Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Photo-induced synthesis and antitumor activity of phakellistatin 18 analog with isoindolinone fragment

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Contents

1. Experimental

General

Commercially available materials were used without further purification. The amino acids (Boc-D-Proline, Boc-L-Isoleucine, Boc-L-Phenylalanine), Phthalylglycyl chloride, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinline(EEDQ), N-[(trimethylsilyl)methyl] benzylamine (BnTMSA), and trifluoroacetic acid (TFA,) were purchased from Energy Chemical. Dichloromethane (DCM), Methanol (MeOH), Ethyl acetate, Petroleum ether, 1,4-dioxane were analytical reagent. Silica gel (300-400 mesh) were bought from Qingdao Haiyang Chemical Co. The agents in bioassay, such as Dulbecco's modied eagle medium (DMEM), Fetal bovine serum (FBS) were purchased from Beijing Dingguo Biotechnology Co. Phosphatebuffered saline (PBS) as a balanced salt solution in cell culture was bought from Invitrogen (10010) was used. HepG-2 cells were obtained from Harbin engineering University. All the above chemicals reagents were used without further purification. ^{1}H and ^{13}C-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.

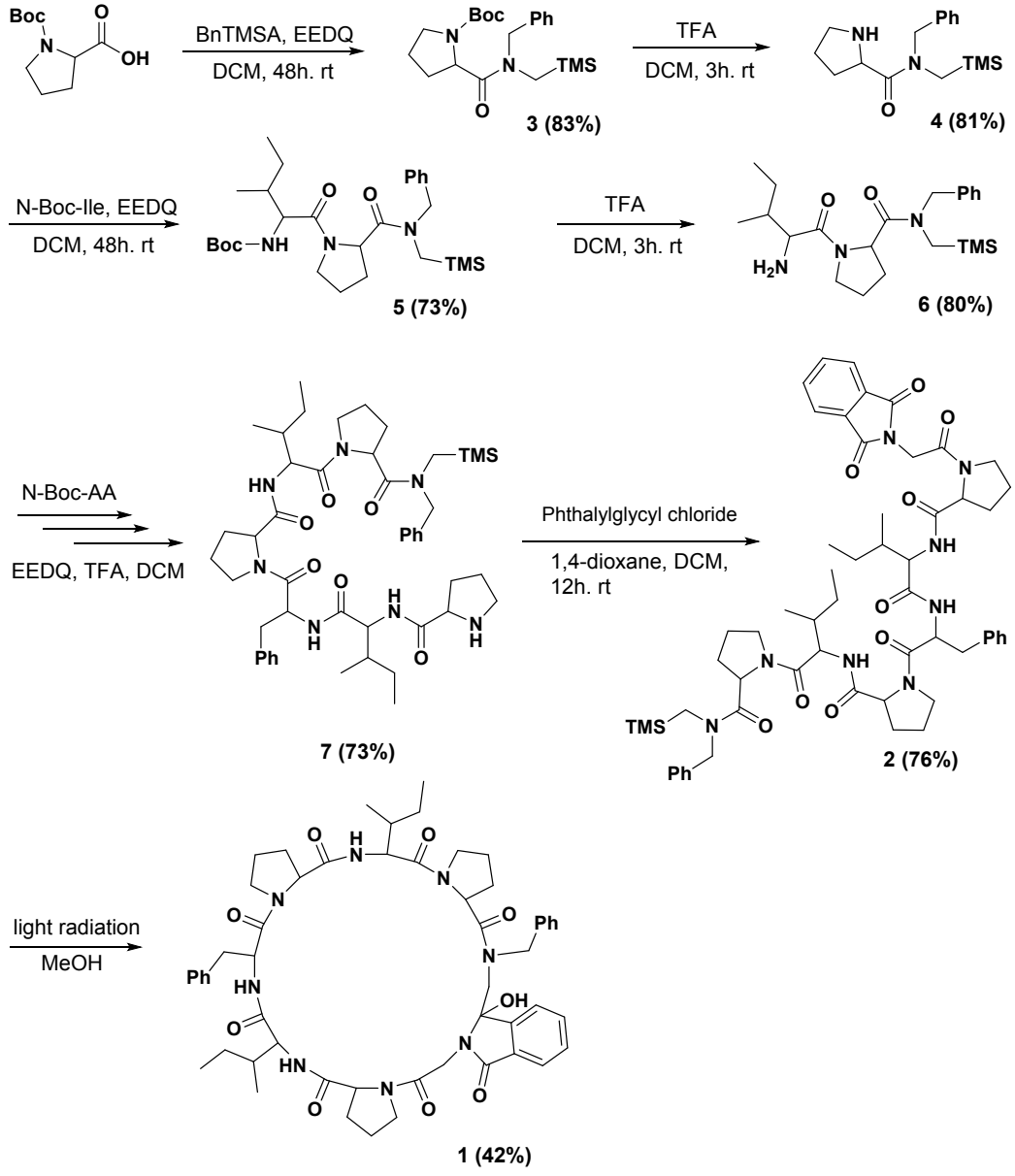
Synthesis of linear peptide N-Phthalimido-Gly-Pro-Ile-Phe-Pro-Ile-Pro-BnTMS (2) (Scheme S1)

