

## Supporting Information for

### Novel PKS/NRPS Tenuazamines A–H from the Endophytic Fungus

#### *Alternaria alternata* FL7 Isolated from *Huperzia serrata*

Hao Zhang<sup>1</sup>, Zhibin Zhang<sup>1</sup>, Yiwen Xiao<sup>1,2,\*</sup>, Wen Wang<sup>1</sup>, Boliang Gao<sup>1</sup>, Yuhao Xie<sup>1</sup>, Jiahao Xie<sup>1</sup>, Xinhua Gao<sup>1</sup>, Du Zhu<sup>1,2,\*</sup>

**Abstract:** In this paper, we present a novel class of hybrid polyketides, tenuazamines A–H (1–8), which exhibit a unique tautomeric equilibrium from *Alternaria alternata* FL7. The elucidation of the structures was achieved through a combination of NMR, HR-ESIMS, ECD diverse methods, with a focus on extensive spectroscopic data analysis. Notably, compounds **1**, **4**, **8–9** exhibited potent toxic effects on the growth of *Arabidopsis thaliana*. This research expands the structural diversity of tenuazonic acid compounds derived from endophytic fungi and provides potential hit compounds for the development of herbicides.

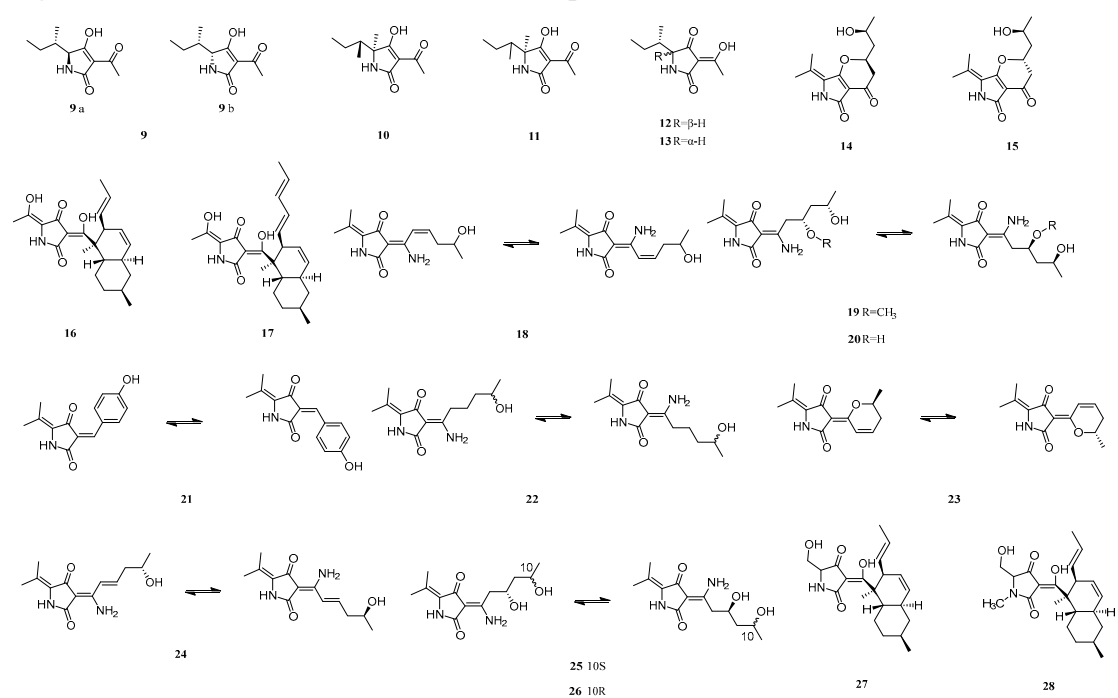
**Keywords:** *Alternaria alternata*; *Huperzia serrata*; tenuazamine; tautomer; herbicides.

# CONTENT

Fig. S1. The chemical structure of TeAs reported.....	1
Fig. S2. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 1.....	1
Fig. S3. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 150Hz) of 1.....	2
Fig. S4. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 1.....	2
Fig. S5. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 1.....	3
Fig. S6. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 1.....	3
Fig. S7. UV spectrum (MeOH) of 1.....	4
Fig. S8. (+)-HR-ESI-MS of compound 1.....	4
Fig. S9. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 2.....	4
Fig. S10. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 100Hz) of 2.....	5
Fig. S11. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 2.....	5
Fig. S12. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 2.....	6
Fig. S13. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 2.....	6
Fig. S14. UV spectrum (MeOH) of 2.....	7
Fig. S15. (+)-HR-ESI-MS of compound 2.....	7
Fig. S16. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 3.....	7
Fig. S17. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 3.....	8
Fig. S18. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 3.....	8
Fig. S19. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 3.....	9
Fig. S20. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 3.....	9
Fig. S21. UV spectrum (MeOH) of 3.....	10
Fig. S22. (+)-HR-ESI-MS of compound 3.....	10
Fig. S23. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 4.....	10
Fig. S24. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 4.....	11
Fig. S25. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 4.....	11
Fig. S26. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 4.....	12
Fig. S27. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 4.....	12
Fig. S28. UV spectrum (MeOH) of 4.....	13
Fig. S29. (+)-HR-ESI-MS of compound 4.....	13
Fig. S30. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 5.....	13
Fig. S31. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 5.....	14
Fig. S32. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 5.....	14
Fig. S33. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 5.....	15
Fig. S34. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 5.....	15
Fig. S35. UV spectrum (MeOH) of 5.....	16
Fig. S36. (+)-HR-ESI-MS of compound 5.....	16
Fig. S37. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 6.....	16
Fig. S38. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 6.....	17
Fig. S39. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 6.....	17
Fig. S40. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 6.....	18
Fig. S41. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 6.....	18
Fig. S42. UV spectrum (MeOH) of 6.....	19

Fig. S43. (+)-HR-ESI-MS of compound 6.....	19
Fig. S44. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 7.....	19
Fig. S45. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 7.....	20
Fig. S46. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 7.....	20
Fig. S47. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 7.....	21
Fig. S48. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 400Hz) of 7.....	21
Fig. S49. UV spectrum (MeOH) of 7.....	22
Fig. S50. (+)-HR-ESI-MS of compound 7.....	22
Fig. S51. <sup>1</sup> H NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 8.....	22
Fig. S52. <sup>13</sup> C NMR spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 8.....	23
Fig. S53. <sup>1</sup> H- <sup>1</sup> H COSY spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 8.....	23
Fig. S54. HMBC spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 8.....	24
Fig. S55. HSQC spectrum (DMSO- <i>d</i> <sub>6</sub> , 600Hz) of 8.....	24
Fig. S56. (+)-HR-ESI-MS of compound 8.....	25

**Fig. S1. The chemical structure of TeAs that reported**



**Fig. S2. <sup>1</sup>H NMR spectrum (DMSO-*d*<sub>6</sub>, 600Hz) of 1**

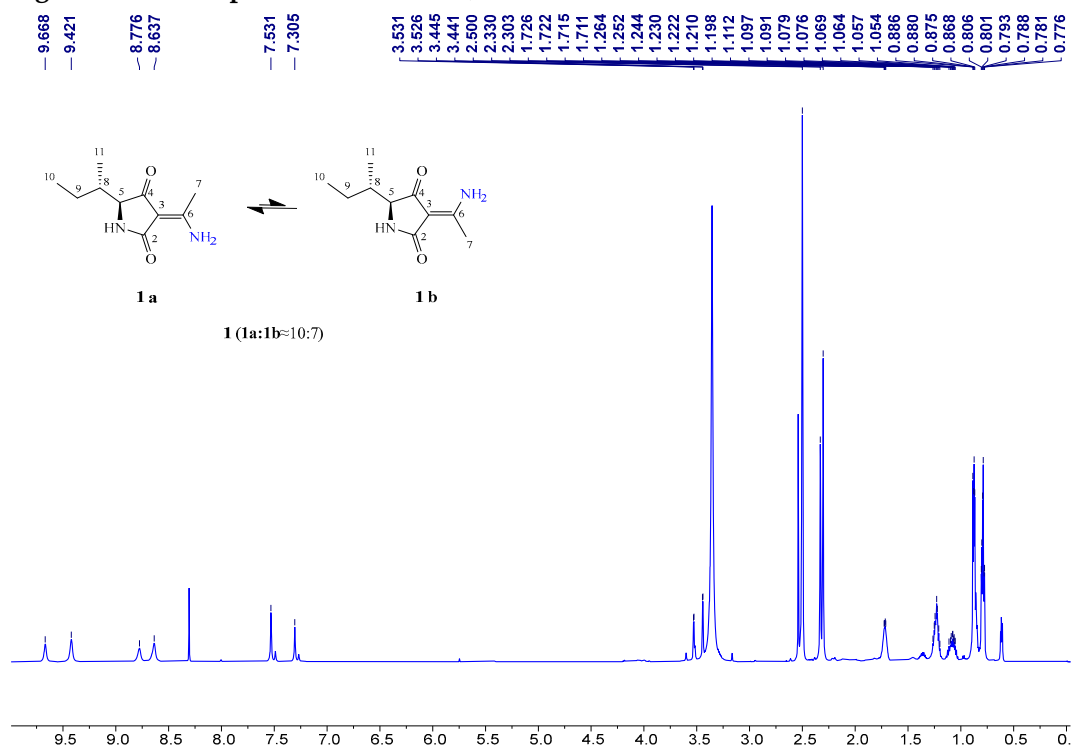


Fig. S3.  $^{13}\text{C}$  NMR spectrum (DMSO-*d*<sub>6</sub>, 150Hz) of 1

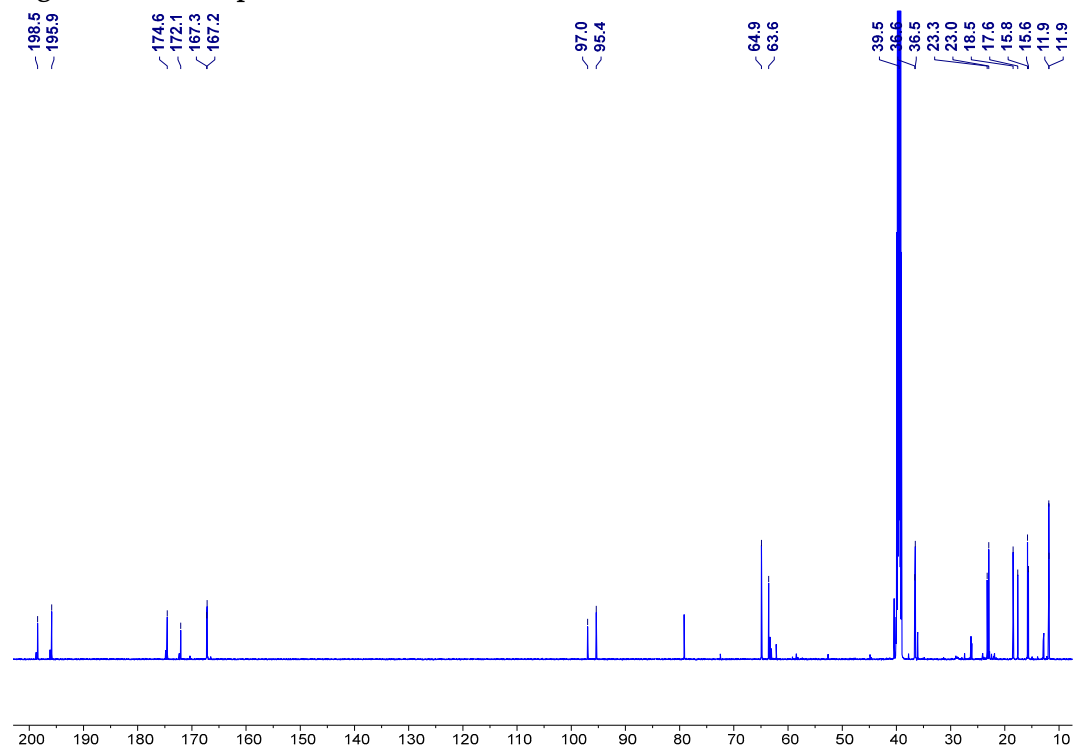


Fig. S4.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO-*d*<sub>6</sub>, 600Hz) of 1

