

Supplementary Material

Design, Synthesis, and Bioactivities of Novel Tryptophan Derivatives Containing 2,5-Diketopiperazine and Acyl Hydrazine Moieties

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Section S1: Copies of NMR spectra

1

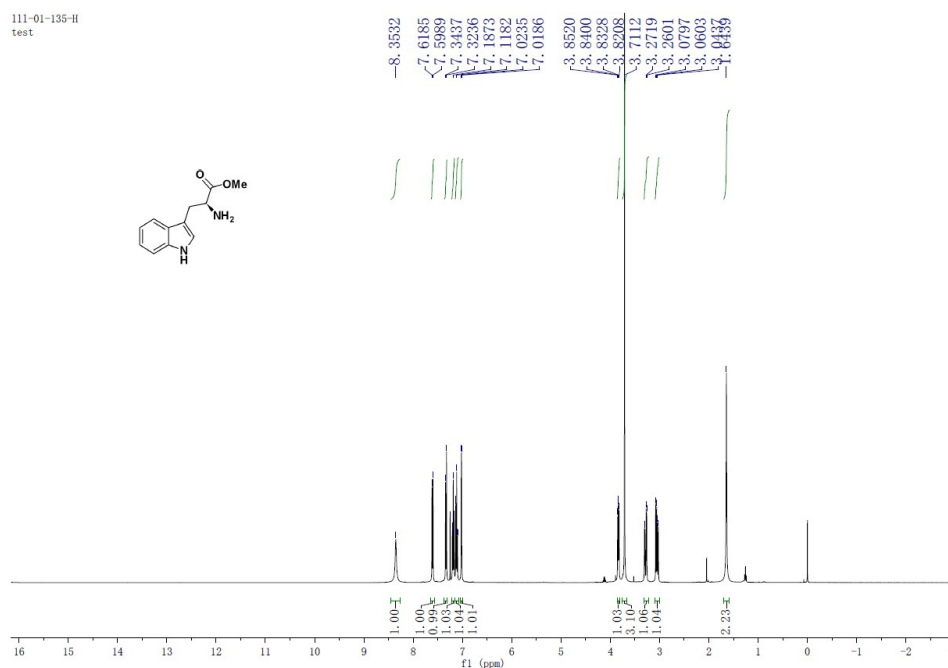


Figure S1 ^1H NMR (400 MHz, CDCl_3) of 1

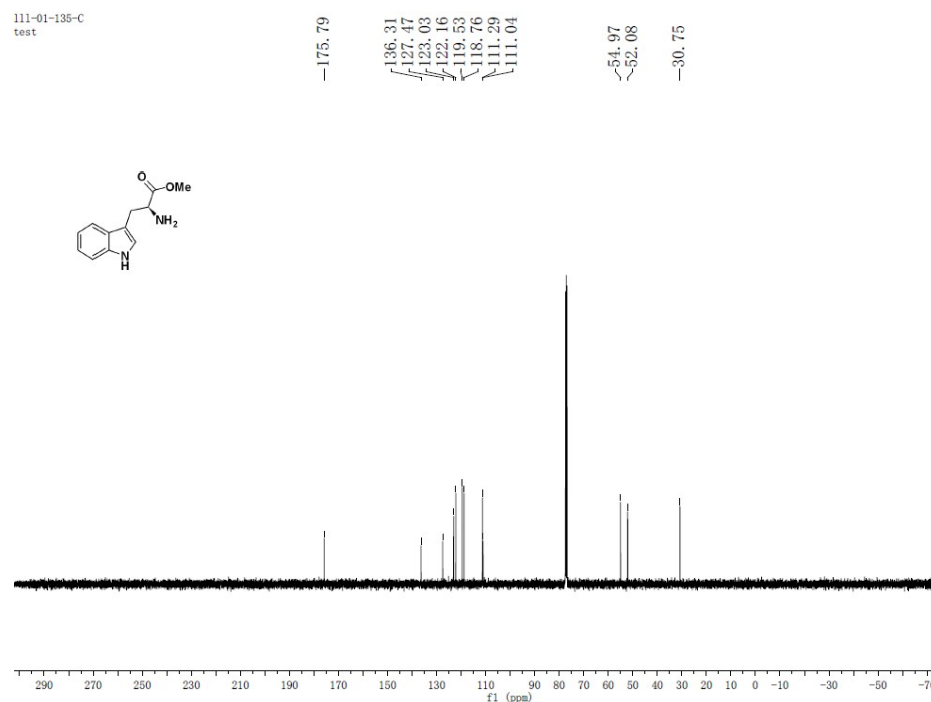


Figure S2 ^{13}C NMR (100 MHz, CDCl_3) of 1

2

111-01-135-H
test

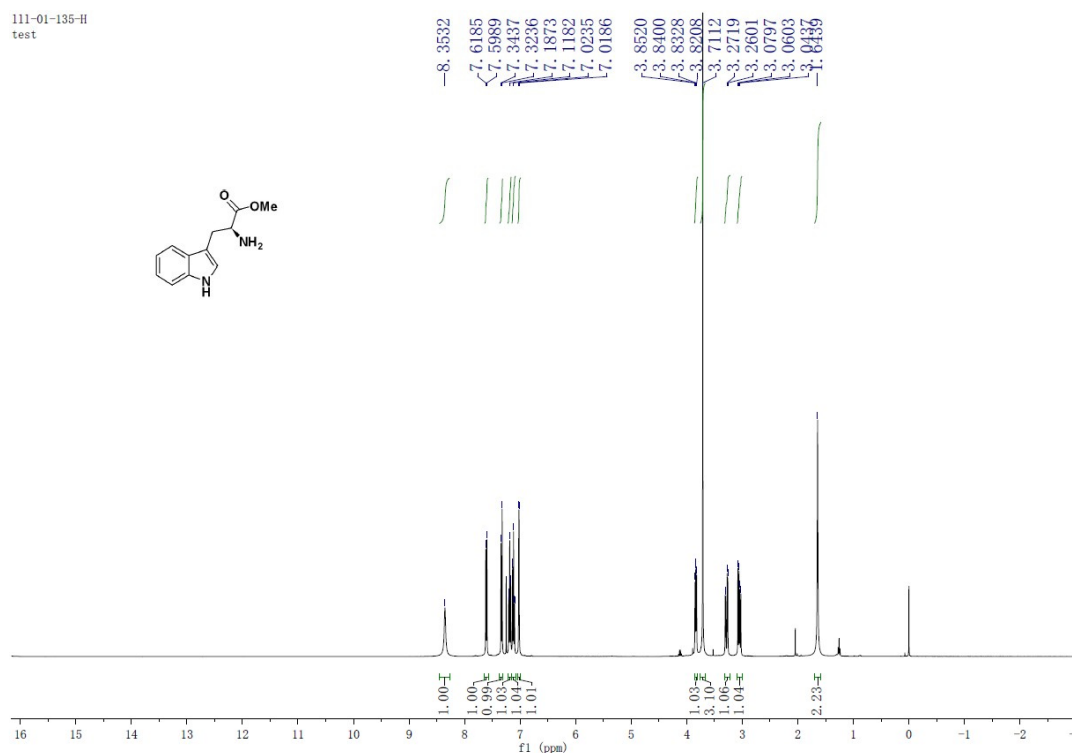


Figure S3 ¹H NMR (400 MHz, CDCl₃) of 2

111-01-139-H
Cl3CPD

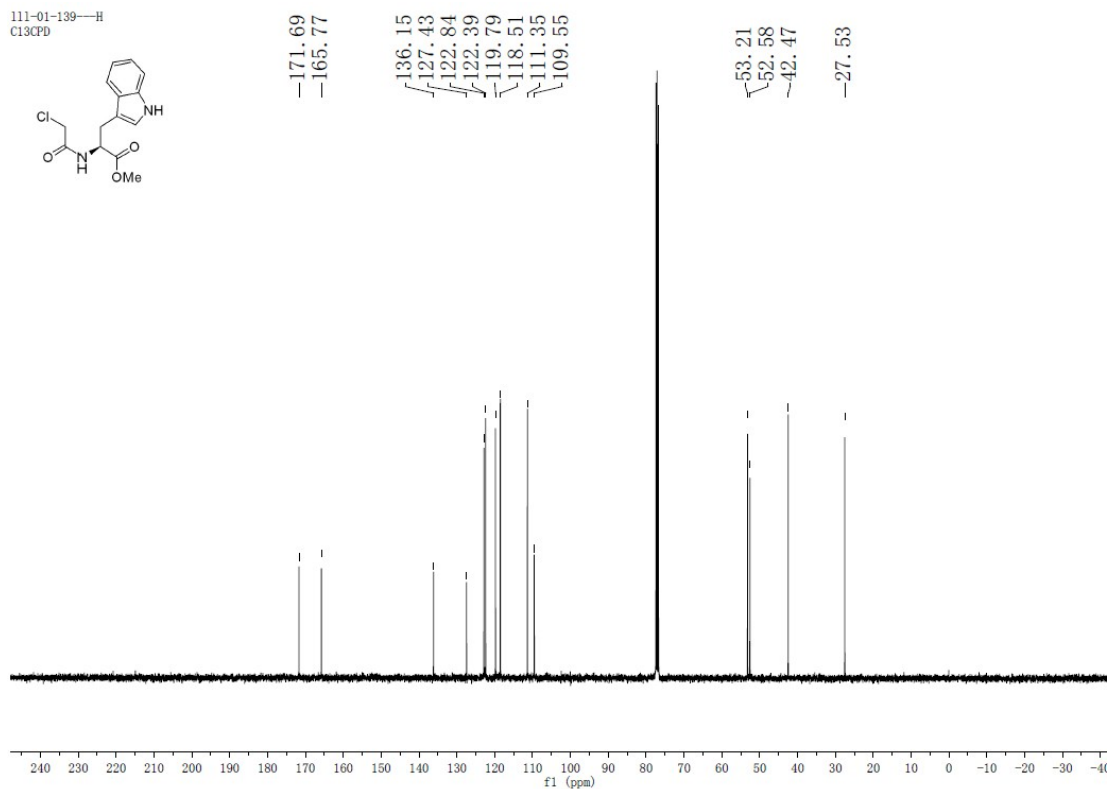


Figure S4 ¹³C NMR (100 MHz, CDCl₃) of 2

3

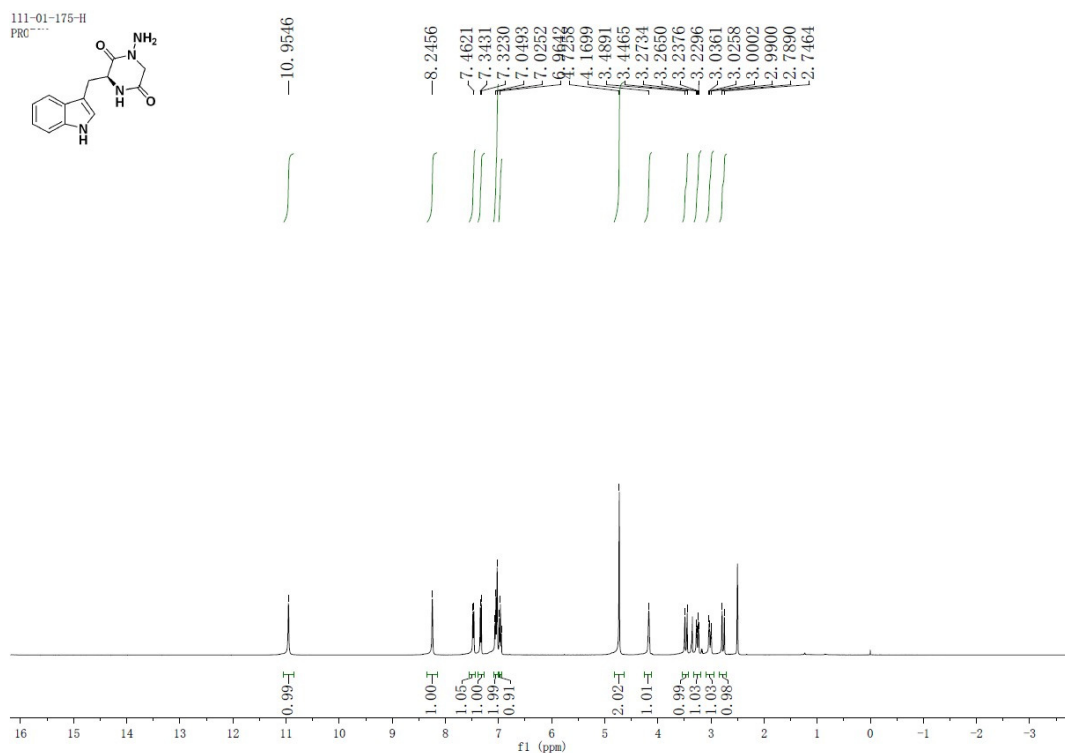


Figure S5 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 3

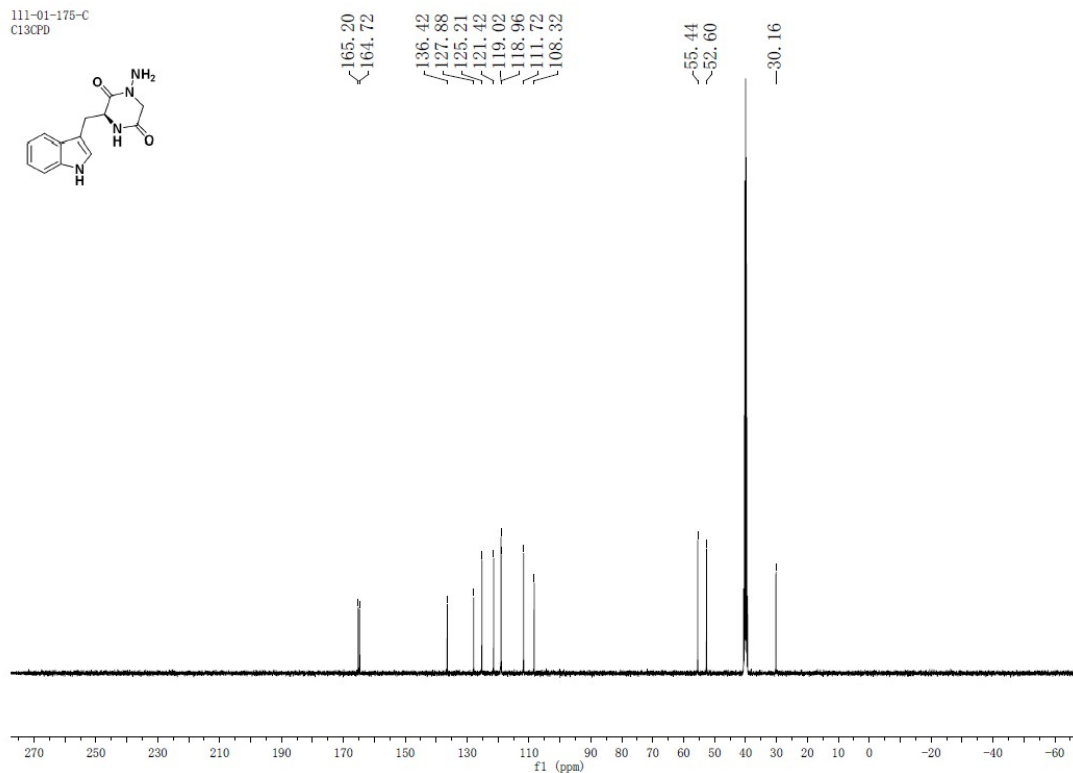


Figure S6 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 3

