Supporting Information for *New journal of chemistry*

**Photo-induced synthesis and in vitro antitumor activity of Fenestin A analogs**

Lishuang Zhao, Hongyue Zhang, Jianing Cui, Meiqi Zhao, Zhiqiang Wang*, Qunfeng Yue*, and Yingxue Jin*

* Key Laboratory of Photochemical Biomaterials and Energy Storage Materials, Heilongjiang Province, College of Chemistry & Chemical Engineering, Harbin Normal University, Harbin, 150025, China. Email: jyxprof@163.com (Y.J); wzq70402@163.com (Z.W); yqprof@163.com (Q.Y).

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Experimental

General

L-leucine (61-90-5), Boc-L-proline (15761-39-4), L-isoleucine (73-32-5), N-[(tert-butoxy carbonyl)-L-alanin (15761-38-3), Di-tert-butyl dicarbonate (24424-99-5), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 16357-59-8), N-[(trimethylsilyl)methyl]benzylamine (53215-95-5), phthalylglycyl chloride (6780-38-7) and trifluoroacetic acid (TFA, 76-05-1) were purchased from Energy Chemical. Dichloromethane, methanol, ethyl acetate, petroleum ether, 1,4-dioxane were analytical reagent. Dulbecco’s modied eagle medium (DMEM), penicillin, fetal bovine serum (FBS), and streptomycin were purchased from Beijing Dingguo Biotechnology Co. Phosphate-buffered saline (PBS) purchased from Invitrogen (10010) was used as a balanced salt solution in cell culture. All the solvents were distilled and puried by standard procedures. All the above chemicals reagents were used without further puriication. 1H and 13C-NMR spectra were recorded at 400 and 100 MHz, respectively, on an AMX400 spectrometer (Bruker, Bremen, Germany) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL JMS-700 spectrometer using the fast atom bombardment (FAB) or electron impact (EI) mode. A 450 W Hanovia medium-pressure mercury lamp surrounded by a Pyrex glass filter (λ > 290 nm) was used for electronic excitation.

Preparation of trimethylsilylbenzylamido dipeptides

The Boc-leucine (2.31 g, 10 mmol) and N-[(Trimethylsilyl) methyl]benzylamine (1.93 g, 10 mmol) was dissolved in 50 mL of anhydrous dichloromethane, EEDQ (3.78 g, 15 mmol in 10 mL of THF) was added dropwise with stirring at room temperature. Bi drops continue stirring 72 h. After the reaction, the reaction solution was washed twice with 16 mL of water, the organic layer was dried over anhydrous sodium sulfate and concentrated, the residue was dispersed in 20 mL of anhydrous dichloromethane, washed twice with 10 mL of water, dried over anhydrous sodium sulfate and concentrated, the residue was dissolved in 10 mL of anhydrous dichloromethane and added dropwise 10 mL of trifluoroacetic acid, then stirred for 3 h. After removal of trifluoroacetic acid and dichloromethane was concentrated, the residue was dispersed in 20 mL of dichloromethane, washed twice with 10 mL of water, dried over anhydrous sodium sulfate and concentrated to give a chemically pure Leu-Si(CH3)3 (2.75 g, yellow oil). The Leu-Si(CH3)3 (2.75 g, 7 mmol) and Boc-Isoleucine (1.32 g, 7 mmol) was dissolved in 20 mL of anhydrous dichloromethane. EEDQ (2.59 g, 10.5 mmol in 10 mL of THF) was added dropwise with stirring at room temperature for 72h. After completion of the reaction, the reaction solution was washed twice with 10 mL of water, the organic layer was washed over anhydrous sodium sulfate and concentrated, and the residue was dissolved in 10 mL of anhydrous dichloromethane, added dropwise 3 mL of trifluoroacetic acid and stirred for 3h. After removal of trifluoroacetic acid methylene chloride, the residue was dispersed in 10 mL of dichloromethane, washed twice with 10 mL of water, dried over anhydrous sodium sulfate and concentrated to give Ile-Leu-Si(CH3)3 2.35 g (a while solid). The same method was used for synthesis of Pro-Leu-Ile-Si(CH3)3, Pro-Pro-Leu-Ile-Si(CH3)3, Leu-Ile- Pro-Si(CH3)3, Pro-Leu-Ile-ProcSi(CH3)3.

