

Supplementary Materials

Discovery of Hyrtinadine A and Its Derivatives as Novel Antiviral and Anti-phytopathogenic-fungus Agents

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Section S1: Detailed bio-assay procedures

Phytotoxic activity. The phytotoxic activity test was carried out in a climate chamber at a temperature of 25 °C. The growing 3–5 leaf stage tobaccos (*Nicotiana tabacum* var *Xanthi nc*) were selected. The compound solution (100 µg/mL or 500 µg/mL) was sprayed on the leaves and then tested the plant height and weight changes after 0, 3, 7 and 10 days respectively. There are three replicates for each compound.¹

Antiviral Biological Assay.²

Purification of Tobacco Mosaic Virus.

Using Gooding's method³, the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000 g, treated with PEG twice, and centrifuged again. The whole experiment was processed at 4 °C. Absorbance value was estimated at 260 nm by ultraviolet spectrophotometer.

$$\text{Virus concn} = (A_{260} \times \text{dilution ratio}) E_{1\text{cm}}^{0.1\%, 260\text{nm}}$$

Protective Effect of Compounds against TMV in Vivo.

The compound solution was smeared on growing *Nicotiana. tabacum* L. leaves (at least 3 leaves) of the same age. In another pot, the leaves were smeared with the solvent as a control. After 12 h, the leaves were inoculated with TMV with the juice-leaf rubbing method and then washed with water. The total local lesion numbers appearing on the leaves 3–4 days after inoculation were recorded.⁴ There are three replicates for each compound.

Inactivation Effect of Compounds against TMV in Vivo.

To test viral inhibition, equal volumes of the virus and the compound solution were mixed together for 30 min. The mixture was then inoculated into the growing *N. tabacum* L leaves of the same age, and another pot was inoculated with the mixture of solvent and the virus as the control. The local lesion numbers were recorded 3–4 days after inoculation.⁵ There are three replicates for each compound.

Curative Effect of Compounds against TMV in Vivo.

TMV (concentration of 6.0×10^{-3} $\mu\text{g/mL}$) was inoculated on the growing leaves of *N. tabacum* L. of the same age. Then, the leaves were washed with water and dried. The compound solution was smeared on the inoculated leaves, while inoculated leaves in another pot were smeared with the solvent as a control. The local lesion numbers were recorded 3–4 days after inoculation.⁶ There are 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).

Inhibition rate (%) = $[(\text{av local lesion no. of control} - \text{av local lesion no. of drug-treated}) / \text{av local lesion no. of control}] \times 100\%$

Fungicidal activities⁷

The fungicidal activities of compounds were evaluated in mycelial growth tests conducted in artificial media against 14 plant pathogens at a rate of 50 $\mu\text{g/mL}$. Each test compound was dissolved in a suitable amount of acetone and diluted with water containing 0.1% TW-80 to a concentration of 500 $\mu\text{g/mL}$. To each petri dish was

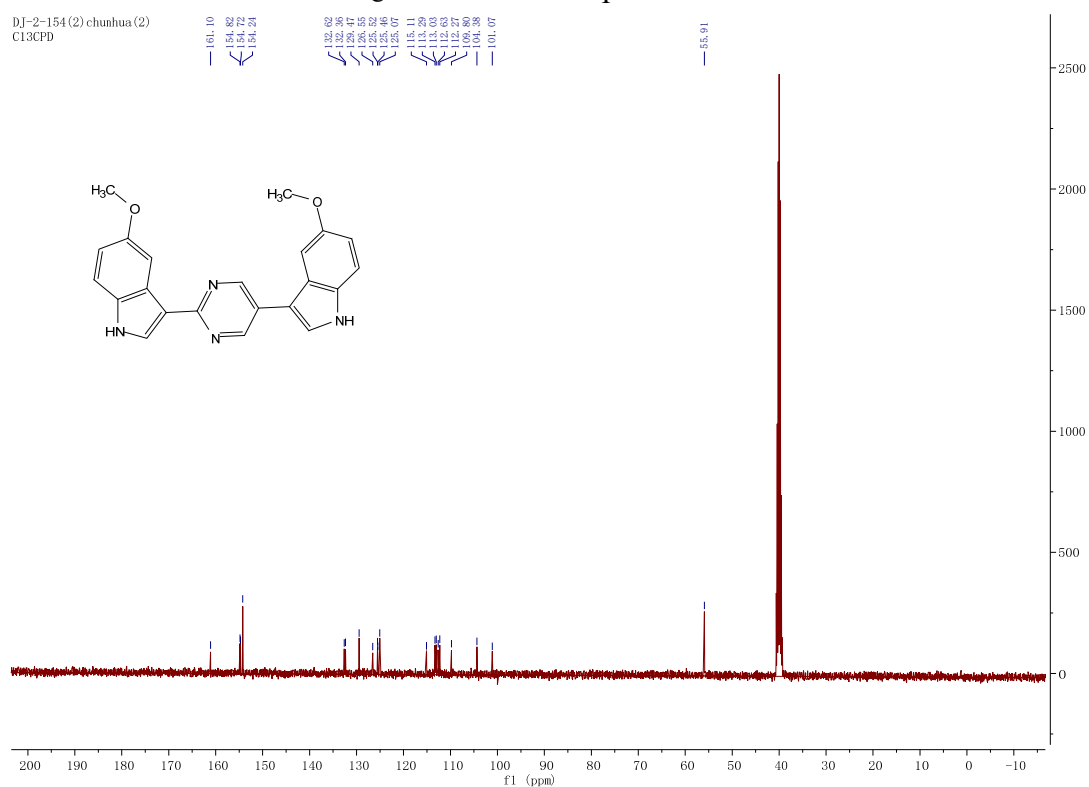
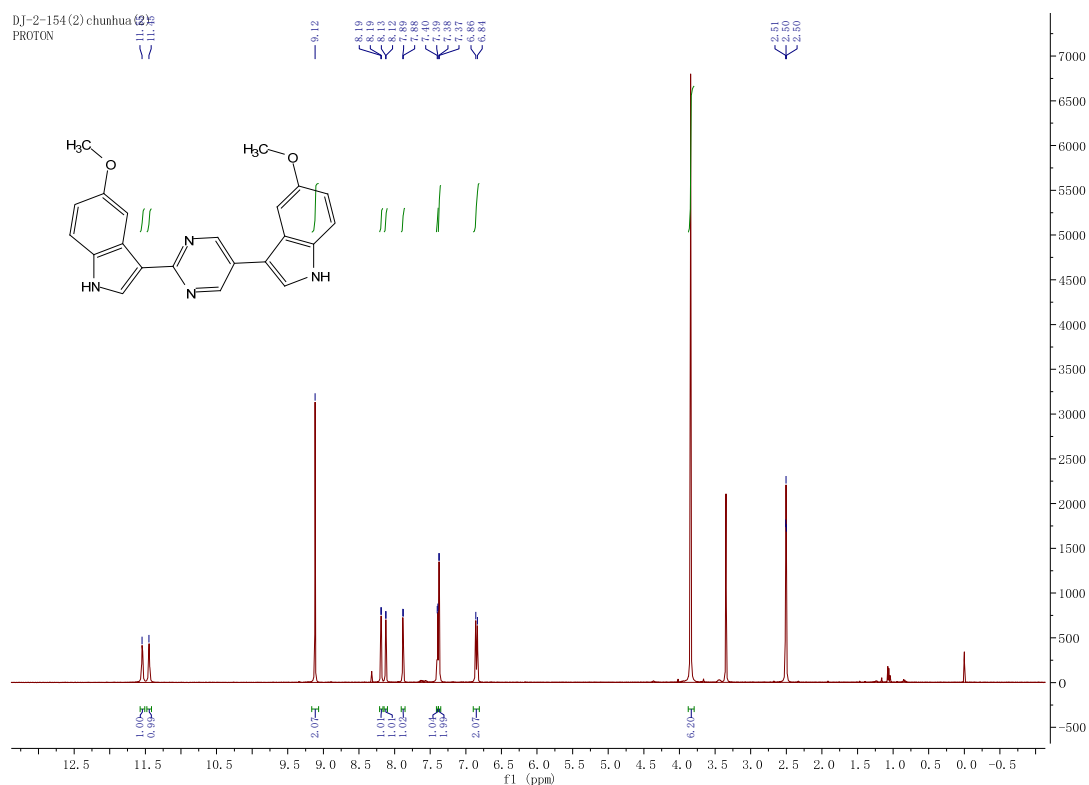
added 1 mL of the test solution and 9 mL of culture medium to make a 50 $\mu\text{g/mL}$ concentration of the test compound, while in another petri dish was added 1 mL distilled water containing 0.1% TW-80 and 9 mL of culture medium as a blank control. A 4 mm diameter of hyphal growth was cut using a hole puncher on a growing fungal S6 culture and the hyphae were moved to the petri dish containing the test compound. Each assay was performed three times. The dishes were stored in controlled environment cabinets ($24\pm 1^\circ\text{C}$) for 4 days, after which the diameter of mycelial growth was measured and the percentage inhibition was calculated using the following equation: Percentage inhibition (%) = (averaged diameter of mycelia in blank controls – averaged diameter of mycelia in medicated tablets) / (averaged diameter of mycelia in blank controls – 4 mm) \times 100.

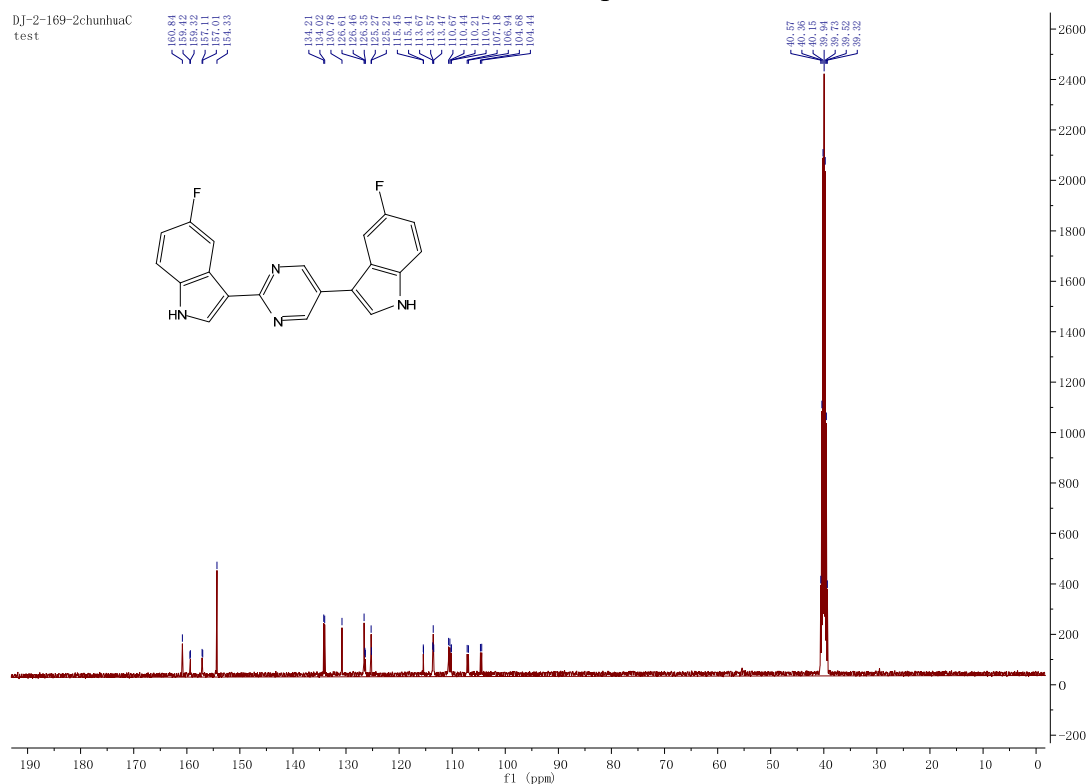
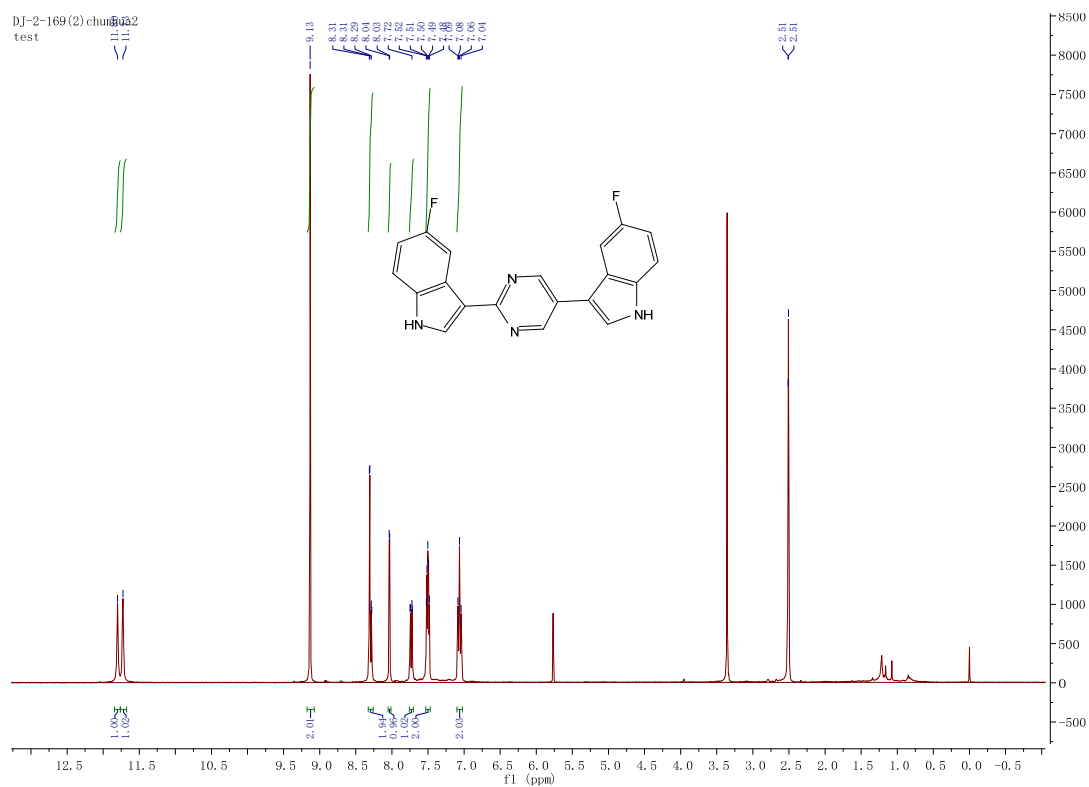
Reference:

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Section S2: Copies of NMR spectra (Figures S1–S50)