4

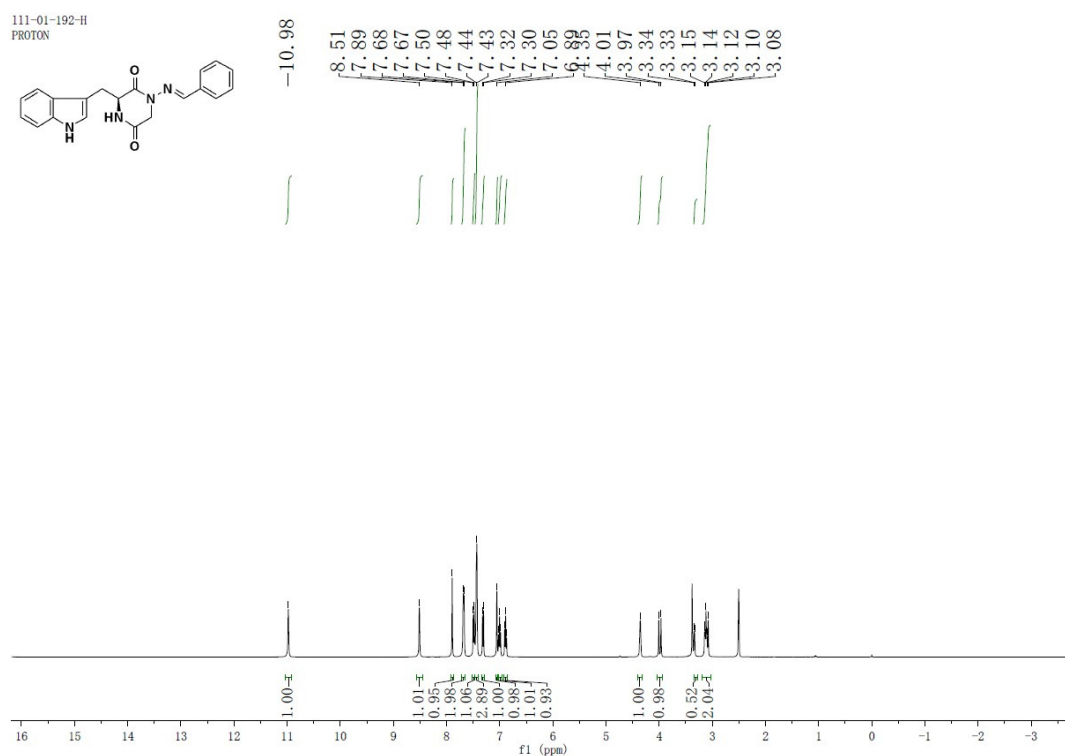


Figure S7 ¹H NMR (400 MHz, DMSO-*d*₆) of 4

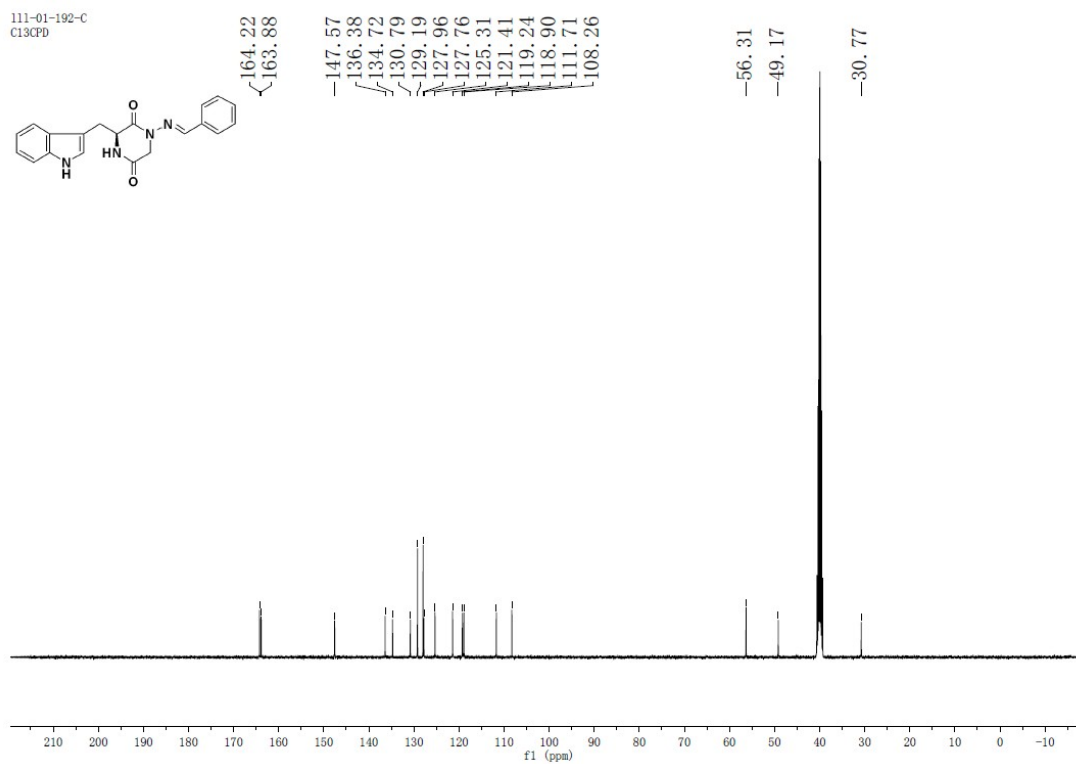


Figure S8 ¹³C NMR (100 MHz, DMSO-*d*₆) of 4

5

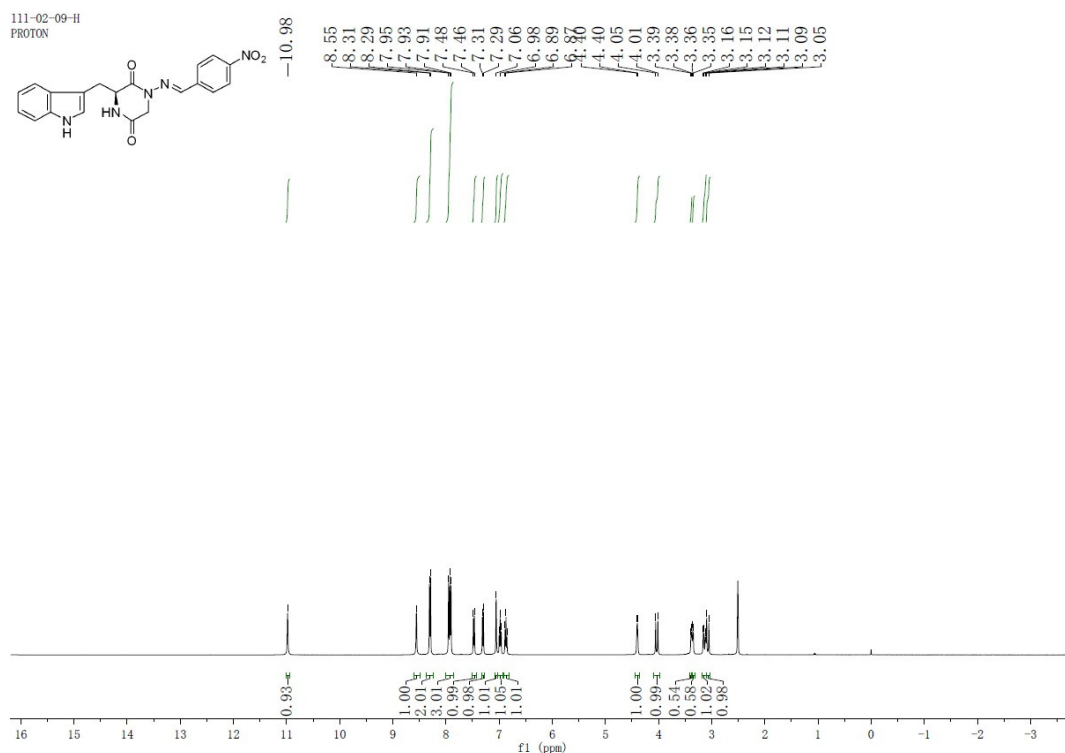


Figure S9 ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of 5

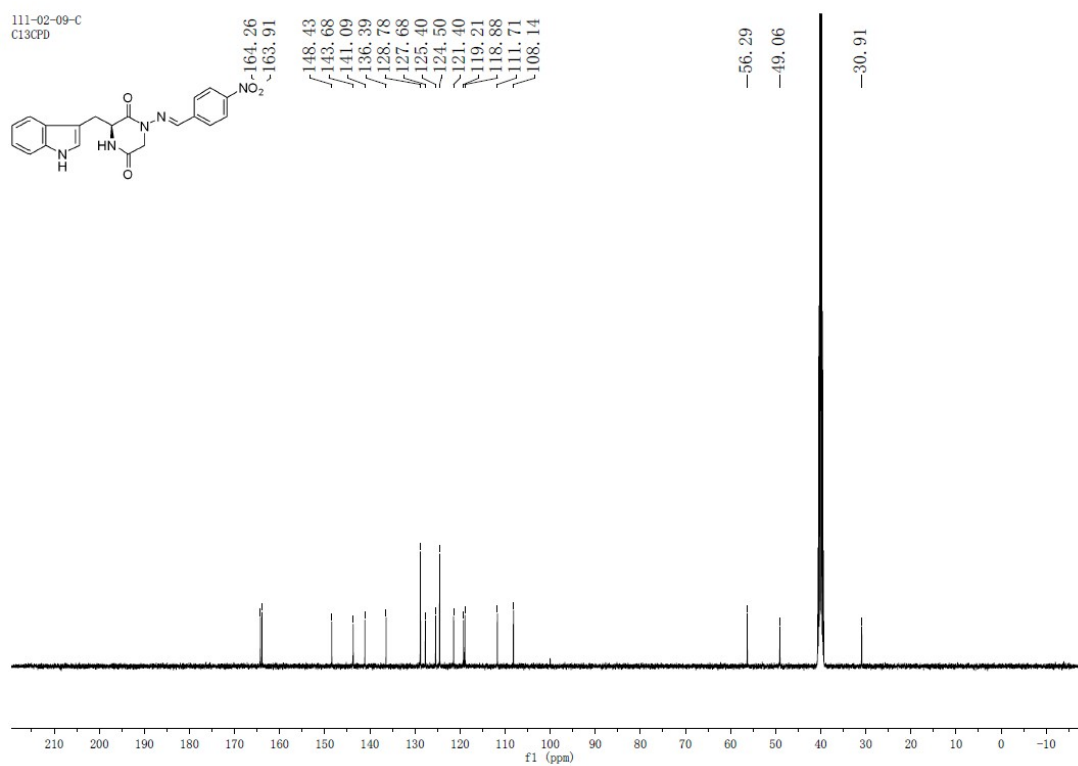
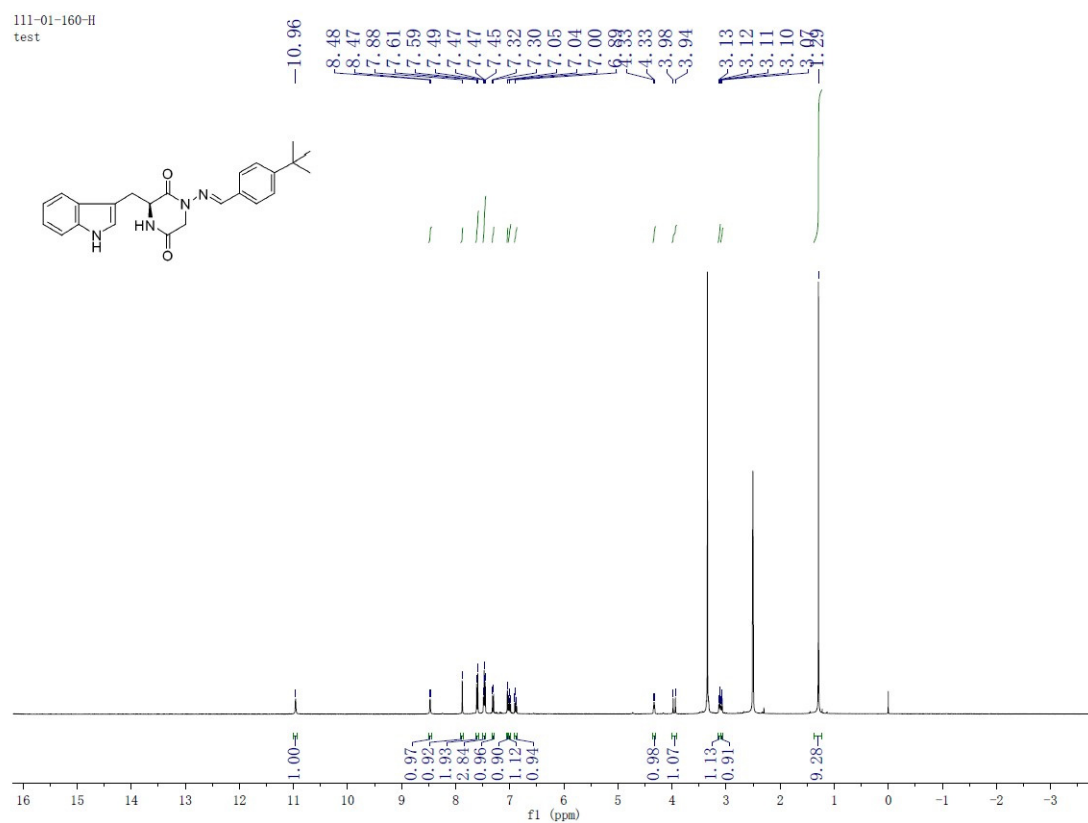
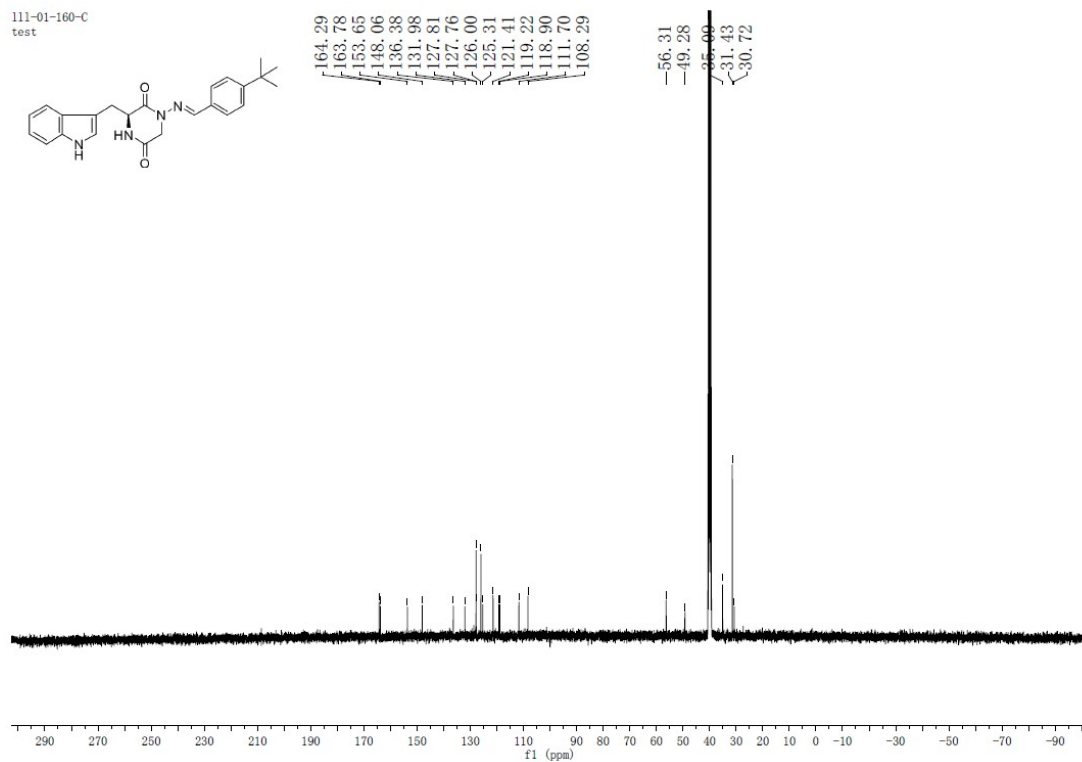
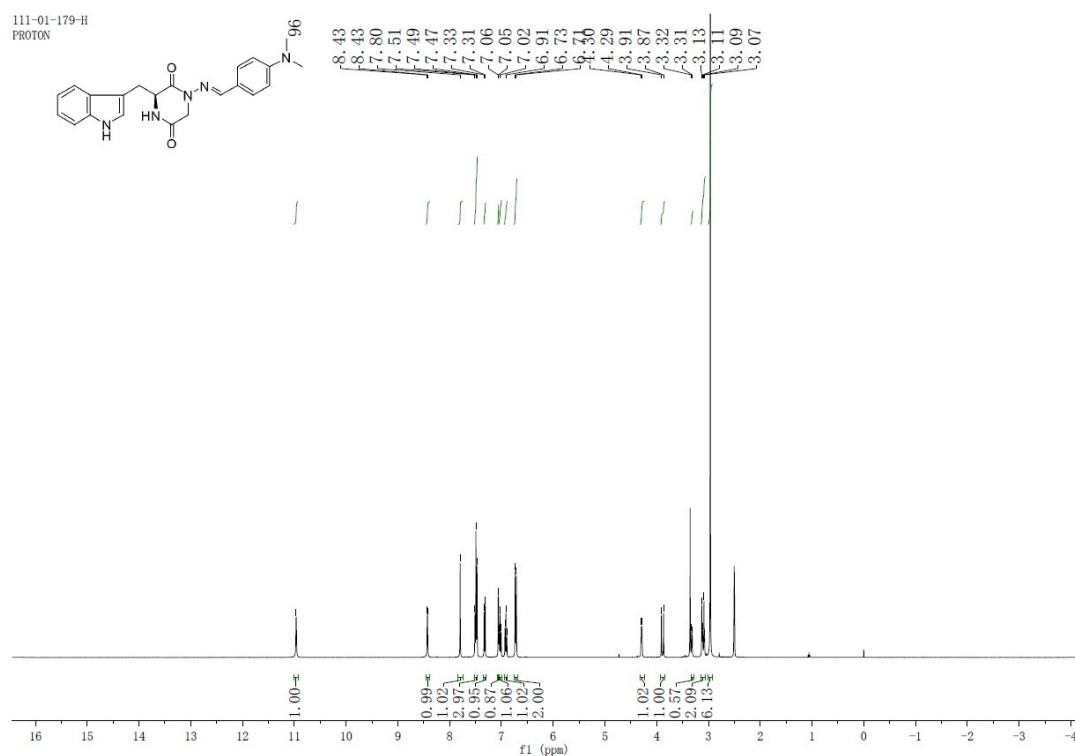
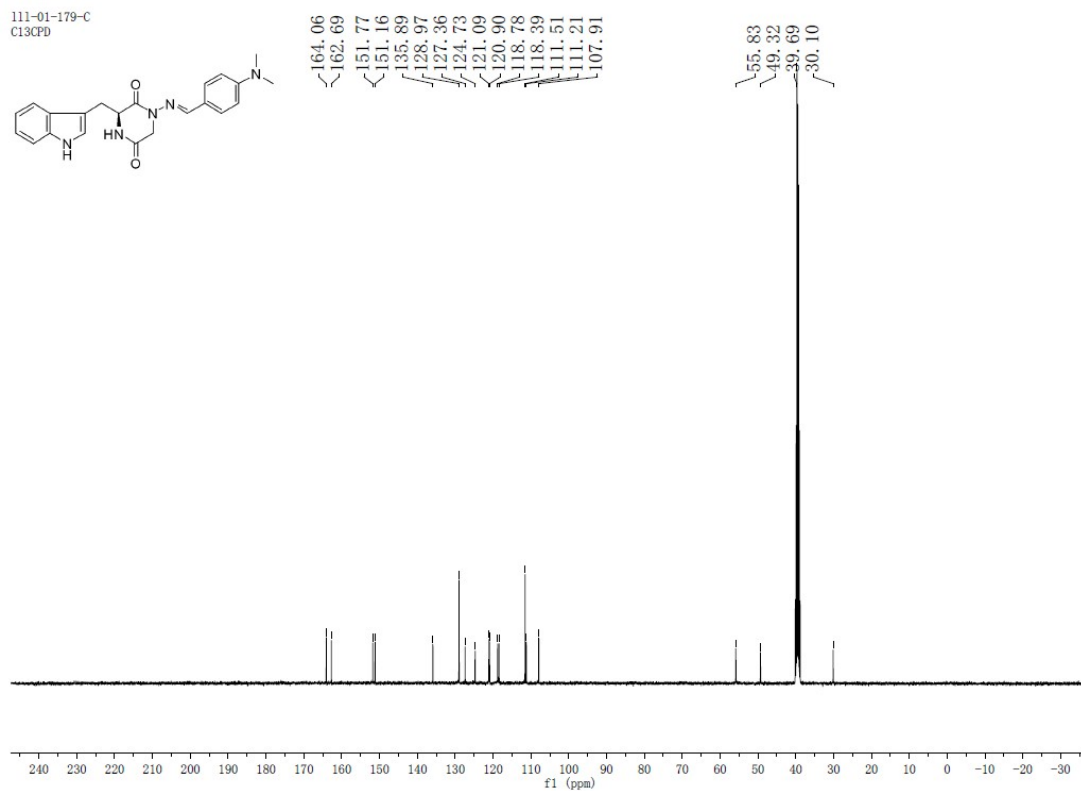


Figure S10 ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) of 5

6

Figure S11 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 6Figure S12 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 6

7

Figure S13 ¹H NMR (400 MHz, DMSO-*d*₆) of 7Figure S14 ¹³C NMR (100 MHz, DMSO-*d*₆) of 7