Preparation of N-Phthalimido-Gly-Pro-Pro-Leu-Ile-Si(CH3)3 and N-Phthalimido-Gly-Pro-Leu-Ile-ProcSi(CH3)3

Pro-Pro-Ile-Leu-Si(CH3)3 (3.61 g, 0.01 mol) and triethylamine (1 mL) were dissolved in anhydrous methylene chloride, then phthalimide acetyl chloride (2.23 g, 0.01 mol, in 10 mL of 1,4-dioxane ) was added dropwise. After stirring at room temperature for 30 min, the reaction solution washed twice with 20 mL of water. The organic layer was dried over anhydrous sodium sulphate, then concentrated and puried by silica gel column chromatography (mobile phase VEA/VP = 2:1) to obtain a while solid. The similar method was used for synthesis of N-Phthaloyl-Gly-Pro-Pro- Leu-Ile-
Nitrogen purged solutions of the substrates in the indicated solvents were irradiated by using Pyrex glass filtered light in an water cooled immersion reactor for time periods required. Concentration of the photoproducts were followed.
by column chromatography to yield the pure products listed below. In brief, 0.5 g of compound 7 in 200 mL of anhydrous methanol were placed in a reactor, then ventilated nitrogen flow for 30 min. Upon maintaining the ventilation of nitrogen, the solutions were irradiated by ultraviolet light (Pyrex tube filtered-light \( \lambda > 290 \text{ nm} \)).

3-Hydroxy-isoidolinone-cyclo-Gly-Pro-Leu-Ile-Pro (1): while solid (yield 30%). \([\alpha]_D^{20} = -37.12^\circ (c = 0.27 \text{ g/100ml}, \text{MeOH}); \text{mp: } 204^\circ \text{C}. \text{H}^1\text{NMR(CDCl}_3\delta ^{1} ) : 0.88-0.93 (m, 12H, CH2), 0.95-1.02 (m, 2H, CH2CH2), 1.09-1.25 (m, 1H, CH(CH2)2), 1.28-1.45 (m, 2H, CHCH2CH), 1.48-1.52 (m, 1H, CHCH2CH), 1.53-1.69 (m, 4H, NCH2CH2CH2CH and NCH2CH2CH2), 1.71-2.04 (m, 4H, NCH2CH2CH2CH and NCH2CH2CH2CH), 3.05-3.56 (m, 2H, NCH2CH2CH2CH), 3.68-3.73 (m, 2H, NCH2CH2CH2CH), 3.88-4.01 (m, 2H, NCH2CH2CH2CH and NCH2CH2CH2CH), 4.11-4.19 (m, 1H, CHCHNH(\text{CO})), 4.28-4.43 (m, 2H, COCH2C(OH)N), 4.48-4.59 (m, 3H, CH2Ph and CH2CHNH(\text{CO})), 4.60-4.76 (m, 2H, NHCH2CON), 7.15-7.87 (m, 9H, ArH); \text{C}^1\text{NMR(CDCl}_3\delta ^{1} ) : 10.7, 14.0, 15.4, 21.3, 22.4, 22.7, 24.2, 24.7, 28.0, 29.7, 37.7, 38.8, 39.4, 40.3, 48.2, 53.2, 55.2, 56.4, 58.5, 60.7, 65.5, 85.1, 122.5, 124.2, 126.3, 127.7, 128.7, 130.2, 130.9, 132.7, 136.6, 144.5, 165.1, 167.0, 170.8, 171.5, 172.0, 172.5, 173.0. (DEPT): 10.7(CH3CH), 14.0(CH2), 15.4(CH2CH2), 21.3(CH2), 22.4(NCH2CH2CH2CH), 22.7(NCH2CH2CH2CH), 24.2(NCH2CH2CH2CH), 24.7(NCH2CH2CH2CH), 28.0(CH2), 29.7(CH2CH2CH), 37.7(CH2CH2CH), 38.8(CH2CH2CH), 39.4(CH2CH2CH2), 40.3(NCH2CON), 48.2(NCH2CON), 53.2(CH2Ph), 55.2(NCH2CH2CH2CH), 56.4(NCH2CH2CH2CH), 58.5(CH2CH2CH2CH), 60.7(NCH2CH2CH2CH), 65.5(NCH2CH2CH2CH), 123.9(Ph), 125.4(Ph), 128.0(Ph), 128.9(Ph), 131.5(Ph) 133.9(Ph). HRMS(ESI) m/z calcd for C60H40N6NaO6+: (M+Na)+ 751.3795, found 751.3737. Anal calcd for C60H42N6O6: C 65.91, H 7.19, N 11.53, found C 65.88, H 7.23, N 11.43.