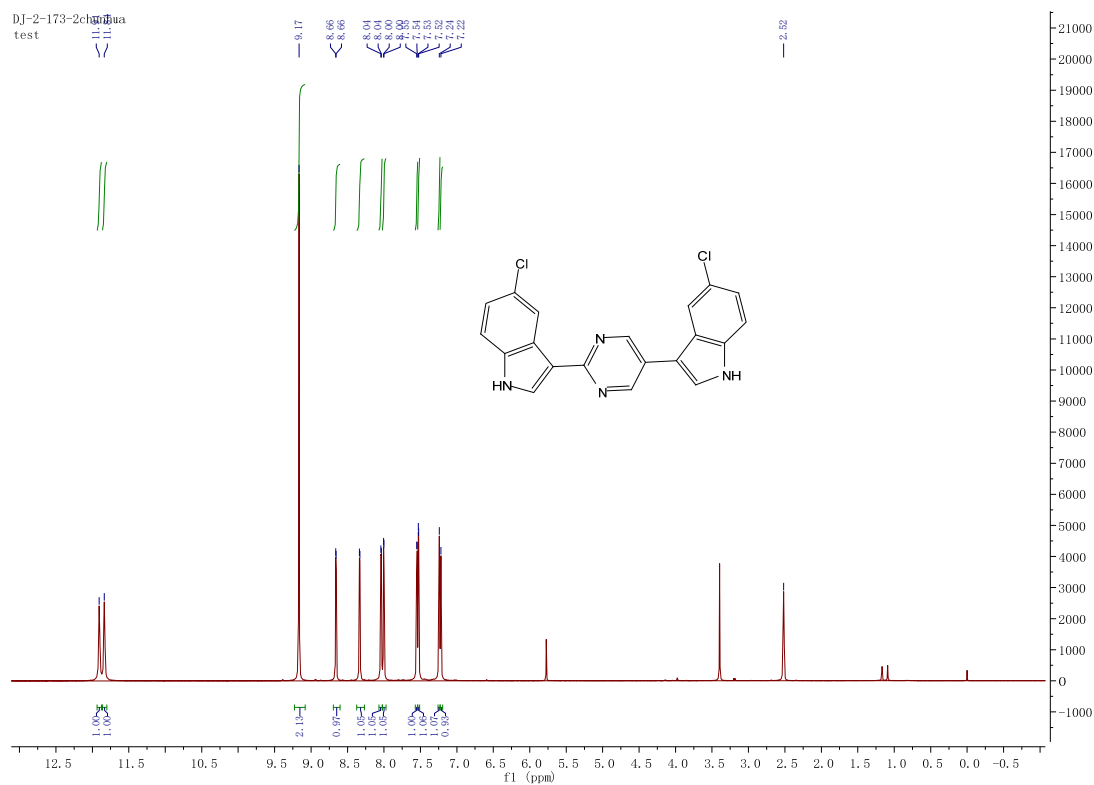


Figure S5. ^1H NMR spectrum of 5c

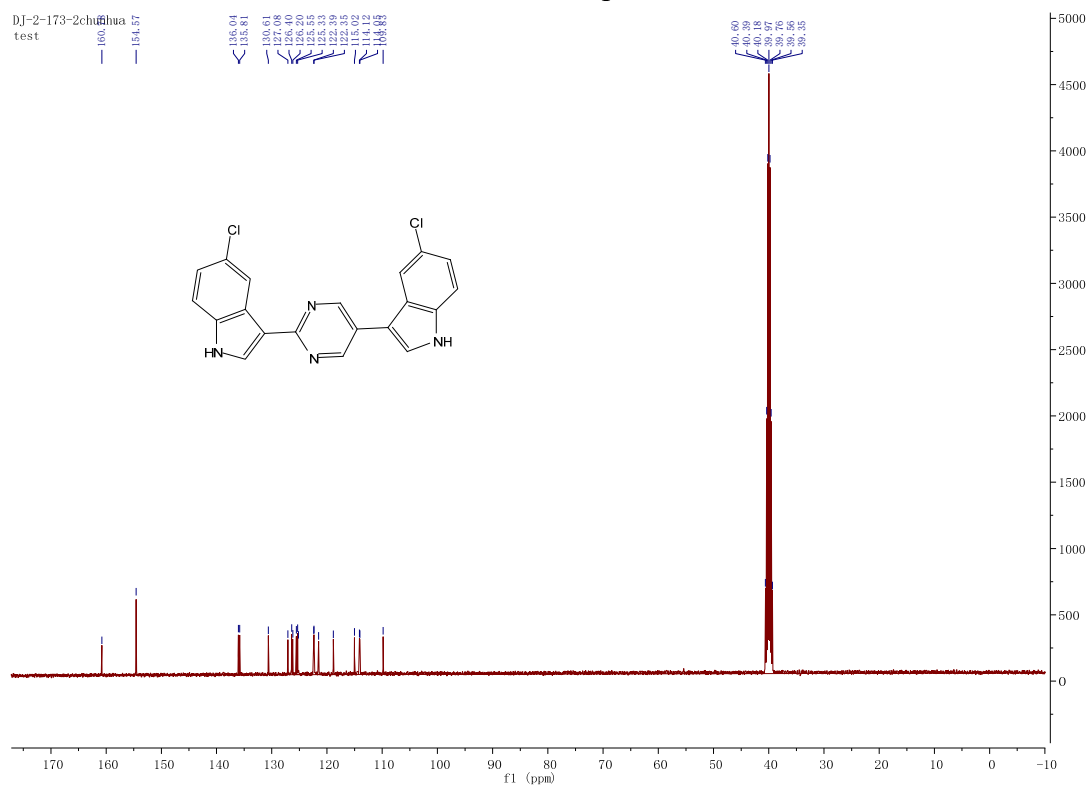


Figure S6. ^{13}C NMR spectrum of 5c

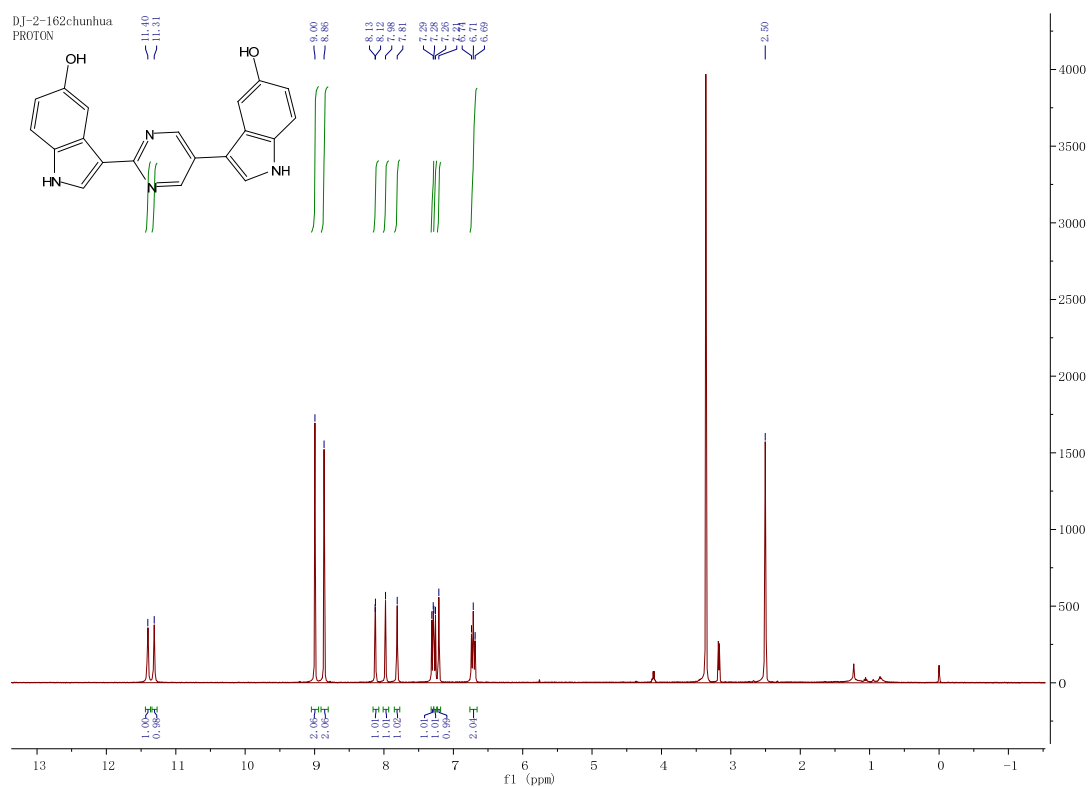


Figure S9. ^1H NMR spectrum of 5e

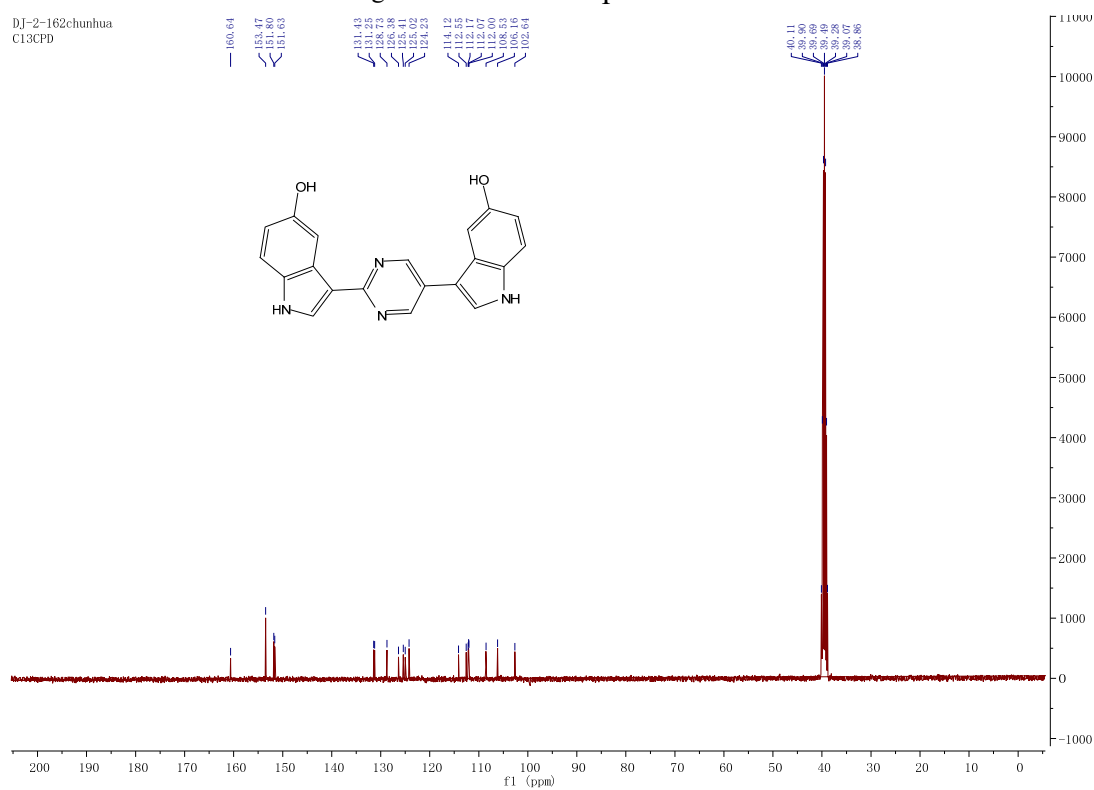


Figure S10. ^{13}C NMR spectrum of 5e

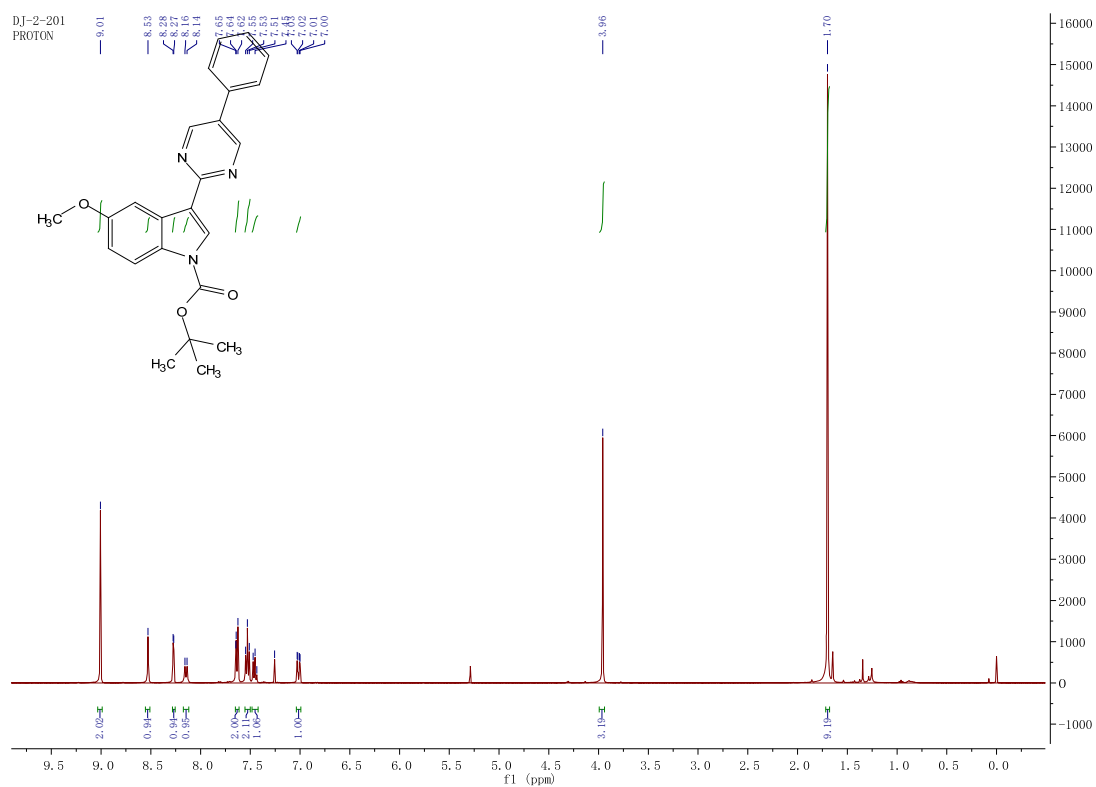


Figure S11. ^1H NMR spectrum of 6a

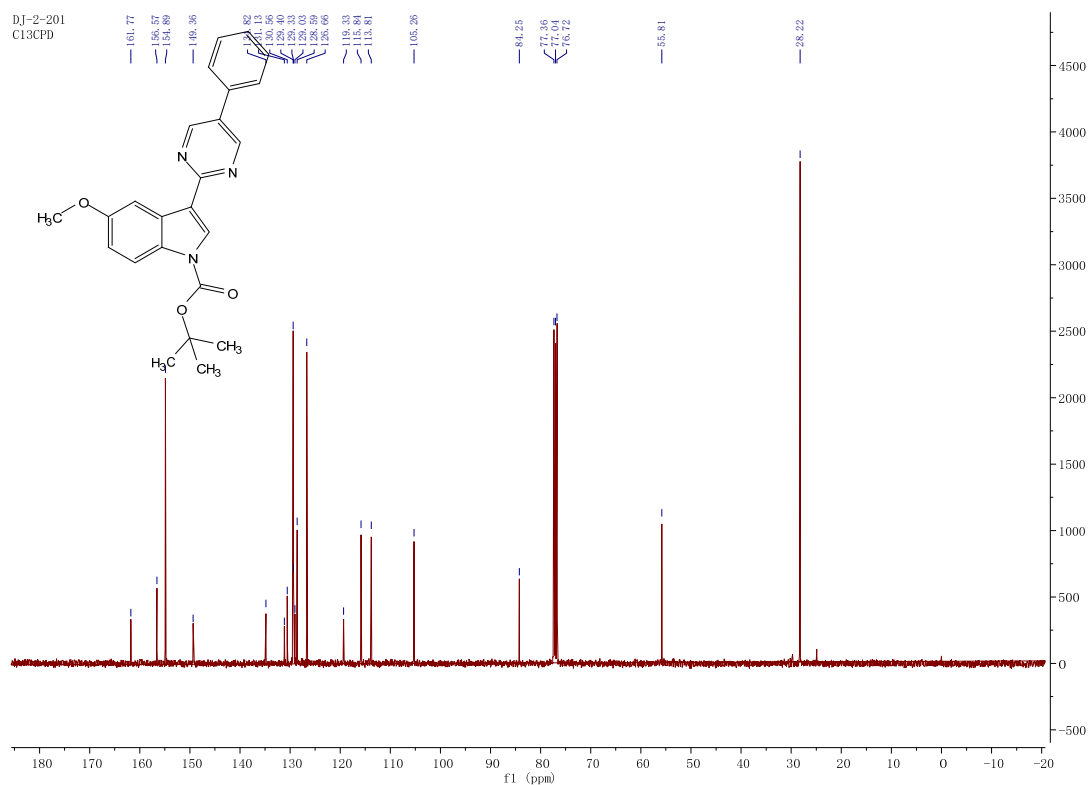


Figure S12. ^{13}C NMR spectrum of 6a