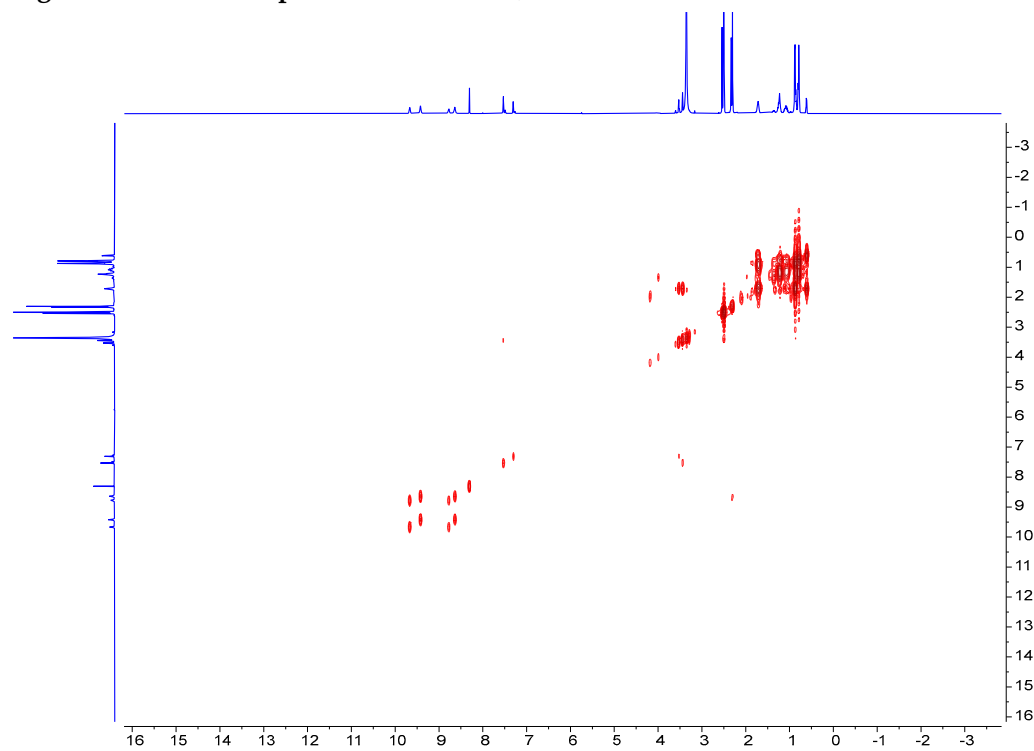


Fig. S5. HMBC spectrum (DMSO-*d*<sub>6</sub>, 600Hz) of 1

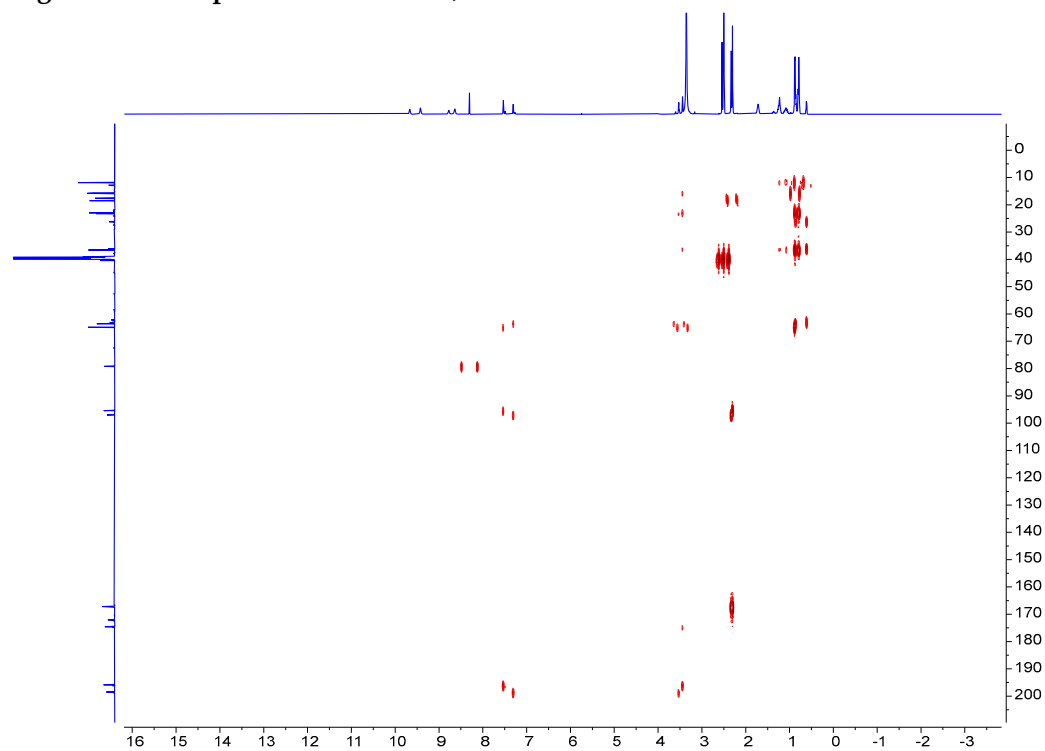


Fig. S6. HSQC spectrum (DMSO-*d*<sub>6</sub>, 600Hz) of 1

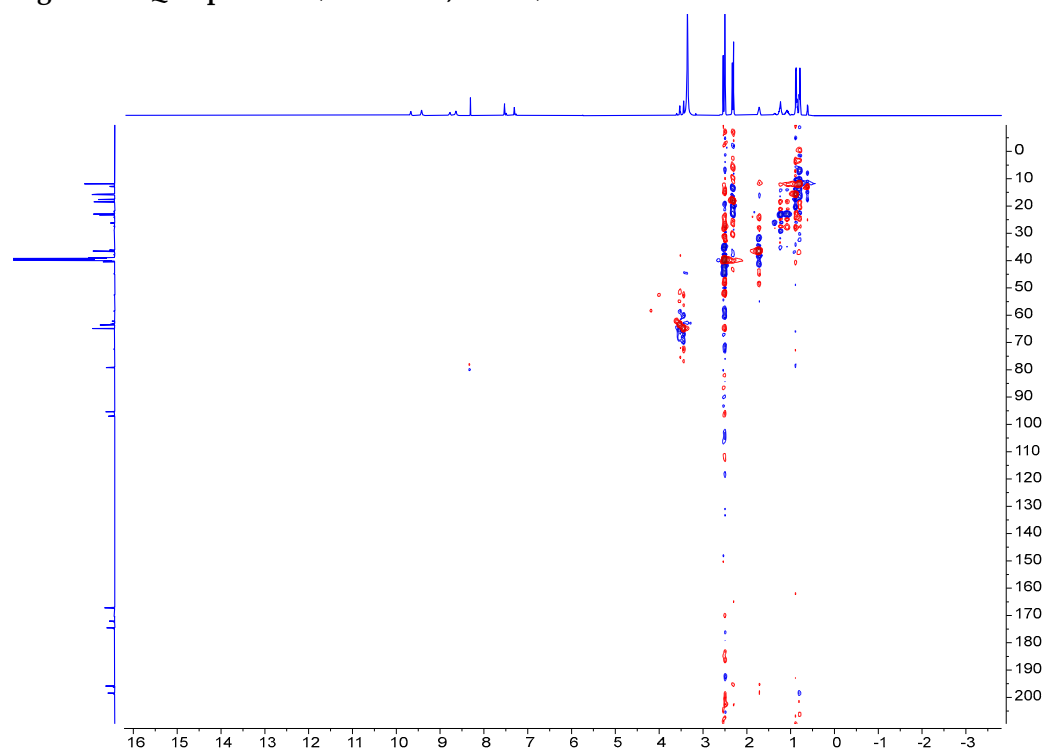


Fig. S7. UV spectrum (MeOH) of 1

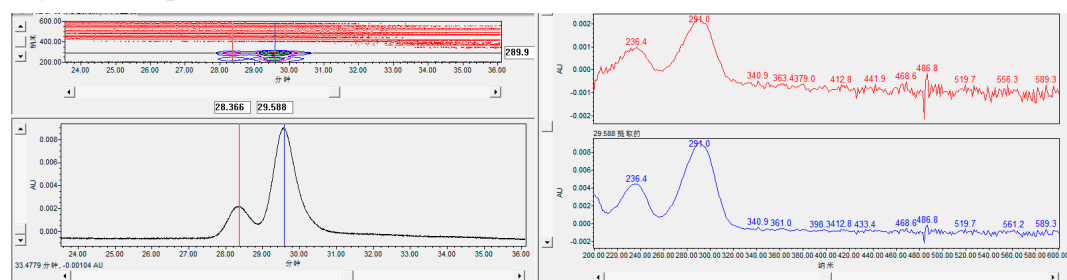


Fig. S8. (+)-HR-ESI-MS of compound 1

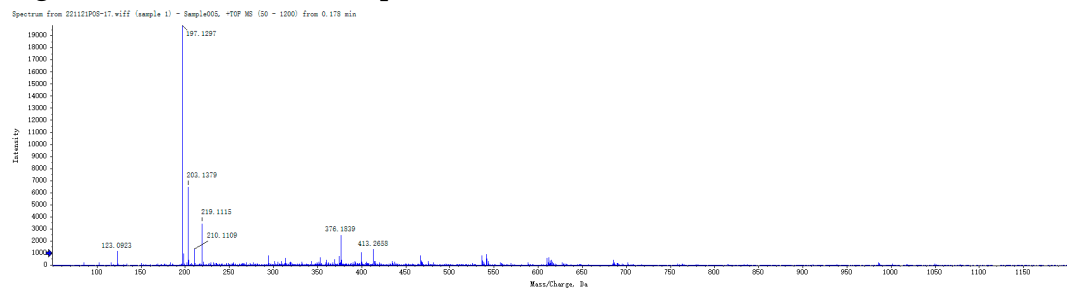


Fig. S9.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 2

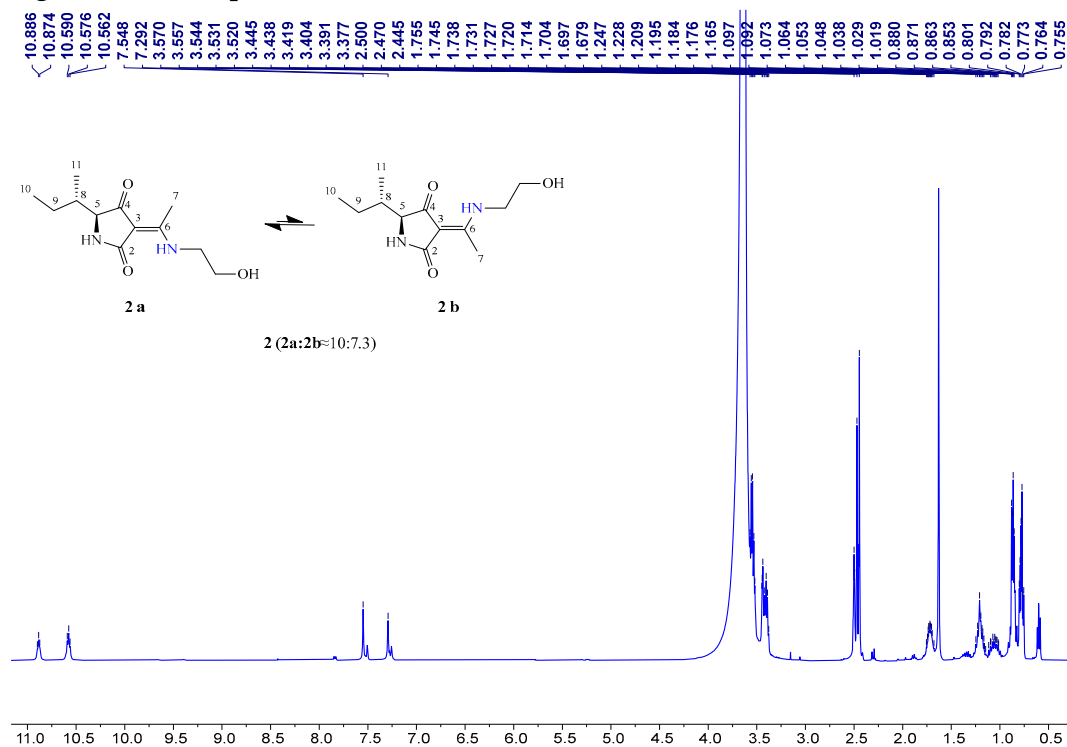


Fig. S10.  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ , 100Hz) of 2

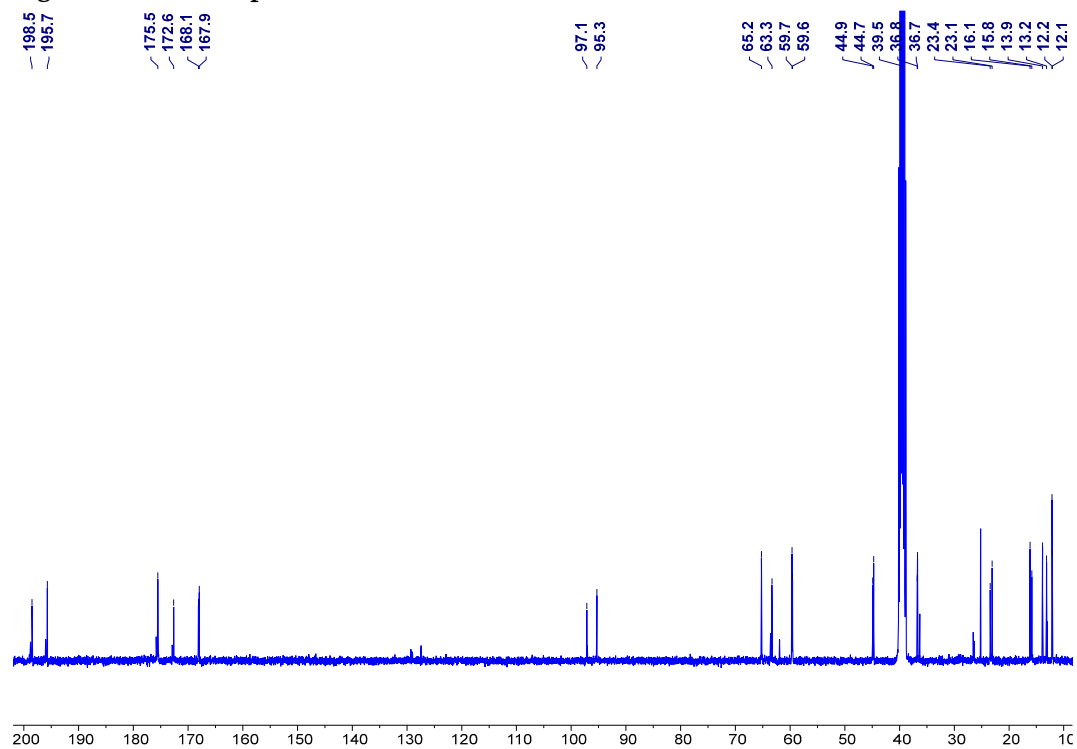


Fig. S11.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO- $d_6$ , 400Hz) of 2