2.15g (10 mmol) of N-Boc-Proline dissolved in 20ml of anhydrous DCM and 3.78g (15mmol) of EEDQ dissolved in 20ml of anhydrous DCM was added into a round-bottom flask, then stirring for 3 hours. 1.93 g (10 mmol) of N-[(trimethylsilyl)methyl]benzylamine was added dropwise to the reaction flask. After 48h of reaction, adjusted the pH to 2 with hydrochloric acid, the reaction solution was washed twice with 15 mL of NaCl aq. The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (mobile phase $V_{Ethyl\ acetate(EA)}/V_{Petroleum\ ether(PE)}$ = 1:4) to obtain pure N-Boc-Pro-BnTMS (**3**) (3.24g, white solid, 83%). Then, N-Boc-Pro-BnTMS (**3**) dissolved in 20 mL of anhydrous DCM were added into a round-bottom flask, then added 10ml TFA dropwise. After stirring for 3 hours, adjusted the pH to 7 with saturated sodium carbonate, the reaction solution was washed twice with 15 mL of 15% NaCl aq., dried over anhydrous sodium sulfate and concentrated to obtain pure Pro-BnTMS (**4**) (1.95 g, yellow oil, 81%). Then, 1.39g (6 mmol) of N-Boc-Isoleucine dissolved in 20ml of anhydrous DCM and 2.27g (9 mmol) of EEDQ dissolved in 20ml of anhydrous DCM was added, then stirring for 3 hours. 1.74 g (6 mmol) of Pro-BnTMS (**4**) was added dropwise to the reaction flask. After 48h of reaction, N-Boc-Ile-Pro-BnTMS (**5**) was obtained after purification. Next, the deprotection reaction was carried out, after which the intermediate (**6**) was obtained. The above experimental procedures were repeated, and the target amino acids were sequentially added to obtain the intermediate (**7**). Then NH_2-Pro-Ile-Phe-Pro-Ile-Pro-BnTMS (**7**) (0.86 g, 1 mmol) and triethylamine (3 mL) were dissolved in anhydrous DCM, phthalylglycyl chloride (0.25 g, 1 mmol, in 3 mL of 1,4-dioxane) was added dropwise. After stirring at room temperature for 12 h, the reaction solution washed twice with 20 mL of 15% NaCl aq. The organic layer was dried over anhydrous Na_2SO_4. The residue was concentrated and purified by silica gel column chromatography (mobile phase V_{EA}/V_{PE} = 1:1) to obtain pure N-Phthalimido-Gly-Pro-Ile-Phe-Pro-Ile-Pro-BnTMS (**2**) (0.80g, white solid, Isolated yield 76%).

Synthesis of 3-Hydroxy-isoindolinone-cyclo-Gly-Pro-Ile-Phe-Pro-Ile-Pro (1) (Scheme S1)

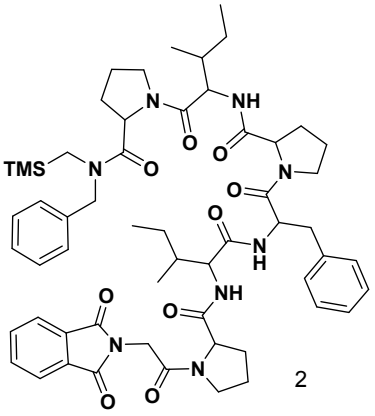
Nitrogen purged solutions of the substrates in the indicated solvents were irradiated by using Pyrex glass filtered light in an water cooled immersion reactor for time periods required to to give >90% coversion (examined by TLC). Concentration of the photoproducts were followed by column chromatography to yield the pure product.

In brief, 0.5 g of linear peptide **2** 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 λ > 290 nm). The solvent was evaporated under reduced pressure, and methanol in the solution was removed to obtain a pale yellow powdery solid as a crude light reaction product of compound **1**. The crude cyclic peptide was dissolved in DCM and purified by silica gel column chromatography (mobile phase EA) to obtain pure 3-Hydroxy-isoindolinone-cyclo-Gly-Pro-Ile-Phe-Pro-Ile-Pro (**1**) (0.2g, white solid, 42%).