8

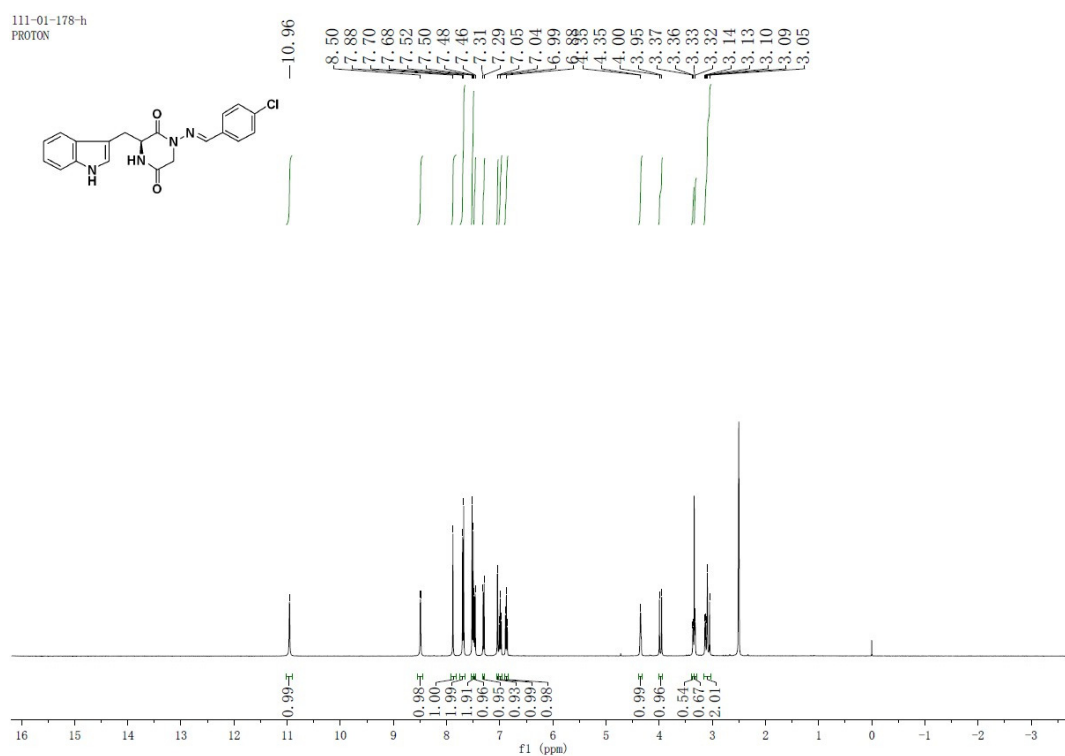


Figure S15 ¹H NMR (400 MHz, DMSO-*d*₆) of 8

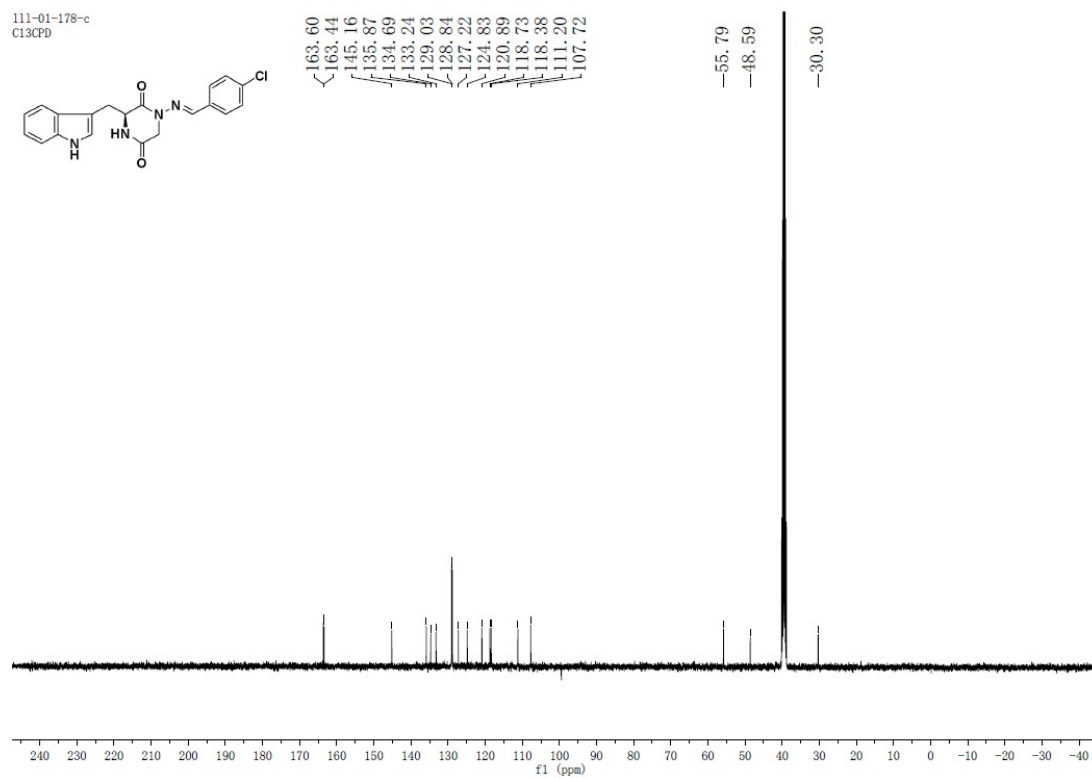


Figure S16 ¹³C NMR (100 MHz, DMSO-*d*₆) of 8

9

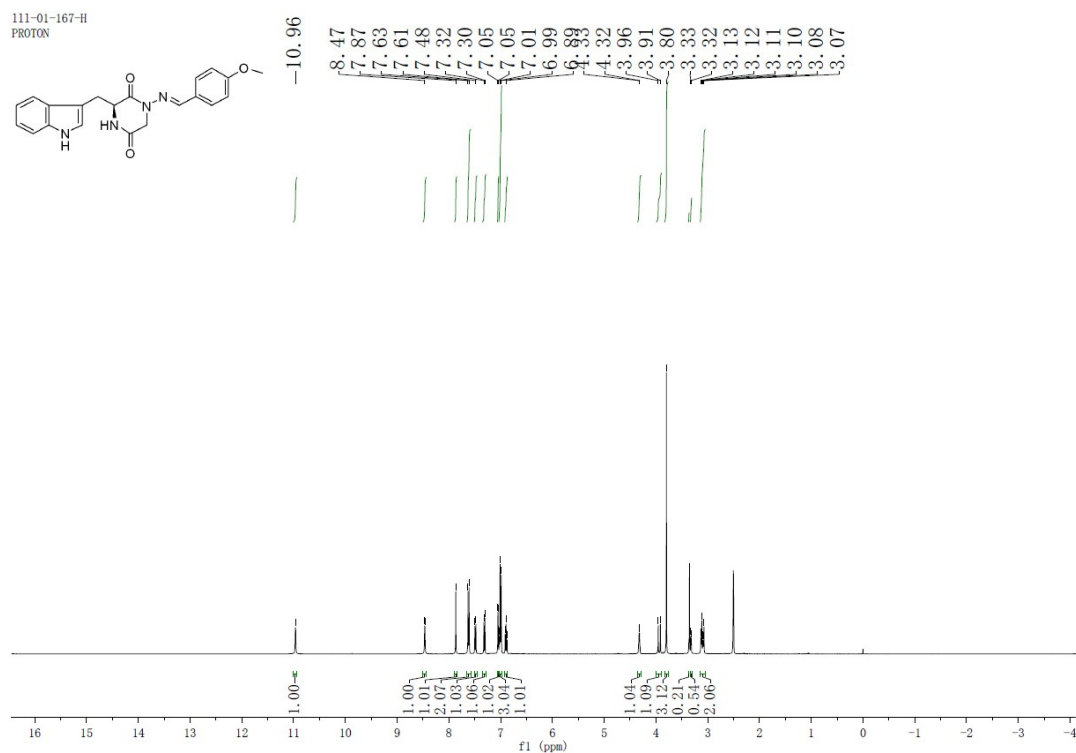


Figure S17 ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of 9

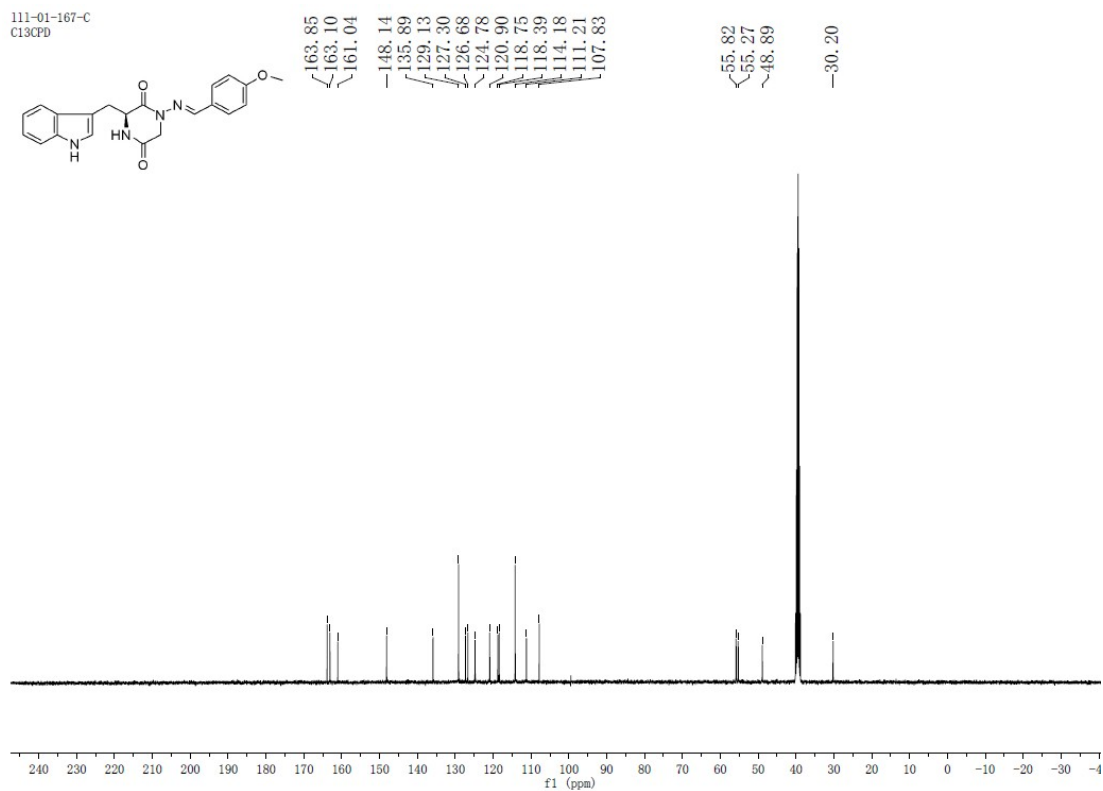


Figure S18 ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) of 9

10

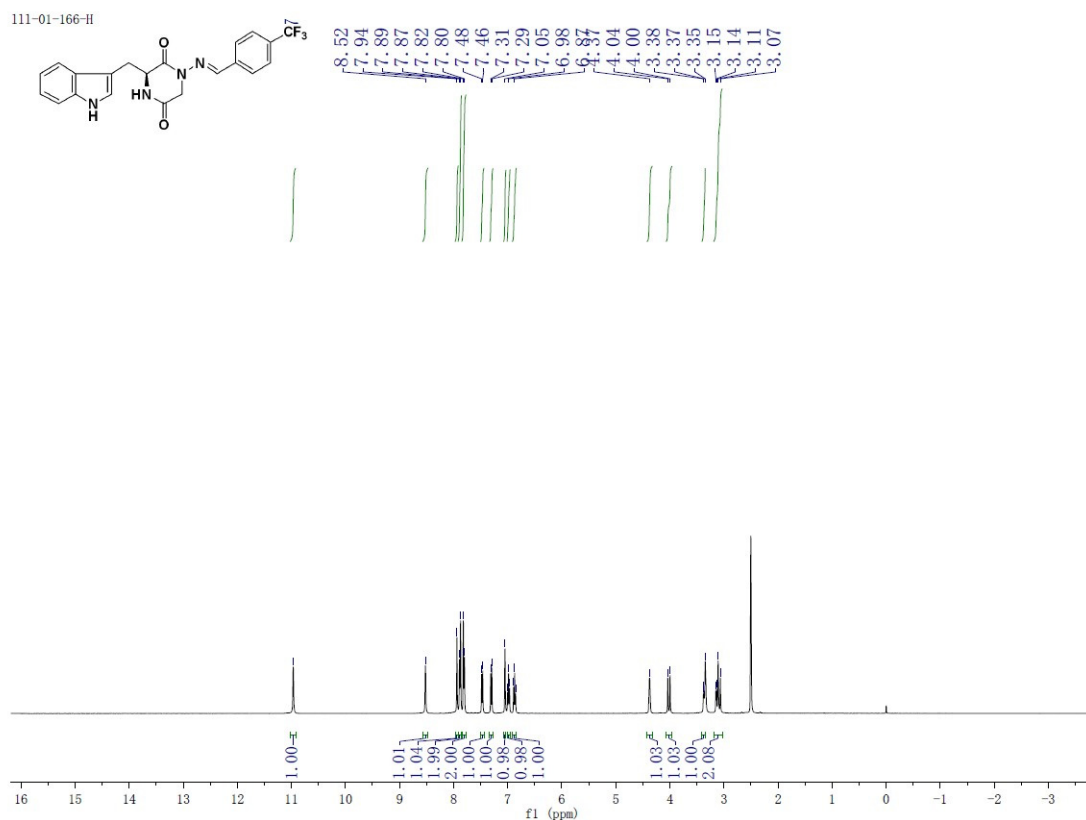


Figure S19 ¹H NMR (400 MHz, DMSO-*d*₆) of 10

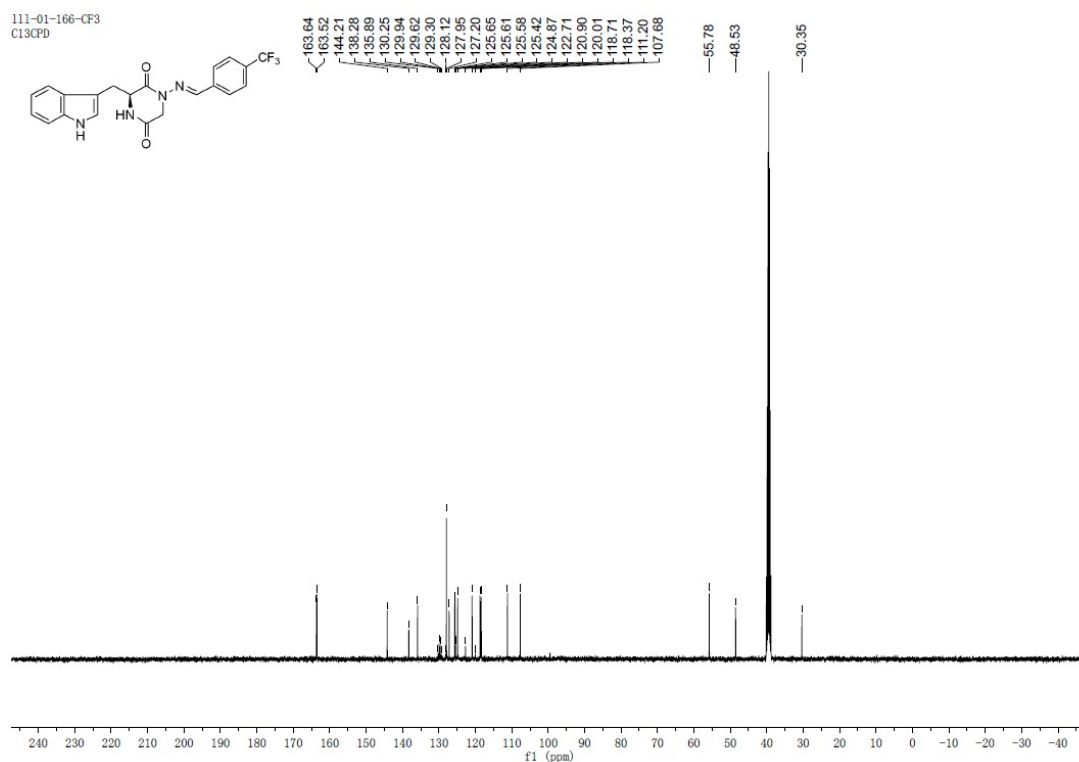
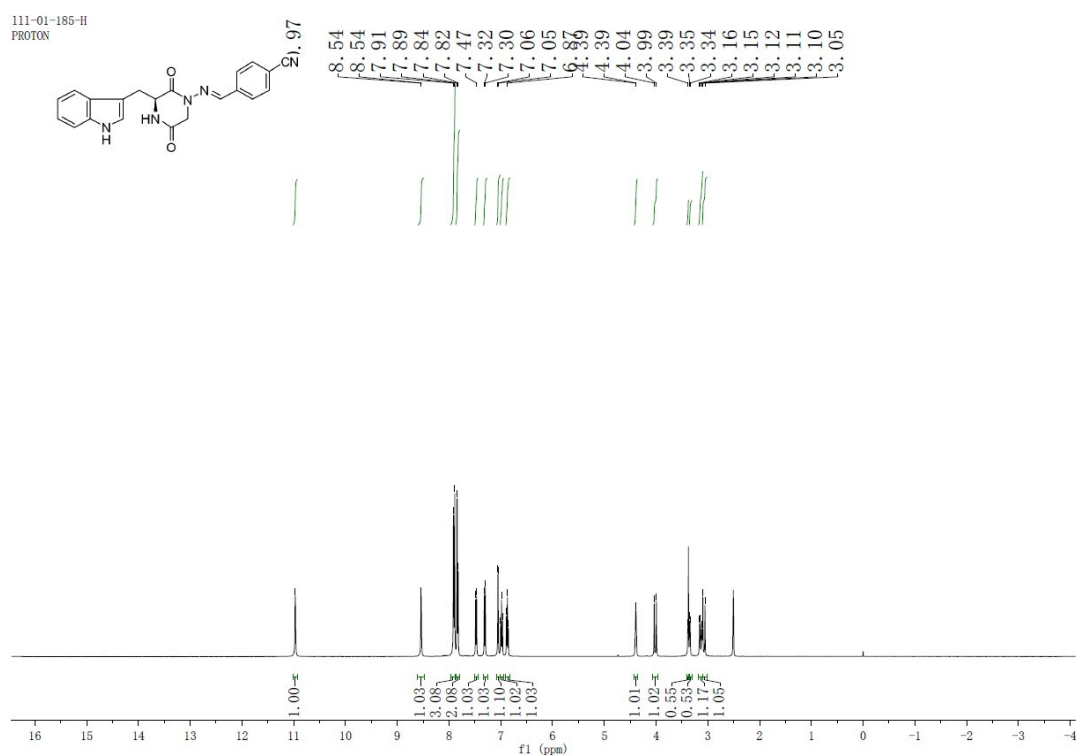
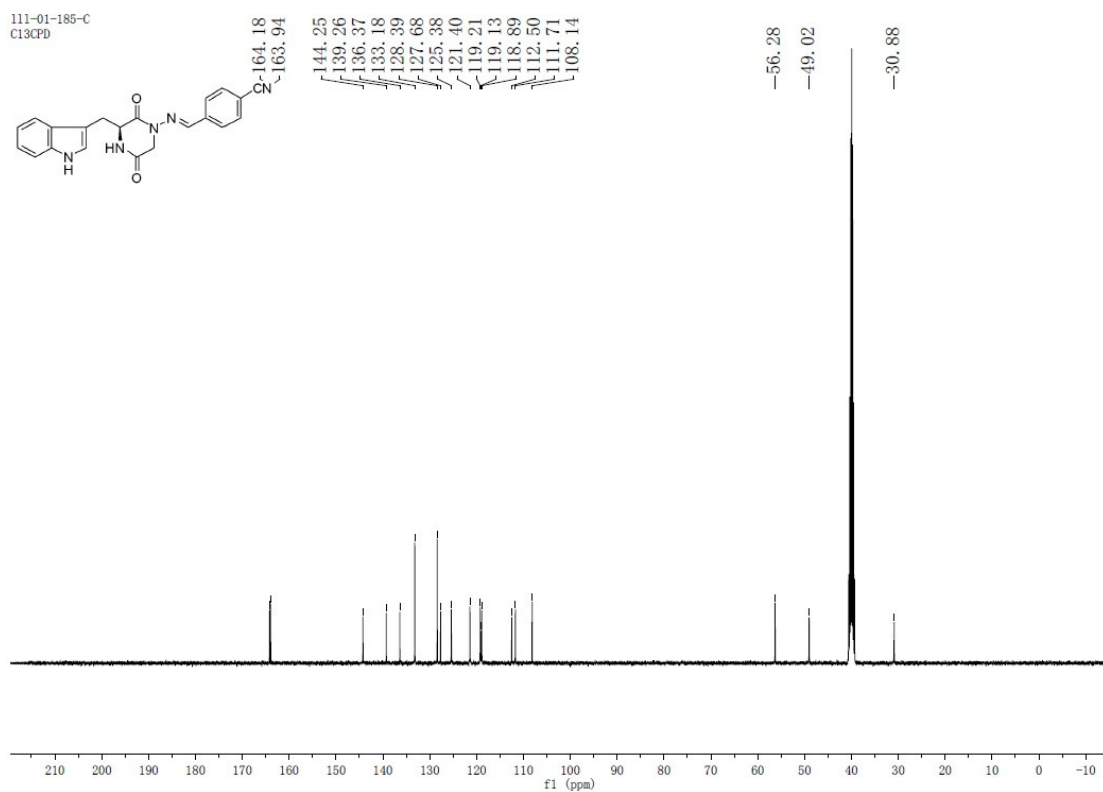


Figure S20 ¹³C NMR (100 MHz, DMSO-*d*₆) of 10

Figure S21 ¹H NMR (400 MHz, DMSO-*d*₆) of 11Figure S22 ¹³C NMR (100 MHz, DMSO-*d*₆) of 11