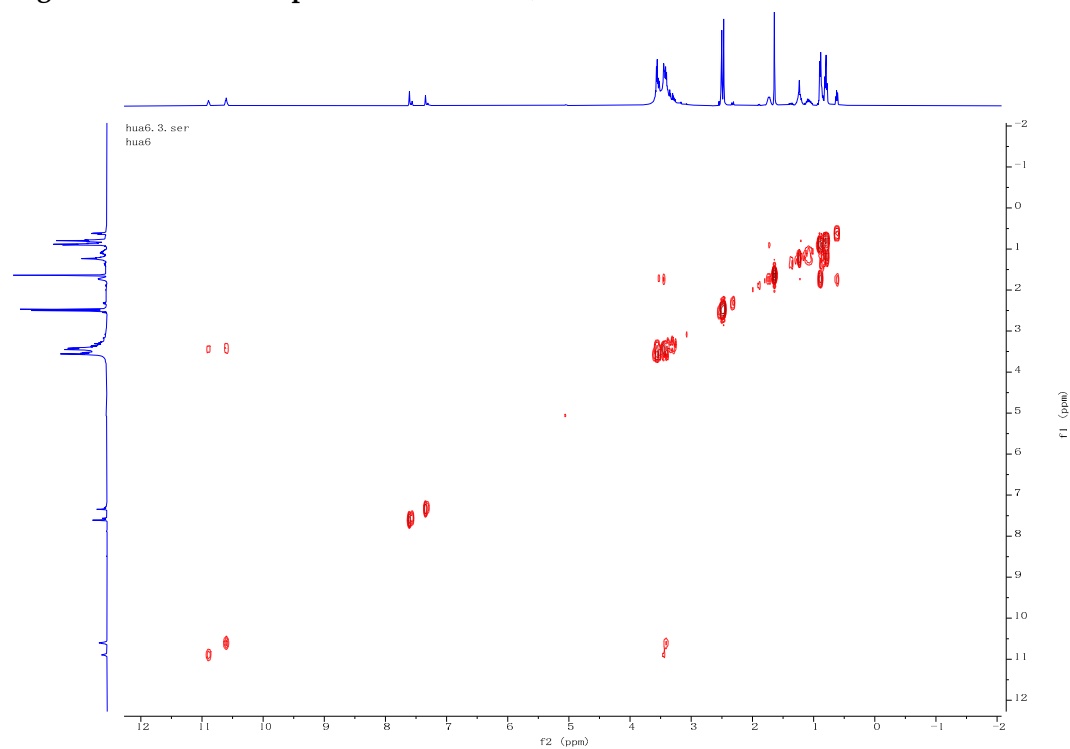




Fig. S12. HMBC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 2

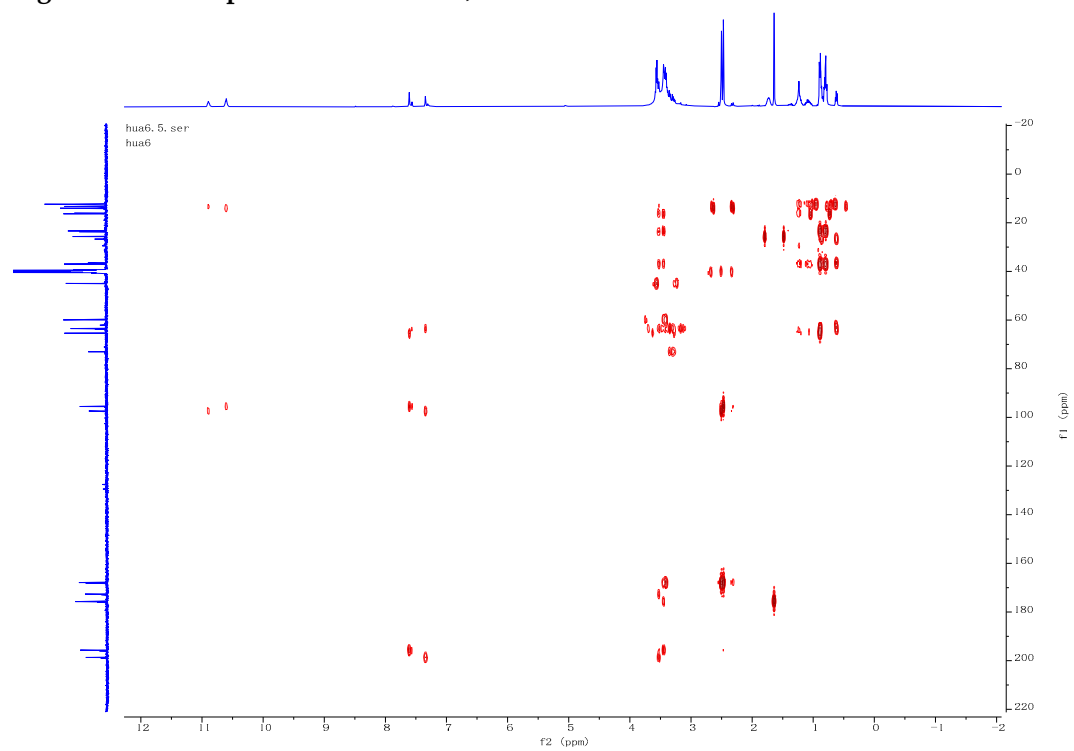


Fig. S13. HSQC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 2

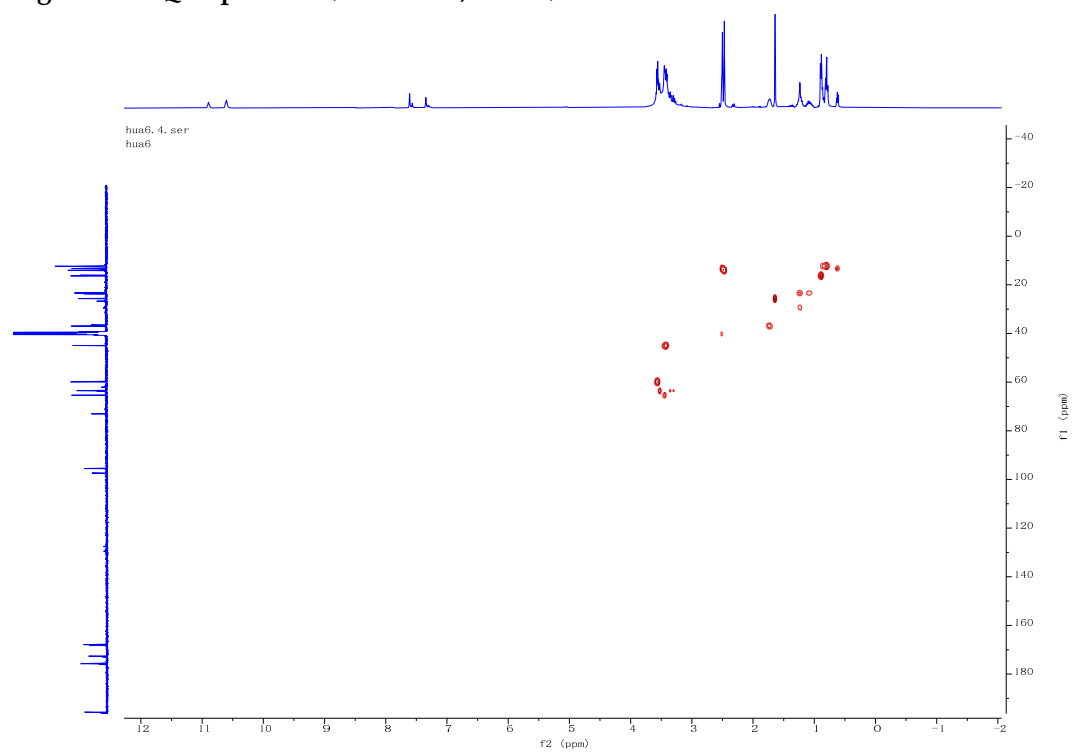


Fig. S14. UV spectrum (MeOH) of 2

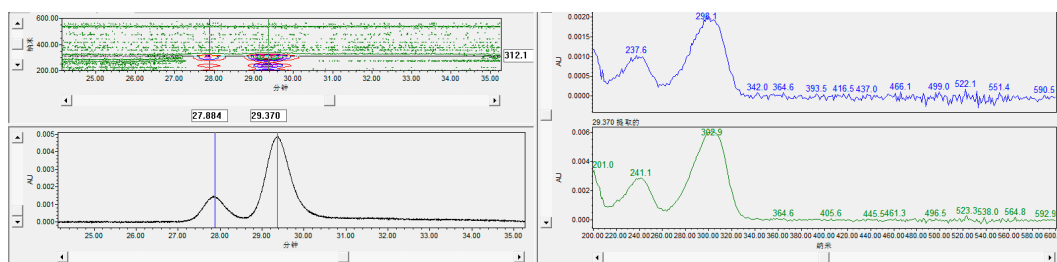


Fig. S15. (+)-HR-ESI-MS of compound 2

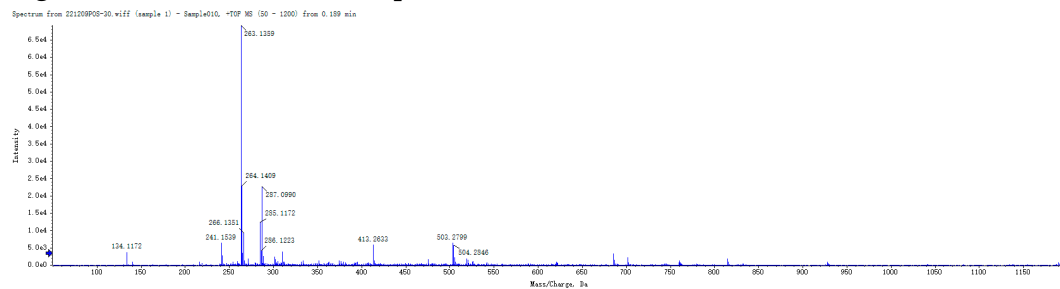


Fig. S16.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 3

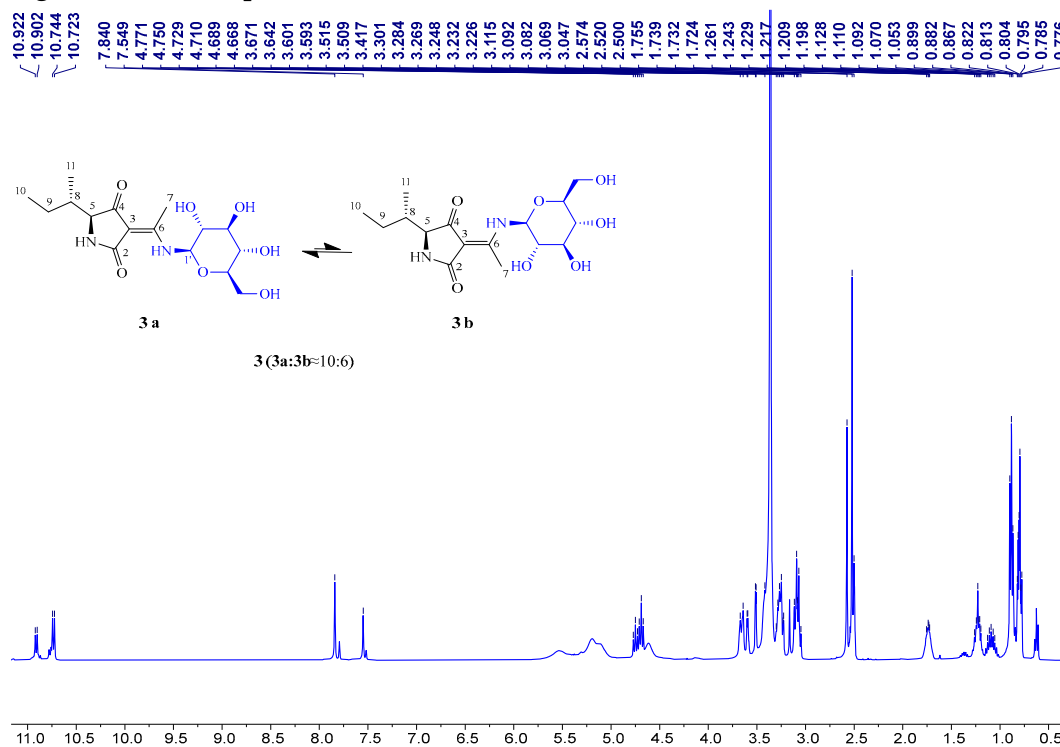


Fig. S17.  $^{13}\text{C}$  NMR spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 3

