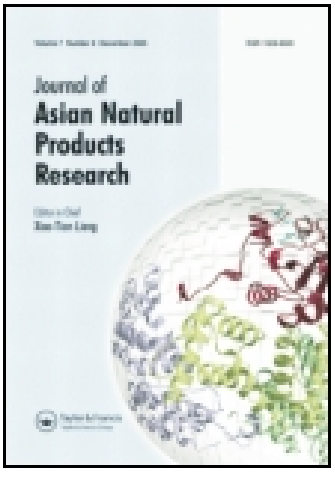

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Synthesis and bioactivity of diketopiperazine PJ147 and its derivatives from Gliocladium sp. YUP08

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Concise total synthesis of diketopiperazine PJ147, obtained from mycelium of *Gliocladium* sp. YUP08, has been achieved in seven steps with 43.5% overall yield. Biological evaluation of PJ147 exhibited strong inhibiting activity against A375-S2, Hela, P388, A-549, HL-60, and BEL-7420 cell lines. Thus, eight derivatives of PJ147 with high water solubility were also synthesized to facilitate the *in vivo* bioassay of this kind of diketopiperazines.

Keywords: diketopiperazines; antitumor; water solubility

1. Introduction

In our ongoing search for bioactive metabolites from marine microorganisms, the new diketopiperazine PJ147 (**11**) was obtained from mycelium of Gliocladium sp. YUP08 isolated from sea mud collected in Rushan [1]. Biological evaluation of PJ147 (**11**) exhibited strong inhibiting activity against A375-S2, Hela, P388, A-549, HL-60, and BEL-7420 cell lines with IC_{50} values of 4.6, 3.4, 2.86, 1.37, 1.73, and 3.2 μmol/l, respectively. Thus, the total synthesis study of PJ147 has been achieved in seven steps with 43.5% overall yield. However, the low water solubility of PJ147 hampers the *in vivo* bioassay. Thus, seven derivatives of PJ147 named PJ1471-1478 (**12**–**18**) with high water solubility were also synthesized to facilitate the *in vivo* bioassay of this kind of diketopiperazines. All the seven derivatives showed strong inhibiting activity against HL-60 cell lines by *in vitro* bioassay. In this paper, we report the synthesis and biological evaluation of all the compounds synthesized.

2. Results and discussion

Derivatives of PJ147 (**12**–**18**) were synthesized according to Scheme 1. The condensation of *N*-carbobenzyloxy-L-alanine (**1**) with glycine methyl ester (**2**) provided dipeptider (**3**). Hereby, a ring closure condensation of **3** established the construction of 3-methylpiperazine-2,5-dione scaffold of **4** by hydrogenation catalyzed by Pd/C. The bromination of the hydroxyl group of **6** with phosphorus tribromide gave **7**, which then reacted with 4-hydroxy benzaldehyde (**8**) to give **5** and reacted with 3,4-dihydroxyl benzaldehyde (**20**) to give **21**. Etherification of the phenolic hydroxyl group of **21** gave **22**, which was then nucleophilically substituted with different amines, leading to the formation of **23**. Finally, the condensation of benzaldehyde derivatives (**5** and **23**) with (*S*)-1,4-diacetyl-3-methylpiperazine-2,5-dione (**9**) gave the target compounds **11**–**18**, respectively.

The cytotoxic activities of synthesized compounds **12**–**18** with better water