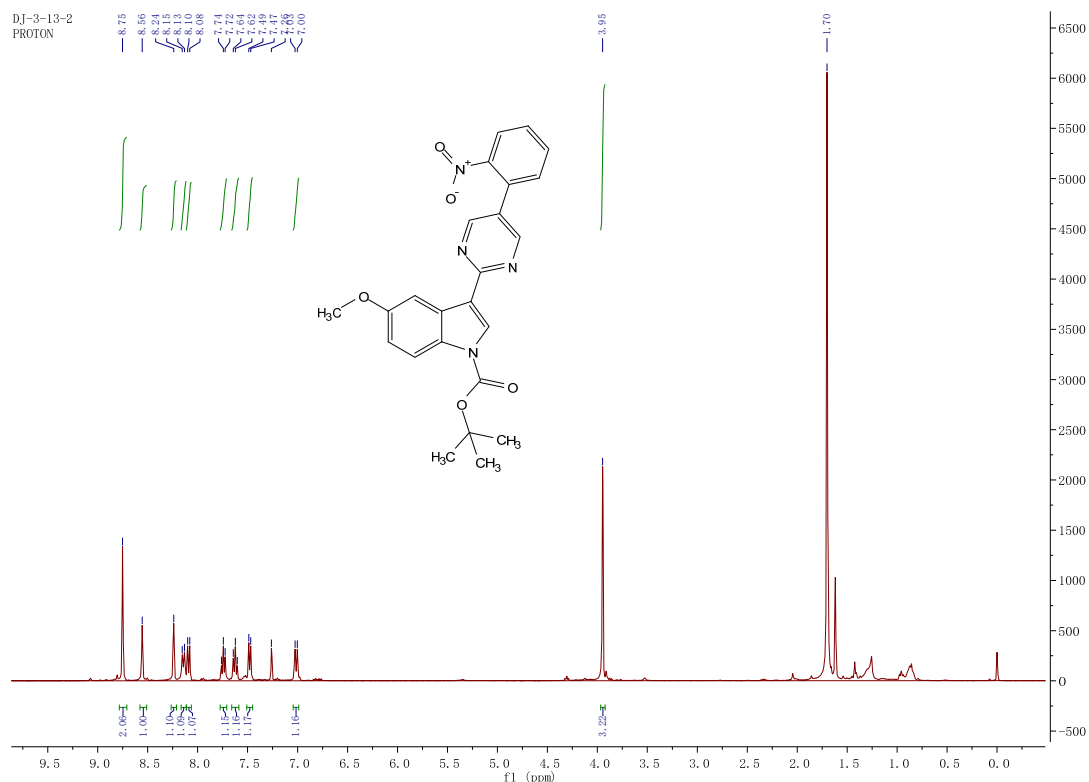


Figure S13. ^1H NMR spectrum of 6b

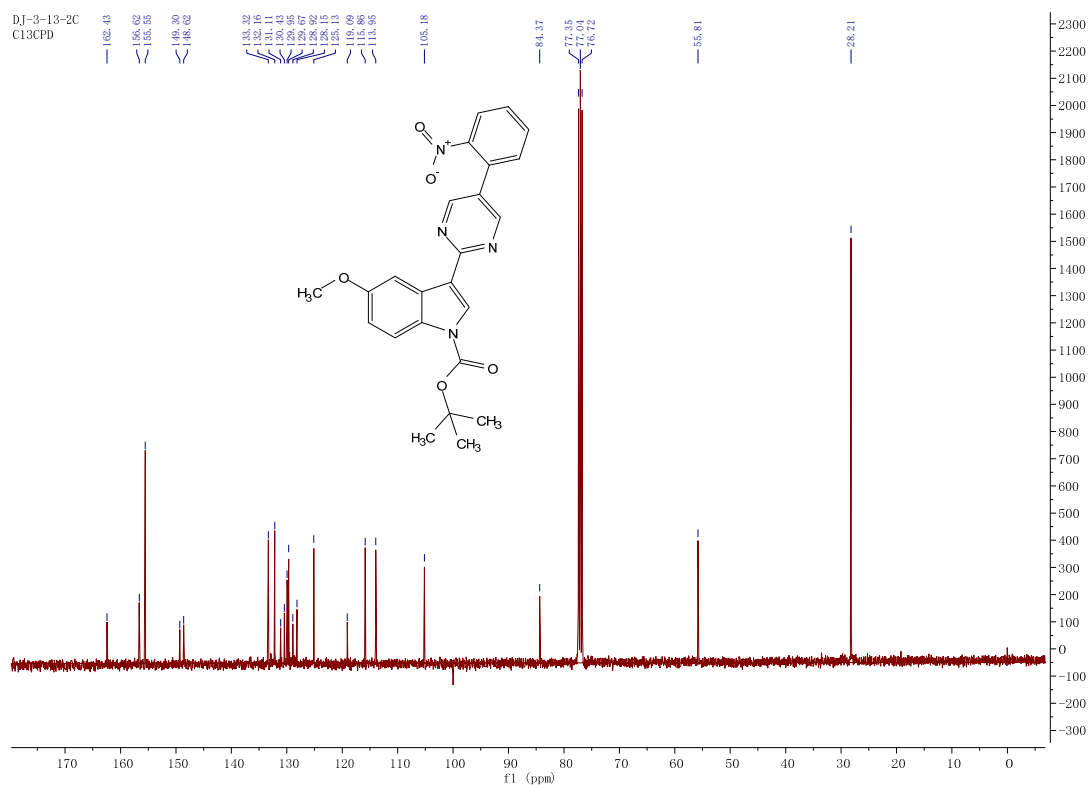


Figure S14. ^{13}C NMR spectrum of 6b

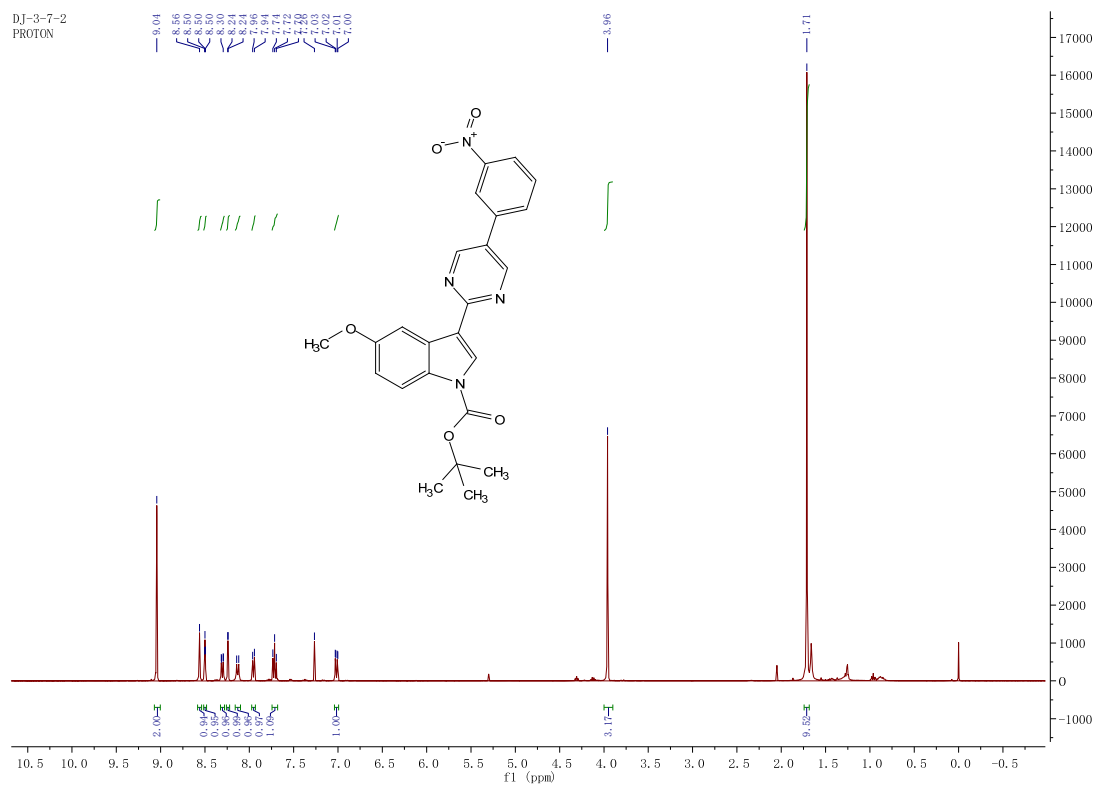


Figure S15. ^1H NMR spectrum of 6c

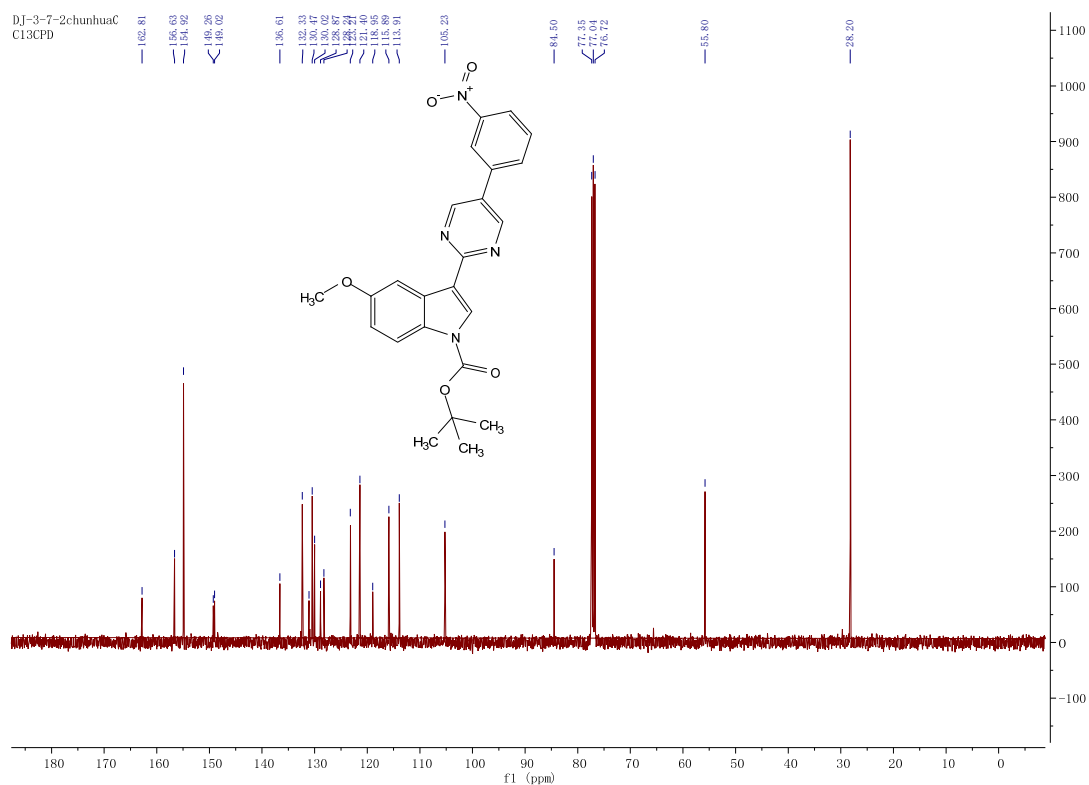


Figure S16. ^{13}C NMR spectrum of 6c

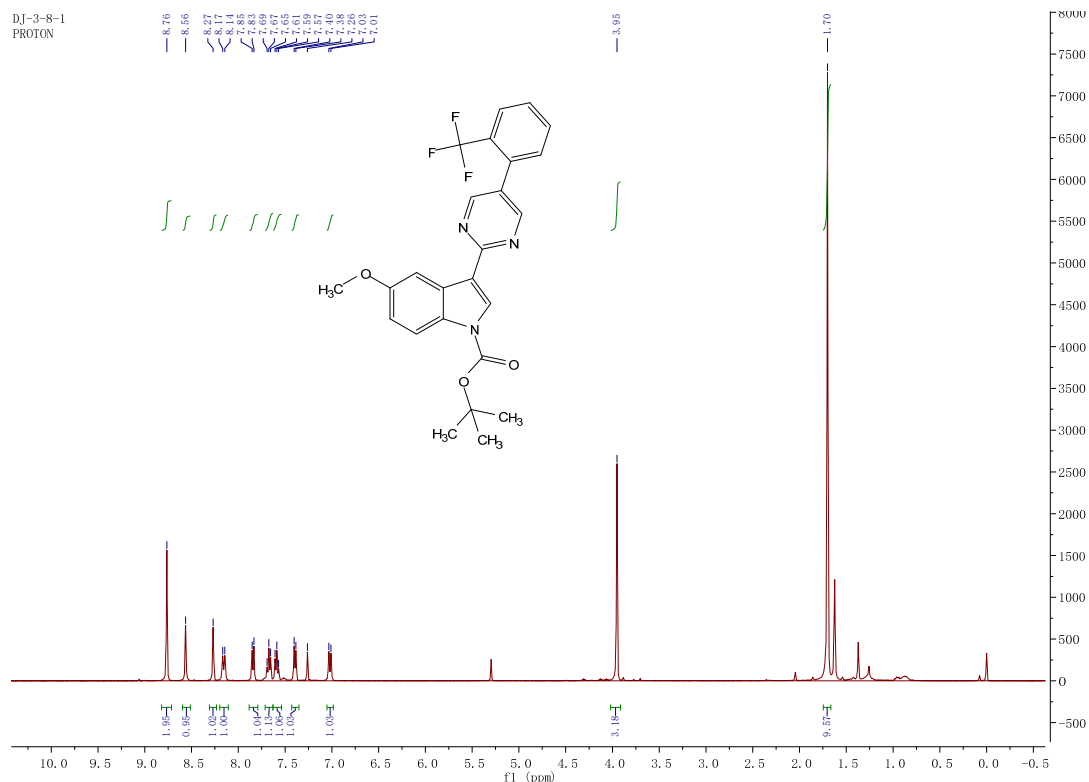


Figure S17. ^1H NMR spectrum of 6d

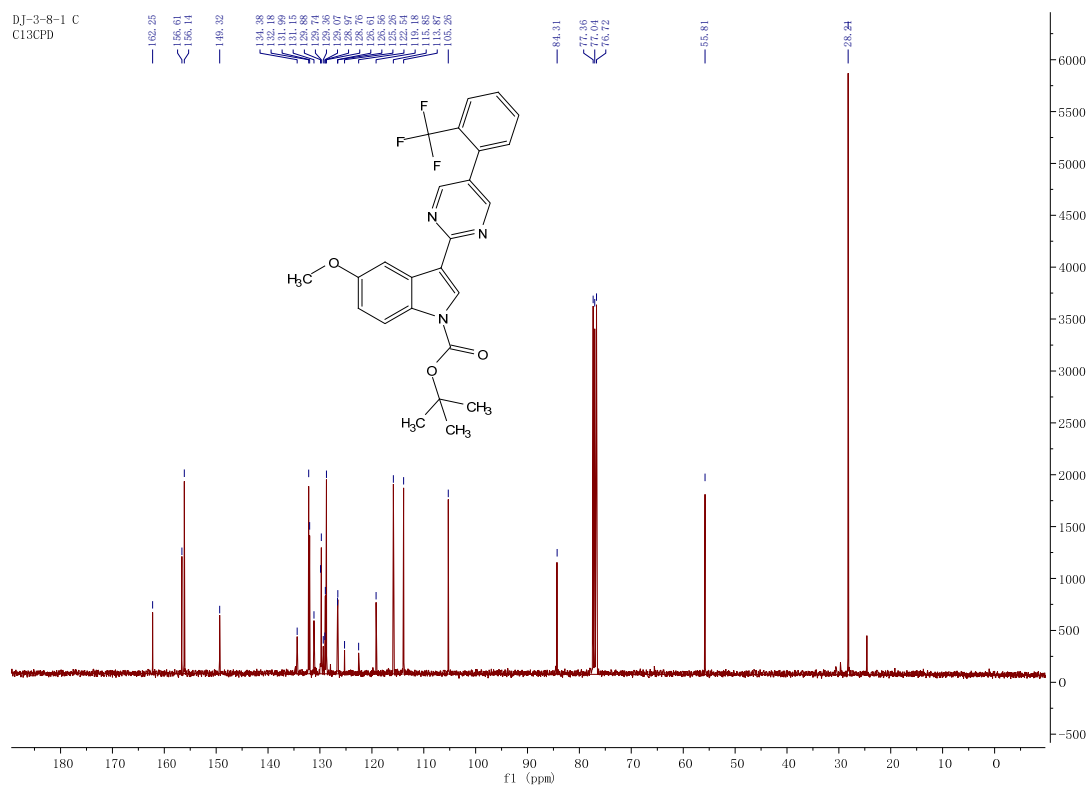


Figure S18. ^{13}C NMR spectrum of 6d

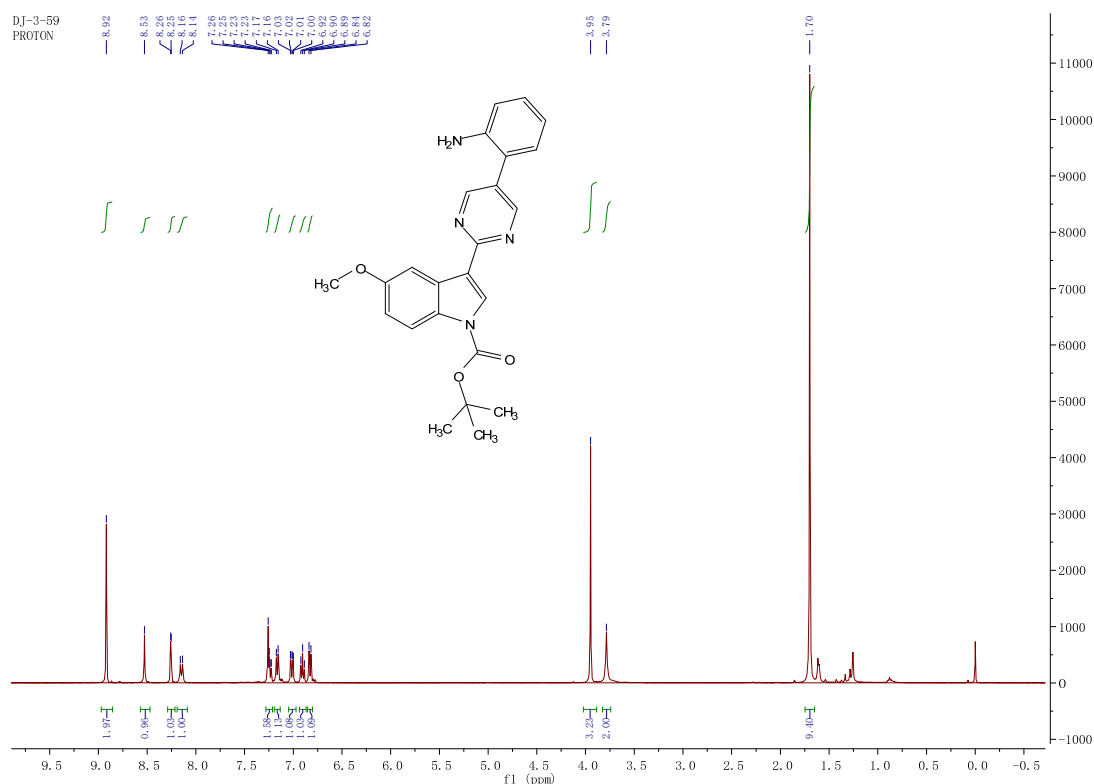