12

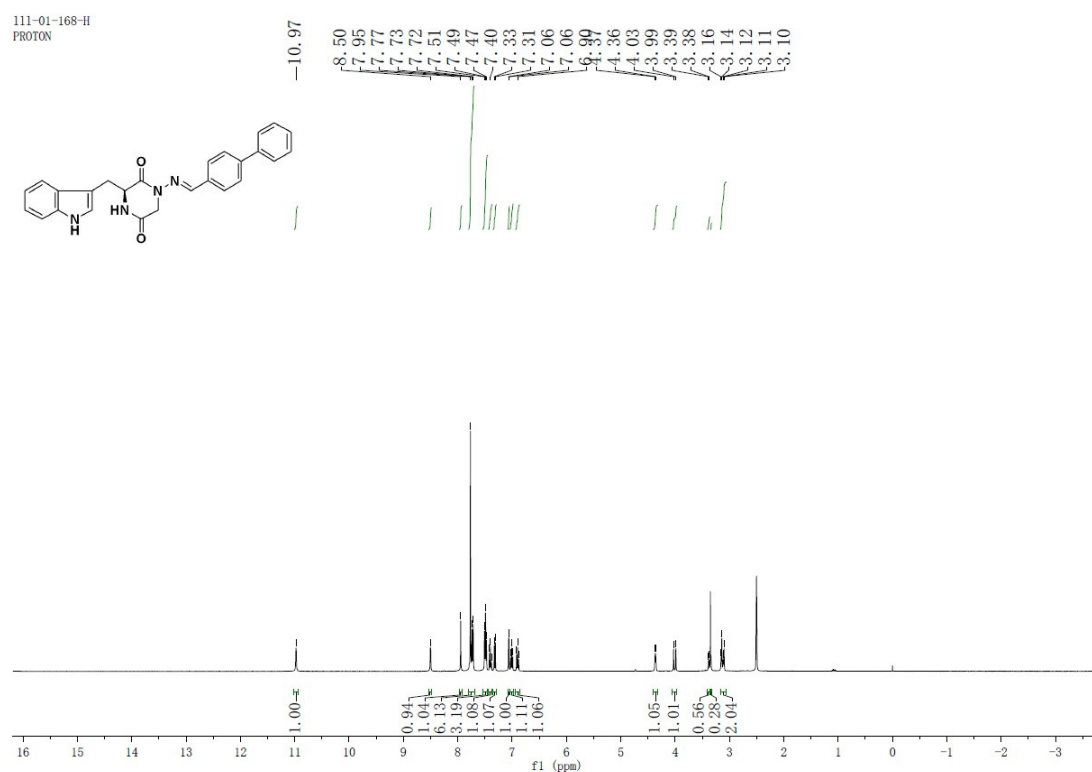


Figure S23 ¹H NMR (400 MHz, DMSO-*d*₆) of 12

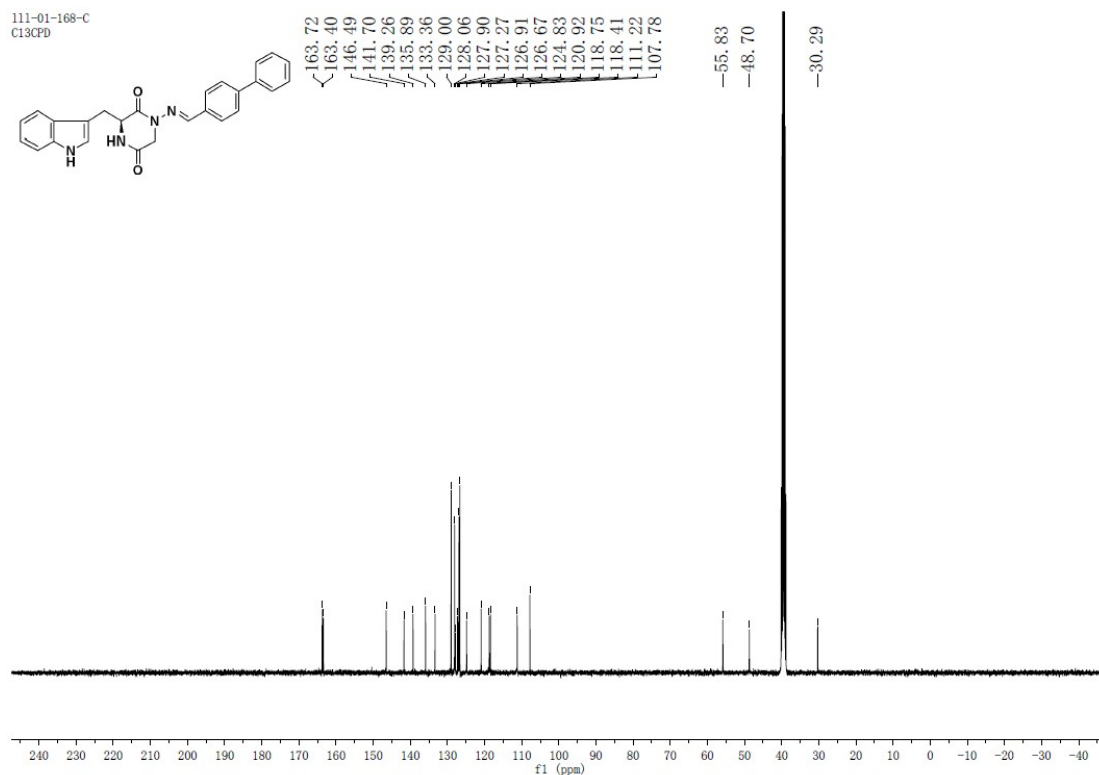


Figure S24 ¹³C NMR (100 MHz, DMSO-*d*₆) of 12

13

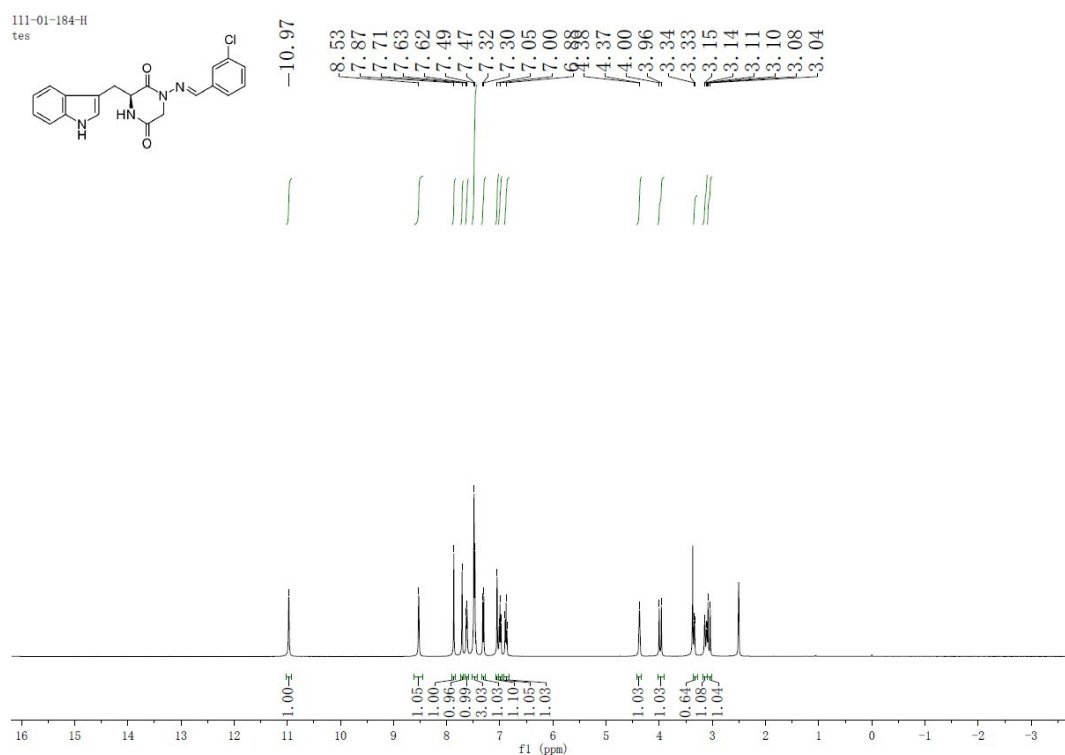


Figure S25 ¹H NMR (400 MHz, DMSO-*d*₆) of 13

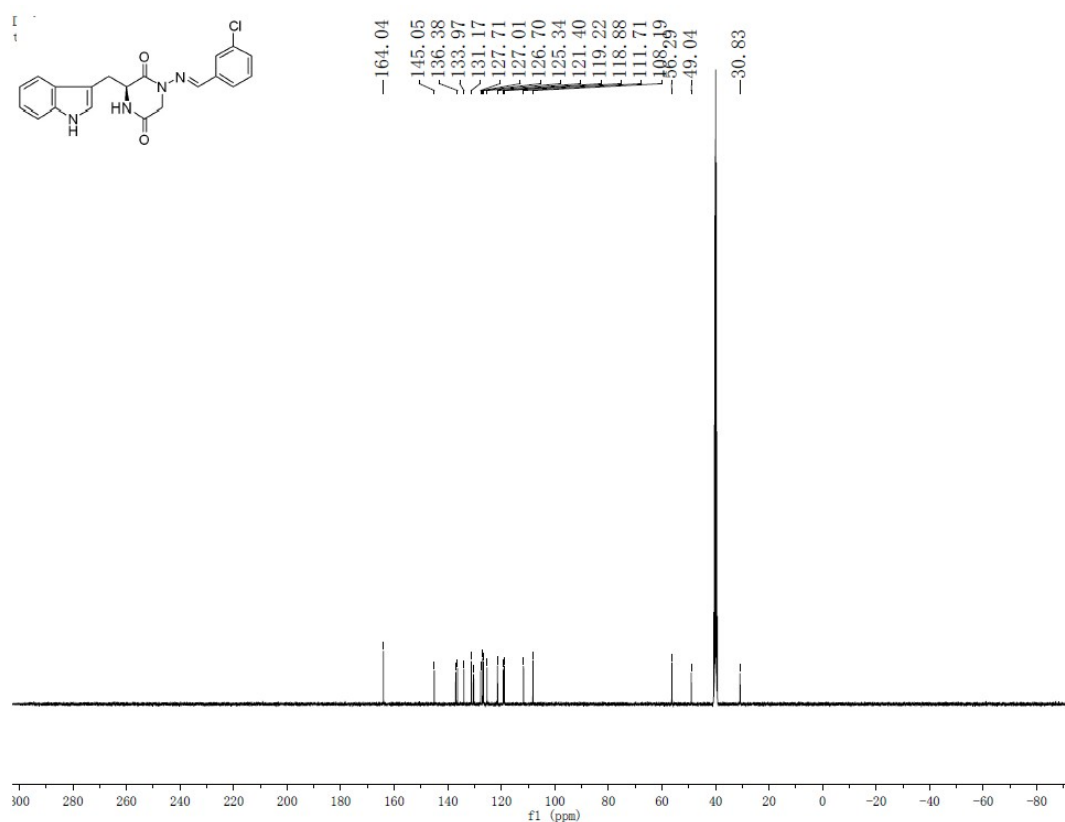


Figure S26 ¹³C NMR (100 MHz, DMSO-*d*₆) of 13

11-01-183-H
C13CPD

O=C1CN(Cc2c[nH]c3ccccc23)C(=O)N1/N=C/c4ccccc4Cl

164.34
164.20
143.53
136.40
134.13
132.32
131.84
130.36
128.02
127.82
127.64
125.50
121.46
119.13
118.90
111.71
108.13

-56.29
-49.56

-30.81

f1 (ppm)

14

15

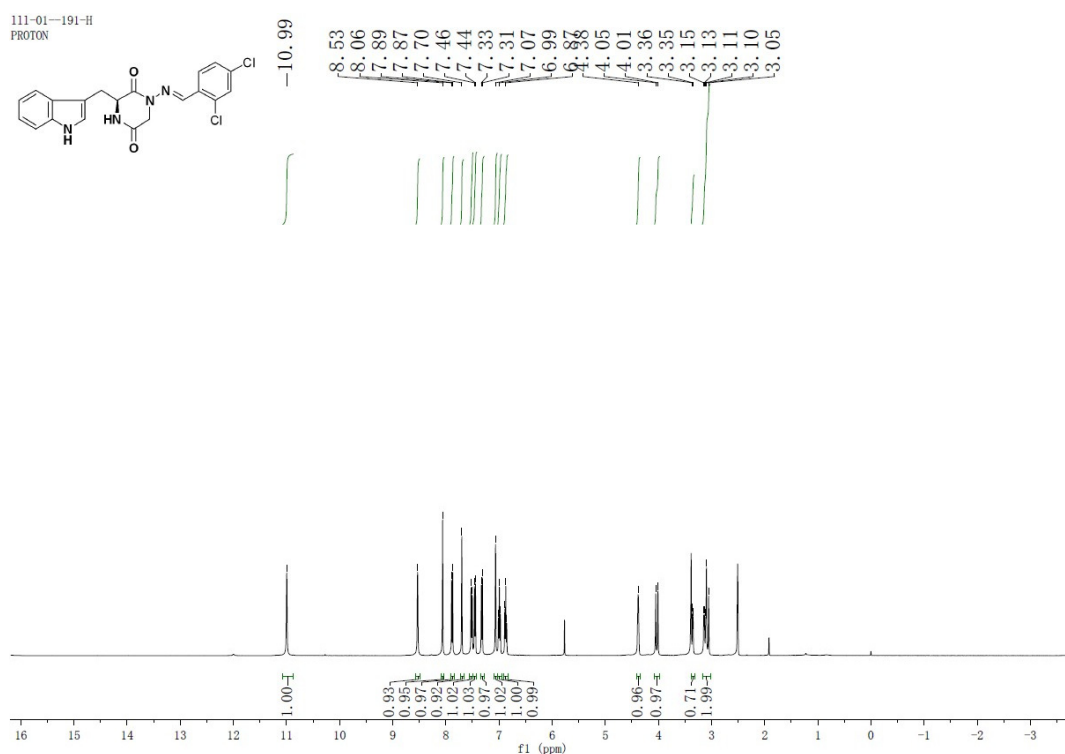


Figure S29 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 15

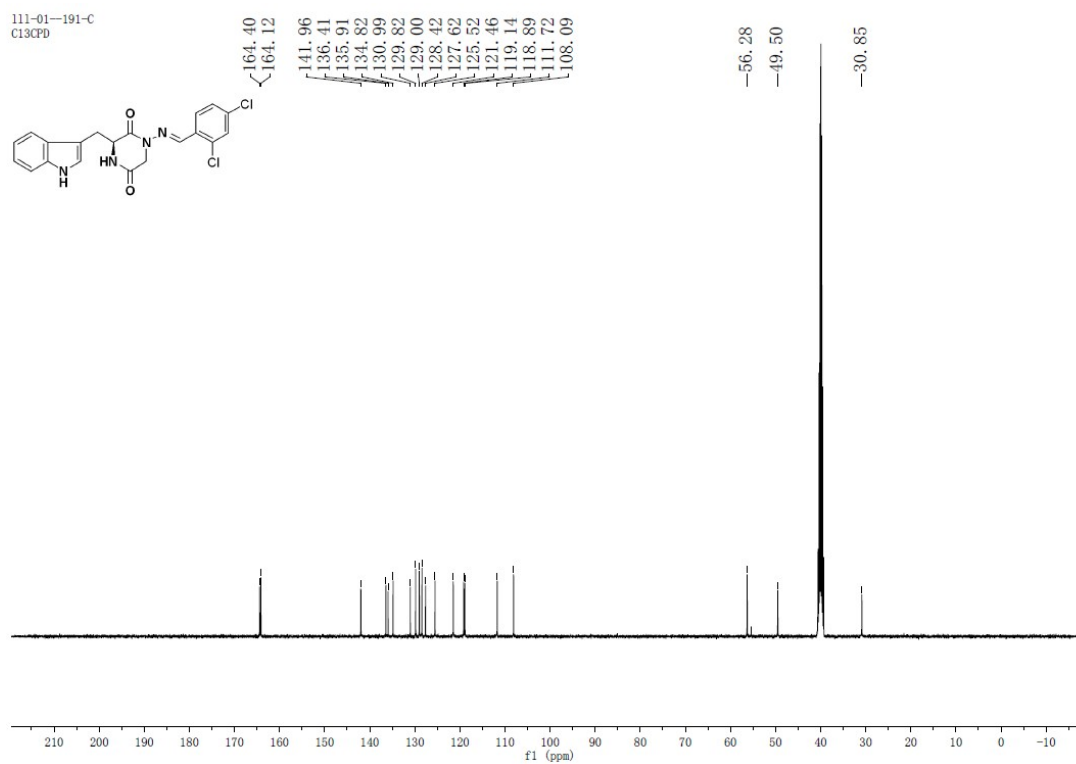
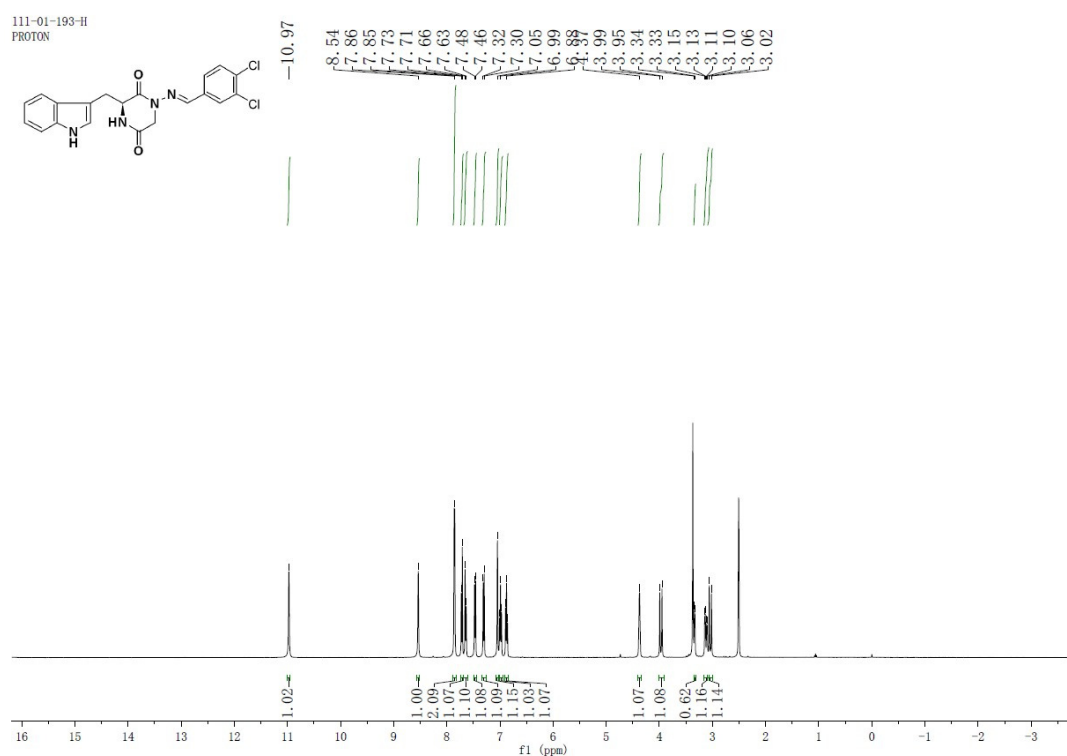
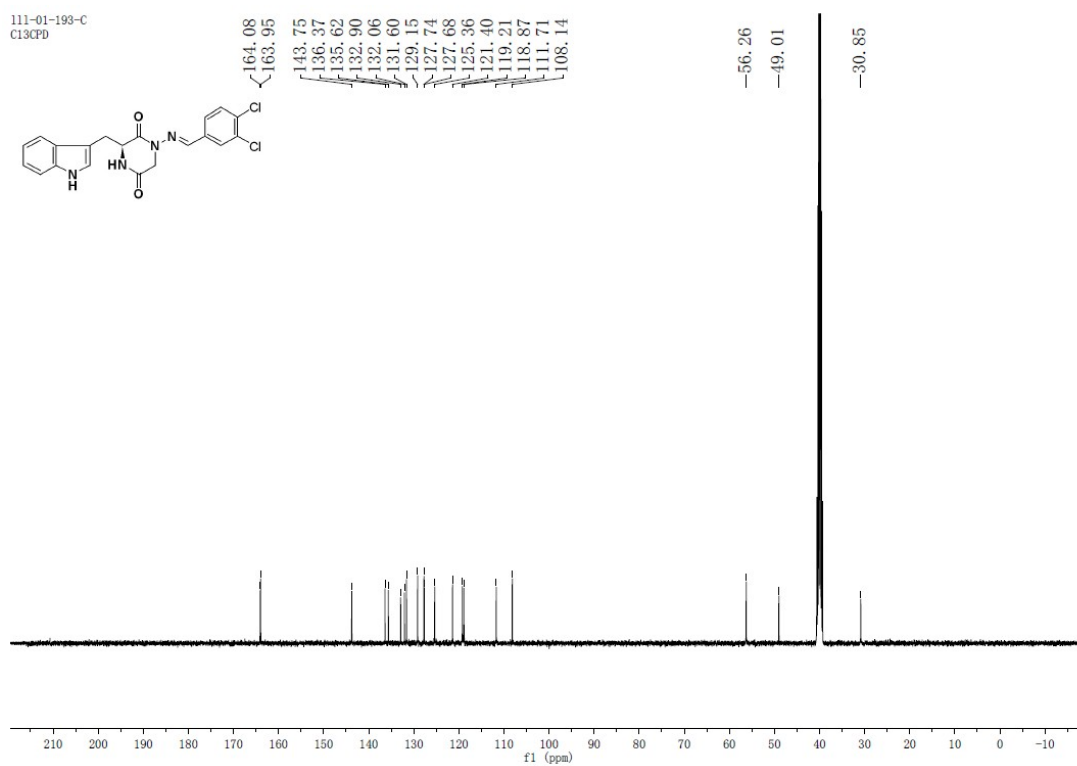


Figure S30 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 15

16

Figure S31 ¹H NMR (400 MHz, DMSO-*d*₆) of 16Figure S32 ¹³C NMR (100 MHz, DMSO-*d*₆) of 16