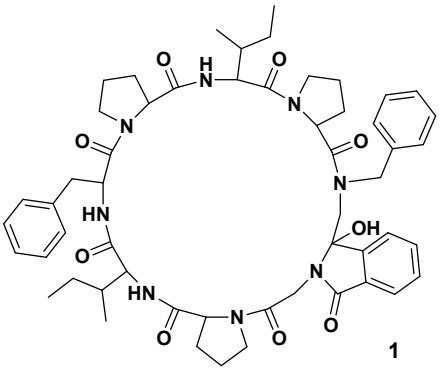


Scheme S1. The synthetic route of the title compound

The detailed NMR data:



N-Phthalimido-Gly-Pro-Ile-Phe-Pro-Ile-Pro-BnTMS (2). ^{1}HNMR (400 MHz, $CDCl_3$) ***δ*** : -0.23~0.03(m, 9H, $SiMe_3$), 0.57~0.87(m, 12H, CH_3), 0.93~1.07(m, 2H, **CH_2**CH_3), 1.07~1.30(m, 3H, CH_2**CH_3** and **CH**CH_3), 1.67~1.86(m, 6H, NCH_2**CH_2**CH_2CH NCH_2**CH_2**CH_2CH and NCH_2**CH_2**CH_2CH), 1.86~2.14(m, 6H, NCH_2CH_2**CH_2**CH, NCH_2CH_2**CH_2**CH and NCH_2CH_2**CH_2**CH), 2.15~2.31(m, 1H, **CH**CH_3), 2.40~2.50(d, 1H, *J* = 14.8 HZ, **CHH**$SiMe_3$), 2.50~2.92(m, 2H, **CH_2**Ph), 2.92~3.22(m, 2H, **CH_2**Ph), 3.22~3.31(d, 1H, *J* = 14.8 HZ, **CHH**$SiMe_3$), 3.31~3.70(m, 5H, N**CH_2**CH_2CH_2CH, N**CH_2**CH_2CH_2CH and **CH**CH_2Ph), 3.72~3.83(m, 1H, $NCH_2CH_2CH_2$**CH**), 3.84~3.96(m, 1H, $NCH_2CH_2CH_2$**CH**), 4.03~4.24(m, 2H, N**CH_2**CH_2CH_2CH), 4.24~4.37(m, 1H, $NCH_2CH_2CH_2$**CH**), 4.37~4.57(m, 2H, HN**CH_2**CO), 4.57~4.74(m, 1H, HN**CH**CO), 4.88~5.13(m, 1H, HN**CH**CO), 6.97~7.78(m, 14H, Ar**H**); ^{13}CNMR(100 MHz, $CDCl_3$) ***δ*** : 0.0, 12.1, 12.4, 16.5, 16.7, 23.3, 24.1, 25.7, 26.4, 29.8, 32.8, 34.0, 36.3, 37.2, 38.7, 39.0, 41.2, 48.3, 48.6, 49.2, 53.8, 54.0, 55.7, 57.4, 59.4, 60.0, 62.1, 124.1, 128.2, 128.5, 128.6, 128.7, 129.8, 129.9, 130.5, 133.8, 134.4, 137.1, 137.7, 166.8, 168.9, 171.3, 171.7, 172.1, 172.4, 172.5, 173.5. ESI-MS revealed that the $[M+H]^+$ (m/z) of compound **2** was 1045.55750, which is consistent with the theoretical molecular weight 1045.55773.



3-Hydroxy-isoindolinone-cyclo-Gly-Pro-Ile-Phe-Pro-Ile-Pro (1). ^{1}HNMR (400 MHz, $CDCl_3$) ***δ*** : 0.55~0.94(m, 12H, CH_3), 0.94~1.18(m, 4H, **CH_2**CH_3 and **CH_2**CH_3), 1.37~1.48(m, 1H, **CH**CH_3), 1.50~1.64(m, 1H, **CH**CH_3), 1.64~1.97(m, 6H, NCH_2**CH_2**CH_2CH, NCH_2**CH_2**CH_2CH and NCH_2**CH_2**CH_2CH), 1.97~2.37(m, 6H, NCH_2CH_2**CH_2**CH, NCH_2CH_2**CH_2**CH and NCH_2CH_2**CH_2**CH), 2.38~2.59(m, 2H, $NCH_2CH_2CH_2$**CH** and $NCH_2CH_2CH_2$**CH**), 2.59~2.90(m, 1H, $NCH_2CH_2CH_2$**CH**), 2.90~3.28(m, 2H, N**CH_2**CH_2CH_2CH), 3.28~3.71(m, 4H, N**CH_2**CH_2CH_2CH and N**CH_2**CH_2CH_2CH), 3.71~3.91(m, 1H, HN**CH**CO), 3.91~4.20(m, 2H, N**CH_2**Ph), 4.20~4.32(m, 1H, HN**CH**CO), 4.32~4.49(m, 2H, N**CH_2**Ph), 4.49~4.87(m, 4H, HN**CH_2**CO and HN**CH_2**C(OH)), 4.87~5.06(m, 1H, **CH**CH_2Ph), 7.03~8.03(m, 14H, Ar**H**); ^{13}CNMR(100 MHz, $CDCl_3$) ***δ*** : 10.7, 11.5, 15.0, 16.7, 23.6, 24.0, 24.7, 25.2, 26.0, 28.7, 29.7, 29.8, 35.5, 35.7, 36.3, 47.2, 47.5, 47.7, 50.1, 53.0, 53.5, 55.2, 55.7, 57.8, 61.4, 62.2, 88.9, 121.6, 124.0, 126.7, 127.1, 127.4, 127.8, 128.6, 128.8, 128.9, 129.5, 132.8, 136.5, 137.3, 148.4, 169.7, 170.0, 170.1, 171.0, 171.9, 173.7. ESI-MS revealed that the $[M+H]^+$ (m/z) of compound **1** was 973.51825, which is consistent with the theoretical molecular weight 973.51820.