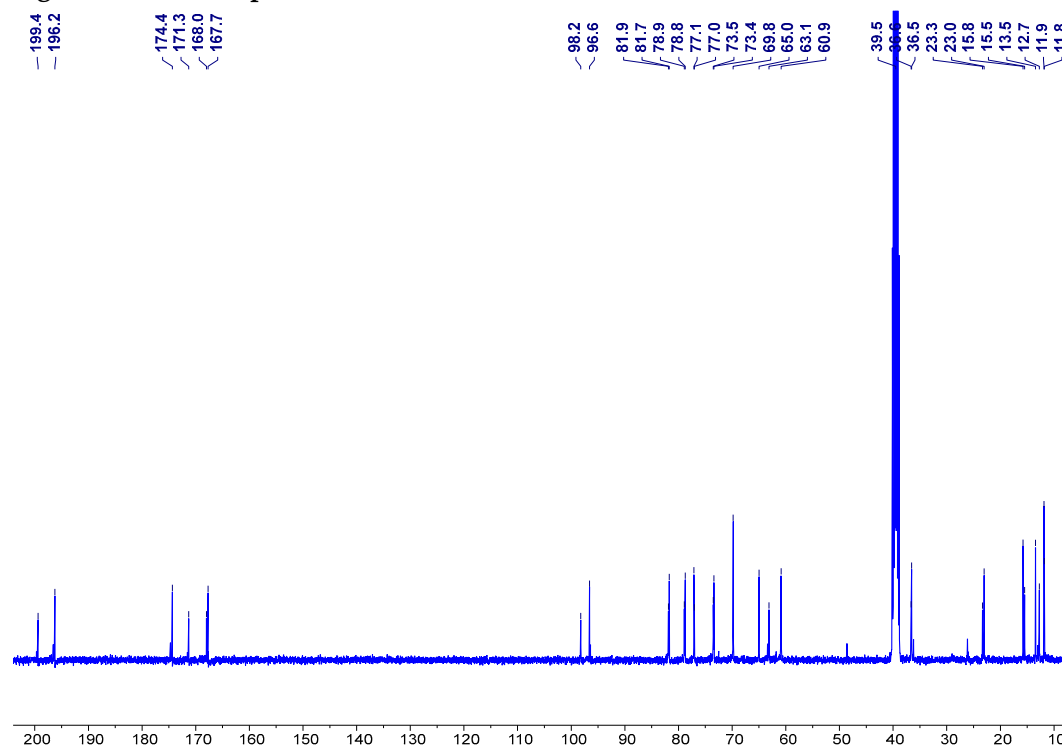


Fig. S18.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 3

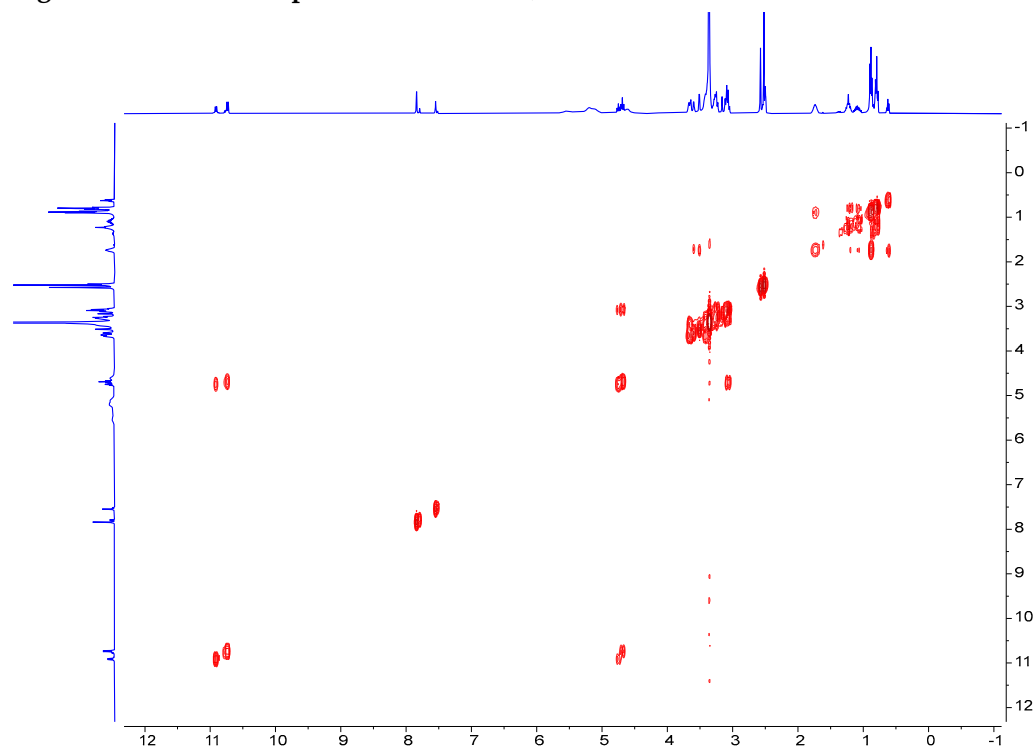


Fig. S19. HMBC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 3

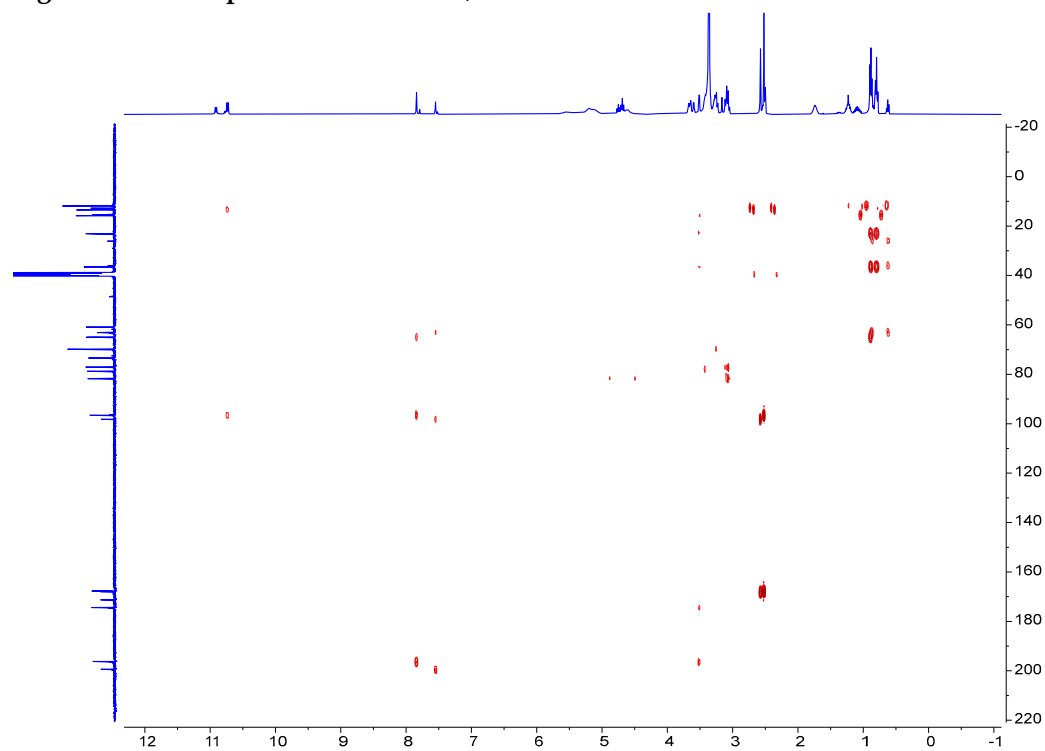


Fig. S20. HSQC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 3

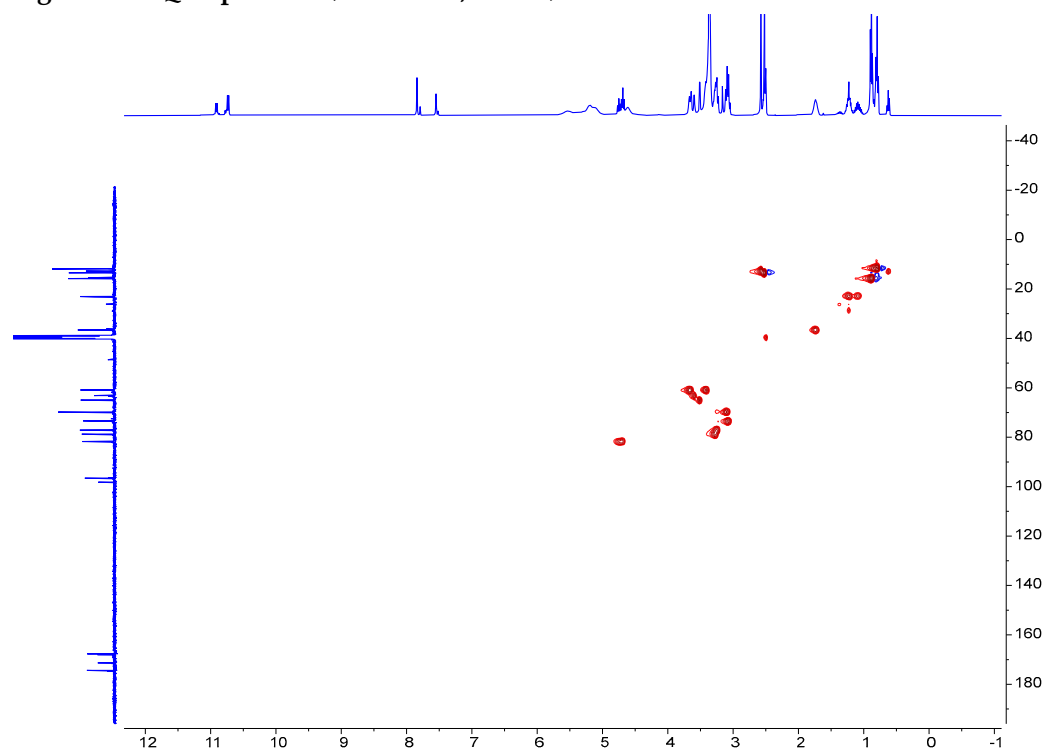


Fig. S21. UV spectrum (MeOH) of 3

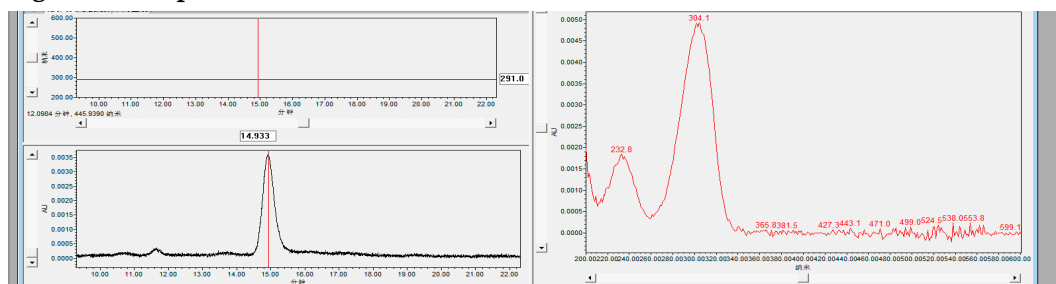


Fig. S22. (+)-HR-ESI-MS of compound 3

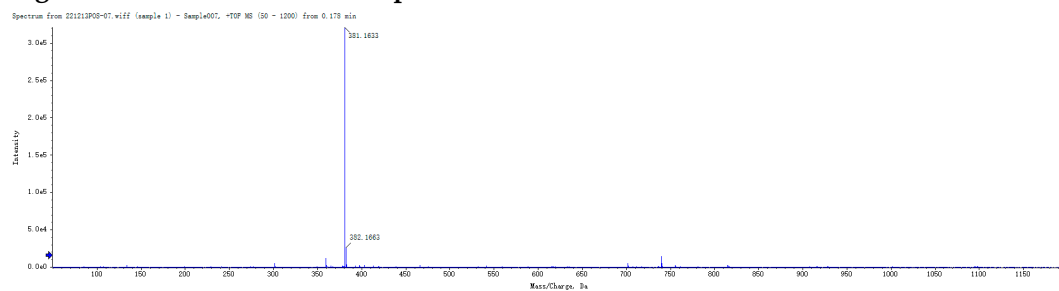


Fig. S23.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 4

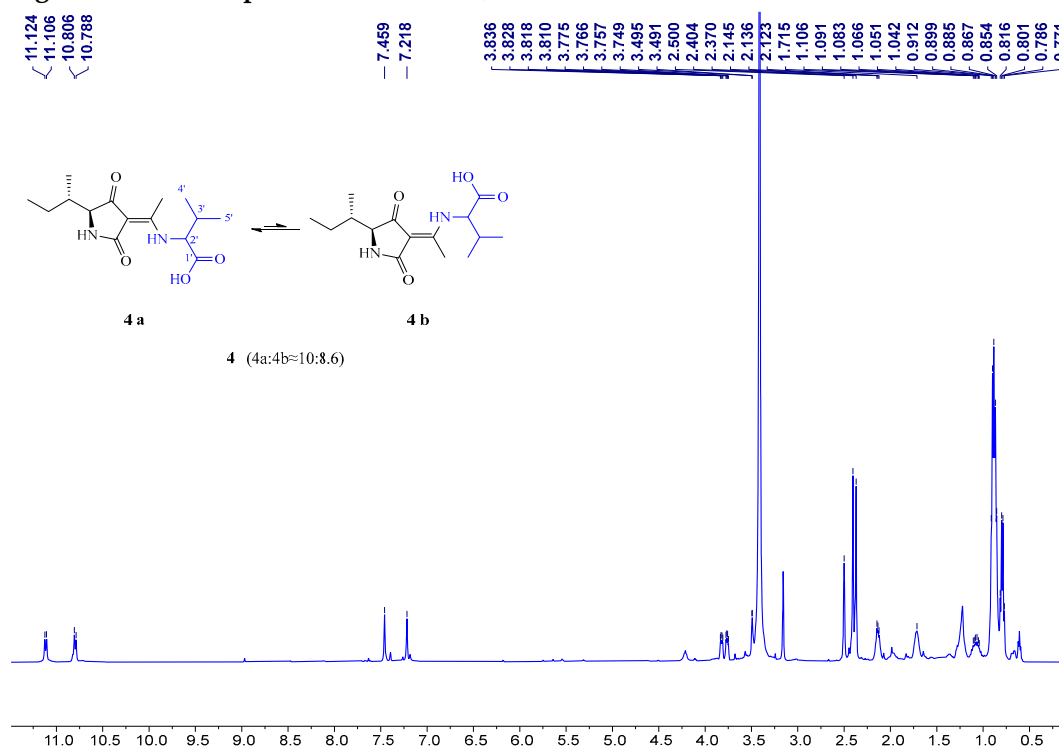


Fig. S24.  $^{13}\text{C}$  NMR spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 4