3-Hydroxy-isoidolinone-cyclo-Gly-Pro-Pro-Leu-Ile (2): while solid (yield 30%). \([\alpha]_D^{20} = -30.12^\circ (c = 0.32 \text{ g/100ml}, \text{MeOH}); \text{mp: } 205^\circ \text{C}. \text{H}^1\text{NMR(CDCl}_3\delta ^{1} ) : 0.85-0.93 (m, 12H, CH2), 1.41-1.49 (m, 2H, CH2CH2), 1.51-1.73 (m, 3H, CH(CH2)2 and CH2CH2CH), 1.83-1.97 (m, 6H, NCH2CH2CH2CH, NCH2CH2CH2CH and NCH2CH2CH2CH), 2.05-2.19 (m, 2H, NCH2CH2CH2CH), 2.20-2.33 (m, 1H, CH(CH2)2), 2.60-3.45 (m, 2H, NCH2CH2CH2CH), 3.48-3.67 (m, 2H, NCH2CH2CH2CH), 3.70-3.78 (m, 2H, NCH2CH2CH2CH and NCH2CH2CH2CH), 4.28-4.42 (m, 2H, COCH2C(OH)N), 4.48-4.55 (m, 3H, CH2Ph and CH2CHNH(\text{CO})), 4.64-4.87 (m, 3H, NHCH2CON and CH2CHNH(\text{CO})), 7.20-7.87 (m, 9H, ArH); \text{C}^1\text{NMR(CDCl}_3\delta ^{1} ) : 11.3, 15.1, 21.6, 23.3, 23.9, 24.2, 24.5, 28.0, 30.0, 31.8, 35.9, 37.7, 38.8, 39.7, 40.3, 42.3, 47.0, 52.8, 55.8, 58.1, 59.6, 86.6, 122.5, 123.7, 126.3, 127.5, 129.0, 130.1, 130.8, 132.5, 136.0, 145.1, 165.2, 166.2, 170.2, 170.9, 171.7, 172.5. (DEPT): 11.3(CH3CH2), 15.1(CH2CH2), 21.6(CH2), 23.3(CH2), 23.9(CH2), 24.2(NCH2CH2CH2CH), 25.9(NCH2CH2CH2CH), 28.0(NCH2CH2CH2CH), 30.0(NCH2CH2CH2CH), 31.8(CH2CH2CH2CH), 35.9(CH2), 37.7(CH2), 38.8(CH2CH2CH2CH), 39.7(NCH2CON), 40.3(NCH2CON), 42.3(CH2), 47.0(NCH2CH2CH2CH), 52.8(NCH2CH2CH2CH), 55.8(CH2CH2CH2CH), 58.1(NCH2CH2CH2CH), 59.6(NCH2CH2CH2CH), 122.5(Ph), 123.7(Ph), 127.5(Ph), 129.0(Ph), 130.1(Ph), 130.8(Ph) 132.5(Ph). HRMS(ESI) m/z calcd for C60H40N6NaO6+: (M+Na)+ 751.3795, found 751.3756. Anal calcd for C60H42N6O6: C 65.91, H 7.19, N 11.53, found C 65.86, H 7.24, N 11.47.
**Phthaloyl-cyclo-Gly-Pro-Pro-Leu-Ile (8):** while solid (yield 5%). \([\alpha]_D^{20} = -35.26^\circ\) (c = 0.37 g/100 ml, MeOH); mp: 209 °C, \(1^1{\text{HNMR(CDCl}_3}\) \(\delta\): 0.71~0.90 (m, 12H, CH₃), 1.02~1.24 (m, 3H, CH₂CH₃ and CH(CH₃)₂), 1.40~1.60 (m, 4H, CH₂CH₂CH and NCH₂CH₂CH₂CH), 1.72~1.83 (m, 2H, NCH₂CH₂CH₂CH), 1.87~2.24 (m, 4H, NCH₂CH₂CH and NCH₂CH₂CH₂CH₂CH), 2.31~2.50 (m, 1H, CH(CH₃)₂), 3.01~3.45 (m, 2H, NCH₂CH₂CH₂CH₂CH), 3.48~3.73 (m, 2H, NCH₂CH₂CH₂CH₂CH), 3.90~4.20 (m, 2H, NCH₂CH₂CH and NCH₂CH₂CH₂CH₂CH), 4.41~4.48 (m, 1H, CH₂CHNNH(CO)), 4.49~4.70 (m, 3H, CH₂Ph and CHCHNH(CO)), 4.94~5.11 (m, 2H, NHCH₂CON), 7.18~7.85 (m, 9H, ArH); \(1^3{\text{CNMR(CDCl}_3}\) \(\delta\): 10.9, 14.7, 21.2, 22.8, 23.4, 23.7, 24.6, 25.5, 27.7, 29.9, 37.1, 38.6, 39.5, 42.4, 46.7, 50.1, 53.2, 55.7, 59.5, 64.4, 66.0, 123.4, 123.8, 126.3, 127.5, 128.5, 132.2, 135.6, 165.4, 166.4, 167.3, 170.5, 171.4, 172.0. HRMS(ESI) m/z calcld for C₄₀H₅₂N₆O₇Na⁺ (M+Na)⁺ 751.3795, found 751.3756. Anal calcd for C₄₀H₅₂N₆O₇: C 65.91, H 7.19, N 11.53, found C 65.87, H 7.22, N 11.48.