Figure S19. ^1H NMR spectrum of 6e

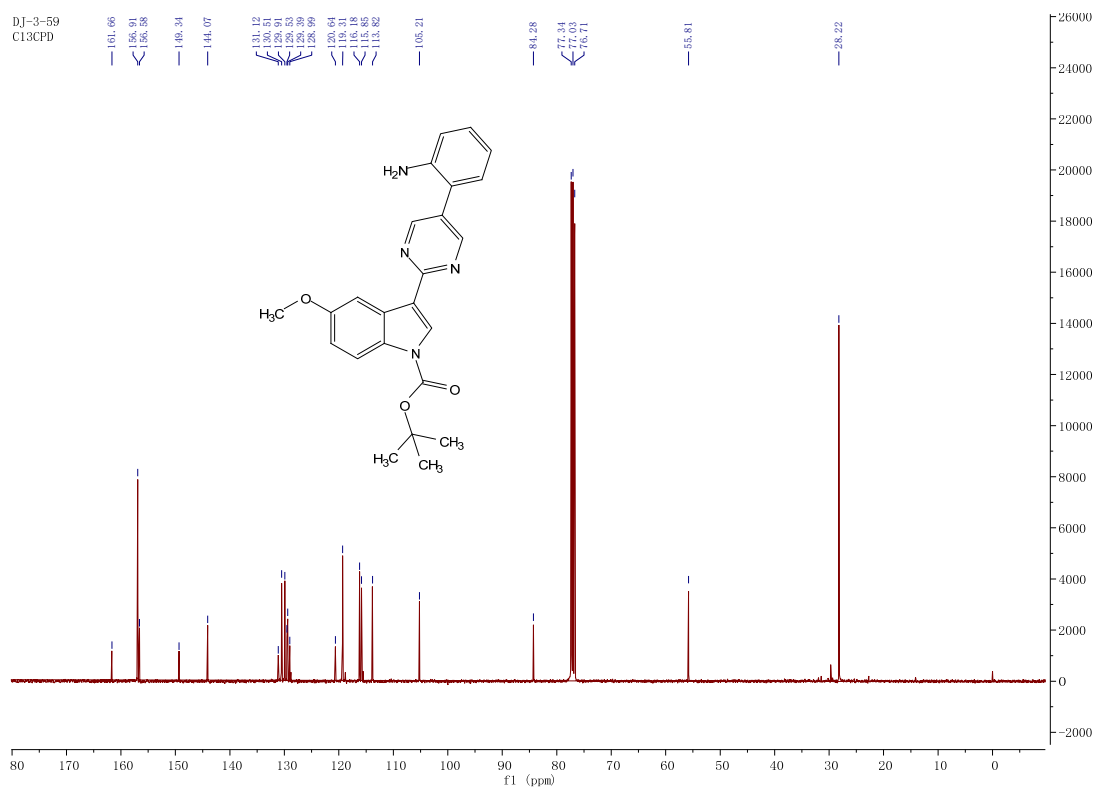


Figure S20. ^{13}C NMR spectrum of 6e

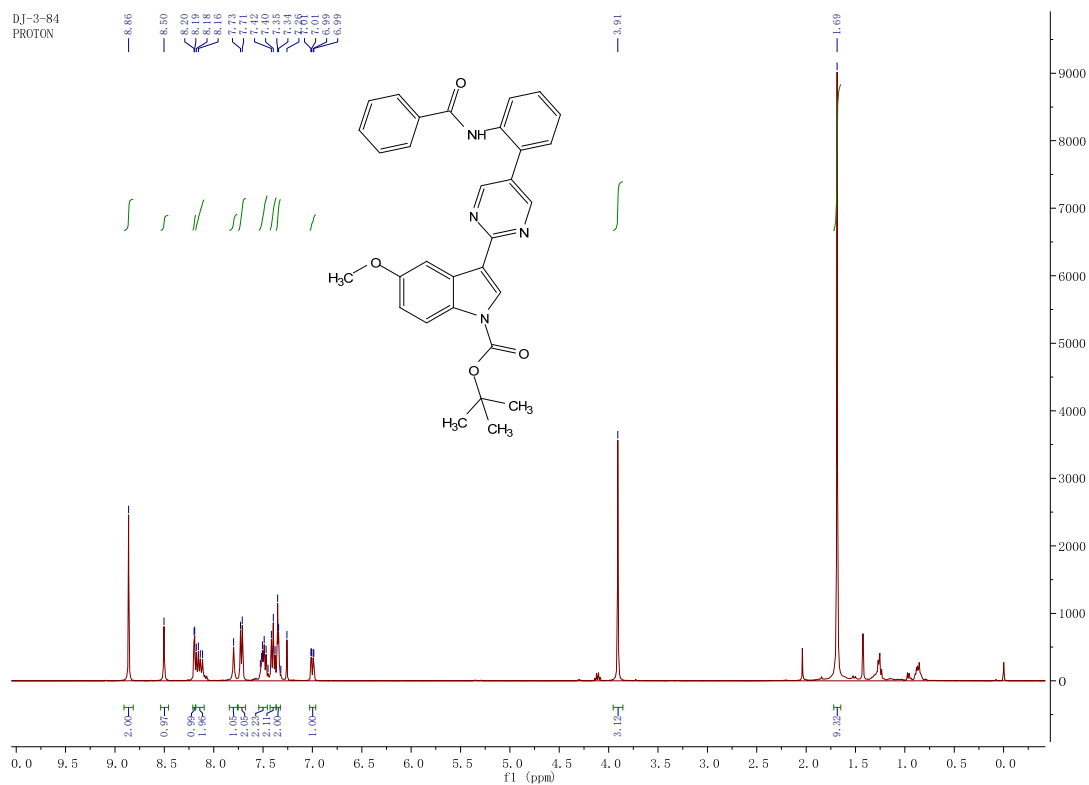


Figure S21. ^1H NMR spectrum of 7a

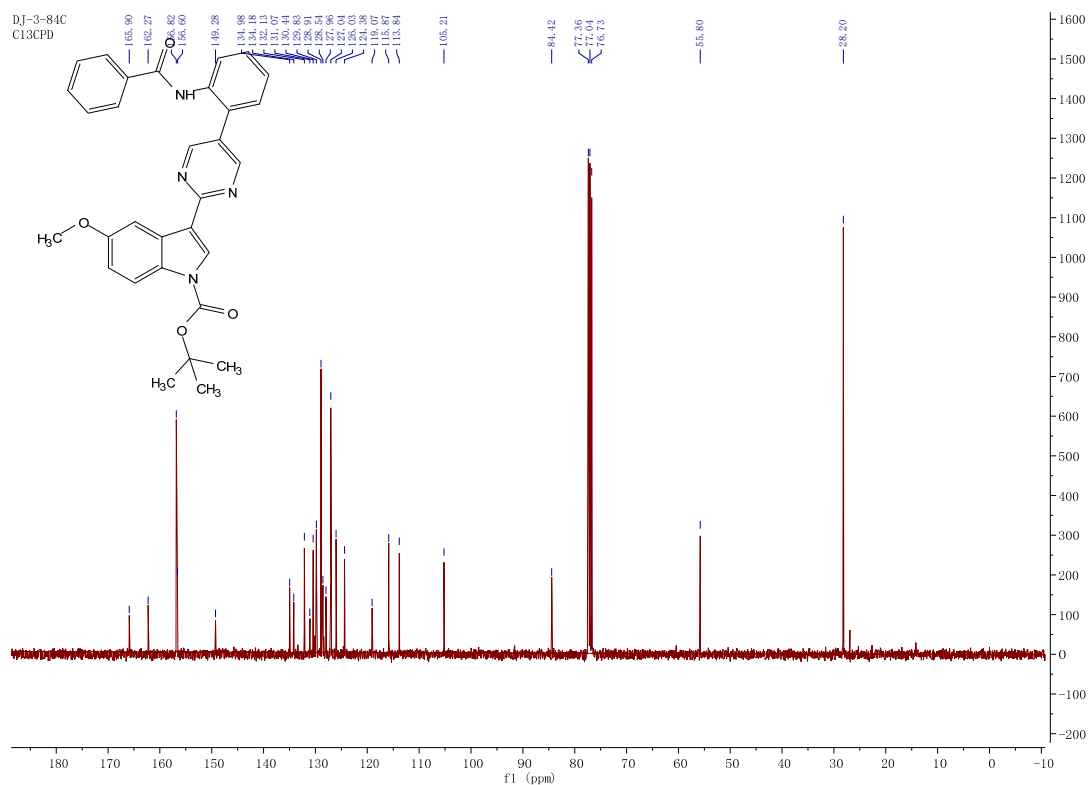


Figure S22. ^{13}C NMR spectrum of 7a

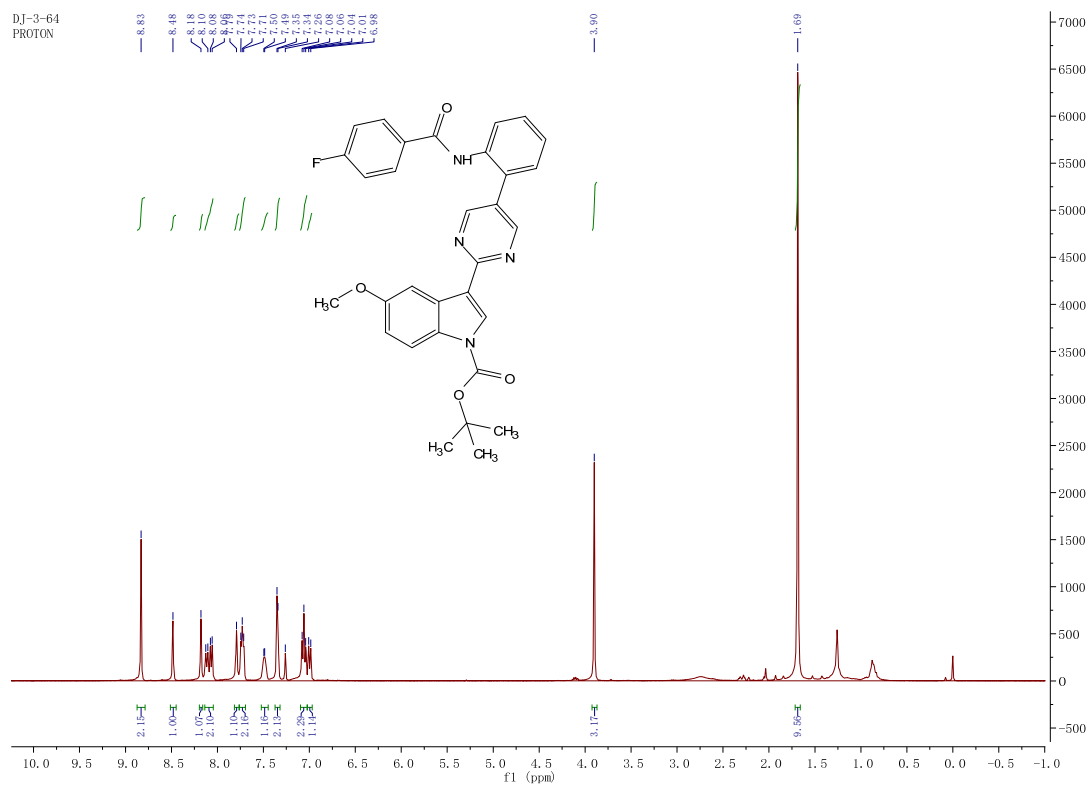


Figure S23. ^1H NMR spectrum of 7b

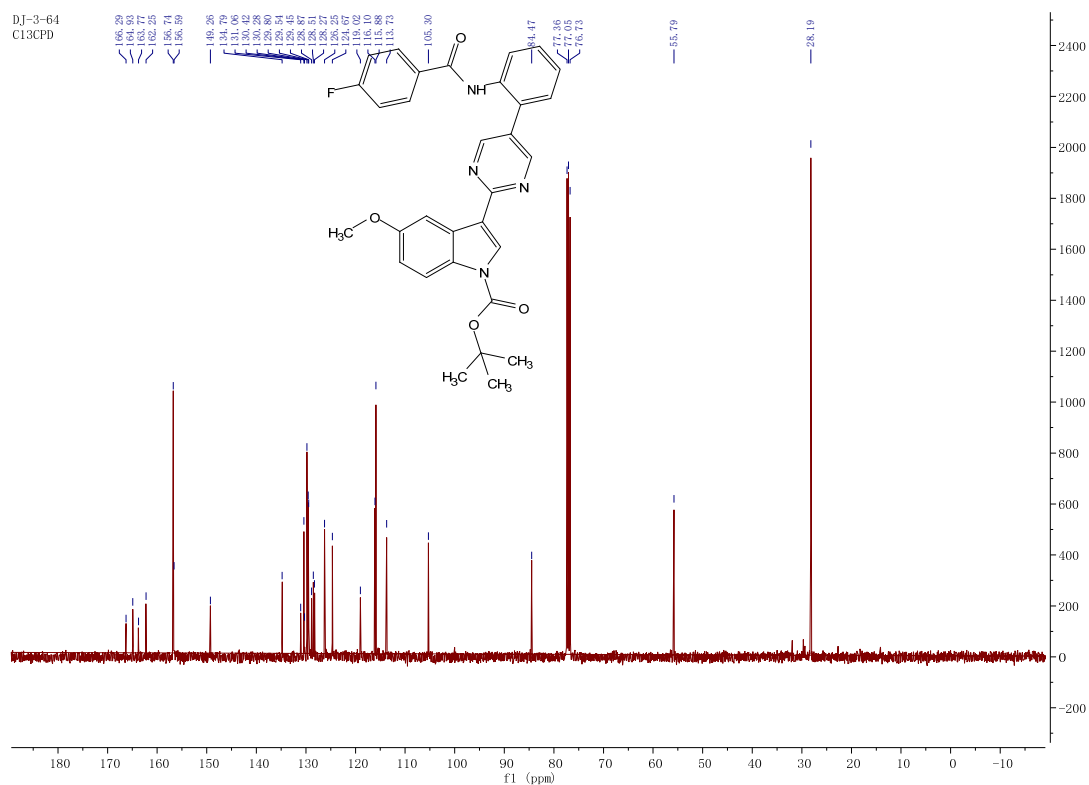
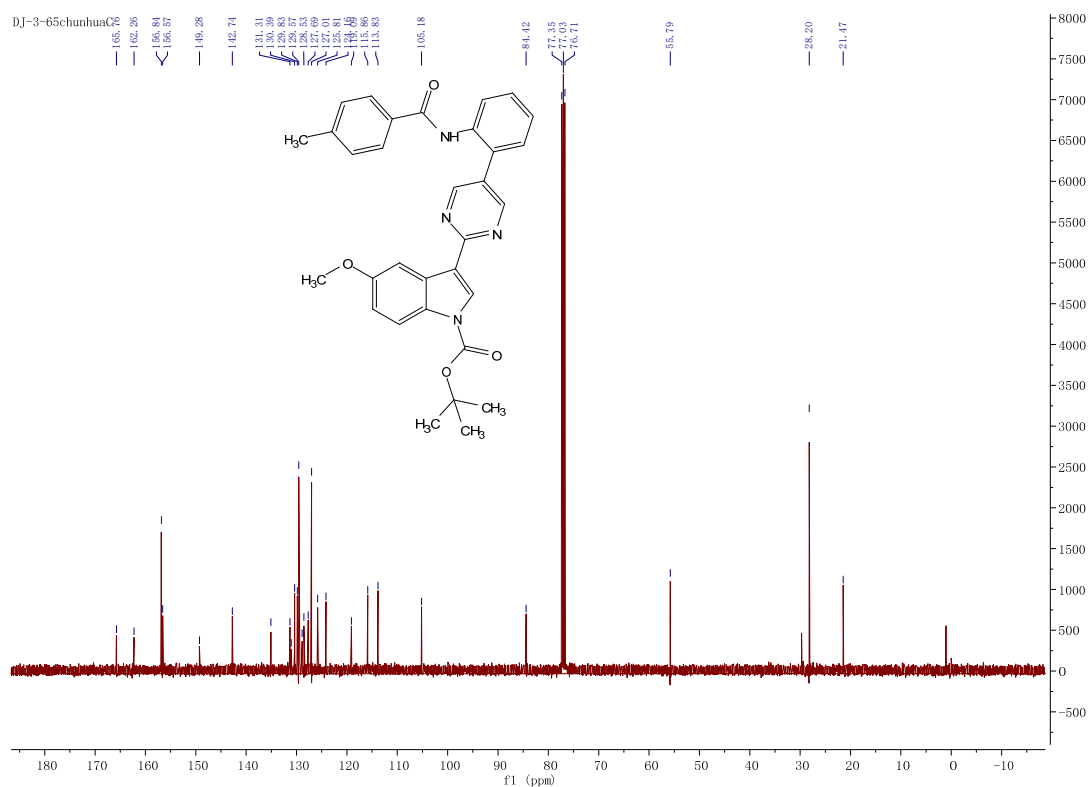
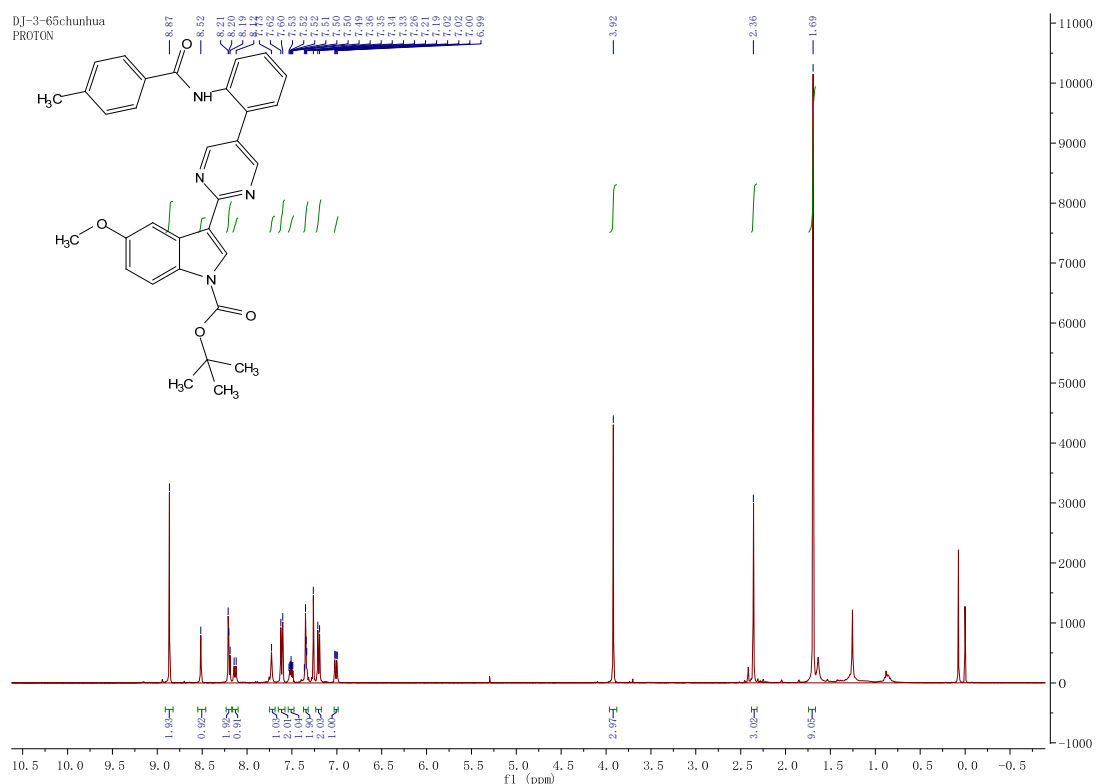