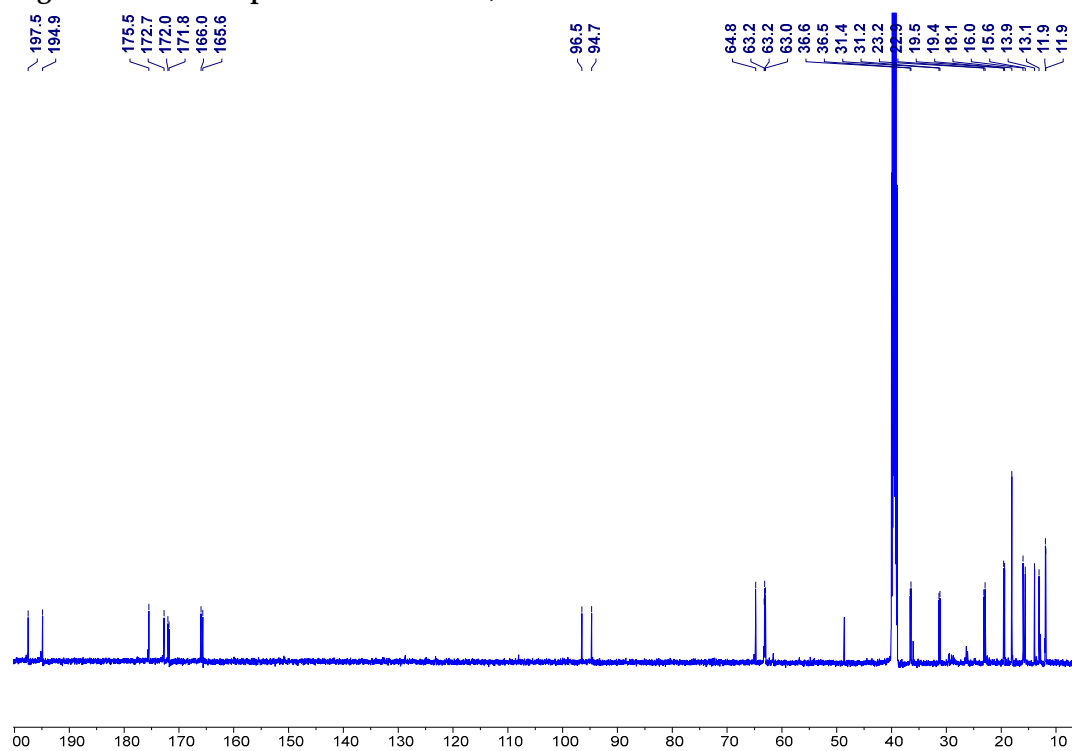


Fig. S25.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 4

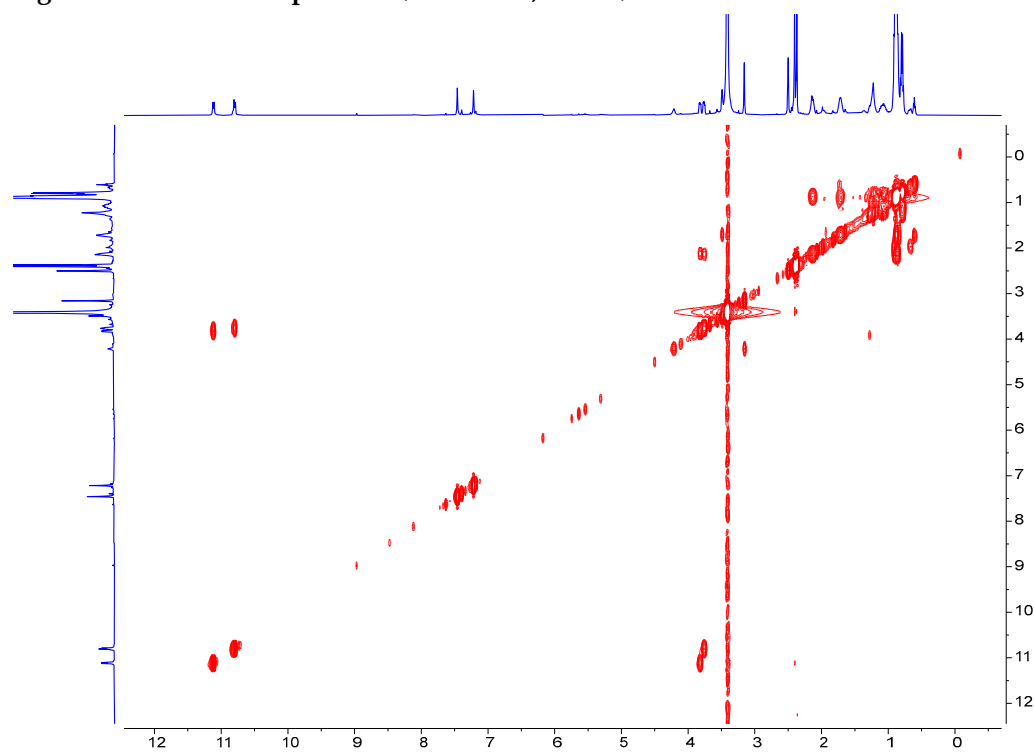


Fig. S26. HMBC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 4

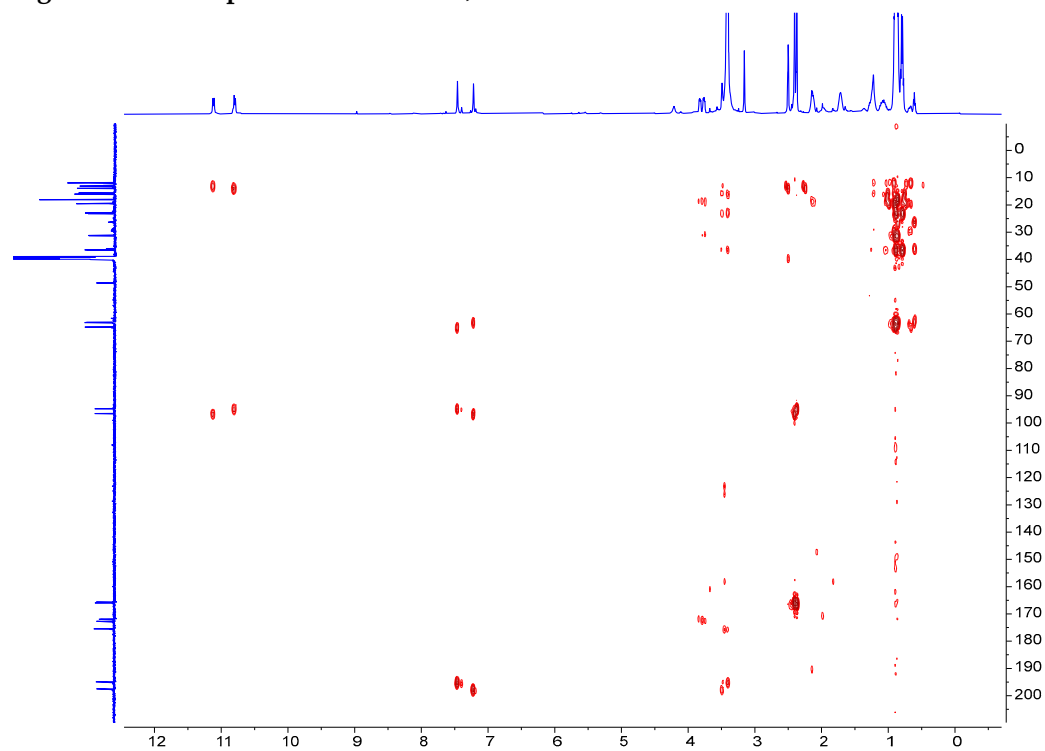


Fig. S27. HSQC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 4

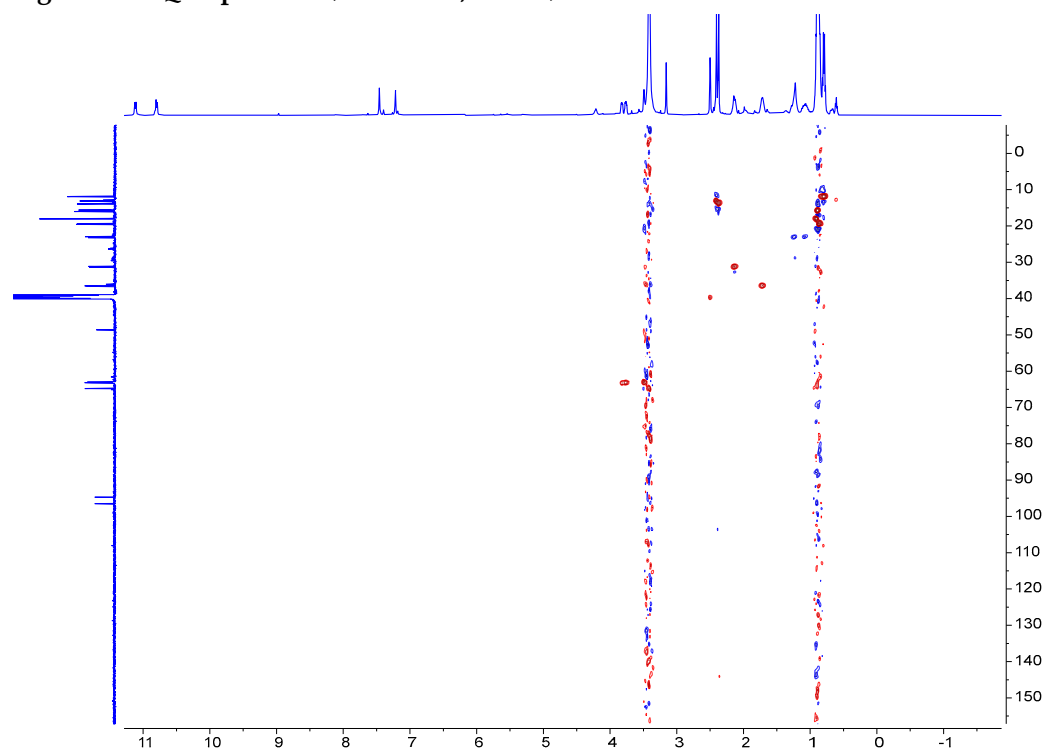


Fig. S28. UV spectrum (MeOH) of 4