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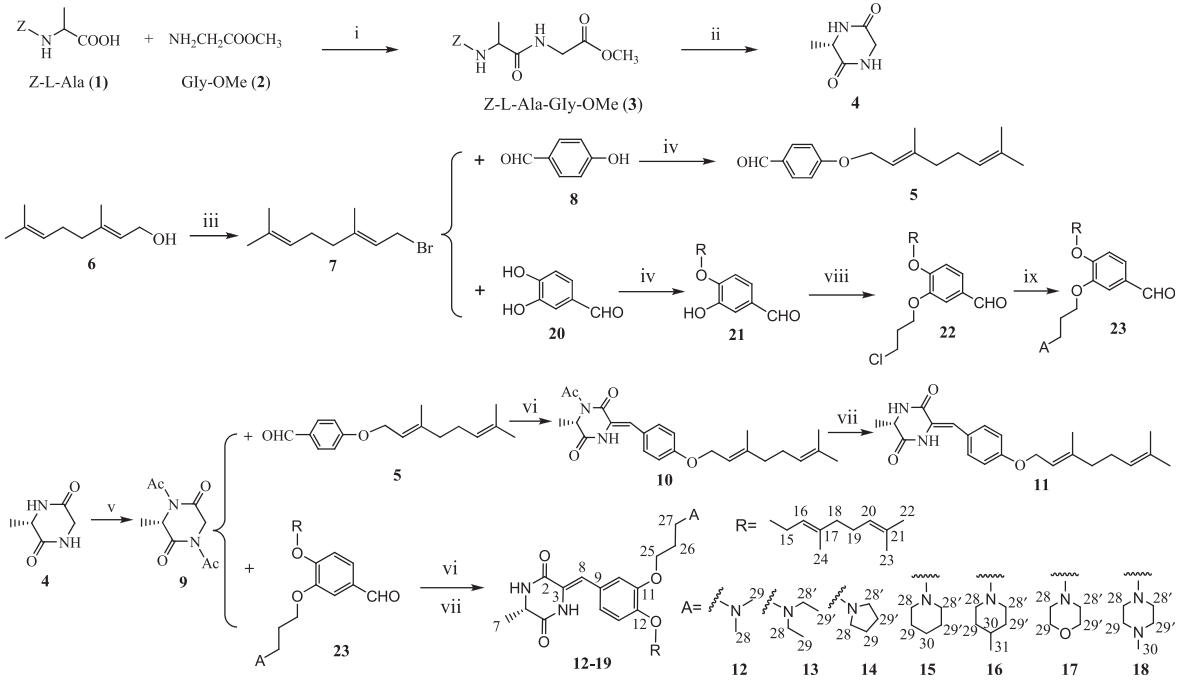

Scheme 1. Synthesis of PJ147 (**11**) and its derivatives (**12**–**18**). Reagents and conditions: (i) DCC, HOBt, Et_3N, CH_2Cl_2, 0°C, 4 h, 82%; (ii) H_2, Pd/C, MeOH, 70°C, reflux, 72 h, 80%; (iii) PBr_3, pyridine, diethyl ether, −15°C, 0.5 h, 75%; (iv) K_2CO_3, acetone, r.t., 5 h, 70%; (v) Ac_2O, 130°C, 8 h; (vi) *t*-BuOK, BuOH, r.t., 8 h, 83%; (vii) $NH_2NH_2{\cdot}H_2O$, DMF, r.t., 3 h, 80%; (viii) $Br(CH_2)_3Cl$, K_2CO_3, r.t., 6 h, 92.4%; (ix) K_2CO_3, KI, 70°C, 10 h.

solubility were bioassayed against HL-60 cell lines and their IC_{50} values were 7.27, 3.84, 4.11, 4.95, 5.4, 6.28, 7.93 μmol/l, respectively. Based on the cytotoxic activities of these synthesized compounds **12–18**, it seemed that the long chain substituent at C-10 does not impact the cytotoxic activity of PJ147 (IC_{50} = 1.73 μmol/l).

3. Experimental

3.1 General experimental procedures

The melting point as recorded on a Fluke 51II apparatus (Fluke Co., American Fork, UT, USA). Optical rotations were measured on a P-E 241 MC (Perkin-Elmer Co., Jena, Germany). IR spectra were recorded on a Bruker IFS-55 infrared spectrophotometer with KBr disks (Bruker Co., Zurich, Switzerland). The NMR spectral data were recorded on Bruker AV-600 (300 MHz for ^{1}H) with TMS as internal standard (Bruker Co.). HR-FAB-MS data were measured on Micross Mass Autospec-Ultima-TOF spectrometer (Bruker Co.). Silica gel GF254 for TLC and silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company, Qingdao, China.