**Cell culture**

HeLa and HepG-2 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM,Gibco) supplemented with 10 % fetal bovine serum (FBS), penicillin G (100 U/mL), and streptomycin (100 g/mL). The HeLa cell lines were received from Harbin engineering University. Cells were cultured at 37 °C in a humidified incubator with 5 % CO₂. Cells were grown on plates and were subcultured after 0.25% trypsin treatment. The experiments were performed when the ratio of cell fusion reached 80%.

**MTT assay for cell viability**

The activity test were grouped into four groups: linear peptide experiment groups, cyclic peptide groups and blank group without peptides, and positive control groups (potassium phthalimide). HeLa and HepG-2 cells were seeded in a 96-well plate at an initial density of 4×10³ cells per well in DMEM complete medium and incubated at 37 °C in 5% CO₂ for 24 h. Then, they were treated with various concentrations (100, 300, and 500 g/mL) of samples. Each dosage was replicated in six wells. After 48h incubation, MTT dyes (100 µL, 0.5 mg/mL) were added to the wells, and incubated for 4 h. The MTT solutions were then removed and 150 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals generated. Then microplate reader was used to detect the absorbance of each well at 490 nm. Cell viability (%) was calculated by the following formula:

\[
\text{Cell viability} \% = \frac{A_{490\text{ (sample)}}}{A_{490\text{ (control)}}} \times 100\%
\]

where \(A_{490\text{ (sample)}}\) represents A values of the wells treated with various concentrations of samples, and \(A_{490\text{ (control)}}\) represents those of the wells treated with DMEM+10% FBS, without any samples. Statistical analyses were performed using the SPSS statistical software version (SPSS Inc., Chicago, IL, USA).