Figure S24. ^{13}C NMR spectrum of 7b



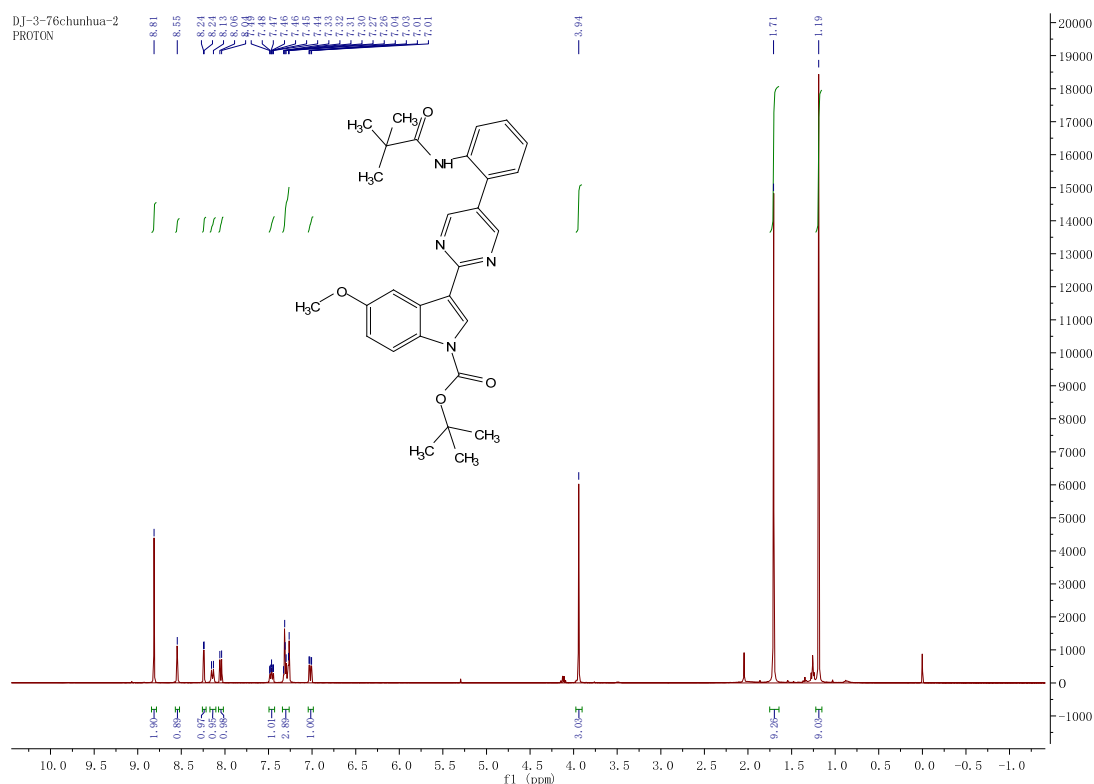


Figure S27. ^1H NMR spectrum of 7d

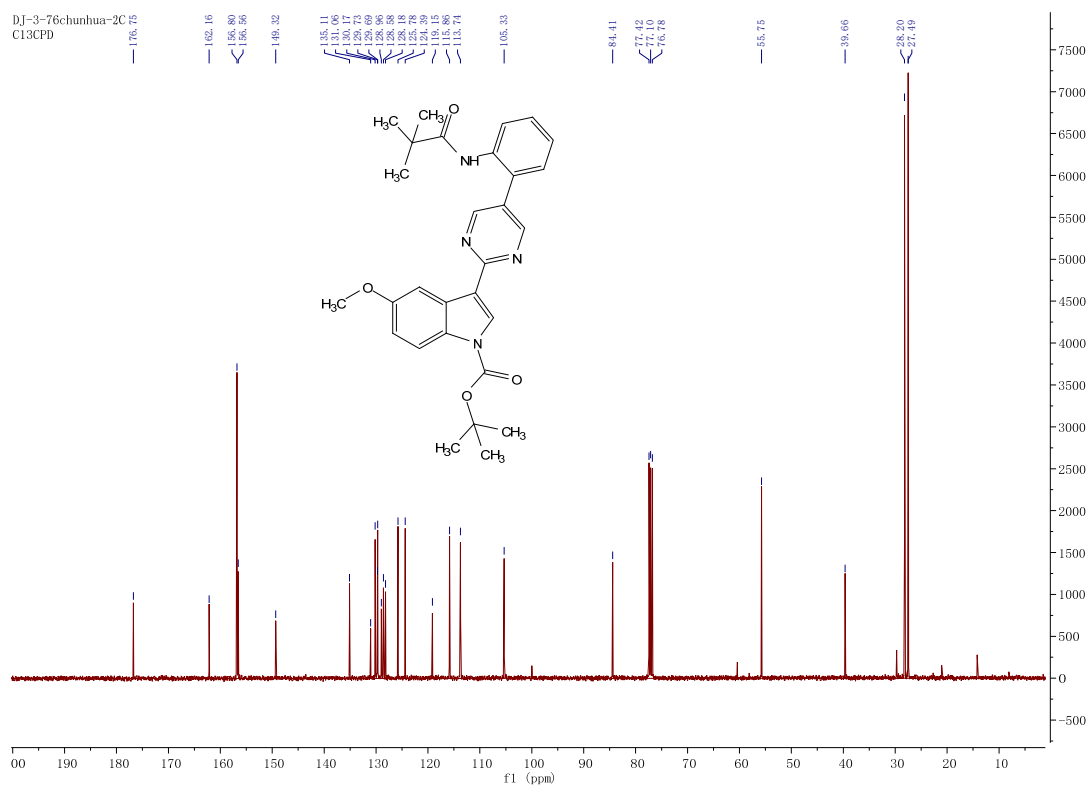
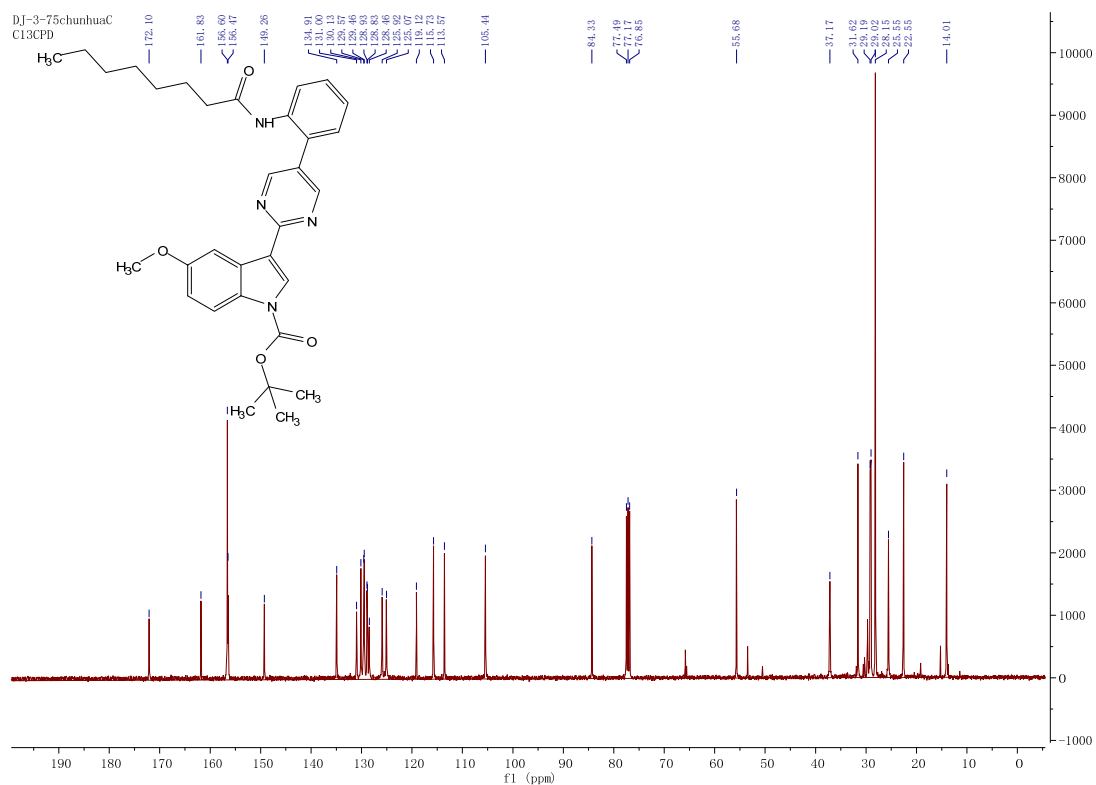
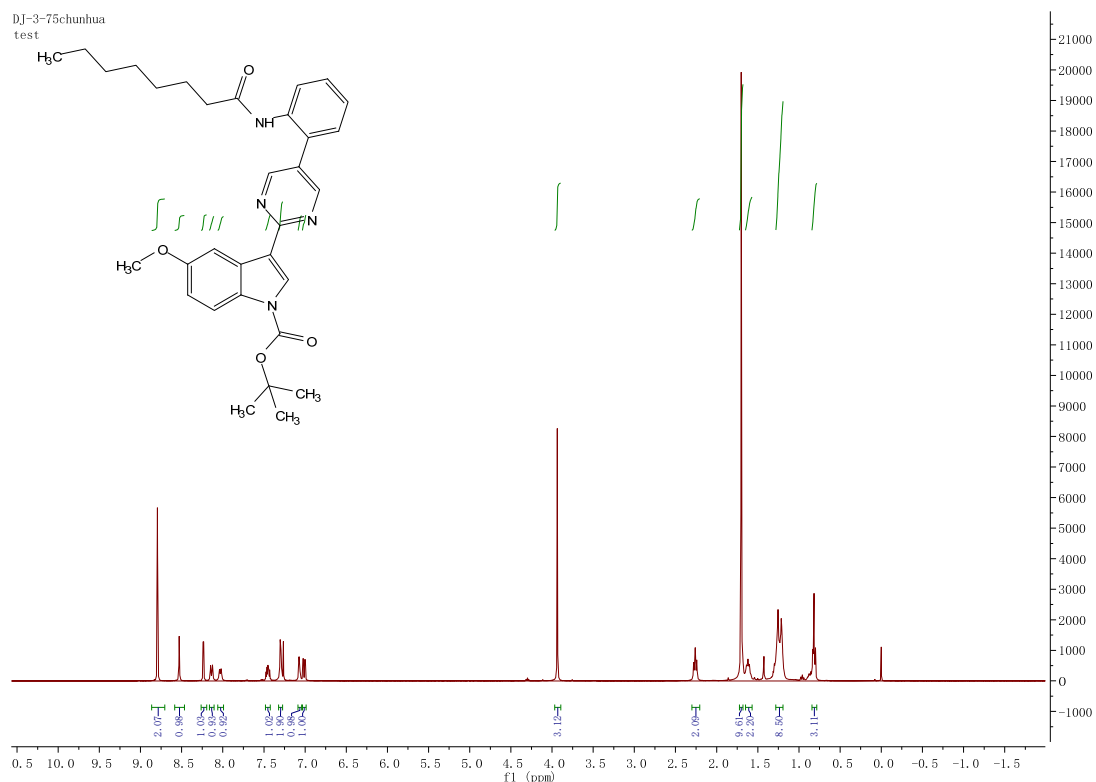