3.2 (E)-4-(3,7-Dimethylocta-2,6-dienyloxy)benzaldehyde (5)

To a solution of *p*-hydroxy benzaldehyde **8** and a.p. carbonate (3.45 g) in acetone, **7** (0.002 mol) was added at room temperature. The reaction mixture was vigorously stirred at reflux for 5 h, and filtered. The filtered solid was diluted with ethyl acetate (50 ml) and dried over anhydrous Na_2SO_4 over night. The reaction mixture was extracted by 10% potassium hydroxide and saturated salt solution, respectively. And the ethyl acetate was removed under reduced pressure to obtain **5**. Brunneus liquid, m.p. 104–106°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.63 (1H, s, —CHO), 7.79 (2H, d, J = 8.7 Hz, H-3′, 5′), 6.98 (2H, d, J = 8.7 Hz, H-2′, 6′), 5.51 (1H, t, J = 6.4 Hz, H-2), 5.10–5.13 (1H, m, H-6), 4.62 (2H, d, J = 6.5 Hz, H-1), 2.13–2.19 (4H, m, H-4, 5), 1.77 (3H, s, CH_3-10), 1.70 (3H, s, CH_3-8), 1.62 (3H, s, CH_3-9); HR-ESI-MS: *m/z* 259.1699 $[M + H]^+$ (calcd for $C_{17}H_{23}O_2$, 259.1698).

3.3 (S,Z)-3-(4-(3,7-Dimethylocta-2,6-dienyloxy)benzylidene)-6-methylpiperazine-2,5-dione (11)

A mixture of **4** (640 mg) and acetic anhydride (10 ml) was refluxed for 8 h at 130°C. Acetic anhydride was removed under reduced pressure to obtain brunneus liquid. The residue was dissolved in DMF (20 ml) and **5** (5.17 g) was added to the solution. To the reaction solution, potassium *tert*-butoxide in *tert*-butyl alcohol (15 ml) was slowly added and then was stirred overnight at room temperature. Then a saturated NaCl solution (40 ml) was added to the reaction mixture and a white precipitate was formed. The mixture was extracted with ether (40 ml), which was then removed under vacuum later to give a yellow liquid.

To a solution of hydrazine hydrate in DMF (20 ml) was added the yellow liquid, and the mixture was allowed to react over 3 h. To the reaction mixture was added saturated salt solution and then precipitation was formed. The mixture was extracted with ether (40 ml), and the solvent was removed under vacuum. The residue was resolved by dichloromethane and filtered, and the filtrate was removed under reduced pressure to obtain **11** (1.20 g, 65.0%). White powder, $[\alpha]_D^{20}$ −56.6 (c = 0.52, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 9.78 (1H, s, NH-1), 8.36 (1H, s, NH-4), 7.45 (2H, d, J = 8.6 Hz, H-10, 10′), 6.96 (2H, d, J = 8.6 Hz, H-11, 11′), 6.64 (1H, s, H-8), 5.42 (1H, t, J = 6.2 Hz, H-14), 5.08 (1H, t, J = 6.3 Hz, H-18), 4.57 (2H, d,

$J = 6.4$ Hz, H-13), 4.11 (1H, q, $J = 6.9$ Hz, H-6), 2.04–2.08 (4H, m, H-16, 17), 1.71 (3H, s, CH_3-22), 1.63 (3H, s, CH_3-20), 1.57 (3H, s, CH_3-21), 1.33 (3H, d, $J = 6.9$ Hz, CH_3-7); HR-ESI-MS: *m/z* 369.2175 $[M + H]^+$ (calcd for $C_{22}H_{29}O_3N_2$, 369.2178).

3.4 PJ1471 (12)

By following the same procedure as described for the preparation of **11**, **12** was obtained from (*E*)-3-(3-(dimethylamino)propoxy)-4-(3,7-dimethylocta-2,6-dienyloxy)benzaldehyde (0.359 g), instead of **5**.

$[\alpha]_D^{20}$ −60.3 ($c = 0.52$, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 9.78 (1H, br s, NH-1), 8.35 (1H, s, NH-4), 7.09 (H, br s, H-10), 7.03 (H, d, $J = 8.4$ Hz, H-14), 6.95 (1H, d, $J = 8.4$ Hz, H-13), 6.61 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.06 (1H, t, $J = 6.0$ Hz, H-20), 4.55 (2H, d, $J = 6.0$ Hz, H-15), 4.09 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 2.34 (2H, t, $J = 7.0$ Hz, H-27), 2.11 (2H, s, H-28, 29), 2.03–2.06 (4H, m, H-18, 19), 1.81–1.83 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.32 (3H, d, $J = 6.9$ Hz, CH_3-7); HR-ESI-MS: *m/z* 470.3021 $[M + H]^+$ (calcd for $C_{27}H_{40}O_4N_3$, 470.3019).