Experiment instruments

^{1}H and ^{13}C-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. Circular dichroism(CD) were recorded on a Chirascan qCD spectrometer (Applied Photophysics Ltd). Hand held UV lamp (254nm, 365nm).

Computational details:

The conformational analysis was performed by arbitrarily fixing the absolute configuration of C-3 for compound **1**, using the Spartan 08 package[1] 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 by the program package Gaussina 09.[2] TDDFT/B3LYP/6311++g(d, p) 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 0.30 eV by Specdis 1.61.[3]

References:

1. Spartan'08, Wavefunction, Inc. Irvine, CA

2. Gaussian 09, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.

3. T. Bruhn, A. Schaumloffel, Y. Hemberger, G. Bringmann, *Chirality,* 2013, **25**, 243.

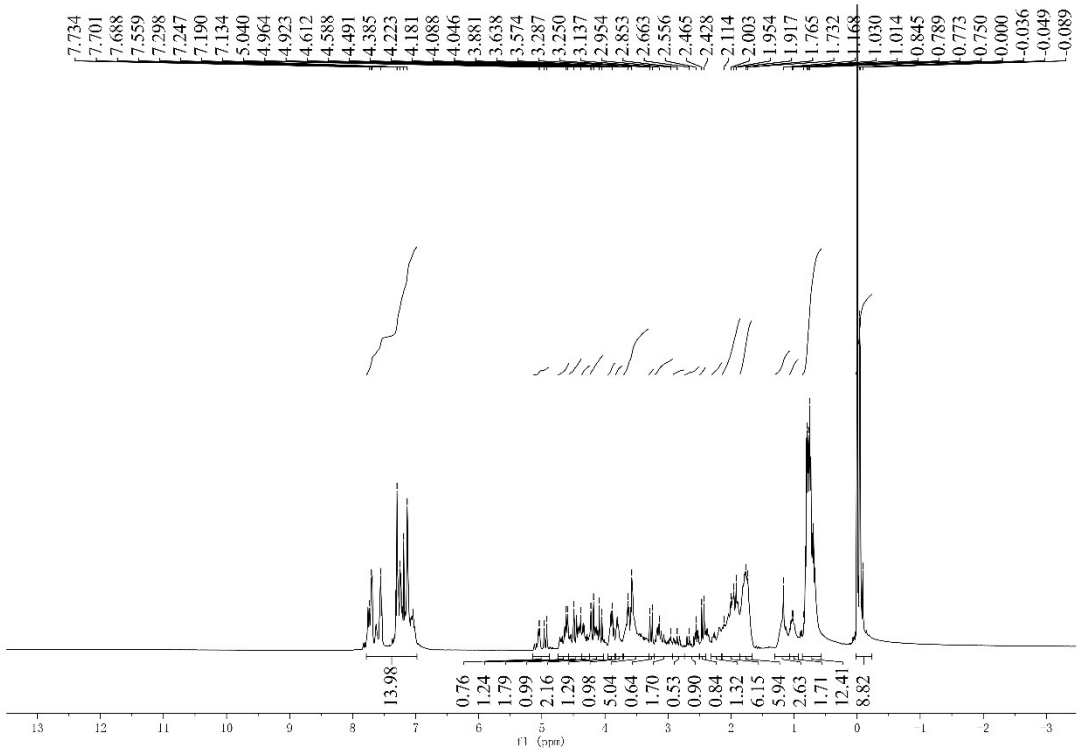
Docking Simulations

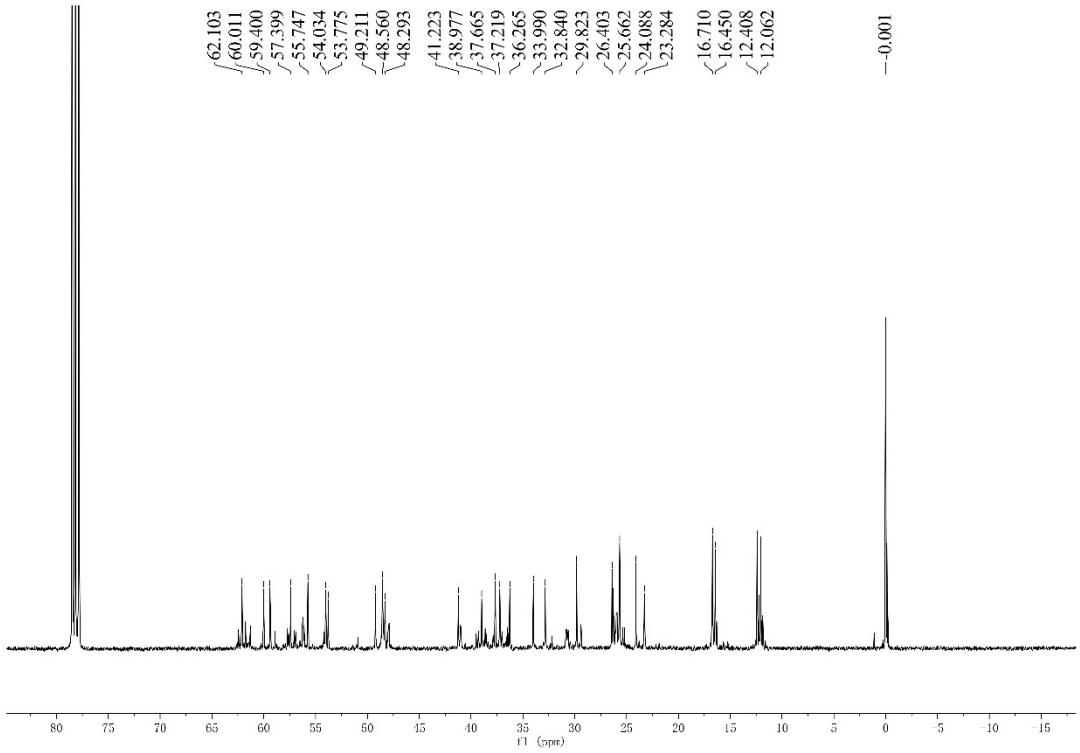
