH=100:1) to give compound 6 in 63% yield.

2.2.2.5 (3R,6R)-3-((1-benzoyl-1H-indol-3-yl)methyl)-6-((R)-1-hydroxyethyl) piperazine-2,5-dione (7)

(S)-methyl-3-(1-benzoyl-1H-indol-3-yl)-2-((2R,3R)-2-((tert-butoxycarbonyl)amino)-3-hydroxy butanamido)propanoate (524 mg, 1 mmol) was dissolved in 5 mL of 1,4-dioxane/H₂O (1:3, v/v) in sealed tube. The solution was stirred at 150 °C for 12 h. After completion of the reaction, the crude products were obtained by evaporation under reduced pressure. The crude product was washed with water, ethanol and CH₂Cl₂ to give compound 7 in 40% yield. White solid.¹H NMR (400 MHz, DMSO-d₆) δ 8.28 (d, J = 7.9 Hz, 1H), 8.14 (d, J = 2.4 Hz, 1H), 8.05 (d, J = 2.4 Hz,
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1H), 7.81 (d, J = 7.2 Hz, 2H), 7.69 (dd, 2H), 7.61 (t, J = 7.5 Hz, 2H), 7.36 (dt, 6.9 Hz, 2H), 7.28 (s, 1H), 5.08 (d, J = 5.7 Hz, 1H), 3.9 (m, 2H), 3.53 (s, 1H), 3.19 (dd, 2 H), 1.02 (d, J = 6.5 Hz, 3H).

13C NMR (100 MHz, DMSO-d6) 20.2, 31.1, 55.3, 60.9, 74.1, 116.3, 117.6, 119.7, 124.1, 125.1, 126.8, 129.2, 129.6, 131.5, 131.5, 132.5, 132.6, 134.7, 136.2, 167.1, 168.1, 168.5.

2.3 Biological assay [6]
K562, Hep2 and HT-29 Cellswere cultured in 96-well plates at a density of 5 x 10^4 cells per well. Cells were treated with different concentrations of compounds in DMSO and incubated at 37 °C for 48 h. Cell viability was measured by a Cell Titer-Blue reagent-based assay. MTT assay was performed using Thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The IC_{50} values were obtained by nonlinear regression using Graph Pad Prism 4.0. IC_{50} measurements for each compound were done three times.

RESULTS AND DISCUSSION

Thirteen synthesized cyclic dipeptides 2a-2l and 7 were evaluated against K562, Hep2 and HT-29 Cells by MTT assay. The observed IC_{50} value is presented in Table 1.

<table>
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<tr>
<th>Compd</th>
<th>IC_{50} (µM)</th>
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<tr>
<td></td>
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<tr>
<td>2a</td>
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<td>&gt;100</td>
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<tr>
<td>7</td>
<td>&gt;100</td>
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*a Results are the average of three independent experiments, each done in duplicate. Standard deviations were below ±20%. *Acarbose is a reference compound.

As is shown in Table 1, compound 2a, containing cyclo(D-Trp-D-Thr), exhibit potent inhibitory activities against Hep2 cell line with IC_{50} = 6.51 µM. However, the activity decreased after the cyclic dipeptide 2a were modified from D-Trp-D-Thr into different sequences [D-Trp-D-Trp (2c), D-Trp-Gly (2d), D-Trp-L-Pro (2e), L-Trp-L-Trp (2g), L-Trp-L-Trp (2h), L-Trp-L-Pro (2j), D-Trp-Gly (2k) and D-Thr-D-Thr (2l)] or various absolute configurations (D-Trp-L-Thr (2b), L-Trp-L-Thr (2g) and L-Trp-D-Thr (2f)). Therefore, the sequence and configuration of 2a were essential for the inhibitory activity. Moreover, replacement of the N-1-H in 2a with the bulky benzoyl group as seen in compound 7 resulted in a decrease in activity.

CONCLUSION

In summary, we have synthesized glioperazine C and its derivatives and tested their antitumor activities against K562, Hep2 and HT-29 cell lines. The results show that glioperazine C has potent inhibitory activities against Hep2 cell line. The preliminary SAR study of these compounds led to the identification of a potent cytotoxic compound 2a. Further modification of other position on glioperazine C and some other derivatives are ongoing.

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REFERENCES