17

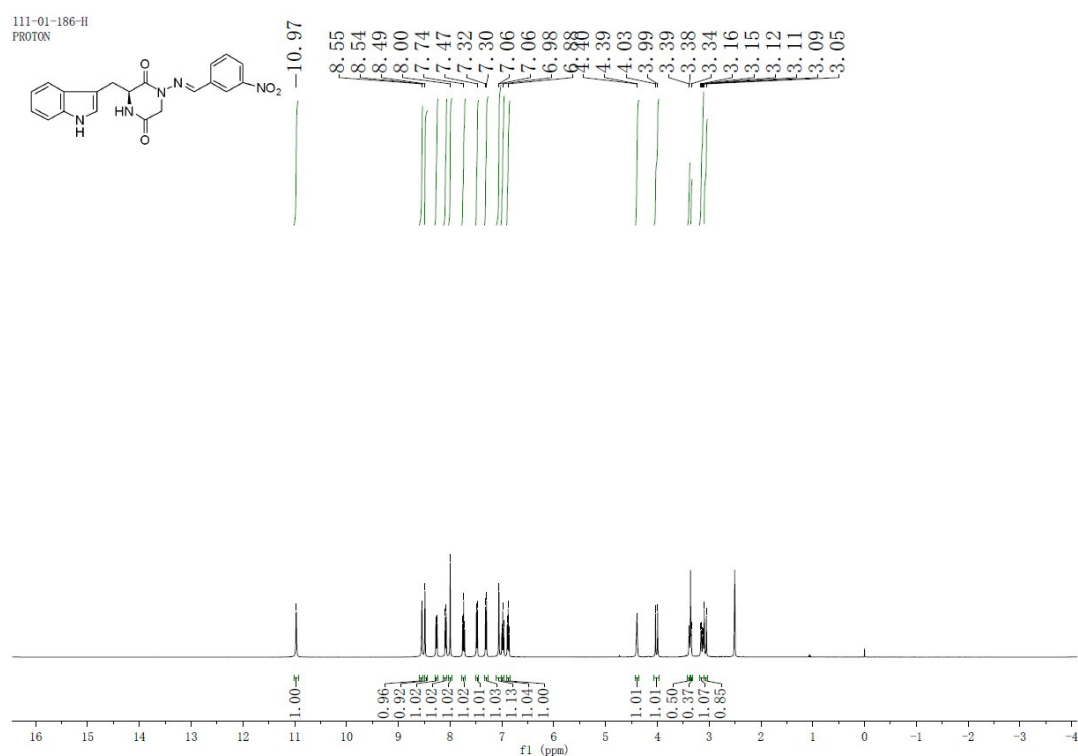


Figure S33 ¹H NMR (400 MHz, DMSO-*d*₆) of 17

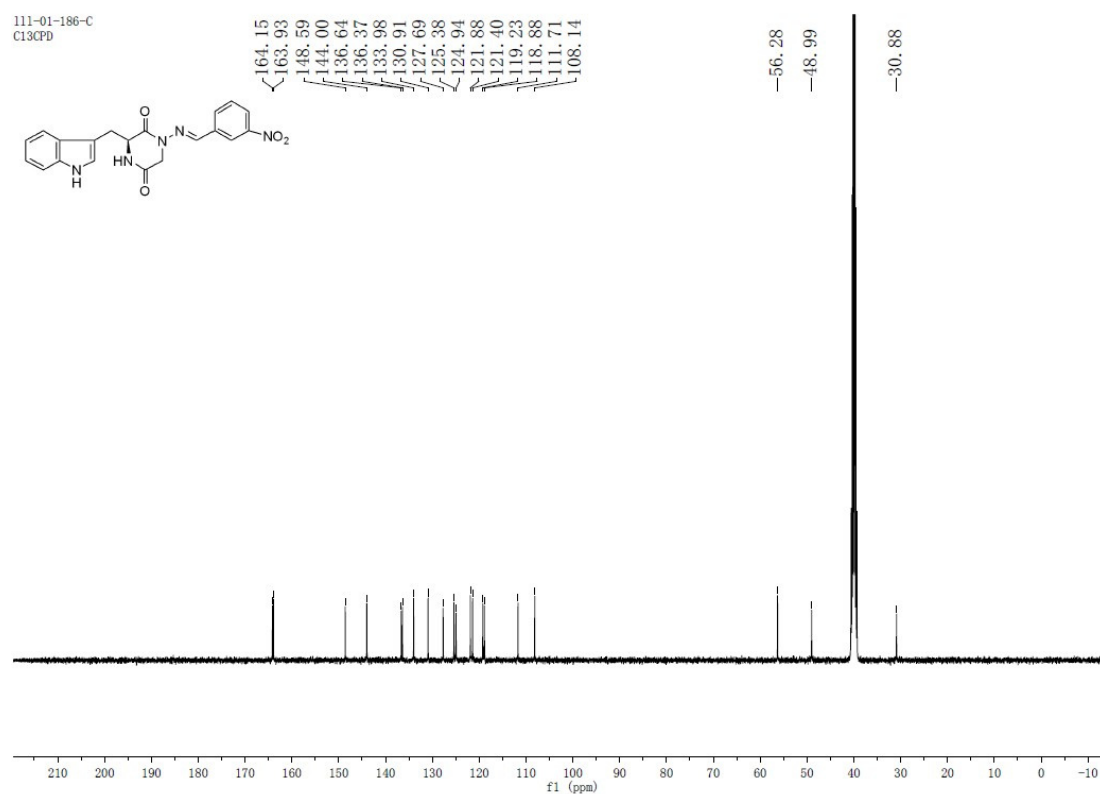


Figure S34 ¹³C NMR (100 MHz, DMSO-*d*₆) of 17

18

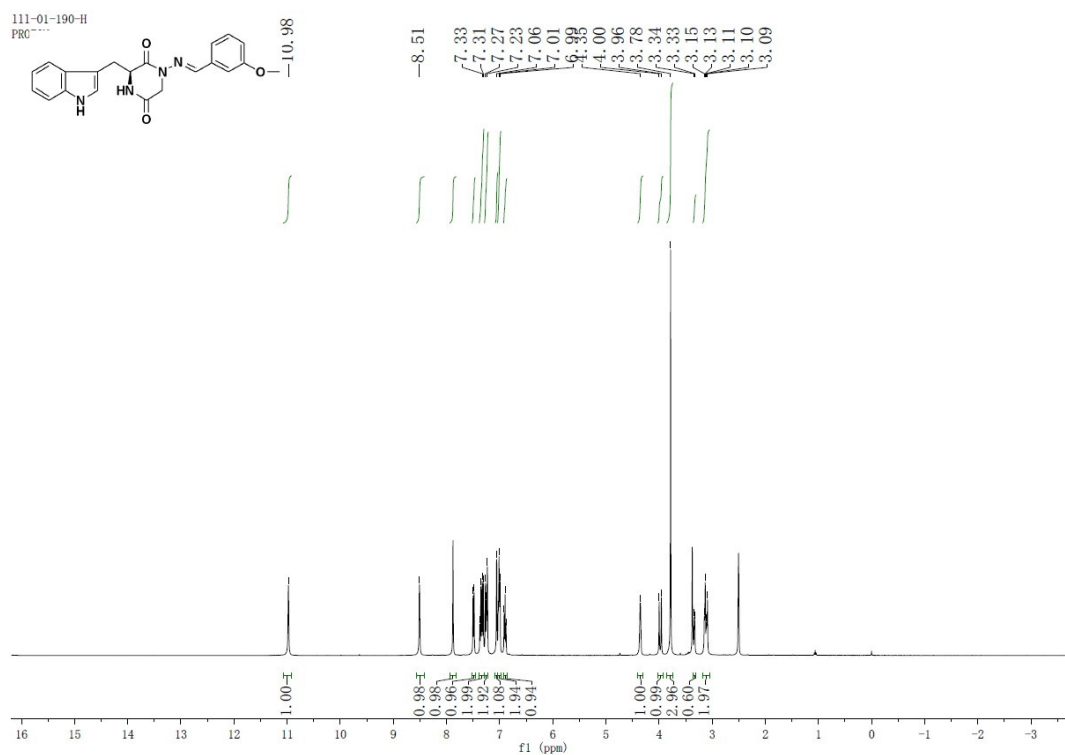


Figure S35 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 18

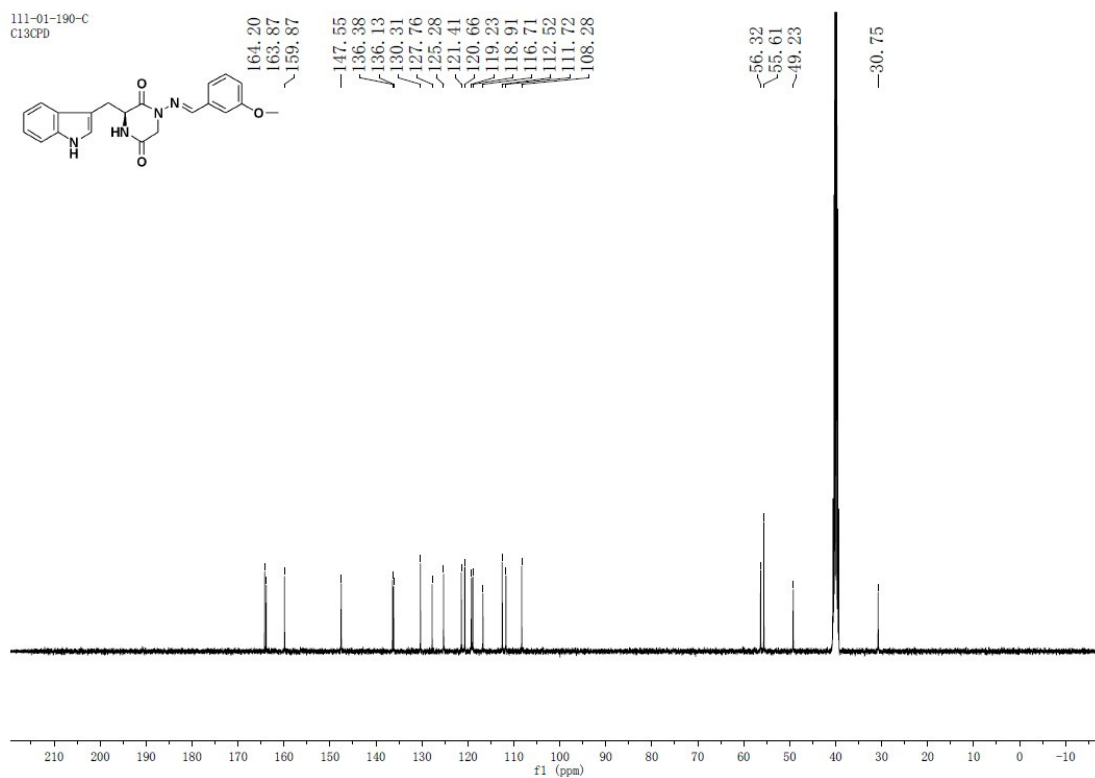


Figure S36 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 18

19

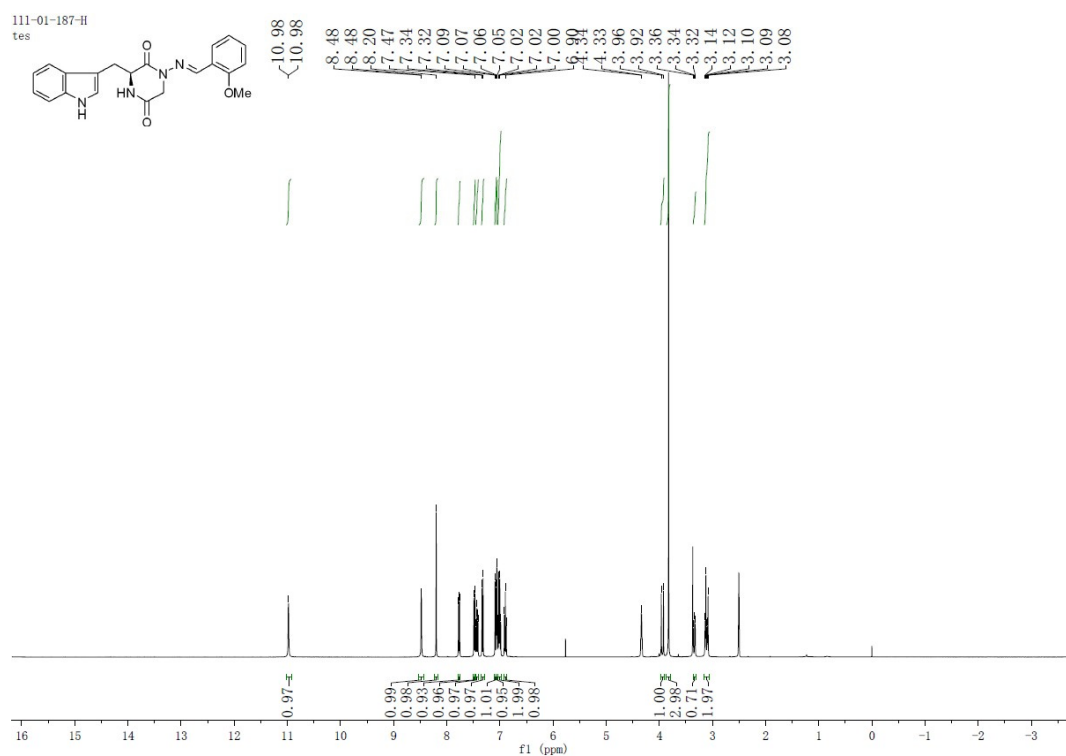


Figure S37 ¹H NMR (400 MHz, DMSO-*d*₆) of 19

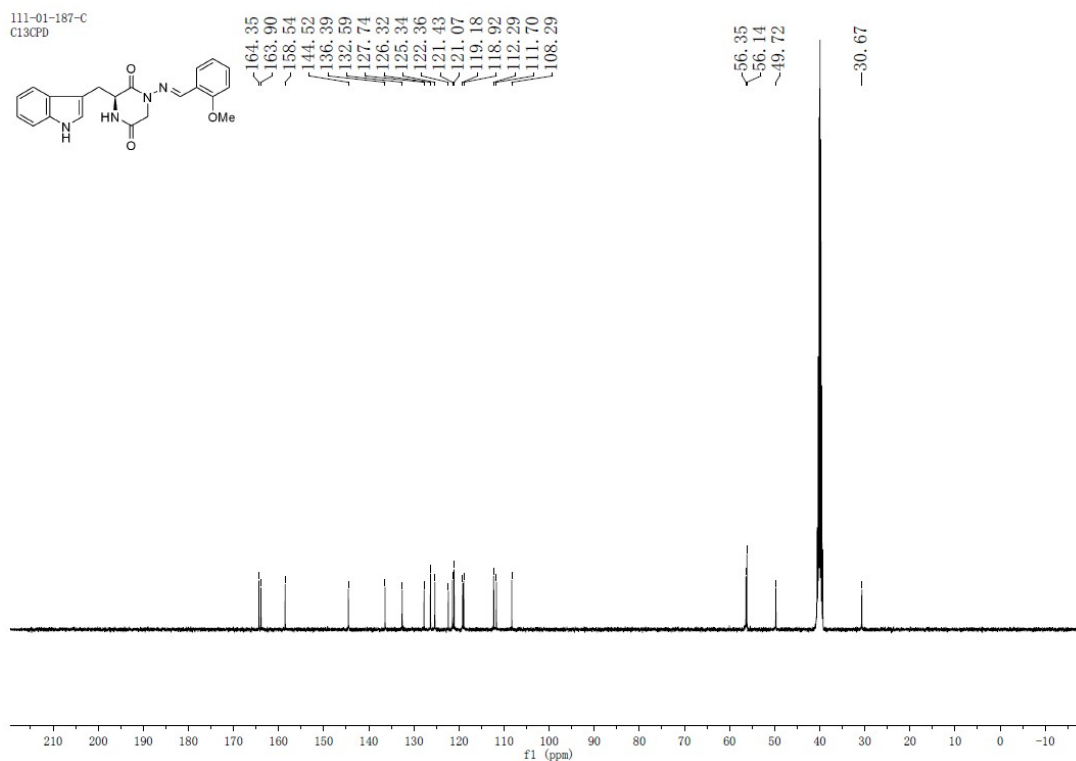


Figure S38 ¹³C NMR (100 MHz, DMSO-*d*₆) of 19

20

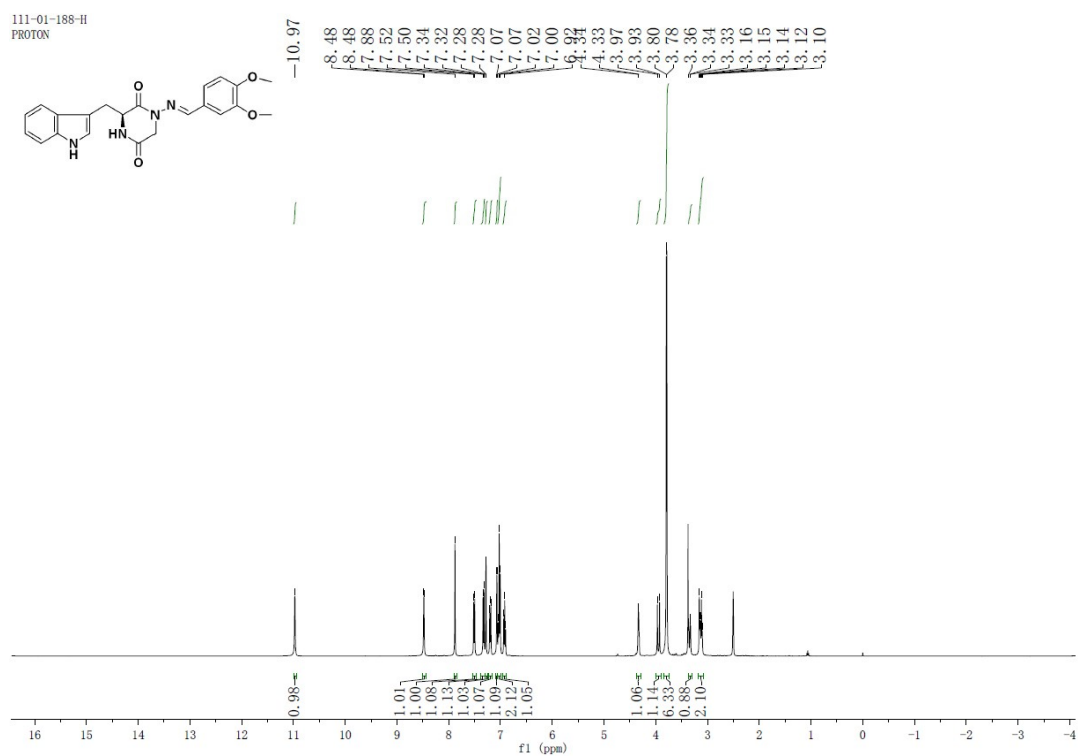