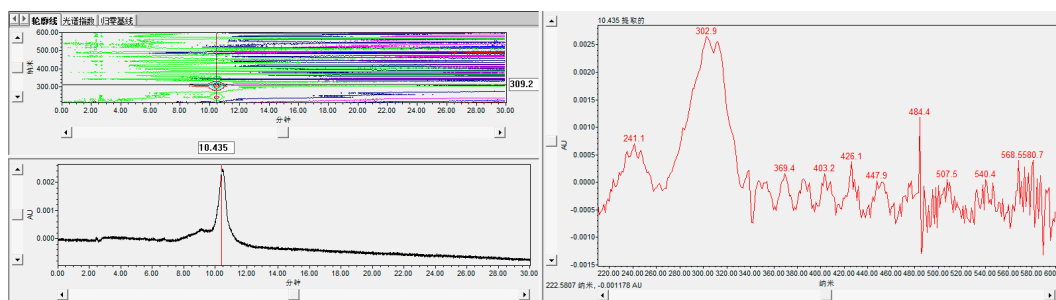


Fig. S29. (+)-HR-ESI-MS of compound 4

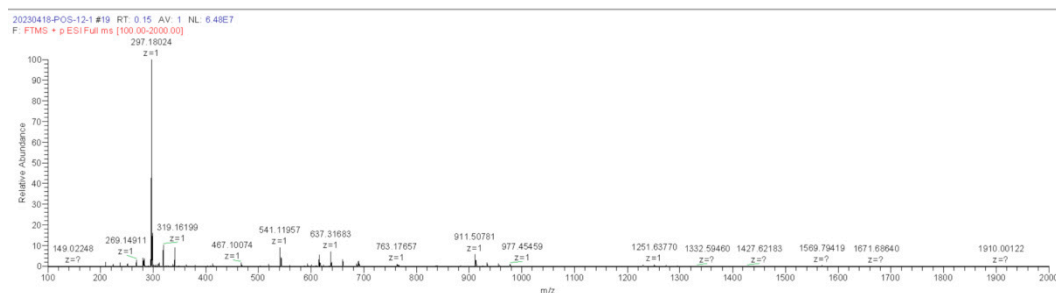


Fig. S30.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 5

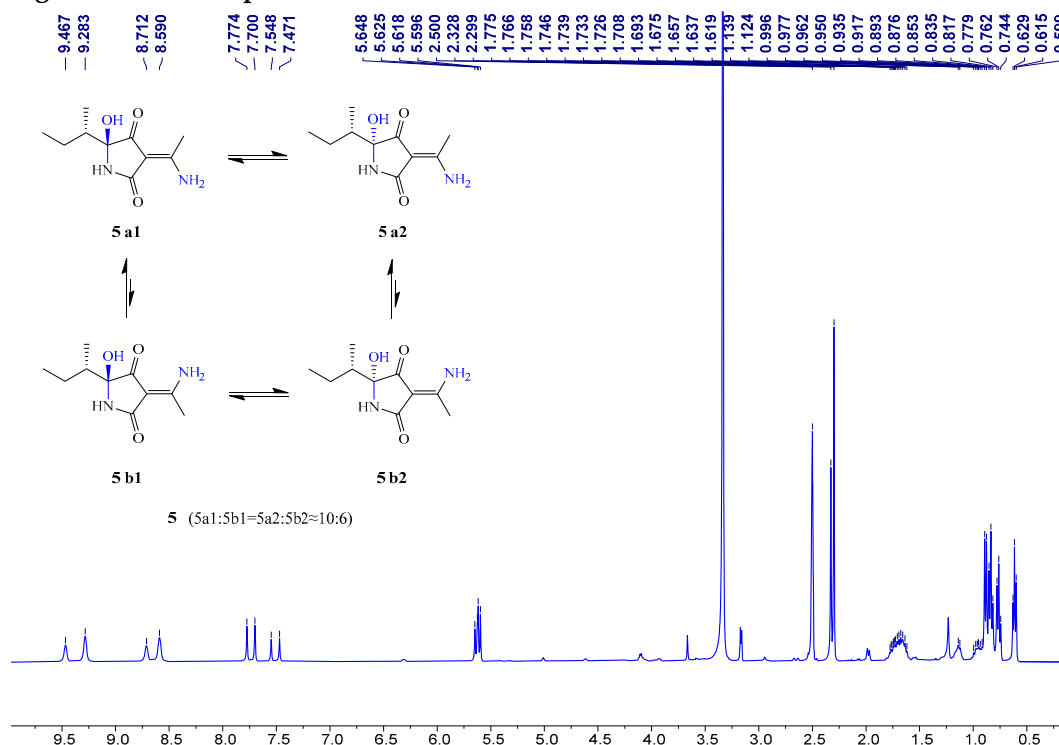




Fig. S31.  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 5

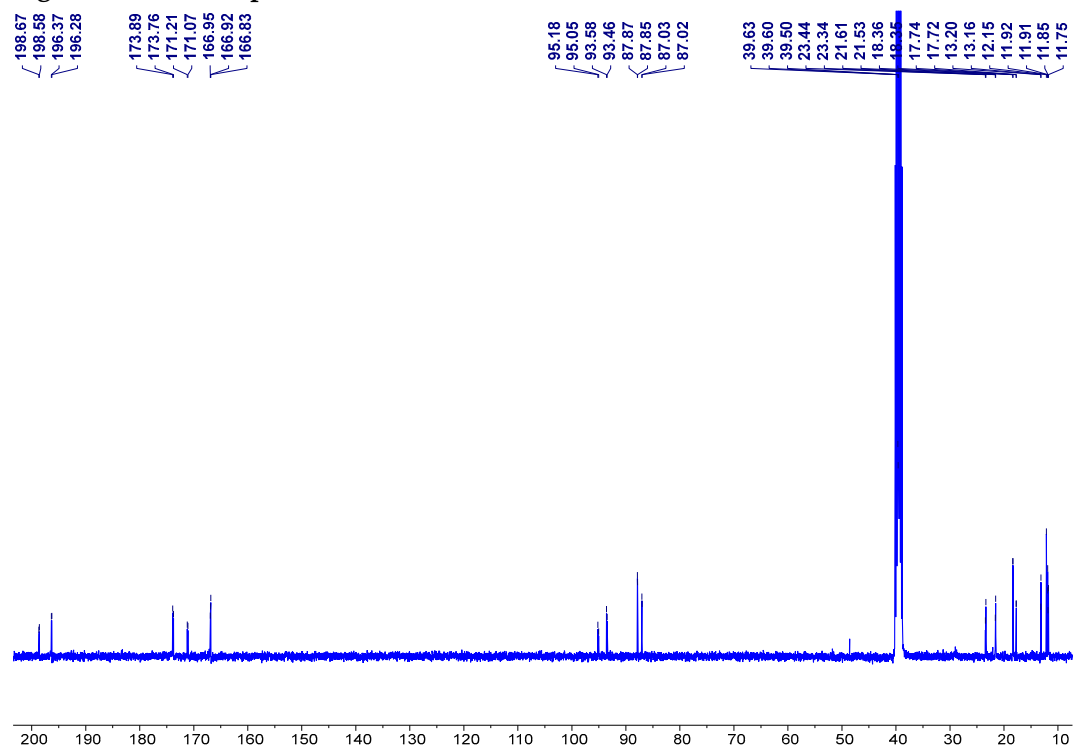


Fig. S32.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO- $d_6$ , 400Hz) of 5