Figure S28. ^{13}C NMR spectrum of 7d



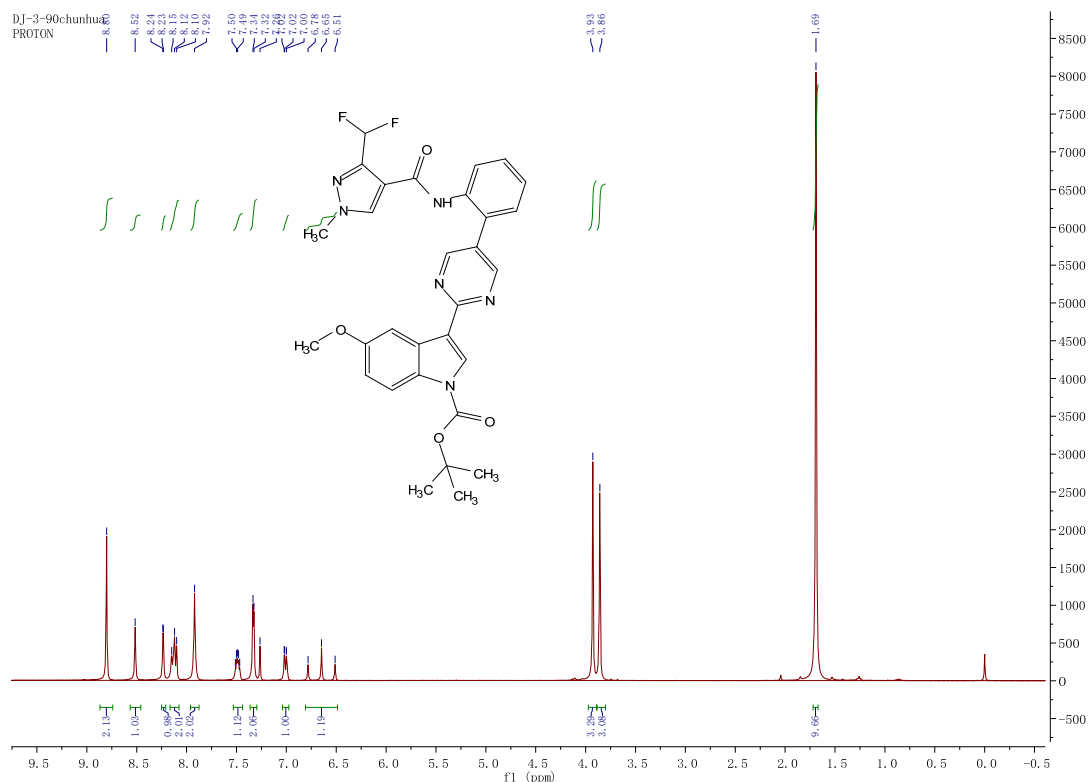


Figure S31. ^1H NMR spectrum of 7f

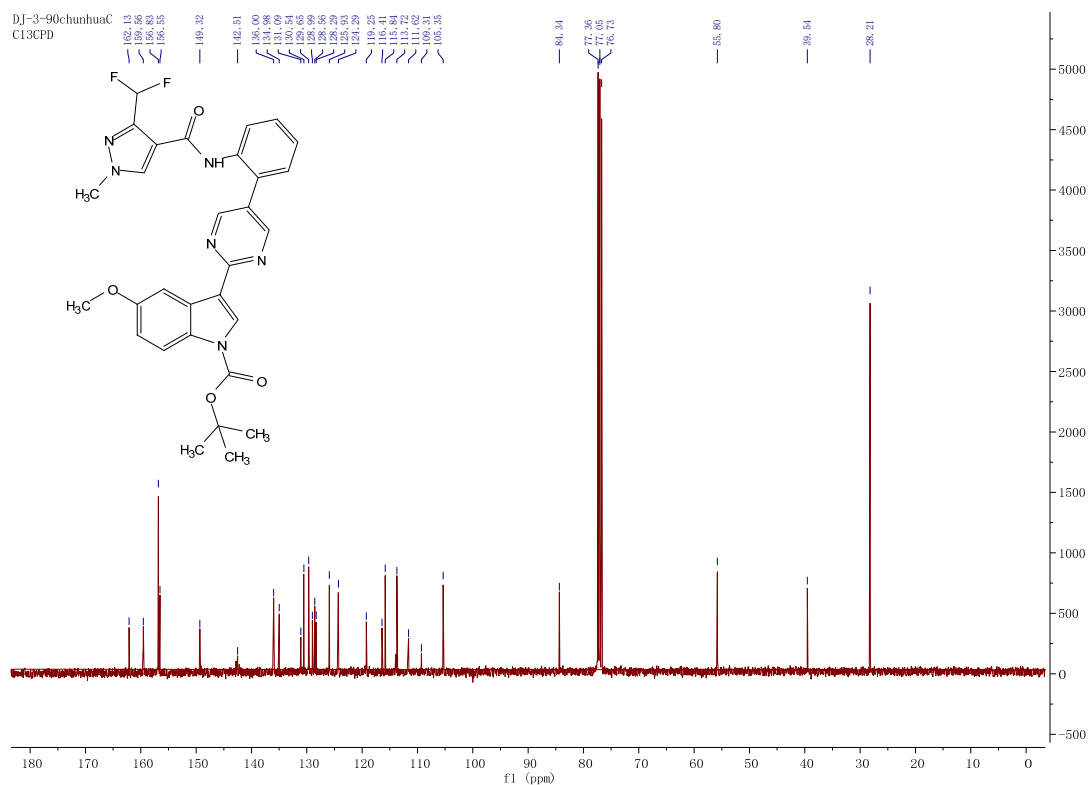


Figure S32. ^{13}C NMR spectrum of 7f

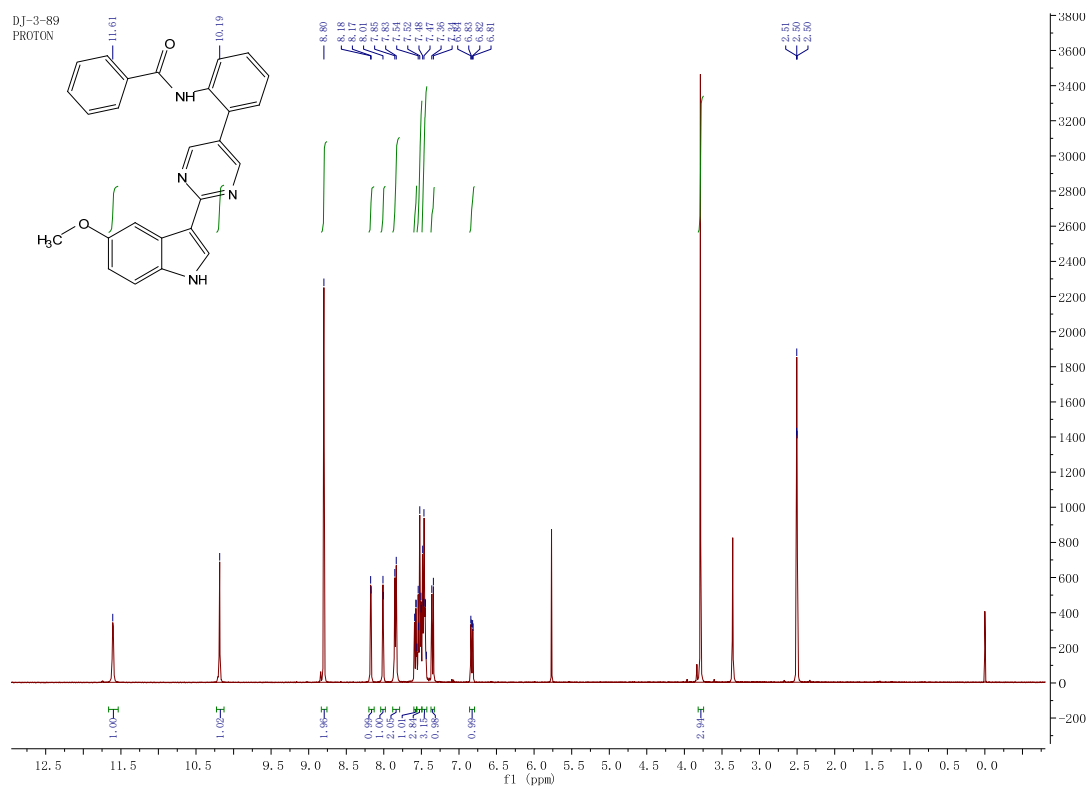


Figure S33. ^1H NMR spectrum of 8a

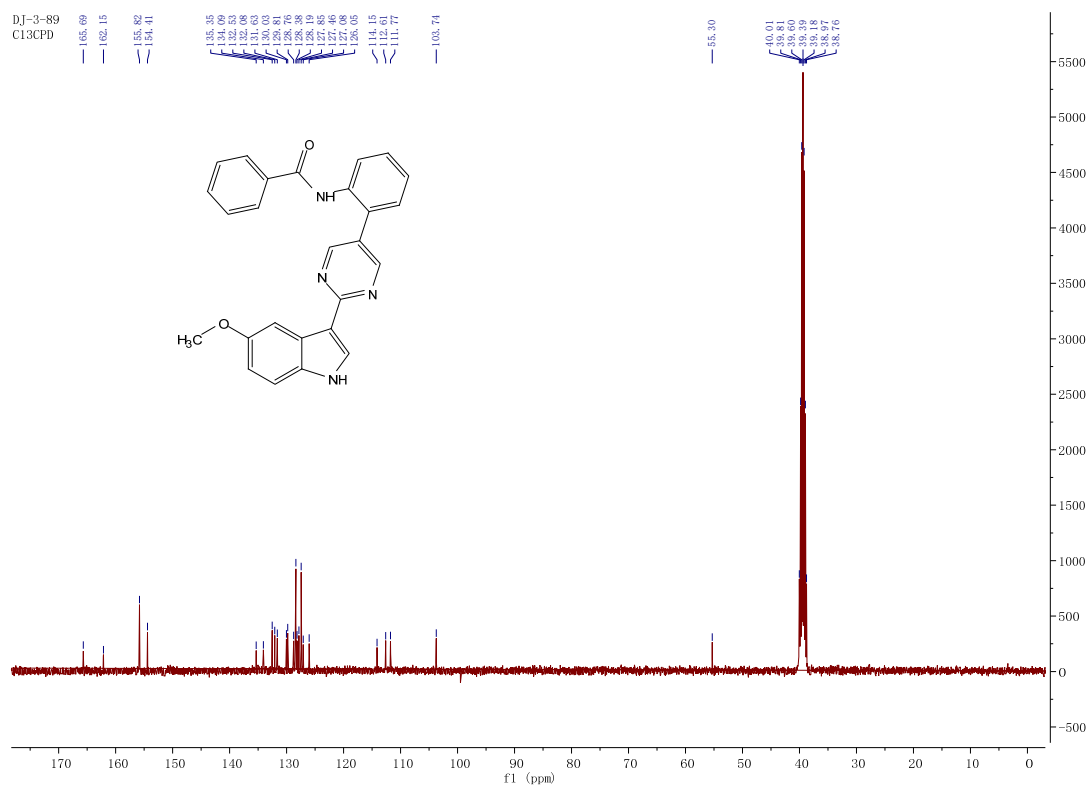


Figure S34. ^{13}C NMR spectrum of 8a

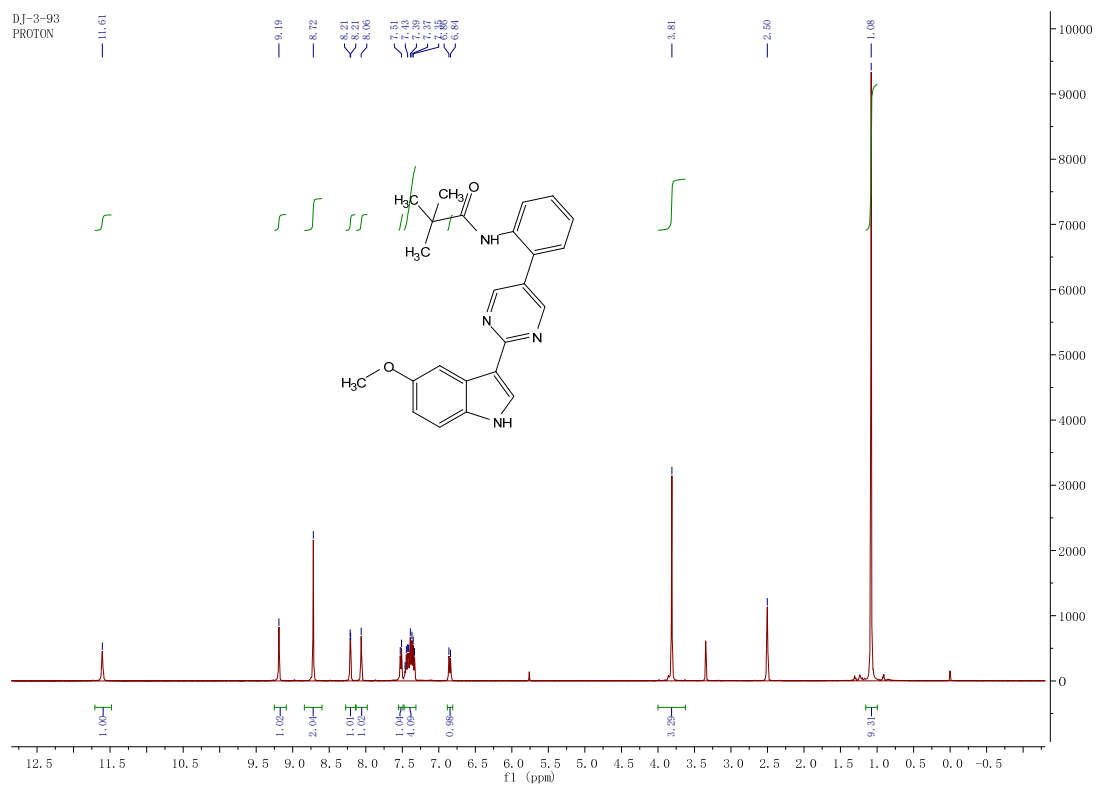


Figure S35. ^1H NMR spectrum of 8b

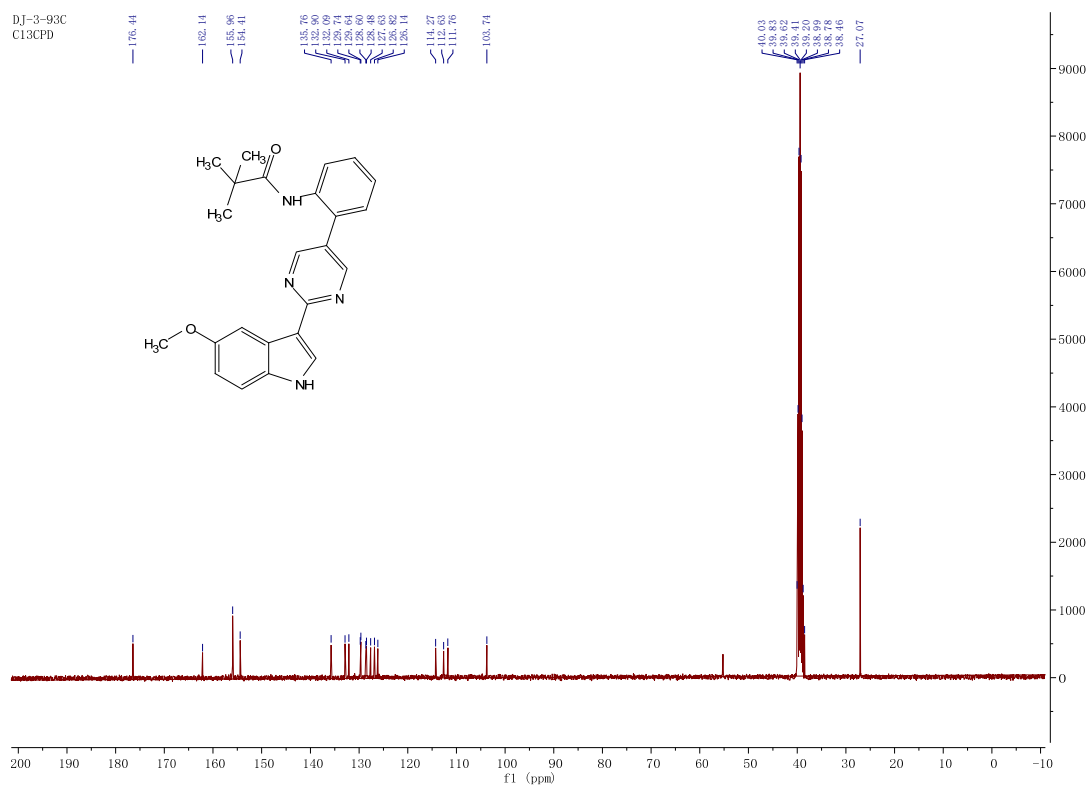


Figure S36. ^{13}C NMR spectrum of 8b

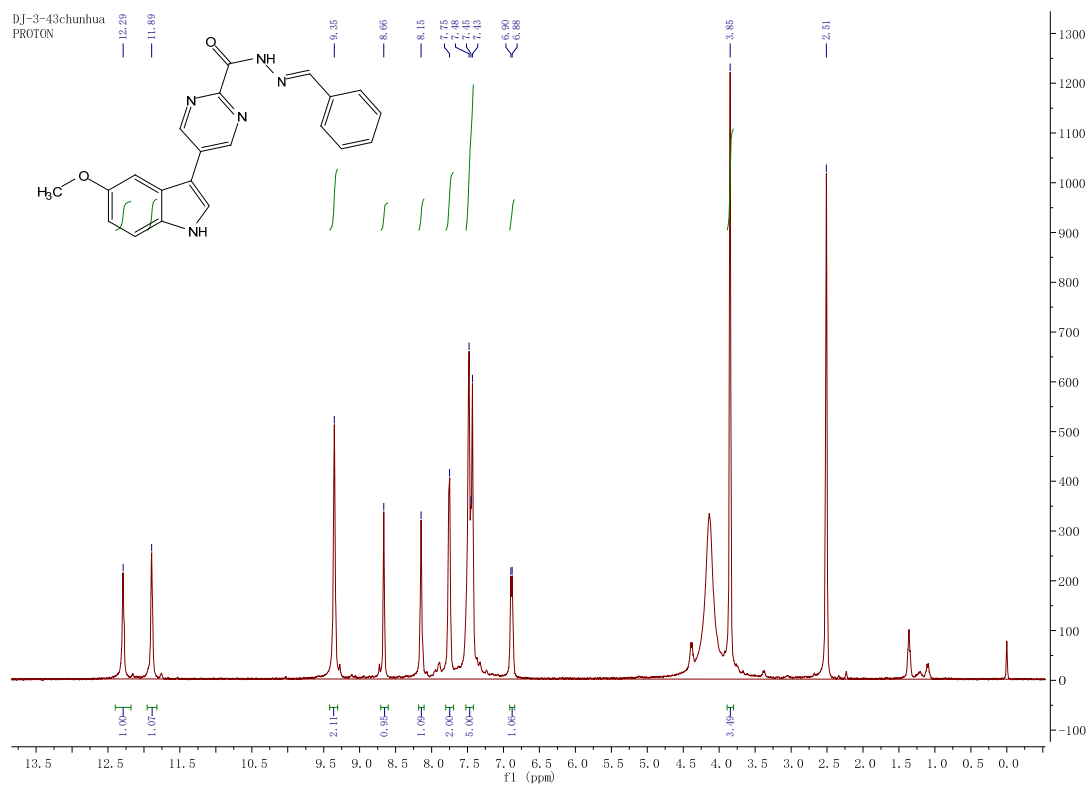


Figure S37. ^1H NMR spectrum of 9a

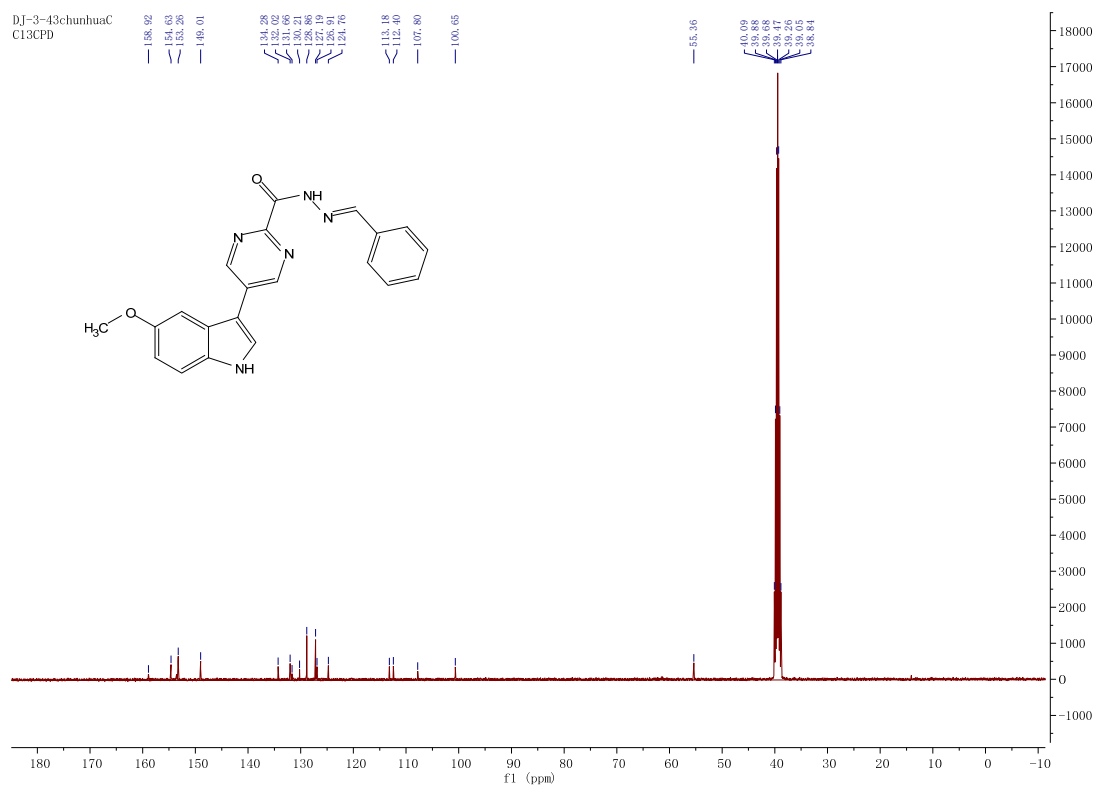
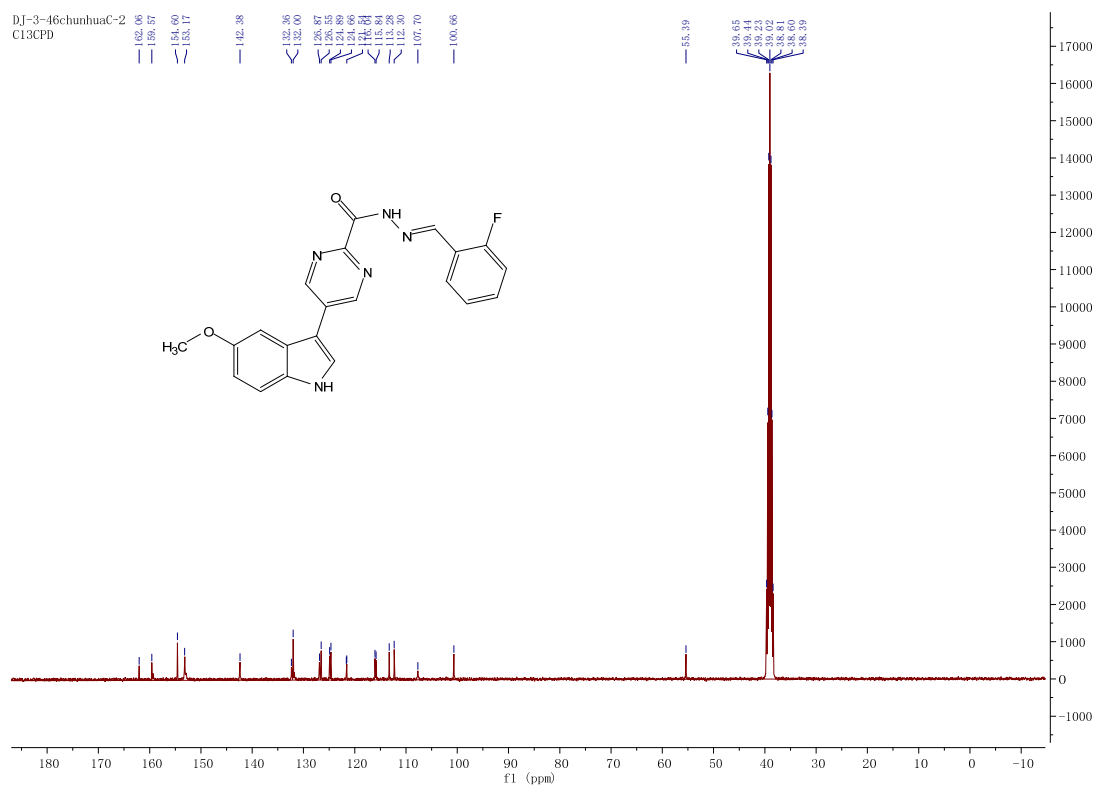
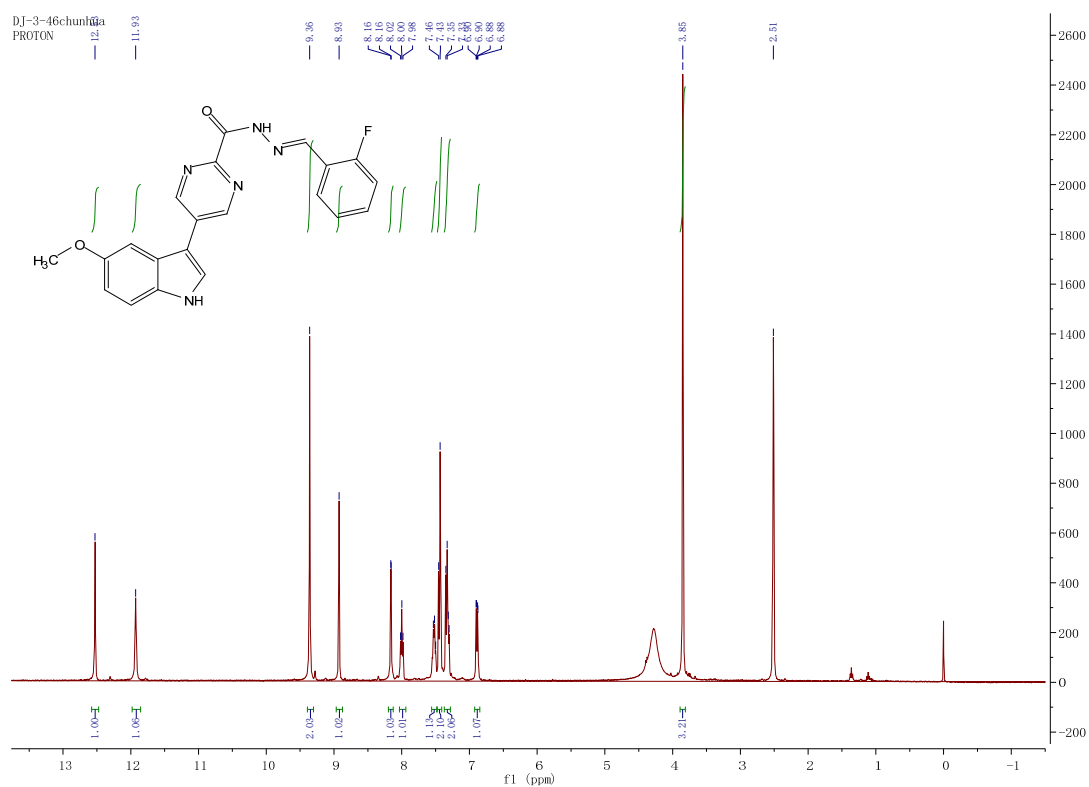