Docking experiments were performed by AutoDock Tools 4.26 (ADT). The crystal structure of MDM2 (PDB ID: 3JZR) was obtained from the Protein Data Bank (PDB). The water molecule of 3JZR and the original ligand pDI6W were deleted with Pymol 1.7, and then the MDM2 molecule was hydrogenated and charged with ADT. Compound **1** was minimized via MDM2 method. Binding pocket was located at a hydrophobic pocket where the original inhibitor binds with MDM2. Docking parameters were shown in Table **S1**. The complex with the lowest energy was chosen as the best binding conformation between MDM2 and the cyclic peptide. Binding sites to MDM2 and interactions were analyzed by Accelrys Discovery Studio Visualizer 2020.

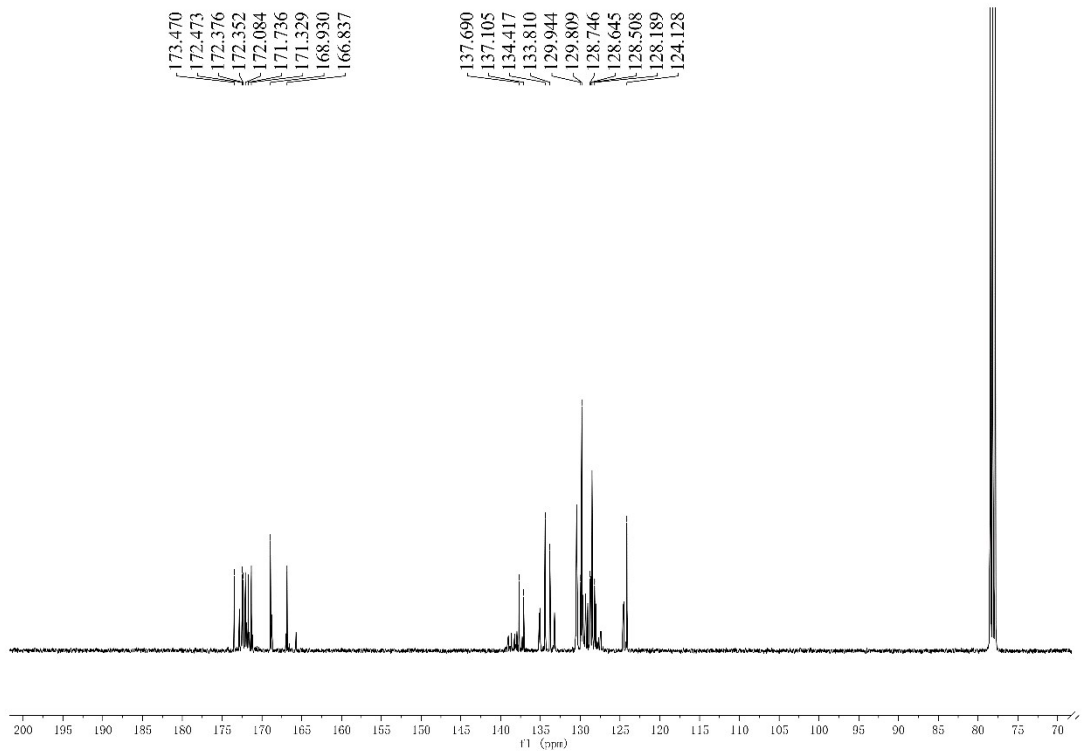
Table S1. Docking process parameter setting.

Parameter	Value
Grid box (nm)	8 × 8 × 8
spacing	0.375
grid center x	11.275
grid center y	-14.574
grid center z	10.266
GA runs	150
population size	150

2. ^{1}H, ^{13}C-NMR and HRMS of linear peptide (2).