Figure S39 ¹H NMR (400 MHz, DMSO-*d*₆) of 20

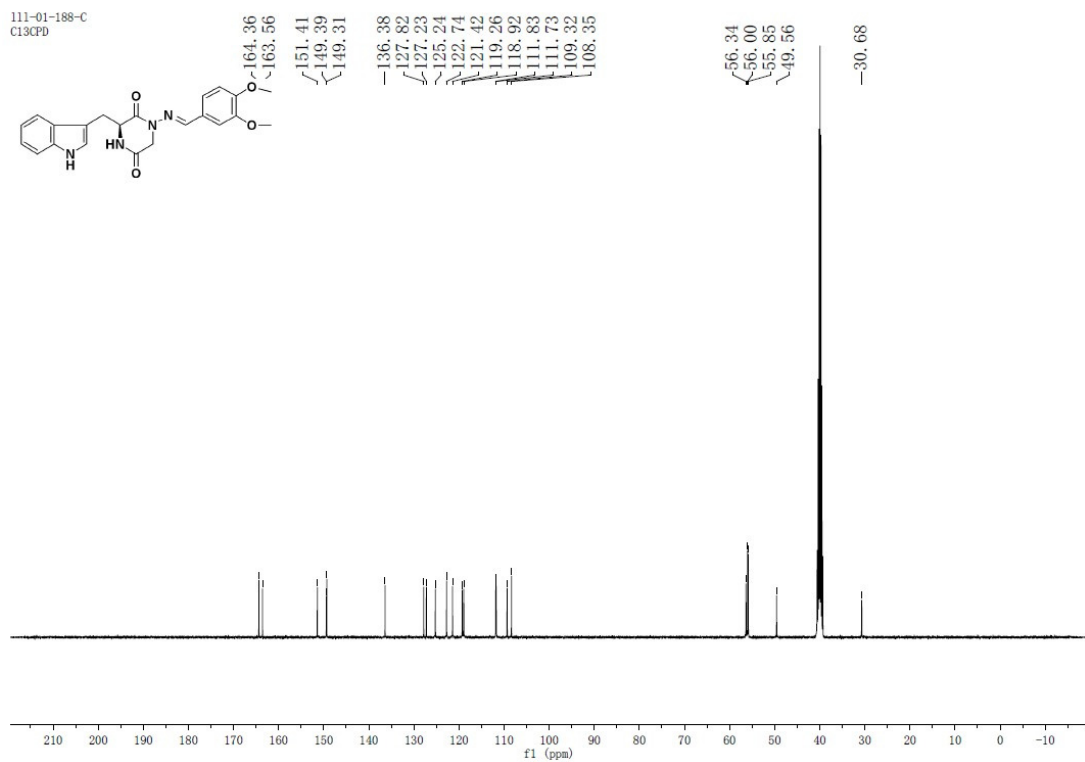


Figure S40 ¹³C NMR (100 MHz, DMSO-*d*₆) of 20

21

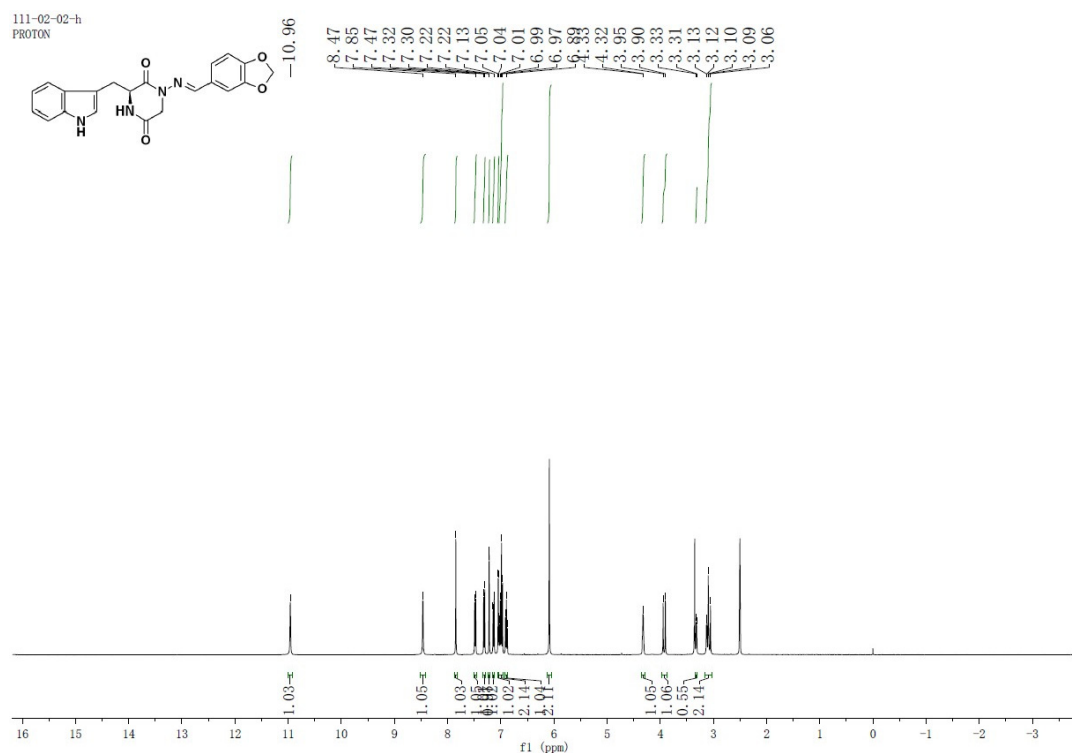


Figure S41 ¹H NMR (400 MHz, DMSO-*d*₆) of 21

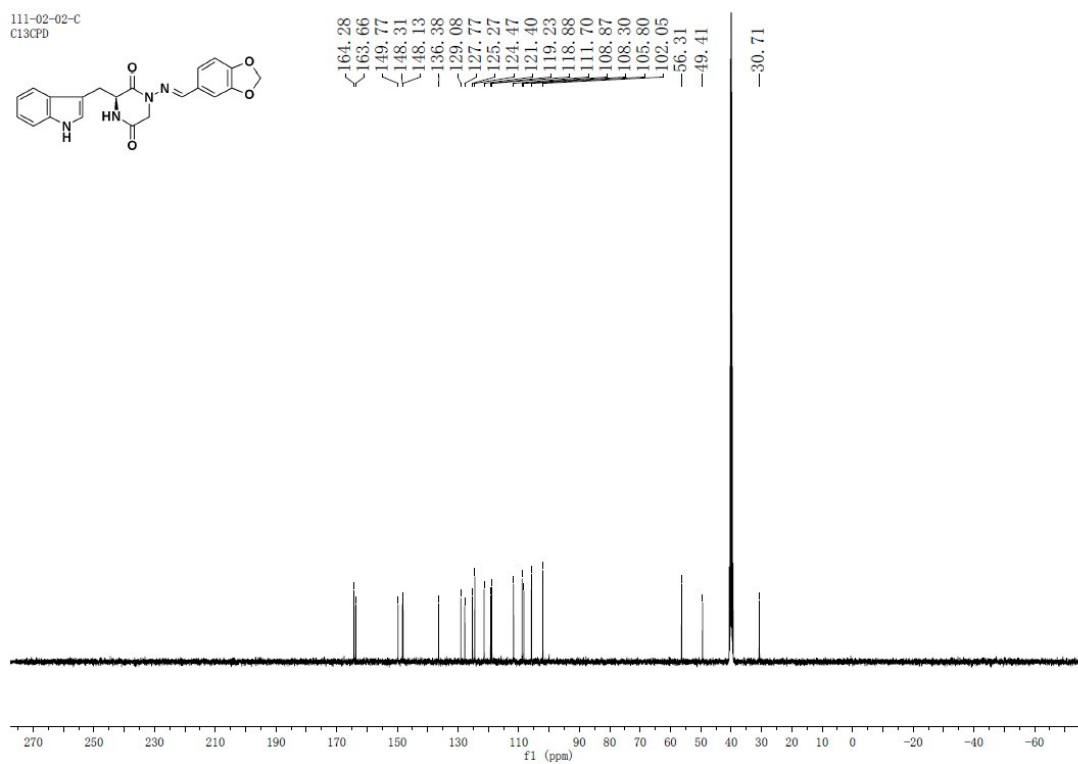


Figure S42 ¹³C NMR (100 MHz, DMSO-*d*₆) of 21

22

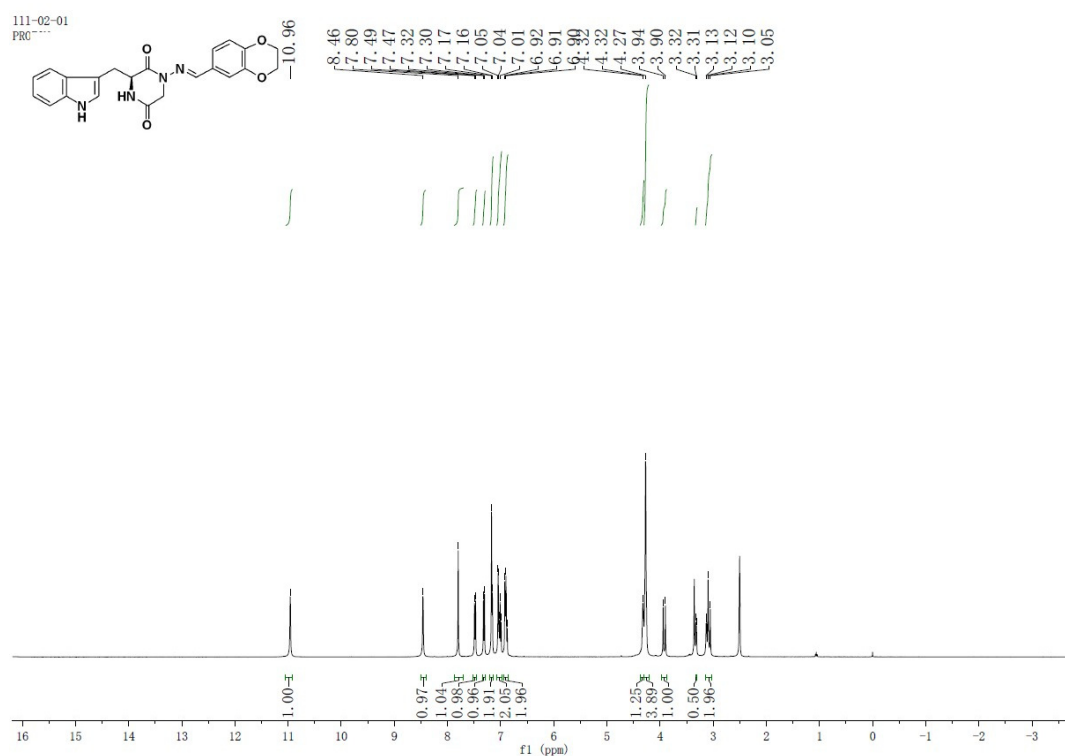


Figure S43 ¹H NMR (400 MHz, DMSO-*d*₆) of 22

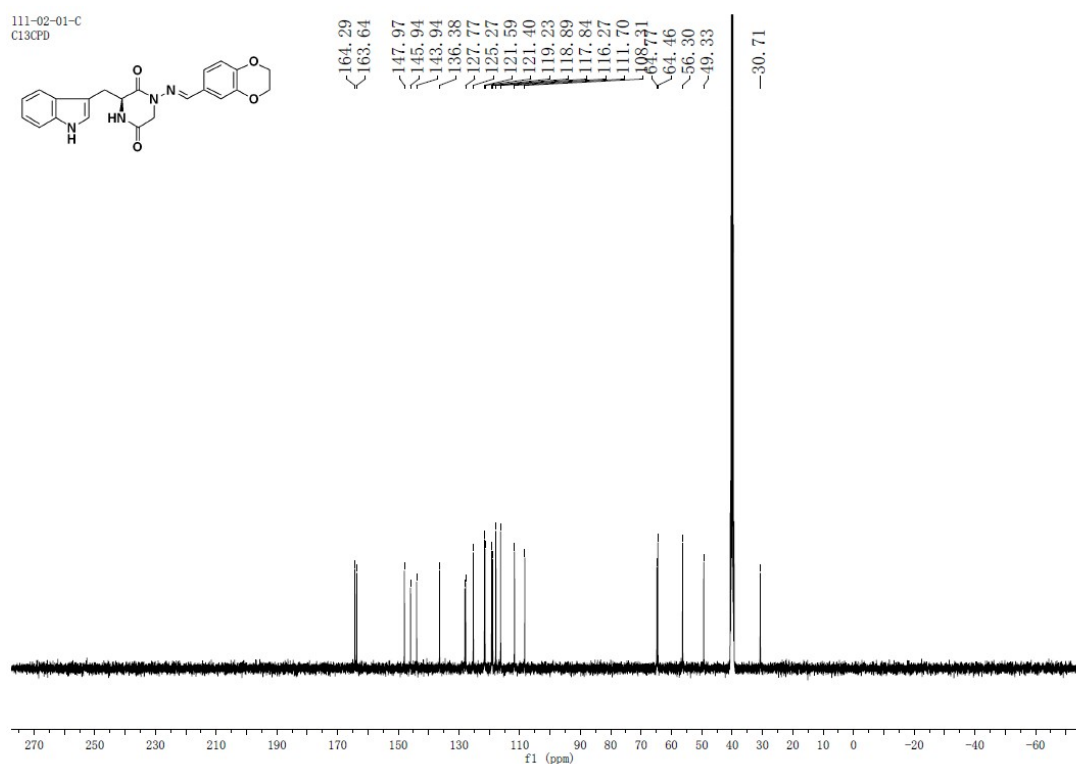
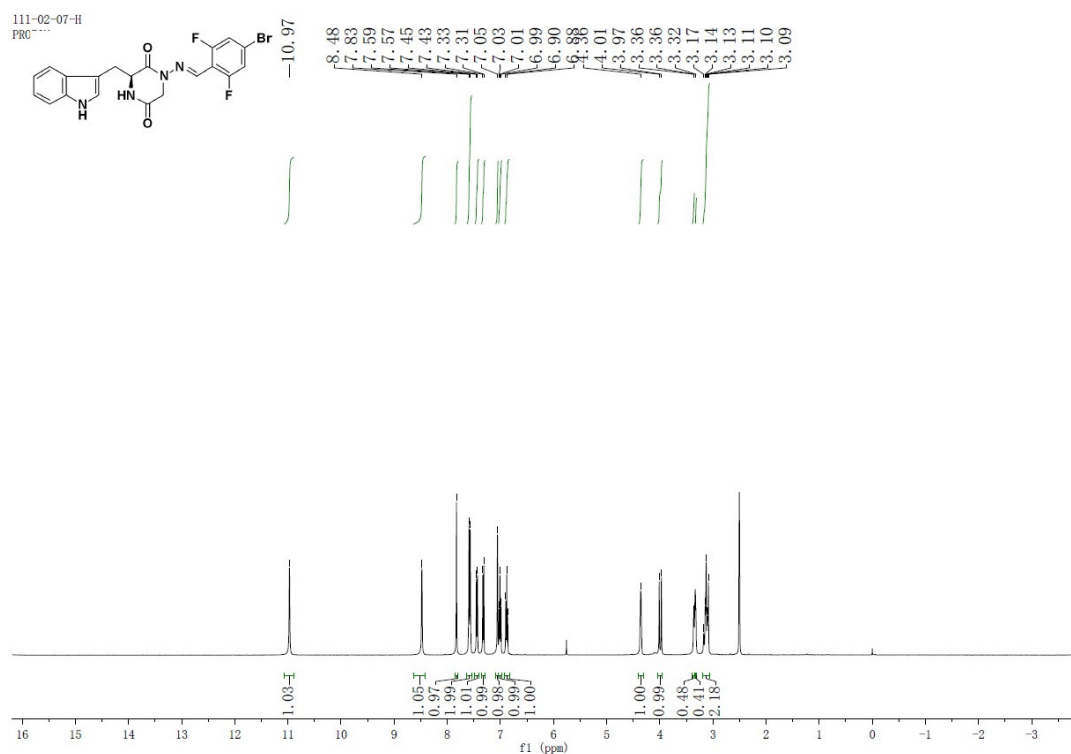
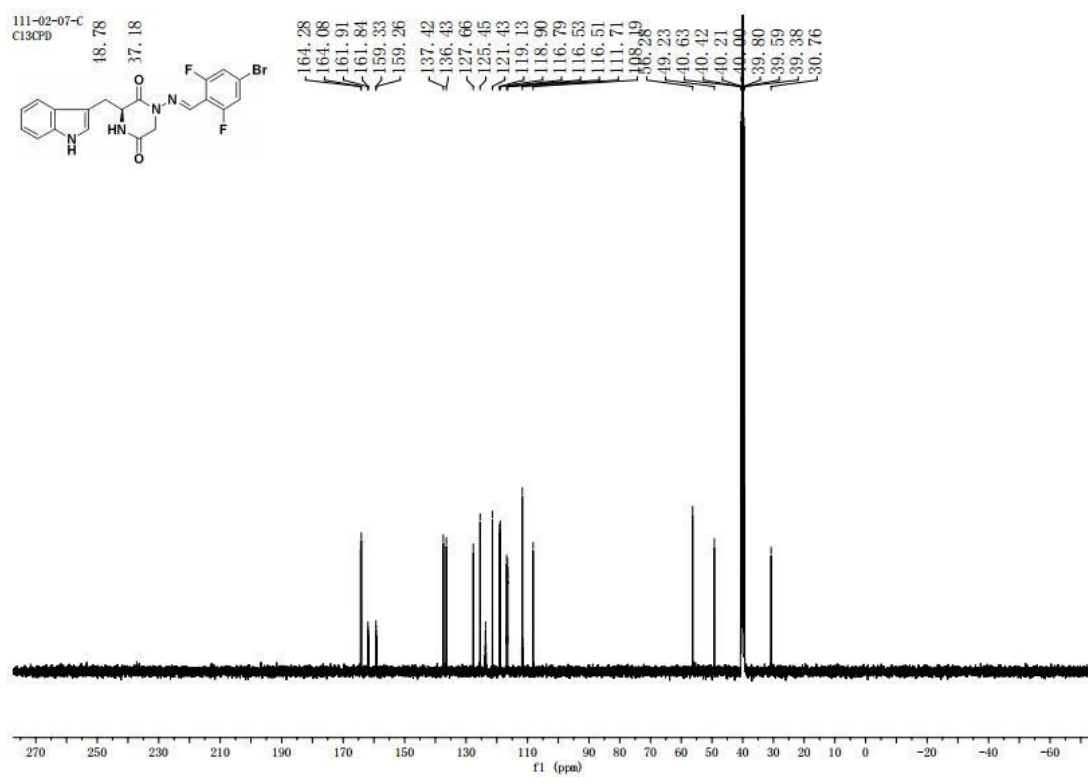


Figure S44 ¹³C NMR (100 MHz, DMSO-*d*₆) of 22

Figure S45 ¹H NMR (400 MHz, DMSO-*d*₆) of 23Figure S46 ¹³C NMR (100 MHz, DMSO-*d*₆) of 23