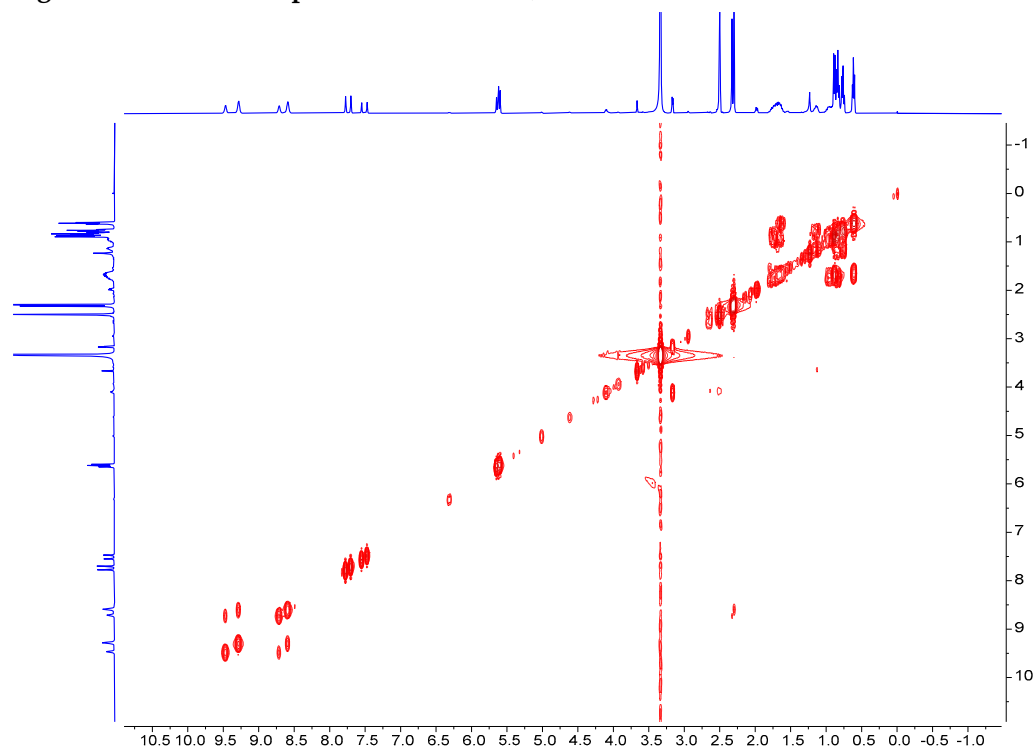


Fig. S33. HMBC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 5

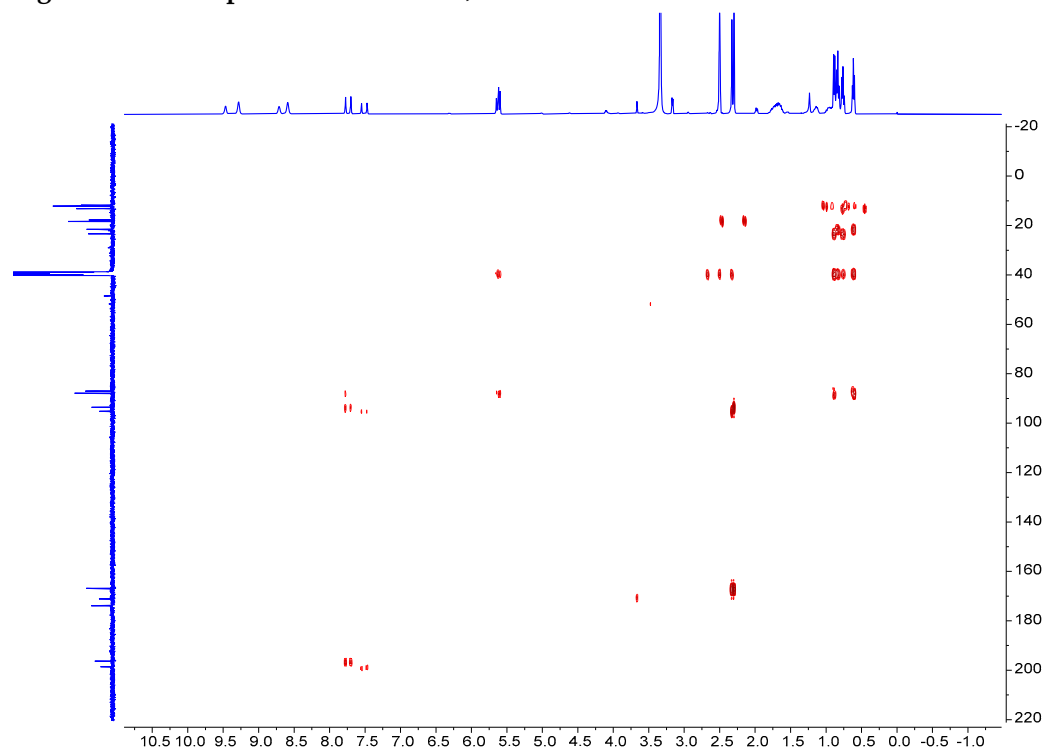


Fig. S34. HSQC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 5

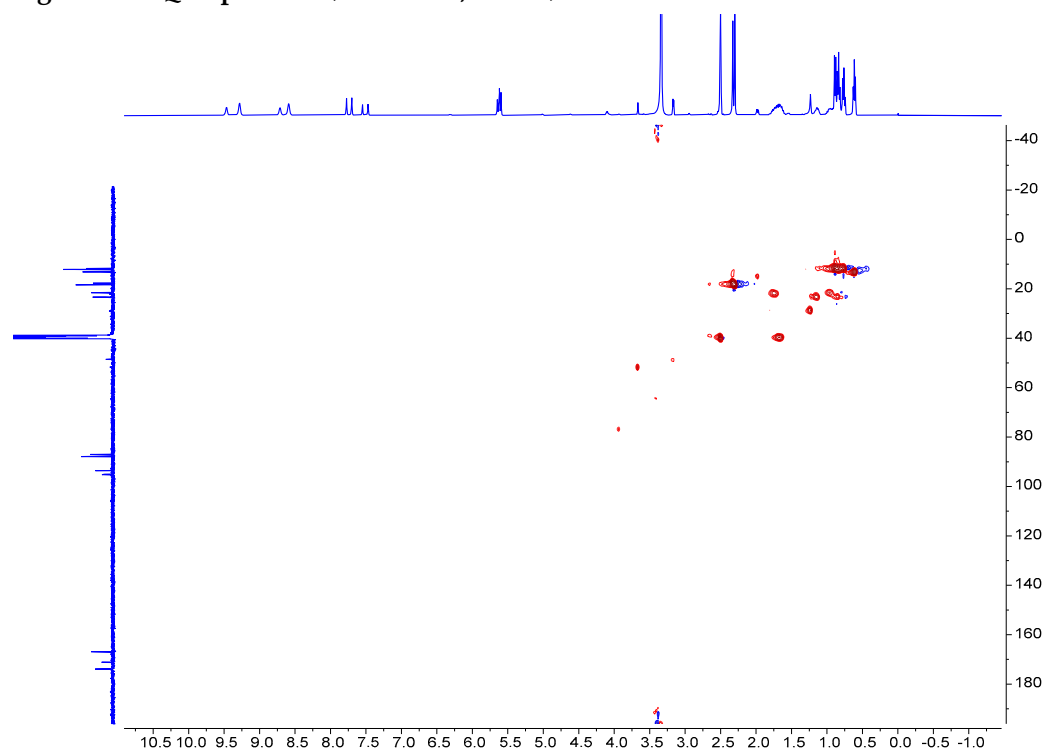


Fig. S35. UV spectrum (MeOH) of 5

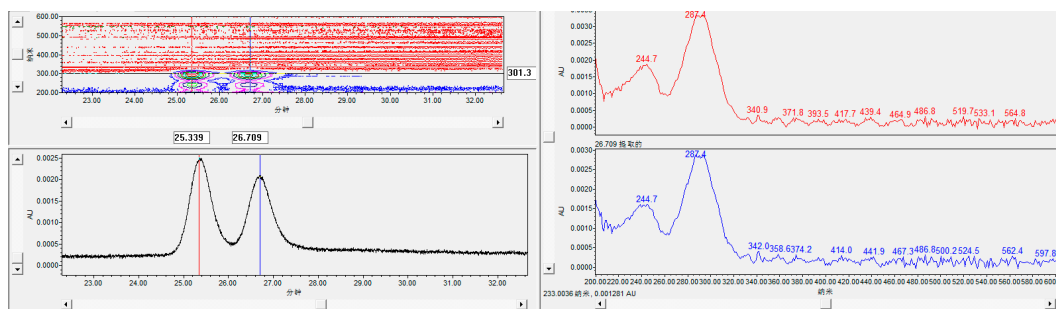


Fig. S36. (-)-HR-ESI-MS of compound 5

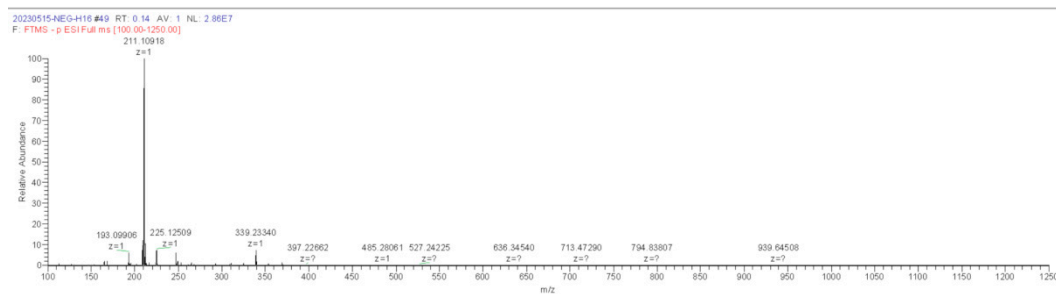


Fig. S37.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 6

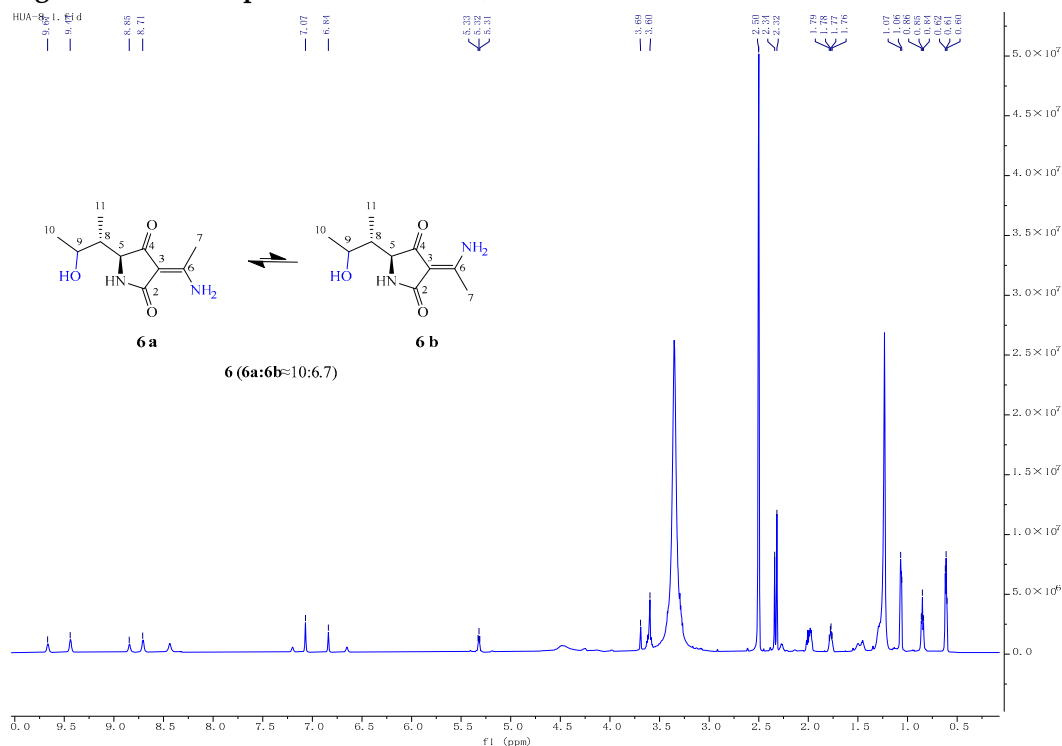


Fig. S38.  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 6

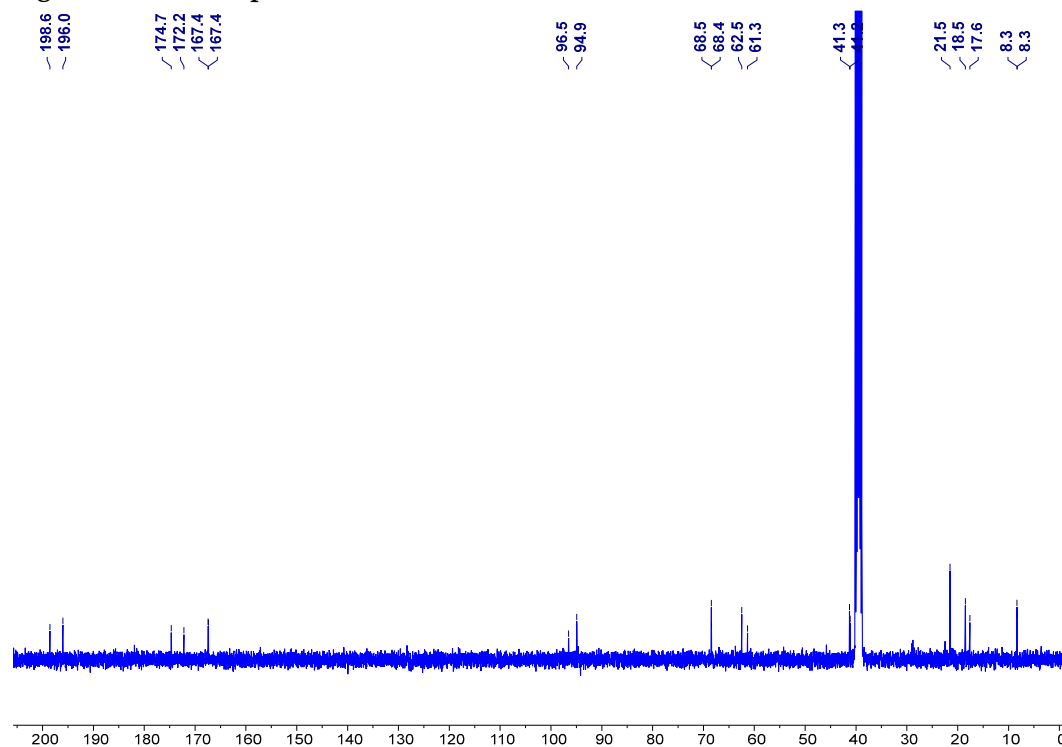


Fig. S39.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO- $d_6$ , 400Hz) of 6