3.5 PJ1472 (13)

By following the same procedure as described for the preparation of **12**, **13** was obtained from (*E*)-3-(3-(diethylamino)propoxy)-4-(3,7-dimethylocta-2,6-dienyloxy)benzaldehyde (0.387 g) (0.165 g, 33.1%). White powder, $[\alpha]_D^{20}$ −63.3 ($c = 0.53$, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 9.78 (1H, br s, NH-1), 8.35 (1H, s, NH-4), 7.07 (1H, br s, H-10), 7.03 (1H, d, $J = 8.4$ Hz, H-14), 6.95 (1H, d, $J = 8.4$ Hz, H-13), 6.61 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.04 (1H, t, $J = 6.0$ Hz, H-20), 4.54 (2H, d, $J = 6.0$ Hz, H-15), 4.09 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 2.51 (2H, t, $J = 6.9$ Hz, H-27), 2.44 (4H, q, $J = 7.2$ Hz, H-28, 28′), 2.03–2.06 (4H, m, H-18, 19), 1.77–1.79 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.32 (3H, d, $J = 6.9$ Hz, CH_3-7), 0.92 (6H, t, $J = 7.2$ Hz, H-29, 29′); HR-ESI-MS: *m/z* 498.3329 $[M + H]^+$ (calcd for $C_{29}H_{44}O_4N_3$, 498.3332).

3.6 PJ1473 (14)

By following the same procedure as described for the preparation of **12**, **14** was obtained from (*E*)-4-(3,7-dimethylocta-2,6-dienyloxy)-3-(3-(pyrrolidin-1-yl)propoxy)benzaldehyde (0.385 g) (0.163 g, 32.8%). White powder, $[\alpha]_D^{20}$ −59.3 ($c = 0.50$, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 9.78 (1H, br s, NH-1), 8.35 (1H, s, NH-4), 7.07 (1H, br s, H-10), 7.04 (1H, d, $J = 8.4$ Hz, H-14), 6.95 (1H, d, $J = 8.4$ Hz, H-13), 6.62 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.04 (1H, t, $J = 6.0$ Hz, H-20), 4.55 (2H, d, $J = 6.0$ Hz, H-15), 4.10 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 2.51 (2H, t, $J = 7.2$ Hz, H-27), 2.39–2.41 (4H, m, H-28, 28′), 2.03–2.06 (4H, m, H-18, 19), 1.85–1.97 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.64–1.66 (4H, m, H-29, 29′), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.32 (3H, d, $J = 6.9$ Hz, CH_3-7); HR-ESI-MS: *m/z* 496.3177 $[M + H]^+$ (calcd for $C_{29}H_{42}O_4N_3$, 496.3175).

3.7 PJ1474 (15)

By following the same procedure as described for the preparation of **12**, **15** was obtained from (*E*)-4-(3,7-dimethylocta-2,6-dienyloxy)-3-(3-(piperidin-1-yl)propoxy)benzaldehyde (0.399 g) (0.170 g, 33.5%). White powder, $[\alpha]_D^{20}$ −61.1 ($c = 0.61$, MeOH); ^{1}H NMR(300 MHz, DMSO-d_6) δ_H: 9.78 (1H, br s, NH-1), 8.35 (1H, s, NH-4), 7.06 (1H, br s, H-10), 7.03

(1H, d, $J = 8.4$ Hz, H-14), 6.95 (1H, d, $J = 8.4$ Hz, H-13), 6.62 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.05 (1H, t, $J = 6.0$ Hz, H-20), 4.55 (2H, d, $J = 6.0$ Hz, H-15), 4.10 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 2.36 (2H, t, $J = 7.2$ Hz, H-27), 2.25–2.30 (4H, m, H-28, 28′), 2.02–2.07 (4H, m, H-18,19), 1.81–1.83 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.44–1.47 (4H, m, CH_3-29, 29′), 1.35–1.37 (2H, m, CH_3-30), 1.33 (3H, d, $J = 6.9$ Hz, CH_3-7); HR-ESI-MS: m/z 510.3330 $[M + H]^+$ (calcd for $C_{30}H_{44}O_4N_3$, 510.3332).