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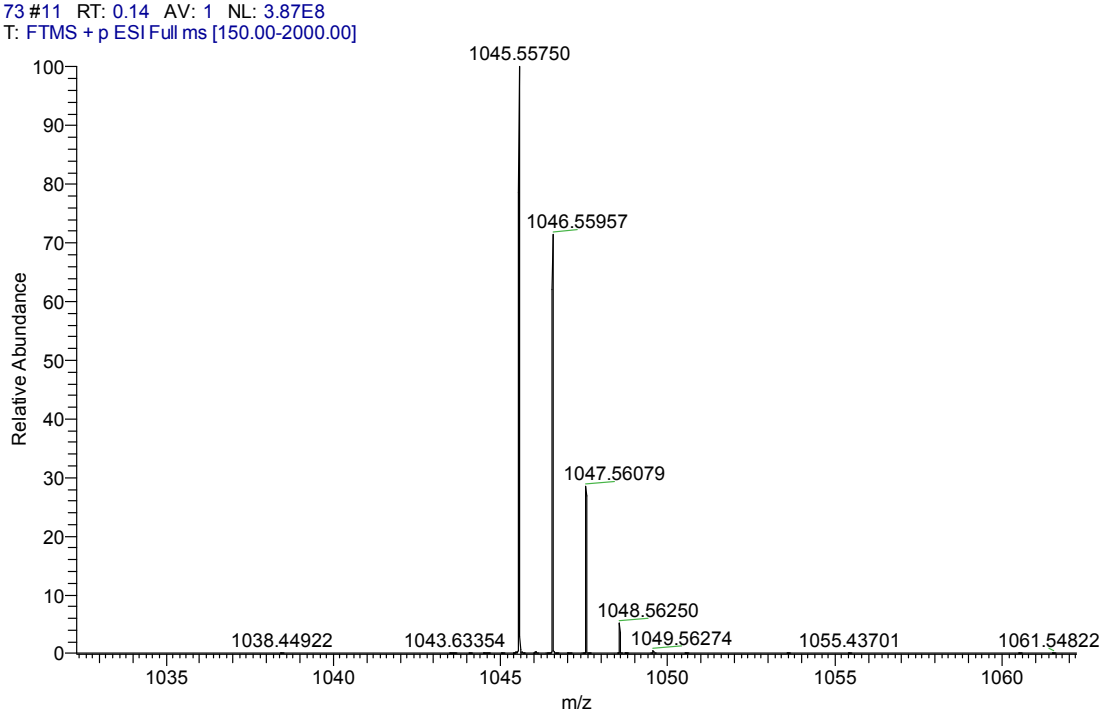
172.473
172.376
172.352
172.084
171.736
171.329
168.930
166.837
137.690
137.105
134.417
133.810
129.944
129.809
128.746
128.645
128.508
128.189
124.128
200 195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70
f1 (ppm)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T: FTMS + p ESI Full ms [150.00-2000.00]