Figure S38. ^{13}C NMR spectrum of 9a



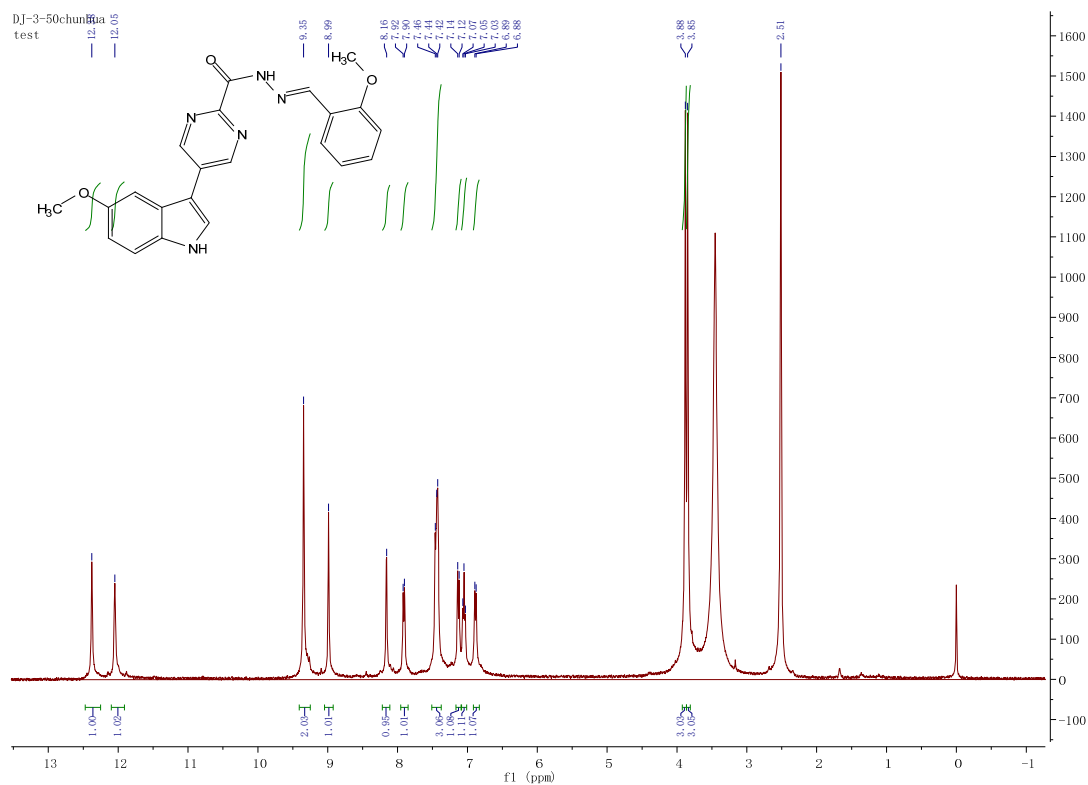


Figure S41. ^1H NMR spectrum of 9c

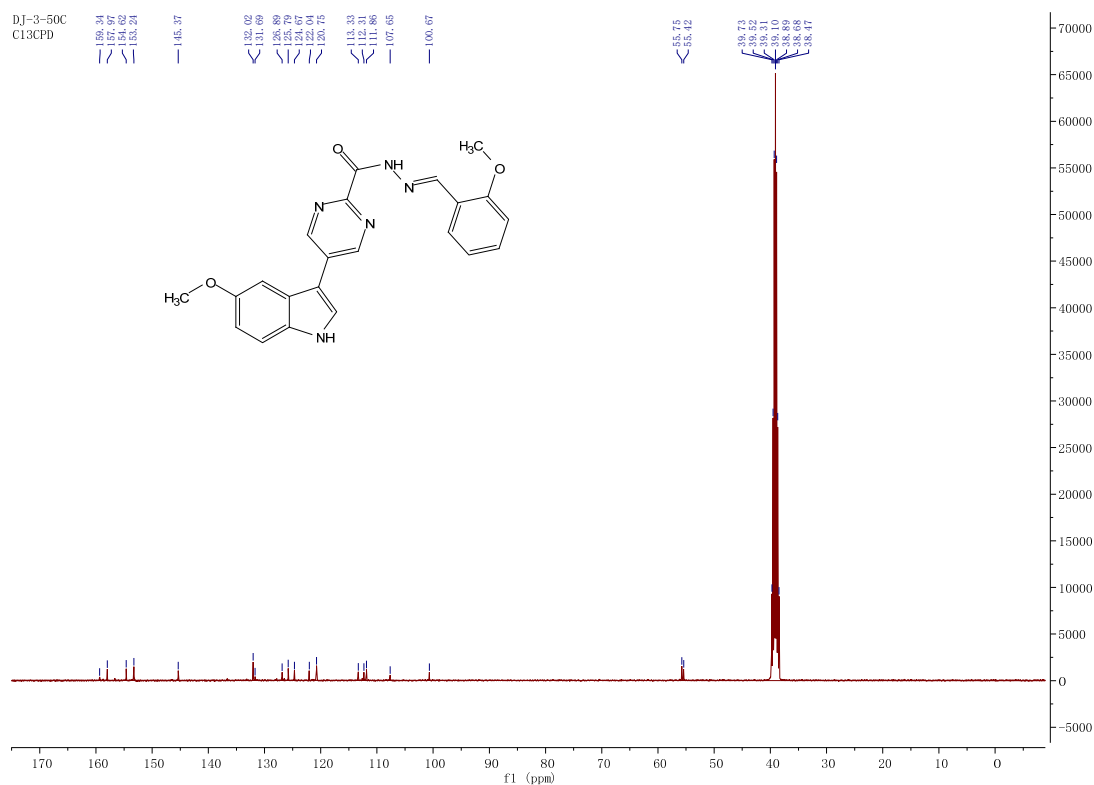


Figure S42. ^{13}C NMR spectrum of 9c

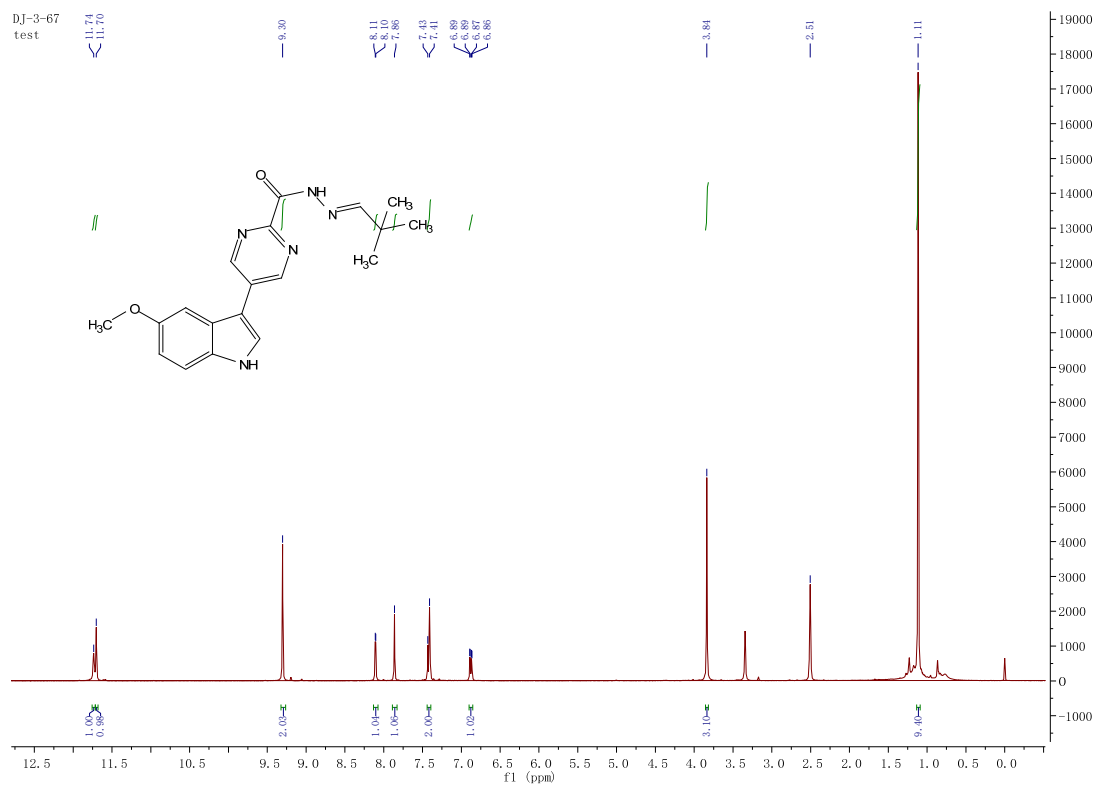


Figure S43. ^1H NMR spectrum of 9d

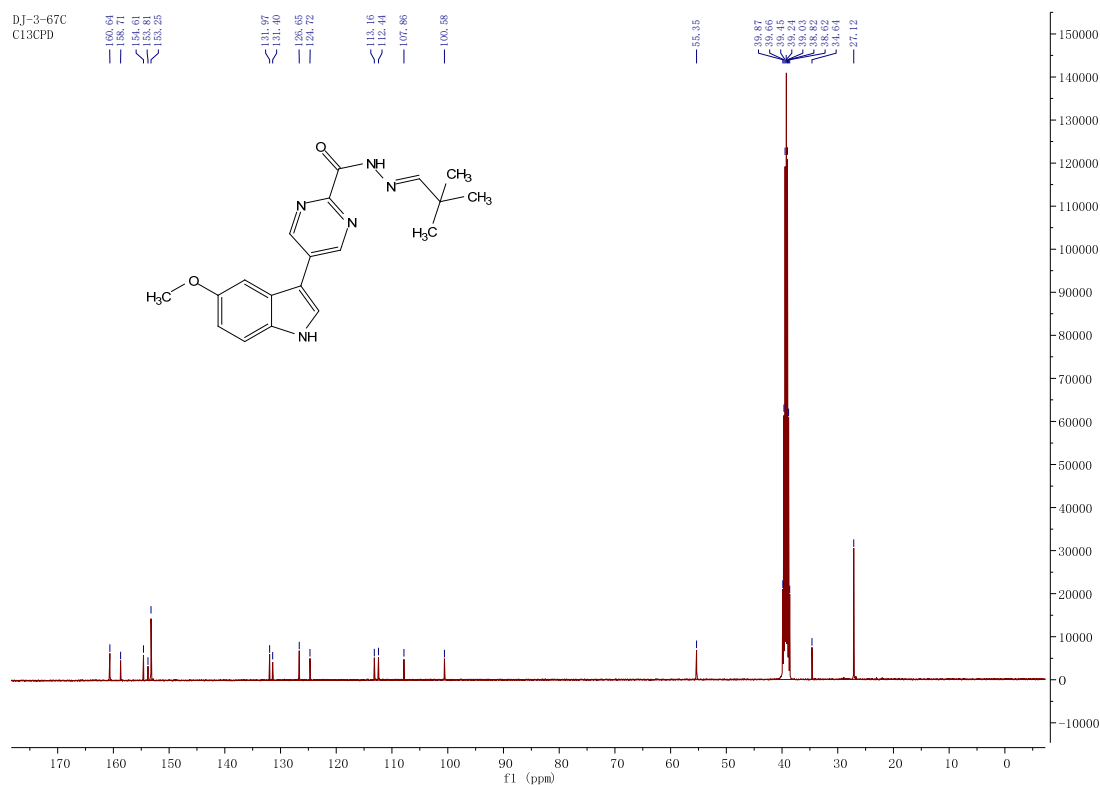
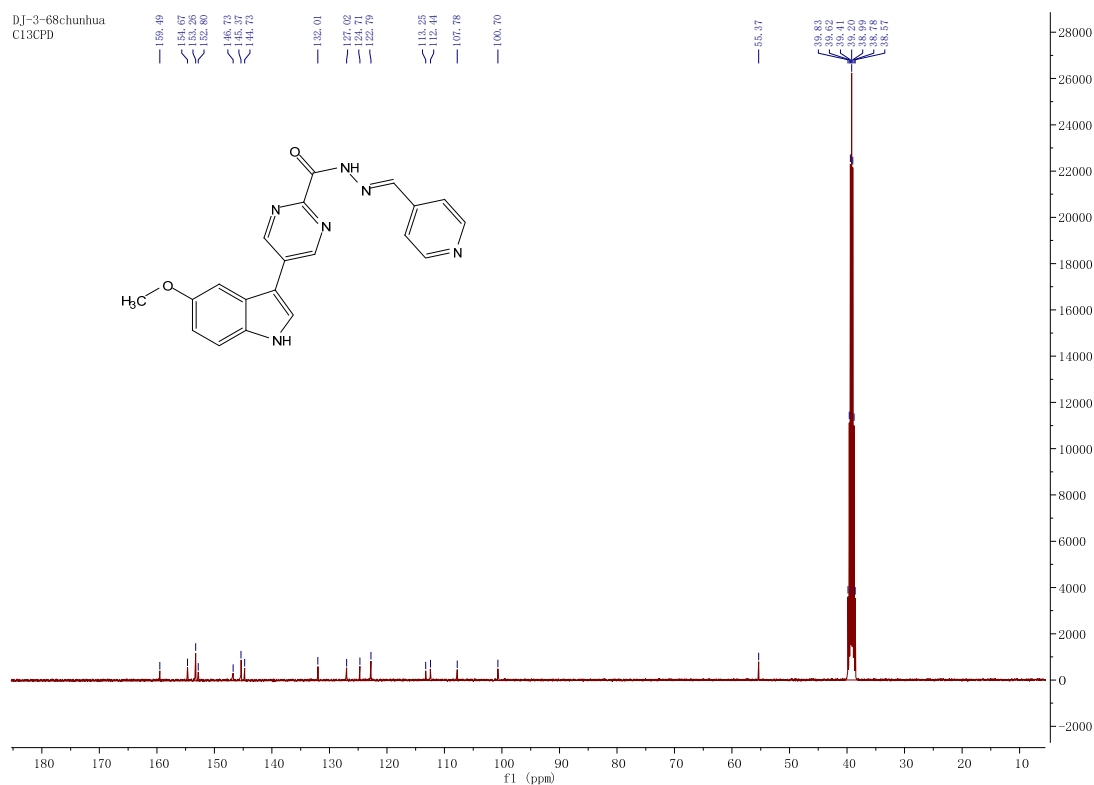