3.8 PJ1475 (16)

By following the same procedure as described for the preparation of **12**, **16** was obtained from (*E*)-4-(3,7-dimethylocta-2,6-dienyloxy)-3-(3-(4-methylpiperidin-1-yl)propoxy)benzaldehyde (0.399 g) (0.155 g, 30.1%). White powder, $[\alpha]_D^{20}$ −60.8 ($c = 0.49$, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.78 (1H, br s, NH-1), 8.35 (1H, s, NH-4), 7.06 (1H, br s, H-10), 7.03 (1H, d, $J = 8.4$ Hz, H-14), 6.95 (1H, d, $J = 8.4$ Hz, H-13), 6.62 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.05 (1H, t, $J = 6.0$ Hz, H-20), 4.55 (2H, d, $J = 6.0$ Hz, H-15), 4.10 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 2.79 (2H, t, $J = 11.3$ Hz, H-28, 28′), 2.38 (2H, t, $J = 7.2$ Hz, H-27), 2.03–2.07 (4H, m, H-18, 19), 1.81–1.83 (2H, m, H-28, 28′), 1.82–1.84 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.52 (2H, br s, CH_3-29, 29′), 1.32 (3H, d, $J = 6.9$ Hz, CH_3-7), 1.29–1.31 (1H, m, H-30), 1.08–1.11 (2H, m, H-29, 29′), 0.86 (3H, d, $J = 6.3$ Hz, CH_3-31); HR-ESI-MS: m/z 524.3485 $[M + H]^+$ (calcd for $C_{31}H_{46}O_4N_3$, 524.3488).

3.9 PJ1476 (17)

By following the same procedure as described for the preparation of **12**, **17** was obtained from (*E*)-4-(3,7-dimethylocta-2,6-dienyloxy)-3-(3-morpholinopropoxy)benzaldehyde (0.401 g) (0.179 g, 35.0%). White powder, $[\alpha]_D^{20}$ −62.9 ($c = 0.60$, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 9.78 (1H, br s, NH-1), 8.26 (1H, s, NH-4), 7.17 (1H, br s, H-10), 7.07 (1H, d, $J = 8.4$ Hz, H-14), 6.94 (1H, d, $J = 8.4$ Hz, H-13), 6.55 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.05 (1H, t, $J = 6.0$ Hz, H-20), 4.54 (2H, d, $J = 6.0$ Hz, H-15), 4.08 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 3.55 (4H, t, $J = 4.5$ Hz, H-29, 29′), 2.40 (2H, t, $J = 6.9$ Hz, H-27), 2.31–2.36 (4H, m, H-28, 28′), 2.03–2.07 (4H, m, H-18, 19), 1.84–1.86 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.30 (3H, d, $J = 6.9$ Hz, CH_3-7); HR-ESI-MS: m/z 512.3120 $[M + H]^+$ (calcd for $C_{29}H_{42}O_5N_3$, 512.3124).

3.10 PJ1477 (18)

By following the same procedure as described for the preparation of **12**, **18** was obtained from (*E*)-4-(3,7-dimethylocta-2,6-dienyloxy)-3-(3-(4-methylpiperazin-1-yl)propoxy)benzaldehyde (0.414 g) (0.202 g, 38.6%). White powder, $[\alpha]_D^{20}$ −57.1 ($c = 0.71$, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 9.78 (1H, br s, NH-1), 8.35 (1H, s, NH-4), 7.06 (1H, br s, H-10), 7.03 (1H, d, $J = 8.4$ Hz, H-14), 6.95 (1H, d, $J = 8.4$ Hz, H-13), 6.62 (1H, s, H-8), 5.40 (1H, t, $J = 6.0$ Hz, H-16), 5.05 (1H, t, $J = 6.0$ Hz, H-20), 4.54 (2H, d, $J = 6.0$ Hz, H-15), 4.10 (1H, q, $J = 6.9$ Hz, H-6), 4.00 (2H, t, $J = 6.3$ Hz, H-25), 2.38–2.42 (2H, m, H-27), 2.27–2.33(8H, m, H-28, 28′, 29, 29′), 2.12 (2H, s, CH_3-30), 2.03–2.07 (4H, m, H-18, 19), 1.81–1.83 (2H, m, H-26), 1.68 (3H, s, CH_3-24), 1.62 (3H, s, CH_3-23), 1.55 (3H, s, CH_3-22), 1.32 (3H, d, $J = 6.9$ Hz, CH_3-7); HR-ESI-MS: m/z 525.3441 $[M + H]^+$ (calcd for $C_{30}H_{45}O_4N_4$, 525.3441).

4. Bioactivity studies

The anti-tumor activities of **11**–**18** were determined according to the procedure described in our previous research [2].

Acknowledgments

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