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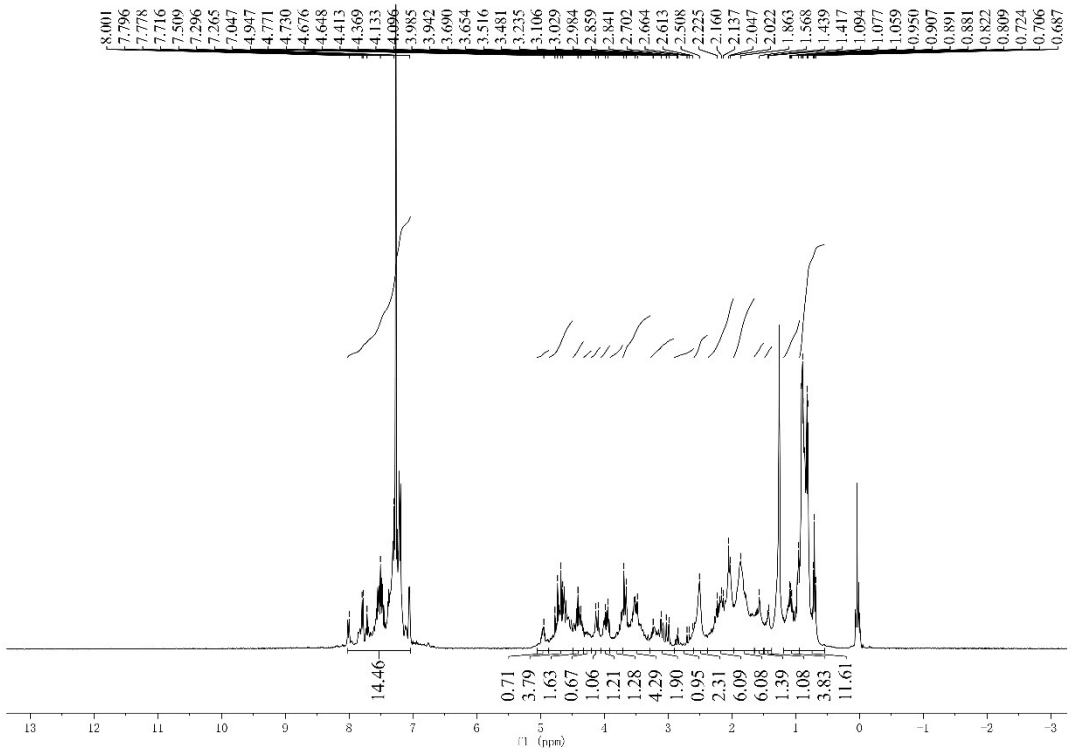
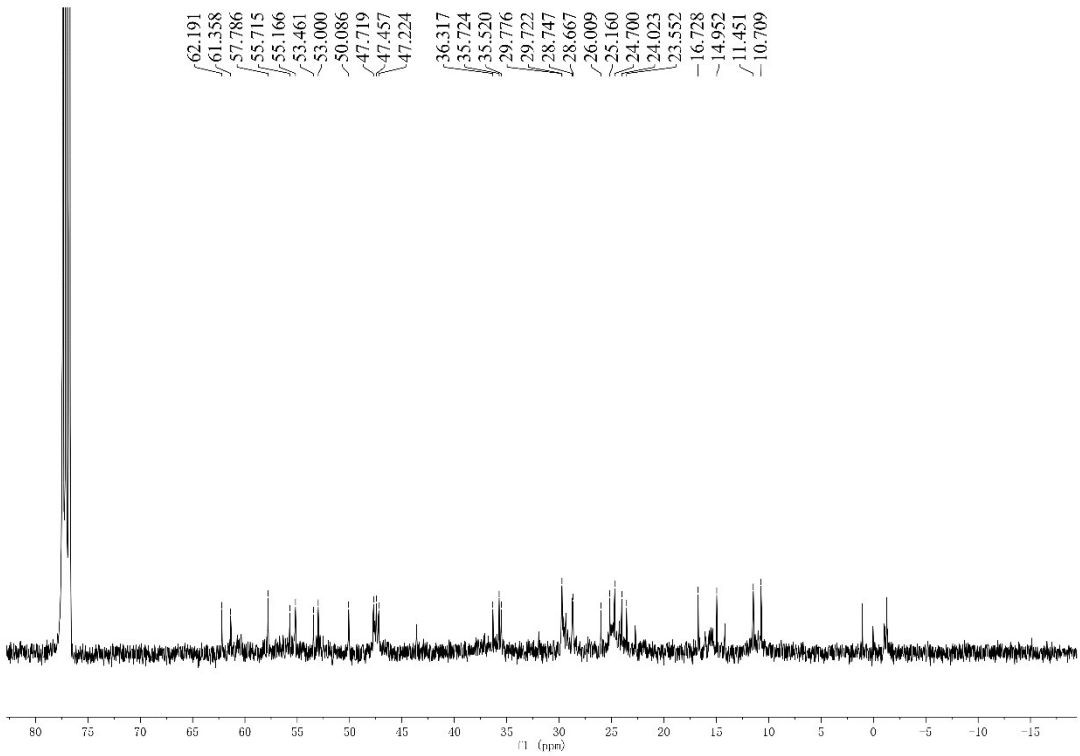
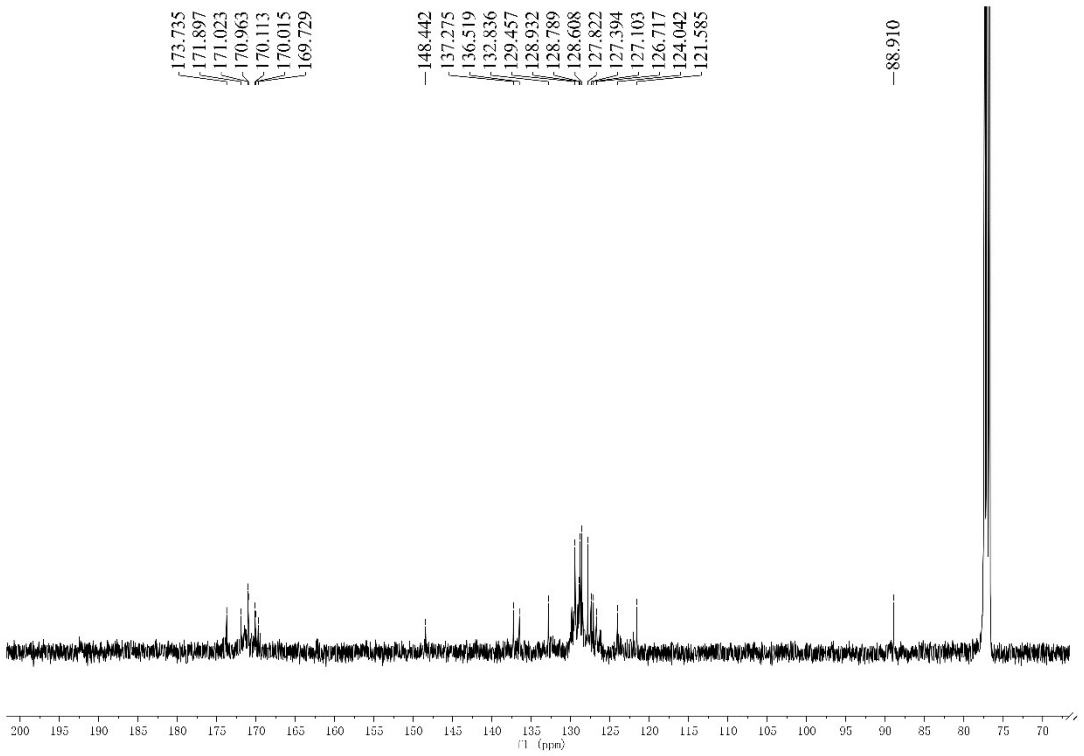
1046.55957
1047.56079
1048.56250
1049.56274
1038.44922
1043.63354
1055.43701
1061.54822
Relative Abundance
m/z
100 90 80 70 60 50 40 30 20 10 0
1035 1040 1045 1050 1055 1060