25

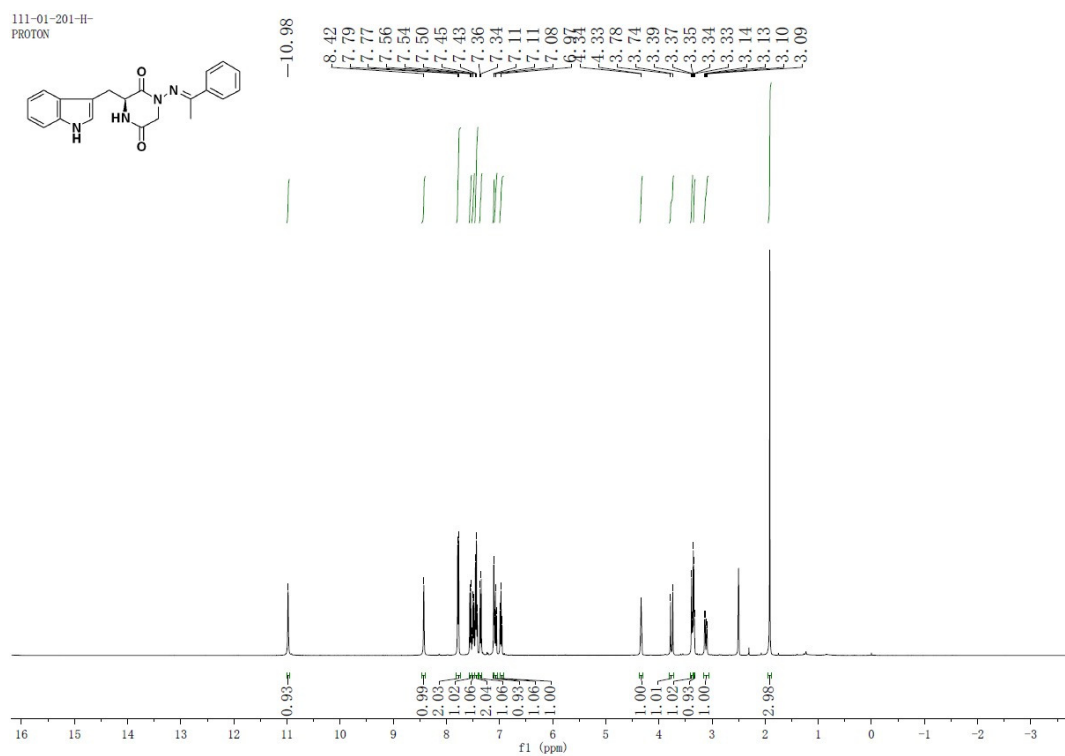


Figure S49 ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of 25

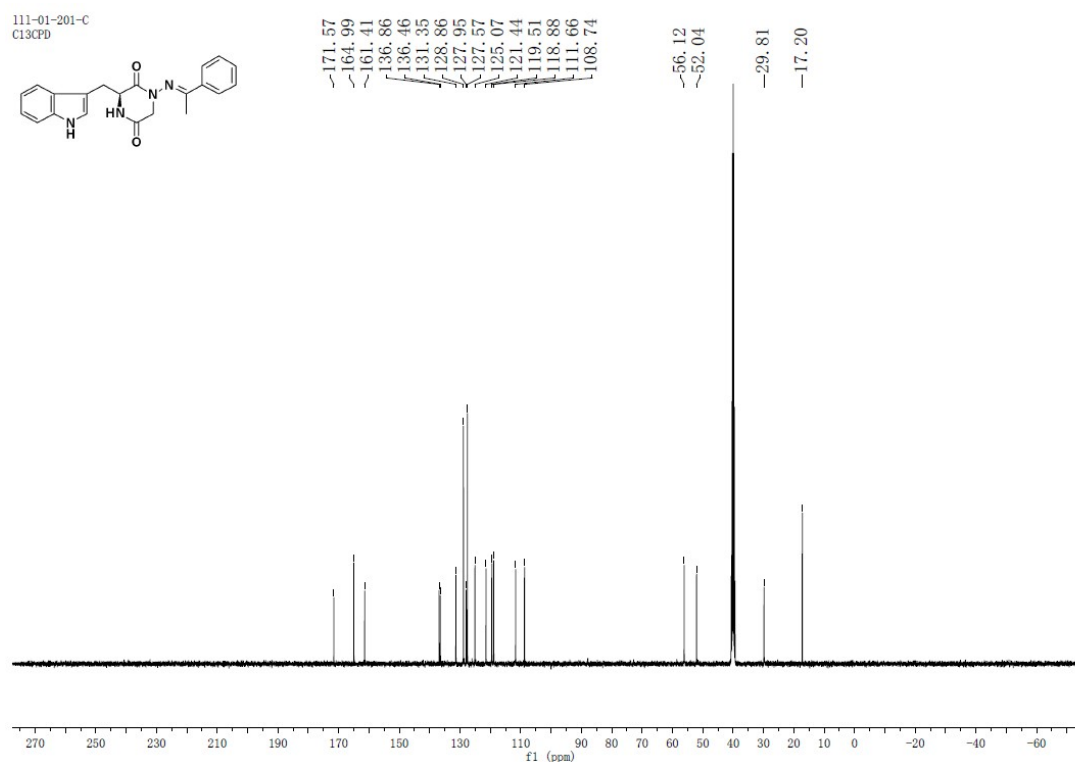
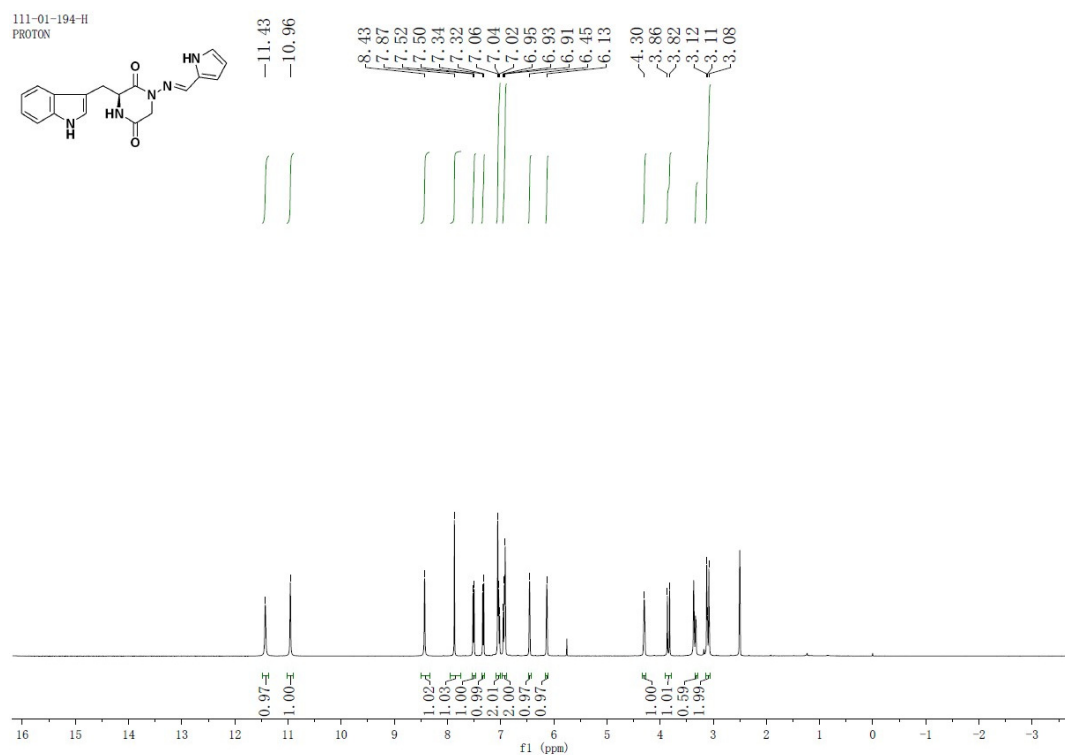
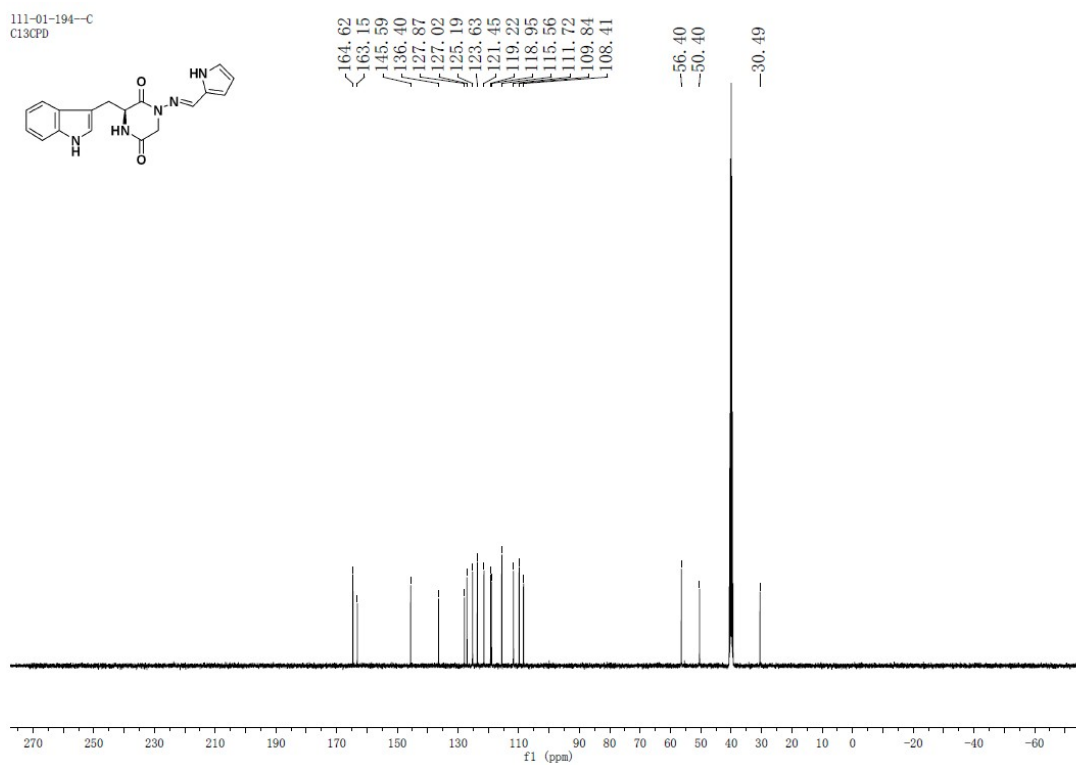


Figure S50 ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) of 25

Figure S51 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 26Figure S52 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 26