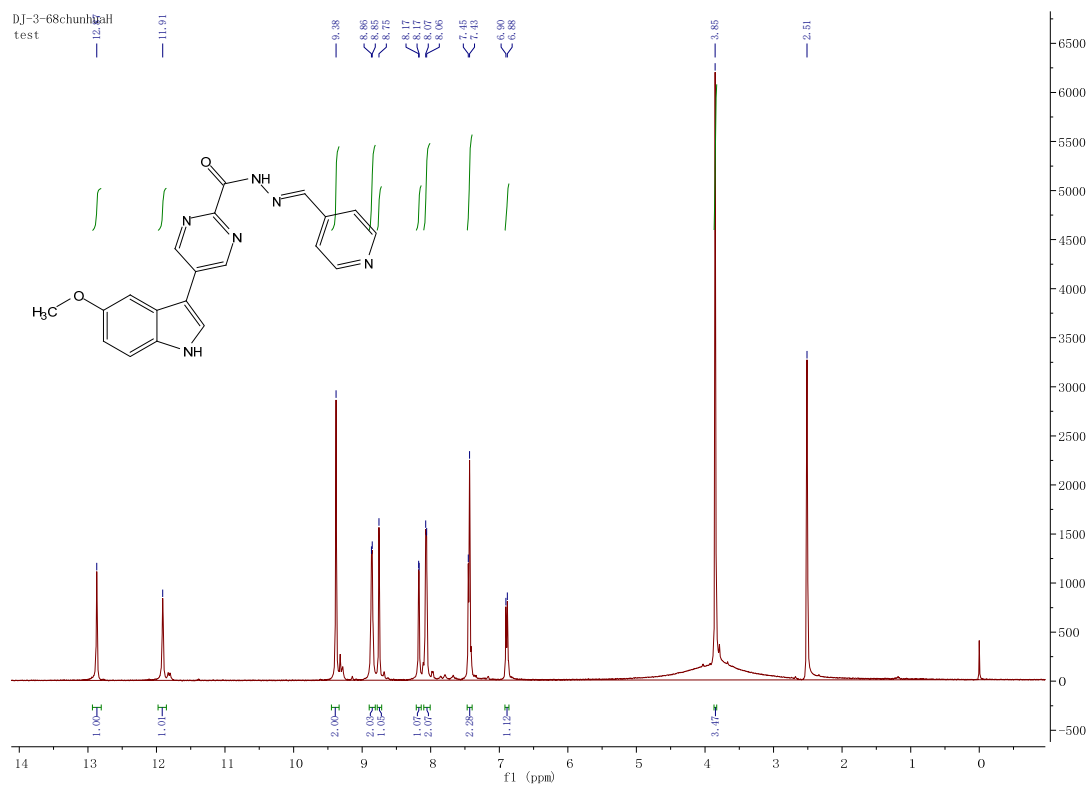
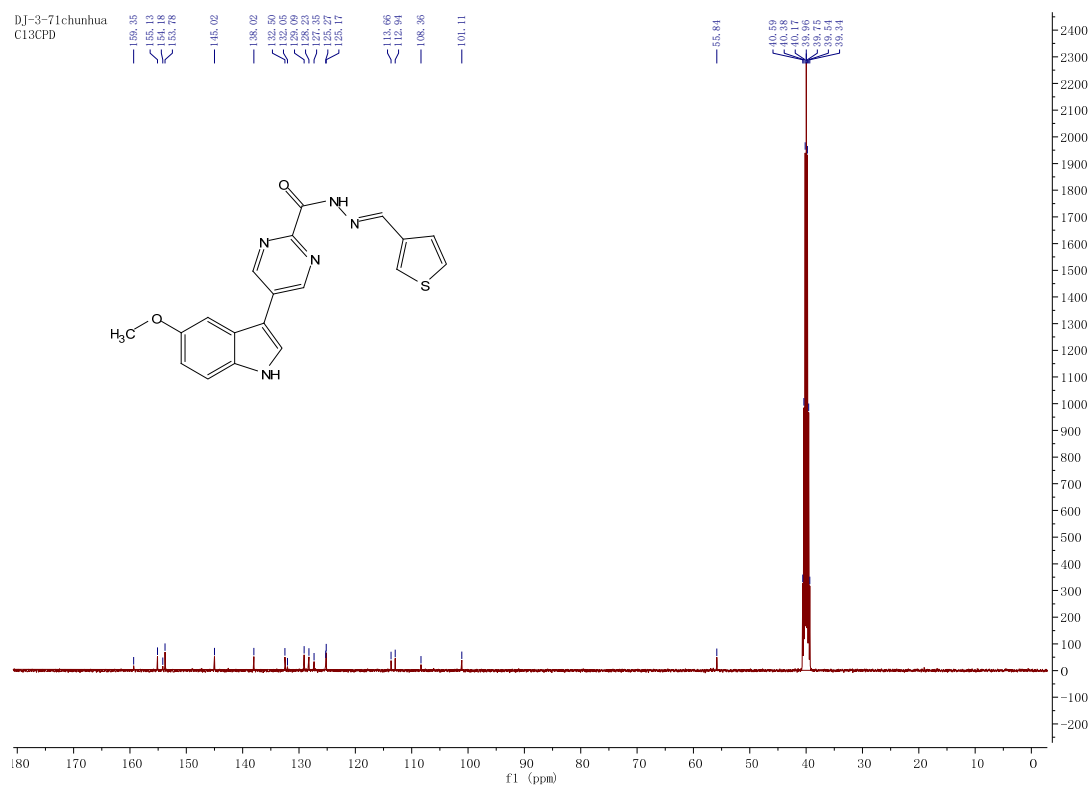
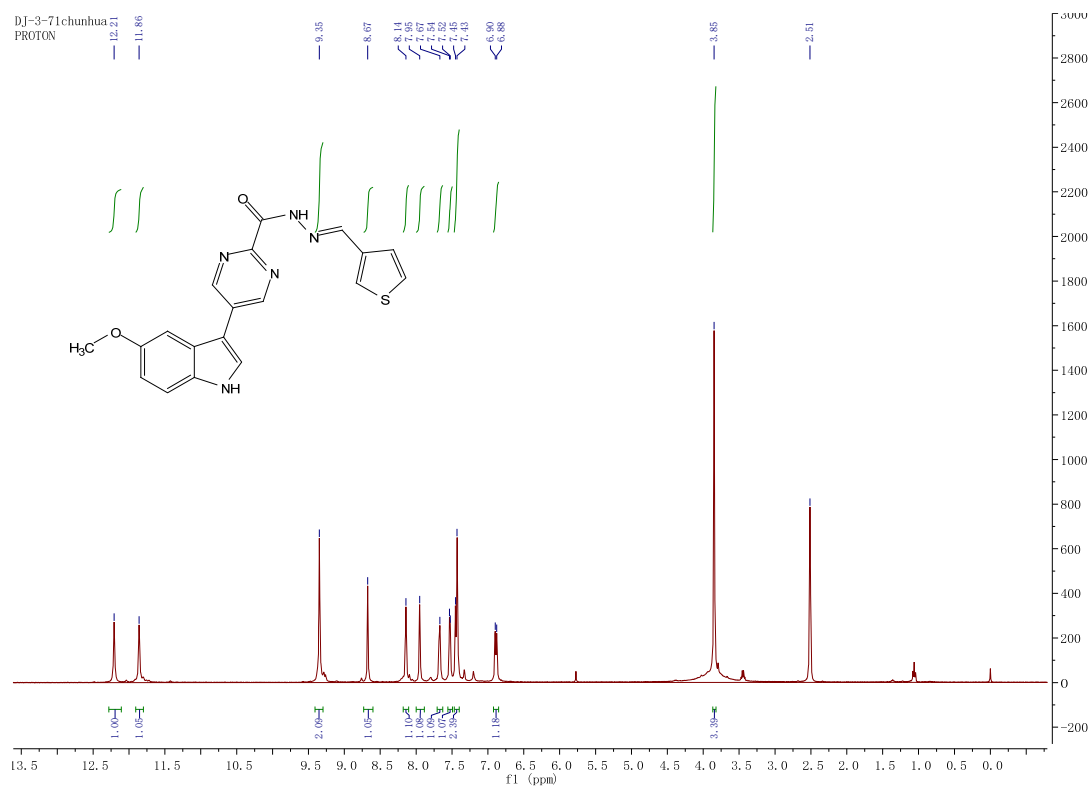


Figure S44. ^{13}C NMR spectrum of 9d





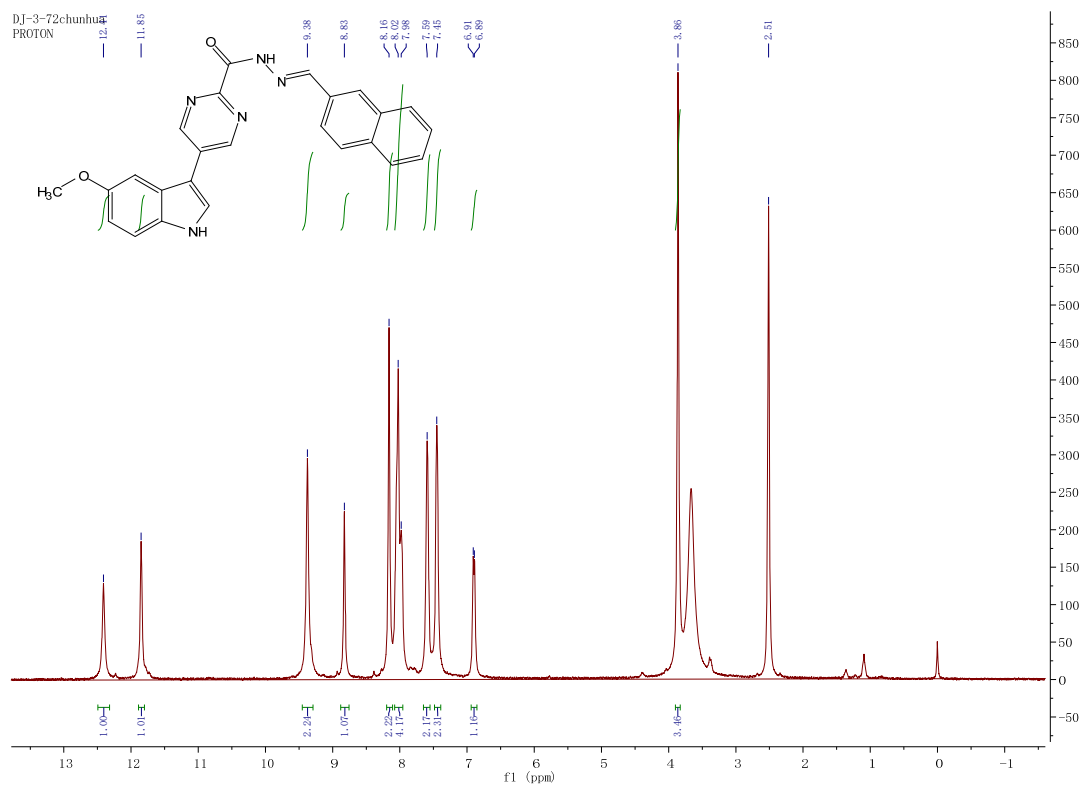


Figure S49. ¹H NMR spectrum of 9g

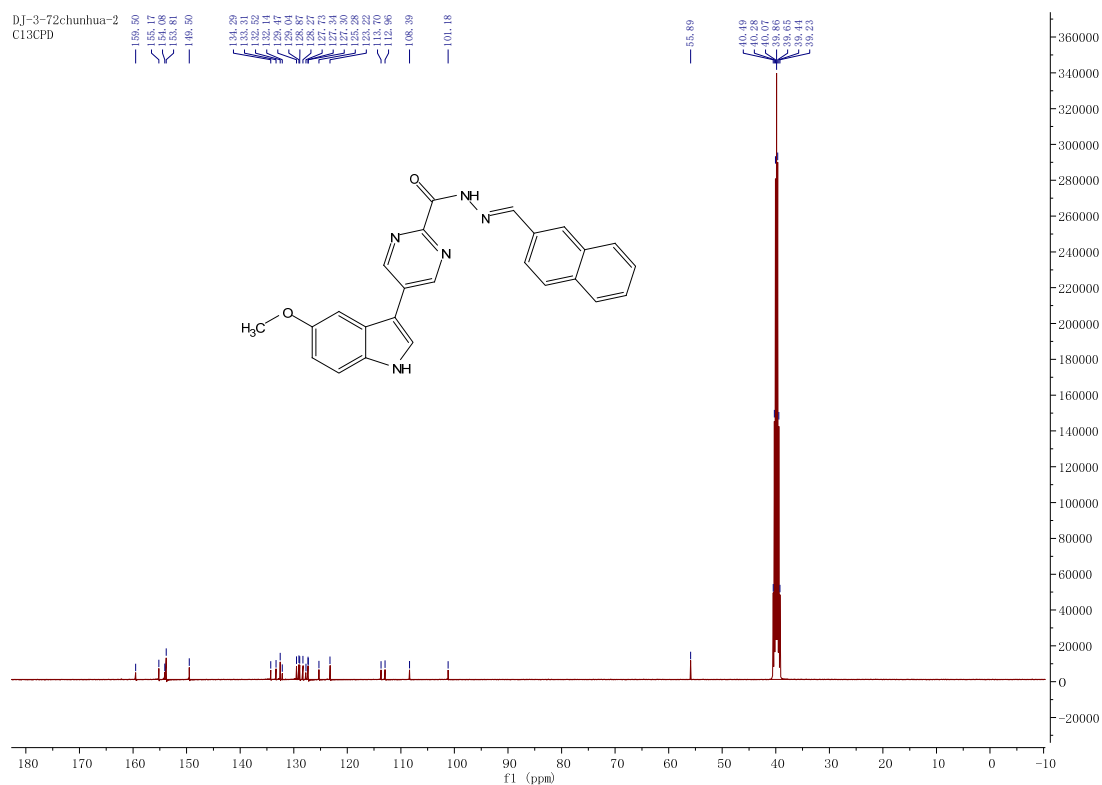


Figure S50. ¹³C NMR spectrum of 9g