3. ^{1}H, ^{13}C-NMR and HRMS of cyclic peptide (1).






173.735
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171.023
170.963
170.113
170.015
169.729
148.442
137.275
136.519
132.836
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128.789
128.608
127.822
127.394
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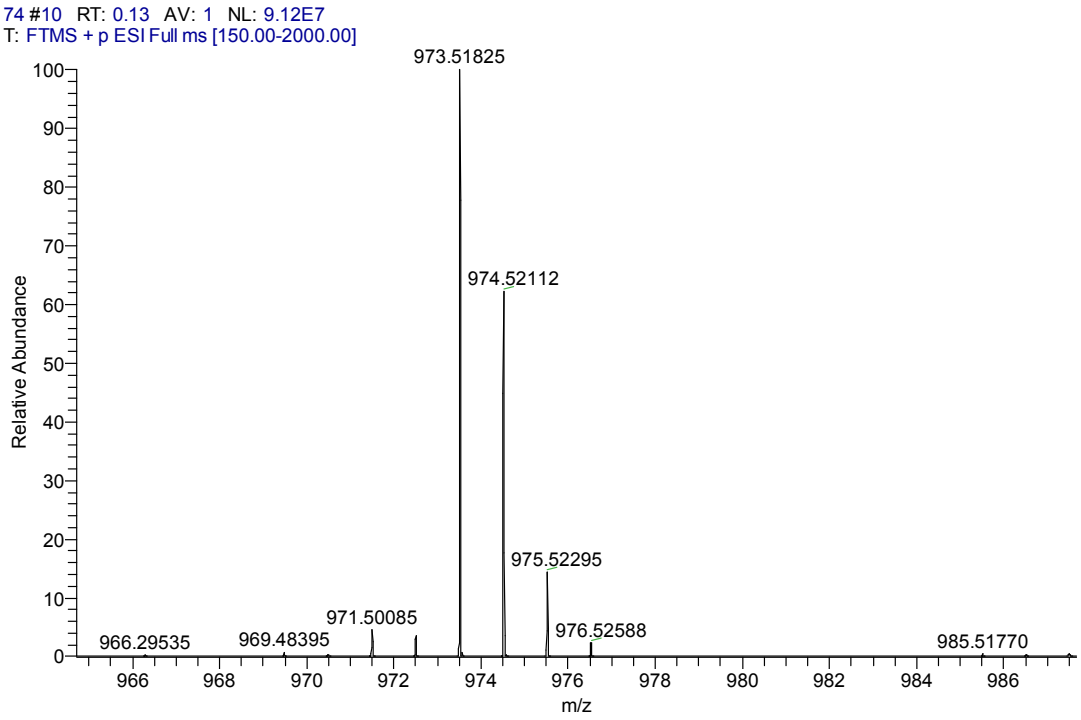
f1 (ppm)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974.52112
975.52295
971.50085
976.52588
966.29535
969.48395
985.51770
Relative Abundance
m/z
100 90 80 70 60 50 40 30 20 10 0
966 968 970 972 974 976 978 980 982 984 986

4. The torsion angles in the secondary structure of the most stable conformer of compound 1.

Table S2. The torsion angles in the secondary structure of the most stable conformer of compound **1** involving H-bondings.

Compd.	compound **1** (the most stable conformer S-1)		
H-B	C-3-O-H⋯ O=C(Gly^1)	(Ile^3)N-H⋯ O=C(Gly^1)	(Ile^6)N-H⋯ O=C(Isoindolinone)
Type	Pseudo-γ-turn	γ-turn	-
Distance Å	1.957	1.925	1.786
$\phi_{Isoindolinone\text{-}N}$	80.99	-	-
$\psi_{Isoindolinone\text{-}N}$	-53.35	-	-
ϕPro^2	-	82.45	-
ψPro^2	-	-60.63	-
Referential characteristic torsion in classical γ-turn [a]	ϕ 75° ψ-64°		
Referential characteristic torsion in classical γ^i-turn [a]	ϕ -79° ψ 69°		

[a] These data were listed as a reference to classify the secondary structures according to literature, the classification of γ-turn was based on the dihedral angles ϕ^{i+1} and ψ^{i+1}.

5. UV of compound 1