**Cell morphological changes of HepG-2 Cells after treatment of sample**

After being cultured with 1 for 0h, 16h, 32h, 48h, respectively, the cell phenotype in bright field were analyzed by Leica DM IL LED Fluorescence inverted microscope (FIM). HepG-2 cell lines were incubated on 6 well plates and incubated 24 h. The 1 (1 mL, 100 µg/mL) was added to each well and then incubated for additional 6 h. Then nucleus’s morphological variation was immediately observed under FIM.
Computational details:

The conformational analysis was performed by arbitrarily fixing the absolute configuration of C-3 for compound 1 and 2, using the Spartan 08 package with the MMFF94 molecular mechanics force field and Monte Carlo searching. The obtained conformers were geometrically optimized at the DFT/B3LYP/6-31G** level of theory in the program package Gaussina 09.2 TD-DFT/CAM-B3LYP/TZVP was employed to calculate excitation energy (denoted by wavelength in nm) and rotatory strength R. ECD curves were calculated based on rotatory strengths using half bandwidth of 0.3 eV with conformers by Specdis 1.61.3

References:
1. Spartan’08, Wavefunction, Inc. Irvine, CA
2. Gaussian 09, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.
1. UV-Vis absorption spectra of 1, 7a and 7b.

**Figure a.** UV-Vis absorption spectra of 1, 7a and 7b. Figure a shows the UV-Vis absorption spectra of 1 (c = 1.08×10⁻⁵ mol/L), a strong band absorption at 203 nm and shoulder band at 250 nm. The UV-Vis absorption spectra of compound 7a (c = 1.05×10⁻⁵ mol/L) and 7b (c = 1.09×10⁻⁵ mol/L) showed a strong band absorption at 217 nm and shoulder band at 292 nm.

2. HPLC of 1 and 2.

Shiseido Capcell PAK C18 (150×4.0 mm, 5 μm) was used as the column at 30 °C, and the mobile phase flow rate was 1 mL/min. During the analytical run, the elution was carried out using mobile phases A (Ultrapure water) and B (acetonitrile), the percentage of mobile phases B was 50%, while the detection wavelength was 192 nm. From HPLC analysis, the retention time of 1 is 12.4 min, and the purity of 1 is approximately 97.2%. The retention time of 2 is 12.0 min, and the purity of 2 is approximately 97.9%.

**Figure b.** HPLC spectrum of 1.

**Figure c.** HPLC spectrum of 2.
3. $^1$H, $^{13}$C-NMR, HRMS and DEPT of 7a.
4. $^1$H, $^{13}$C-NMR, HRMS and DEPT of 7b.
+TOF MS: 0.1712 to 0.5652 m in from Sample 1 (TuneSampleID) of chenzaoxin20170519-2 with different calibrations (DuoSpray)

max. 9537.8 cps.

Intensity, cps
5. $^1$H, $^{13}$C-NMR, HRMS and DEPT of 1.
NMR Spectra: 1.0450 to 1.9702 min from Sample 1 (TuneSampleID) of chenzaoxin20170519-4.wiff different calibrations (DuoSpray()) Max. 2.4e5 cps.
7. $^1$H, $^{13}$C-NMR and HRMS of 8.
TOF-MS: 0.1709 to 1.0961 min from Sample 1 (TuneSampleID) of chenzaoxin20170519-6 with different calibrations (DaSpray ()).

Max. 5.9e4 cps.