27

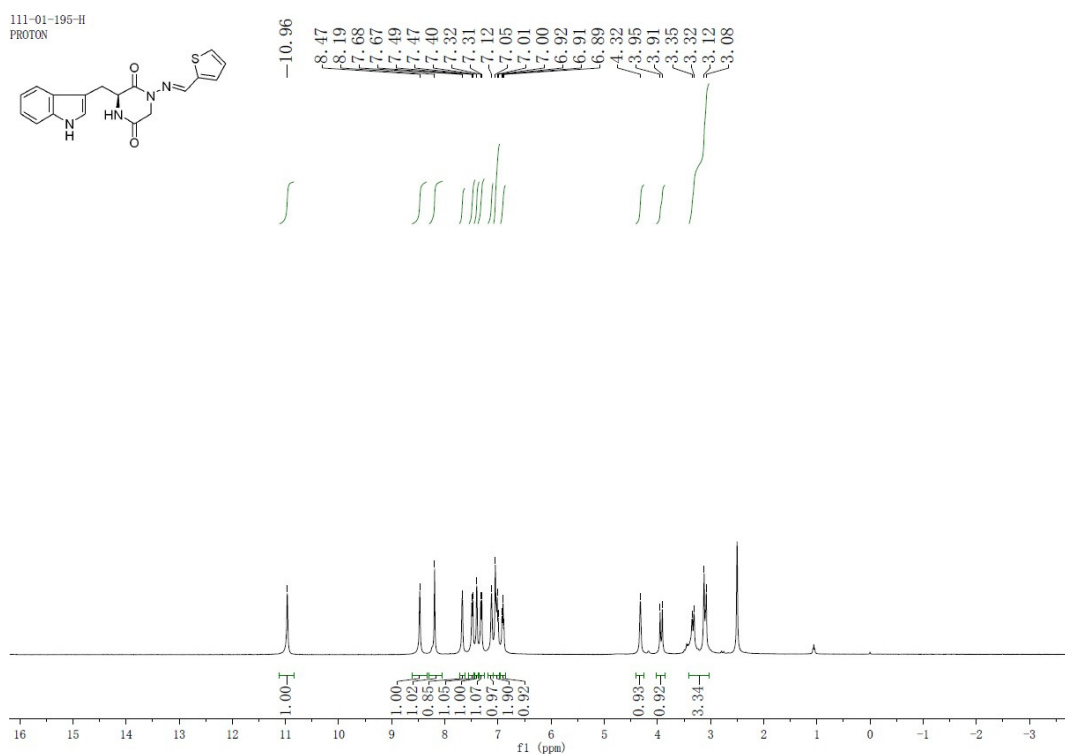


Figure S53 ¹H NMR (400 MHz, DMSO-*d*₆) of 27

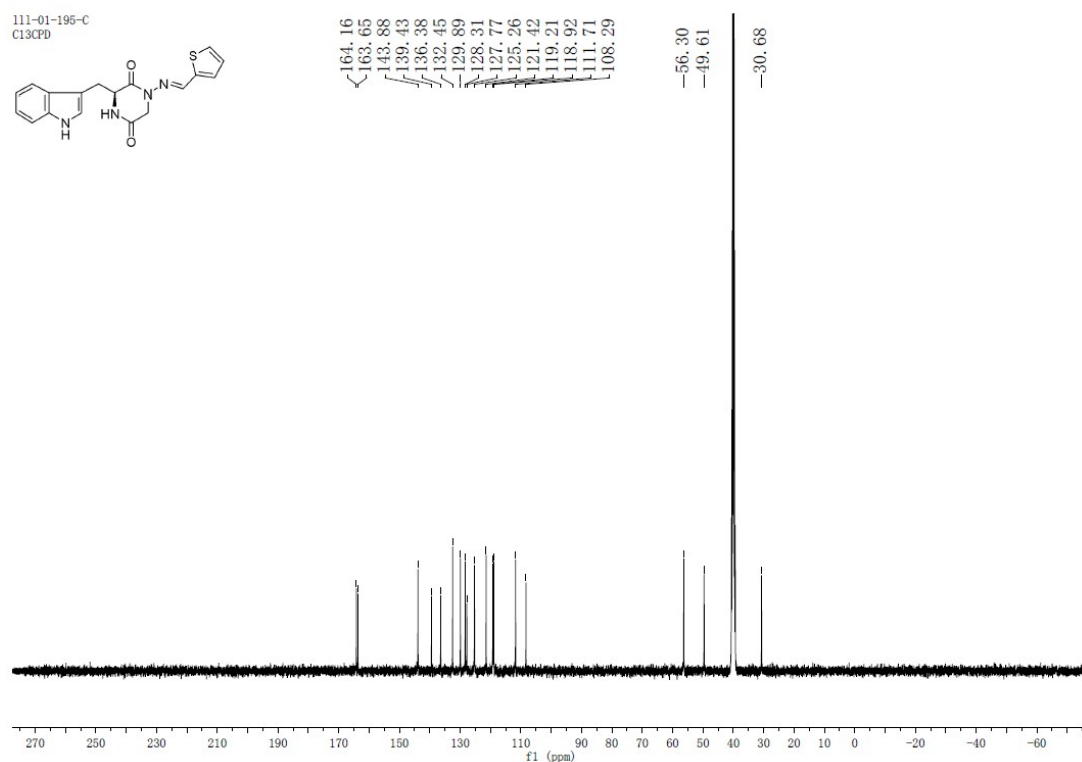


Figure S54 ¹³C NMR (100 MHz, DMSO-*d*₆) of 27

28

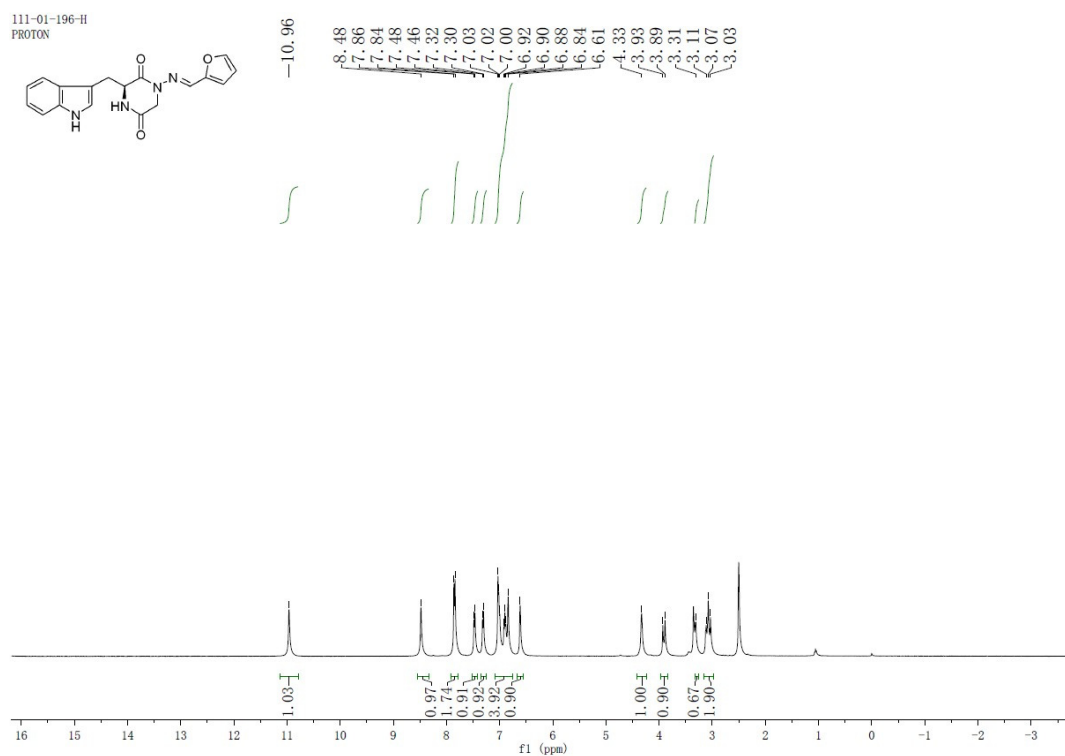


Figure S55 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of **28**

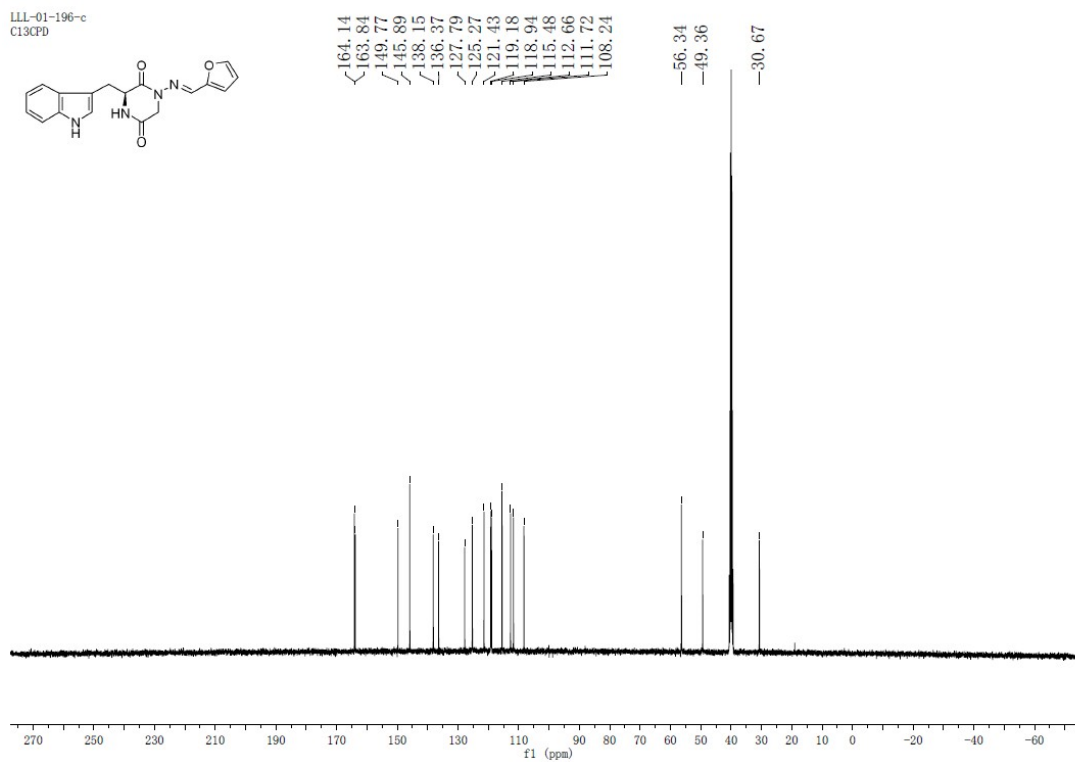


Figure S56 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of **28**

29

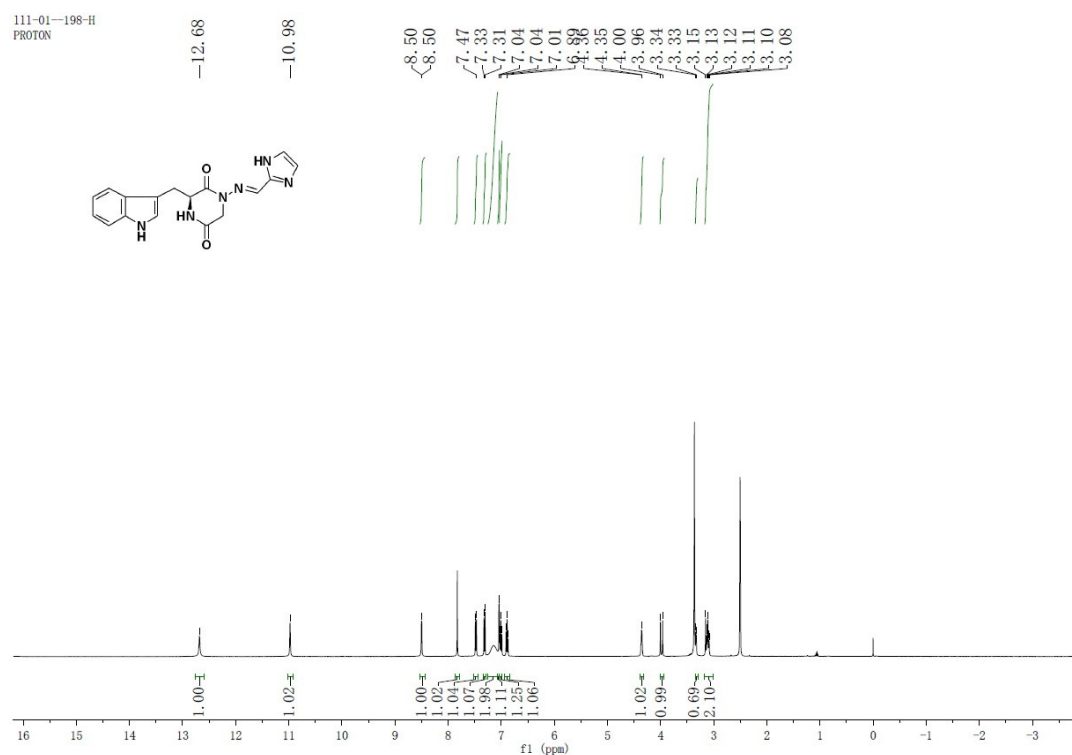


Figure S57 ¹H NMR (400 MHz, DMSO-*d*₆) of 29

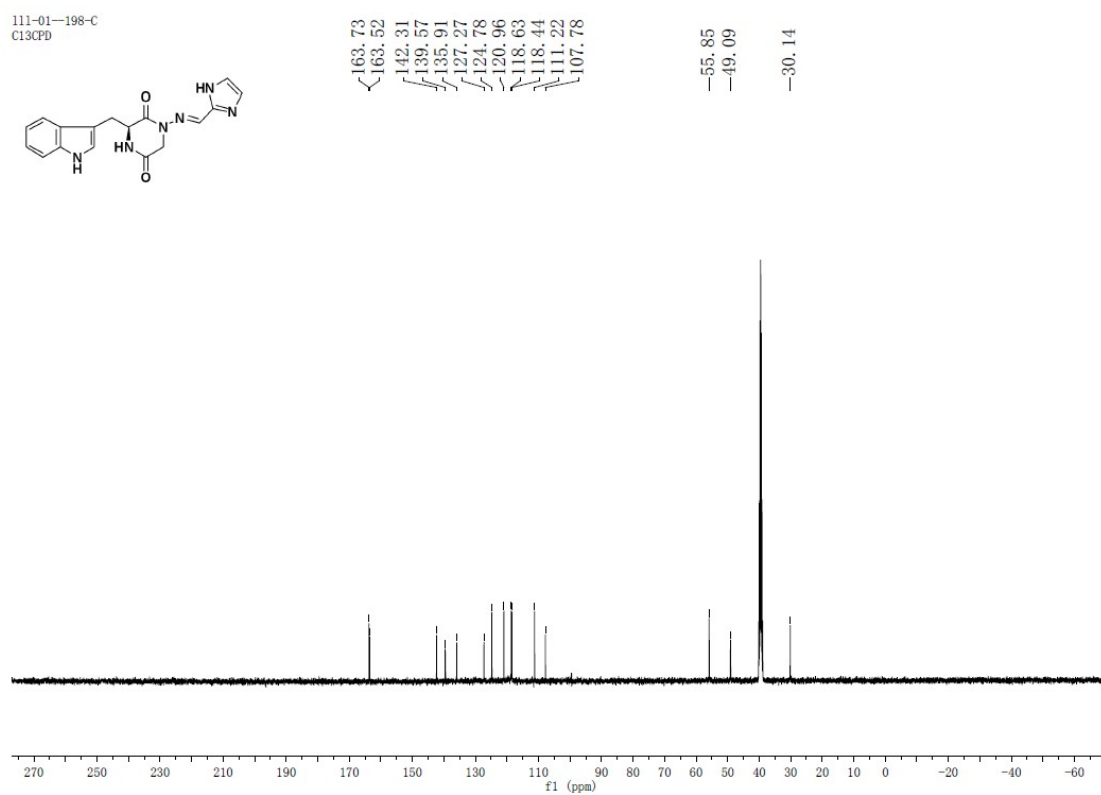


Figure S58 ¹³C NMR (100 MHz, DMSO-*d*₆) of 29

30

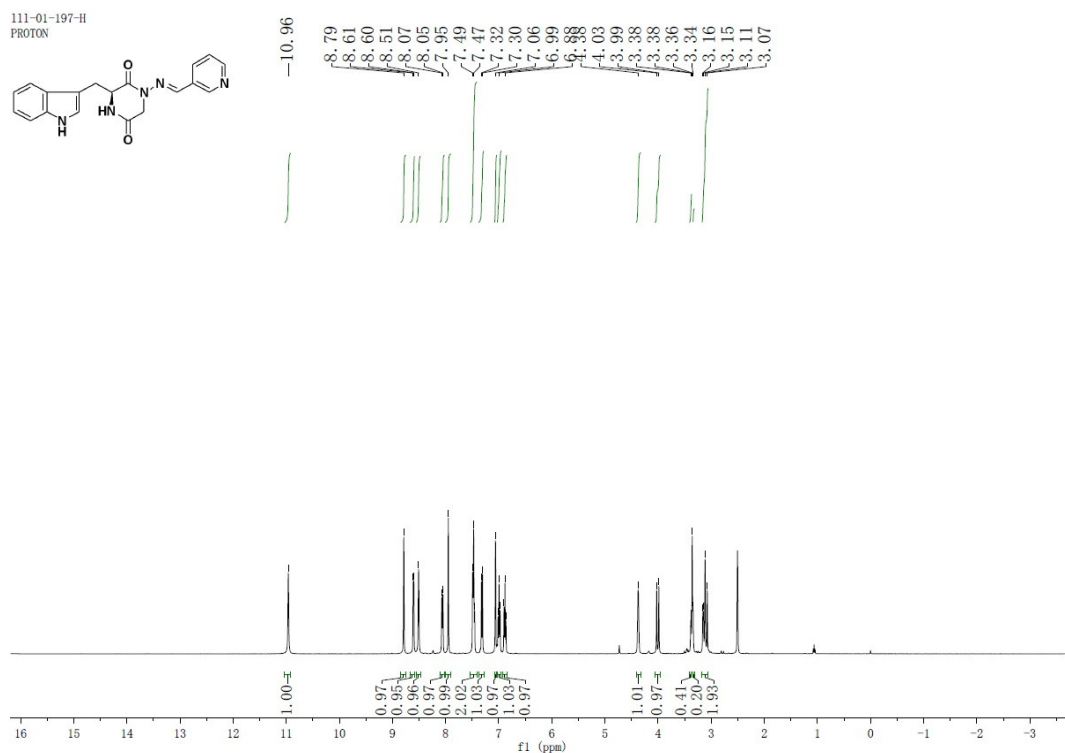


Figure S59 ¹H NMR (400 MHz, DMSO-*d*₆) of 30

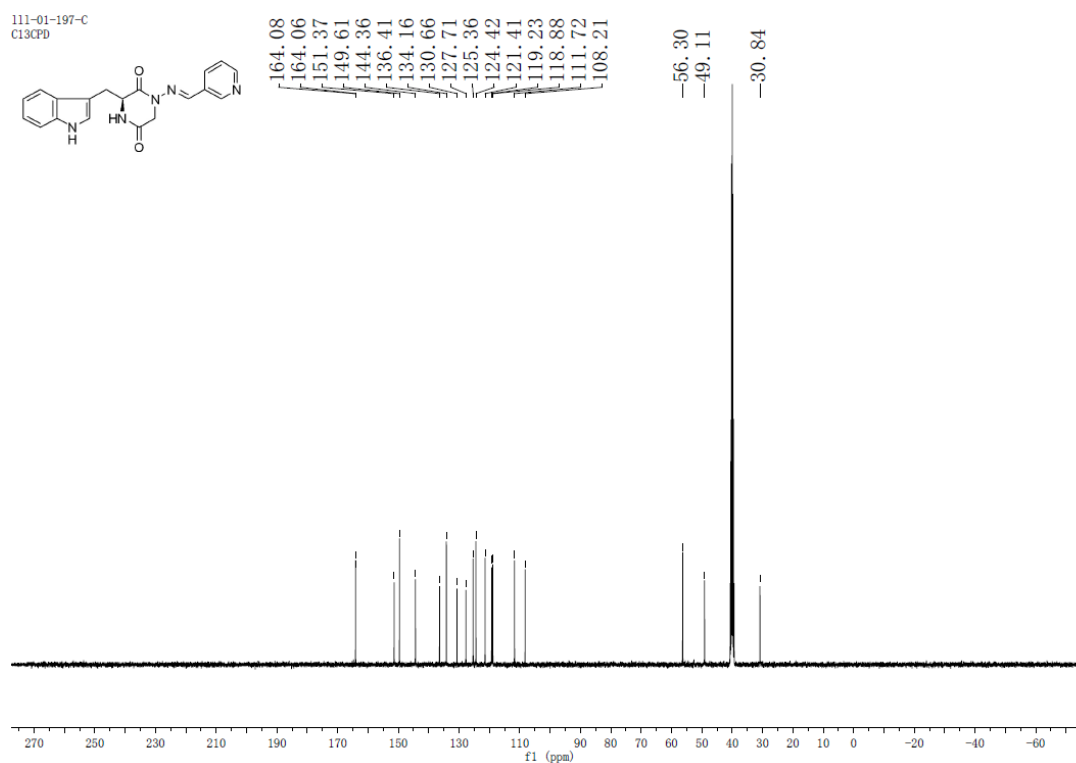


Figure S60 ¹³C NMR (100 MHz, DMSO-*d*₆) of 30

31

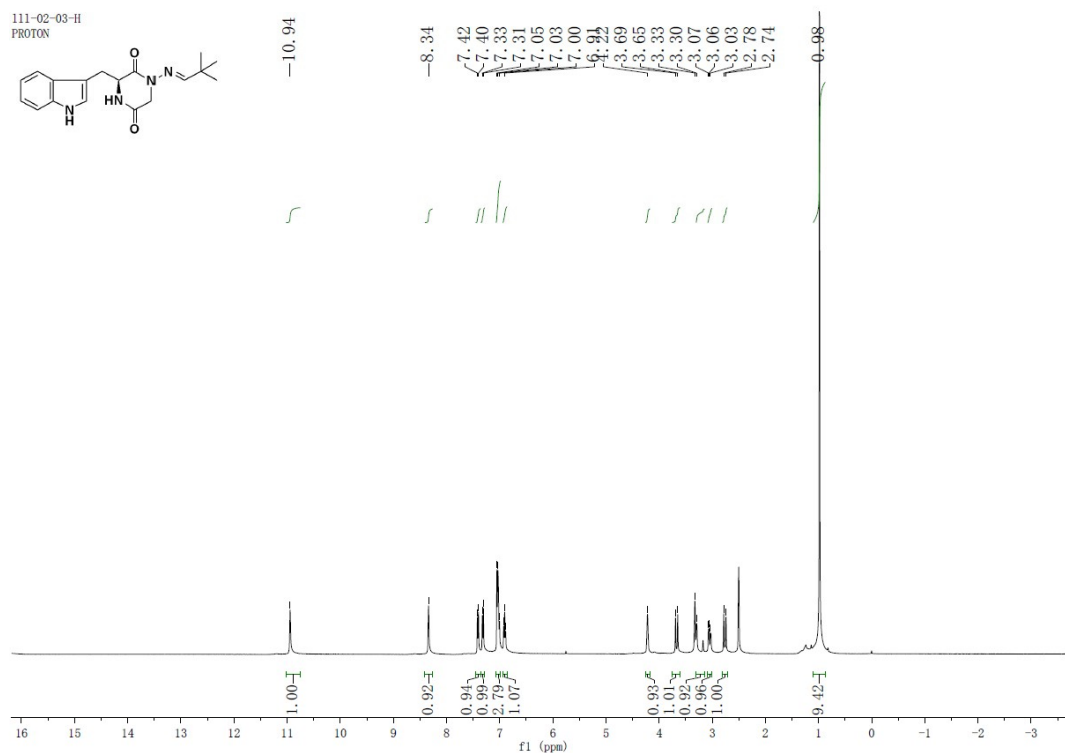


Figure S61 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 31

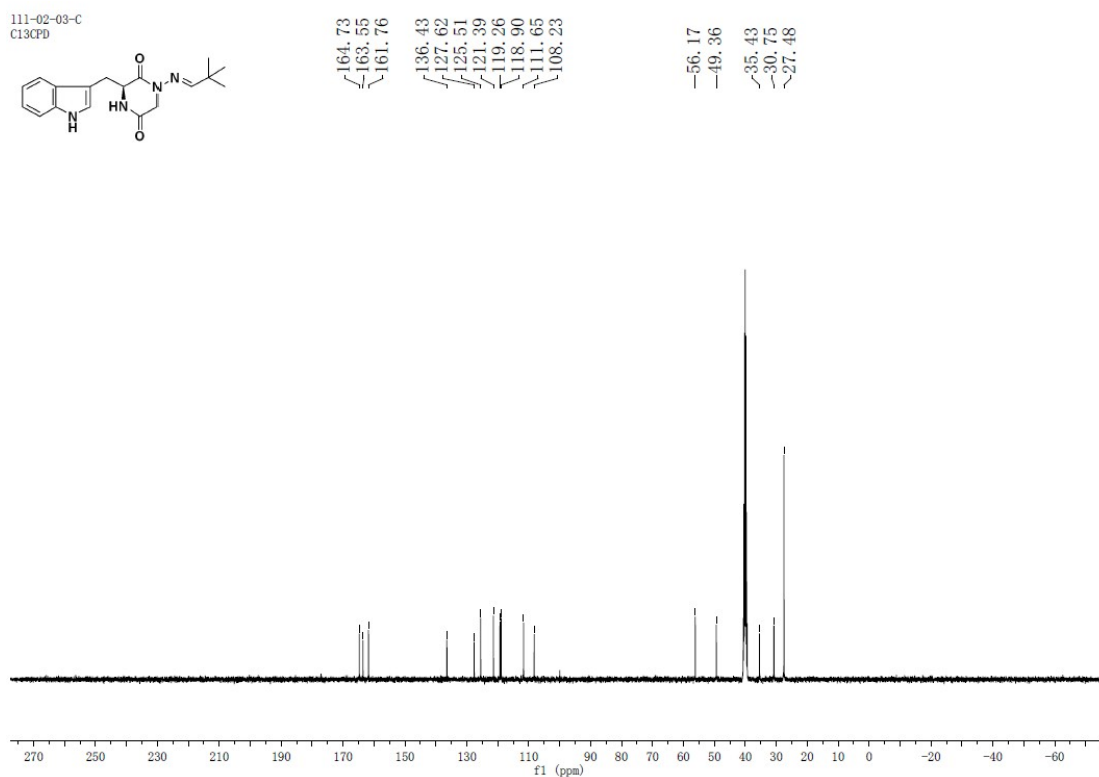


Figure S62 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 31

32

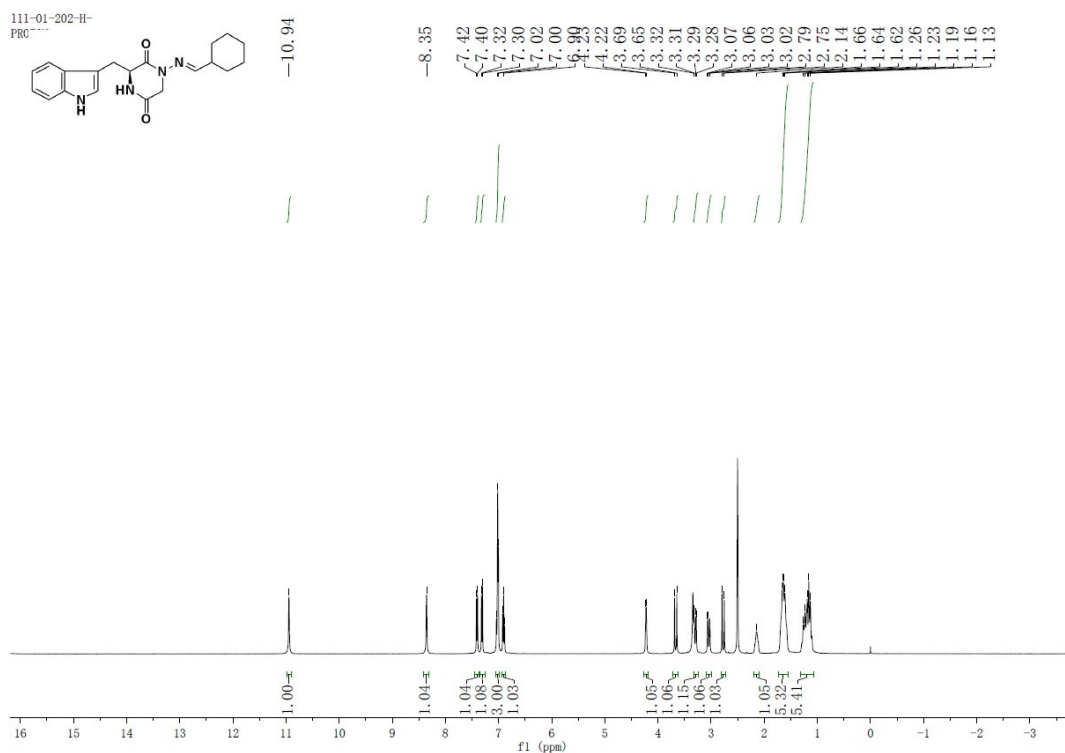


Figure S63 ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of 32

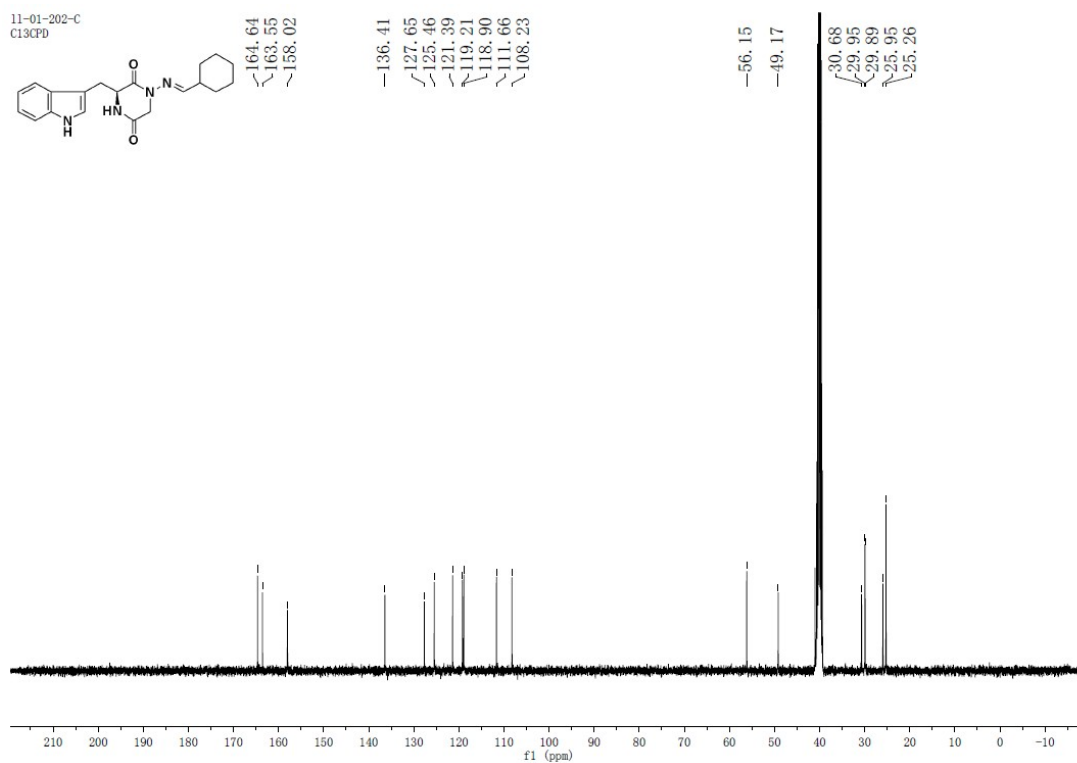


Figure S64 ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) of 32

Section S2: Detailed Bioassay Procedures for Anti-TMV Activities

Compound solution preparation: Test compound was dissolved within a suitable amount of *N,N*-dimethyl formate and diluted with water containing 0.1% TW-80 to make a concentration of 500 µg/mL, and the aqueous solution was diluted to 100 µg/mL.

Antiviral Activity of Compounds against TMV *in Vitro*. Fresh leaf of the 5-6 growth stage of tobacco (*N. tabacum L.*) inoculated by the juice-leaf rubbing method (concentration of TMV was 6×10^{-3} µg/mL) was cut into halves along the main vein. The halves were immersed into the solution of test compounds and solvent (double-distilled water containing 0.1% TW-80) for 30 min, respectively, and then cultured at 25 °C for 72 h. The local lesion numbers were then counted. Each compound was replicated at least 3 times.

Protective Effect of Compounds against TMV *in Vivo*. The compound solution was smeared on the growing *N. tabacum L.* leaves of the same ages. Another pot was smeared with solvent for control. After 12 h, the leaves were inoculated by the juice-leaf rubbing method and then washed with water. The local lesion numbers appearing 3-4 days after inoculation were counted. There were three replicates for each compound.

Inactivation Effect of Compounds against TMV *in Vivo*. The virus was inhibited by mixing with the compound solution at the same volume for 30 min. The mixture was then inoculated on the growing leaves of the same ages, whereas another pot was inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3–4 days after inoculation. There were three replicates for each compound.

Curative Effect of Compounds against TMV *in Vivo*. TMV (concentration of 6.0×10^{-3} mg/mL) was inoculated on the growing leaves of *N. tabacum L.* of the same ages. Then, the leaves were washed with water and dried. The compound solution was smeared on the leaves, whereas another pot was smeared with solvent for control. The local lesion numbers were then counted and recorded 3–4 days after inoculation. There were three replicates for each compound.

The *in vitro* and *in vivo* inhibition rates of the compound were then calculated according to the following formula (“av” means average, and controls were not treated with compound):

$$\text{inhibition rate (\%)} = [(\text{av local lesion number of control} - \text{av local lesion number of drugtreated}) / \text{av local lesion number of control}] \times 100\%.$$

Section S3: Detailed Bioassay Procedures for the Fungicidal Activities

The compounds were evaluated in mycelial growth tests in artificial media against 14 plant pathogens at rate of 50 mg L⁻¹.

Test compound was dissolved within a suitable amount of acetone and diluted with water containing 0.1% TW-80 to the concentration of 500 mg L⁻¹. To each petri dish was added 1 mL such solution and 9 mL culture medium to make a 50 mg L⁻¹ of medicated tablet, whereas to another petri dish was added 1 mL sterilized water and 9 mL culture medium as blank control. A diameter of 4 mm of hyphae was cut by a whole puncher along the hyphae for bacteria to the outer plate and moved to the medicated tablet. Each treatment was performed three times. The dishes were stored in controlled environment cabinets (24 ± 1 °C) for 48 h, after which the diameter of mycelia growth was investigated and percentage inhibition was calculated. There were three replicates for each compound.

Percentage inhibition (%) = (averaged diameter of mycelia in blank controls - averaged diameter of mycelia in medicated tablets) / averaged diameter of mycelia in blank controls.

Section S4: Stomach Toxicity against Lepidopteran Pests

The stomach toxicities of the title compounds against *P. xylostella*, *M. separata*, *H. armigera*, and *P. nubilalis* were tested according to the leaf-dip method using the reported procedure. Leaf disks (about 5 cm) were cut from fresh corn leaves and then were dipped into the test solution for 3–5 s. After air drying, the treated leaf disks were placed individually into a glass-surface vessel (7 cm). Each dried treated leaf disk was infested with 10 third-instar *P. xylostella*, or *M. separata*, or *H. armigera*, or *P. nubilalis*. Percentage mortalities were evaluated 4 days after treatment. Leaves treated with acetone were provided as controls. Each treatment was performed three times.

Section S5: Larvicidal Activities against *Culex Pipiens Pallens*.

10 fourth-instar mosquito larvae were put into the 10 mL of the test solution. Percentage mortalities were evaluated 8 days after treatment. Evaluations were based on a percentage scale of 0–100, where 0 equals no activity and 100 equals total kill. Each treatment was performed three times. Error of the experiments was about 5%.

Section S6: Acaricidal Activity against Larvae of *Tetranychus*

Cinnabarinus

Each test compound was dissolved in acetone, and the acetone solution was diluted with distilled water to the desired concentration (200–0.05 mg/L). Fresh sieva bean leaves infested with mite larvae (60–100 larvae per leaf) were dipped into the test solution and swirled around for 3 s and then placed in a tube (10 cm inner diameter) lined with a piece of filter paper. The bioactivities were evaluated 4 days after treatment and are reported as mortality percentages, where 0% indicates no activity and 100% indicates a total kill.