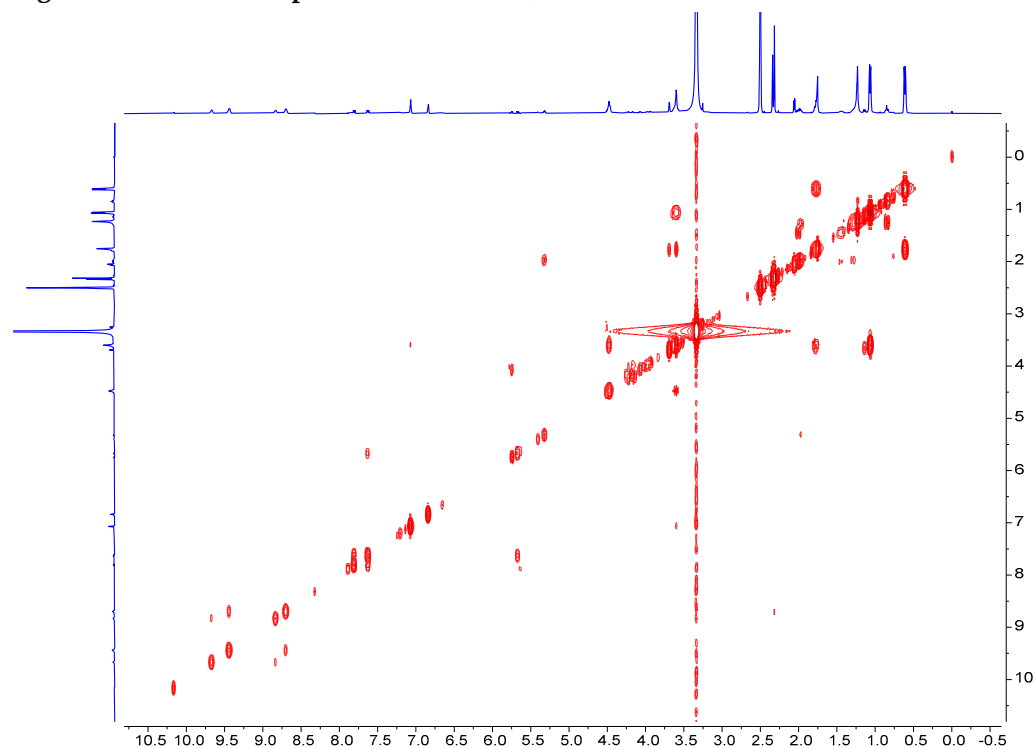


Fig. S40. HMBC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 6

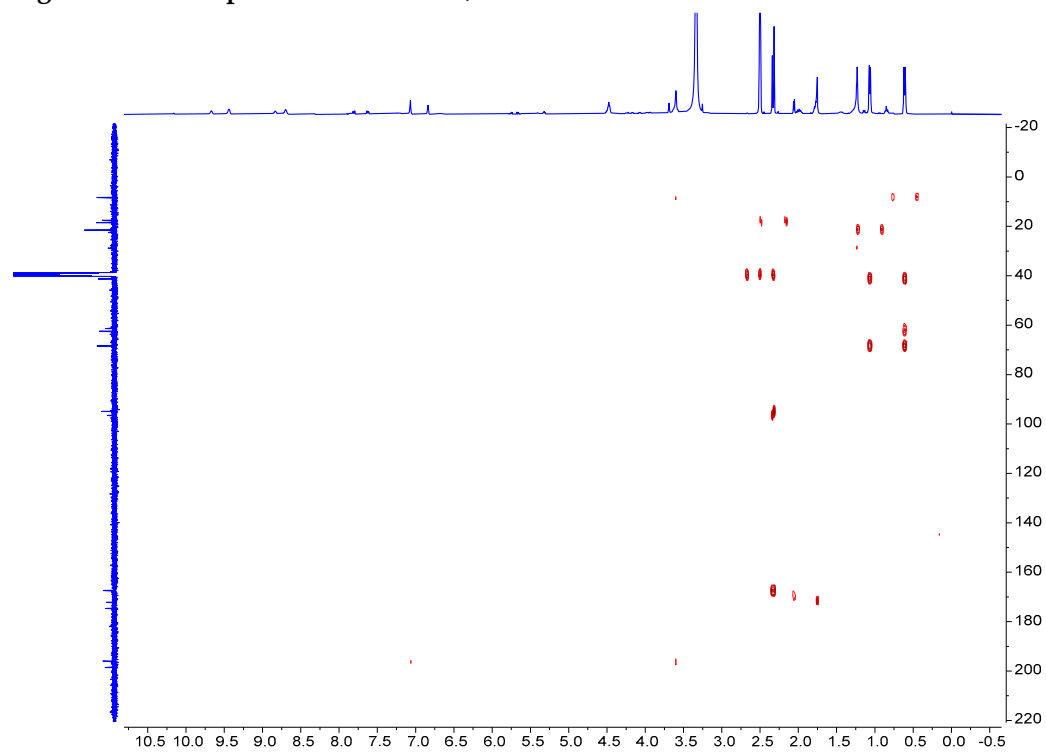


Fig. S41. HSQC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 6

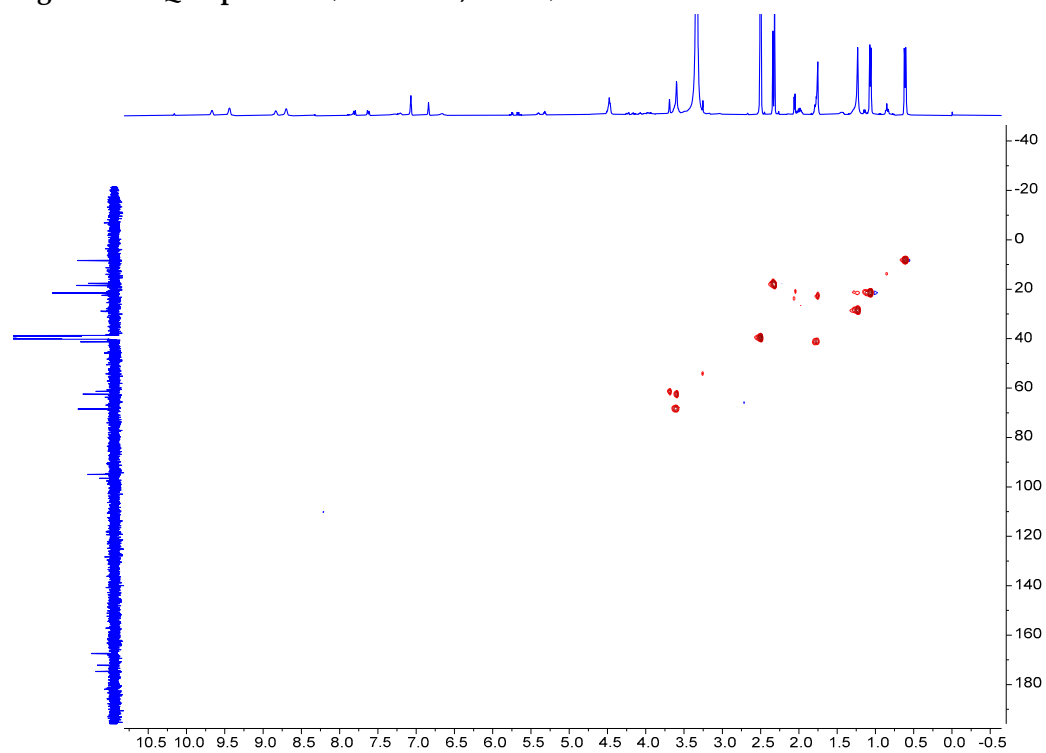


Fig. S42. UV spectrum (MeOH) of 6

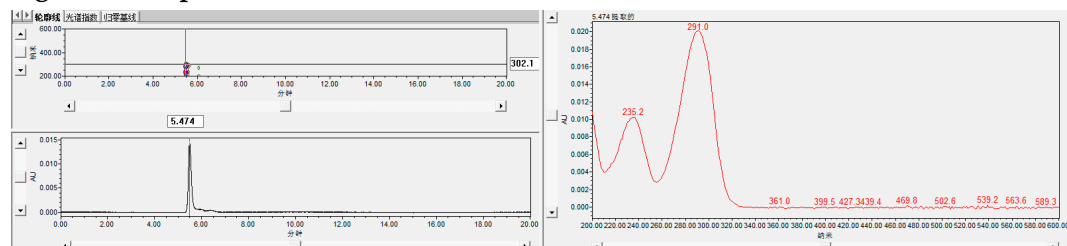


Fig. S43. (+)-HR-ESI-MS of compound 6

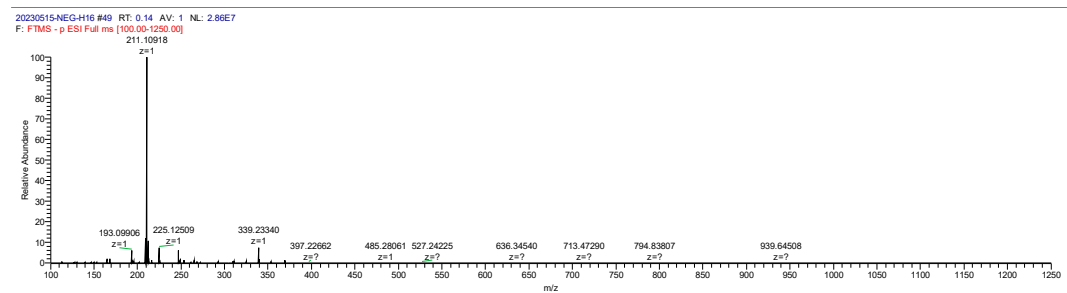


Fig. S44.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 7

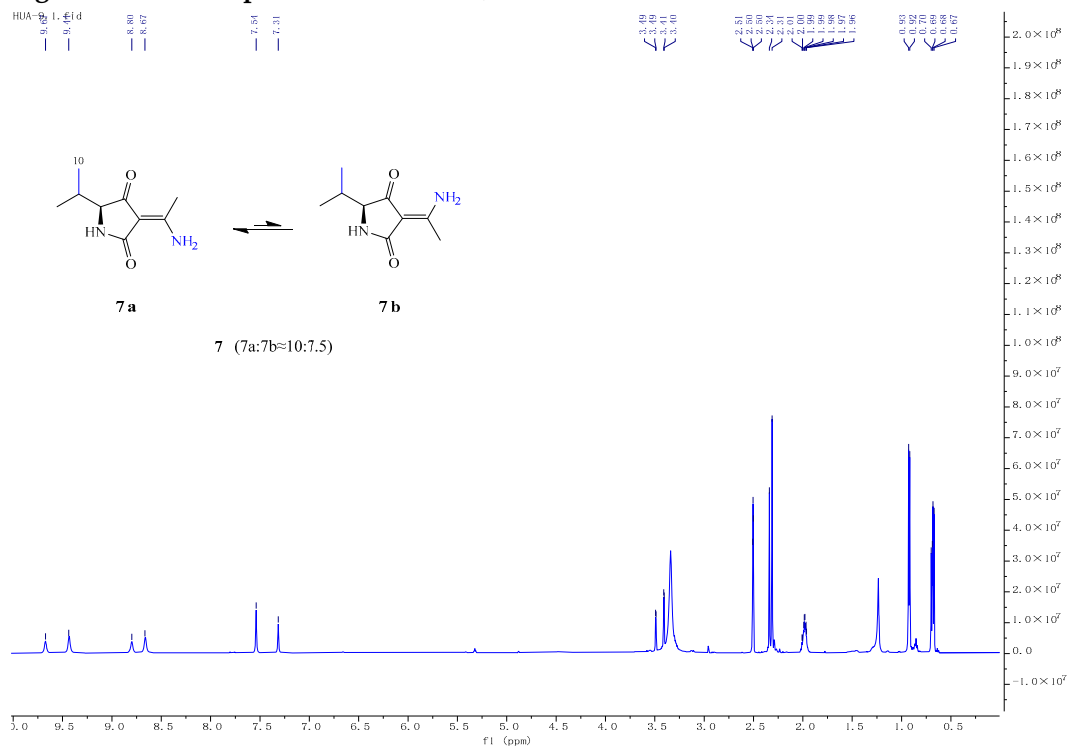


Fig. S45.  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ , 400Hz) of 7

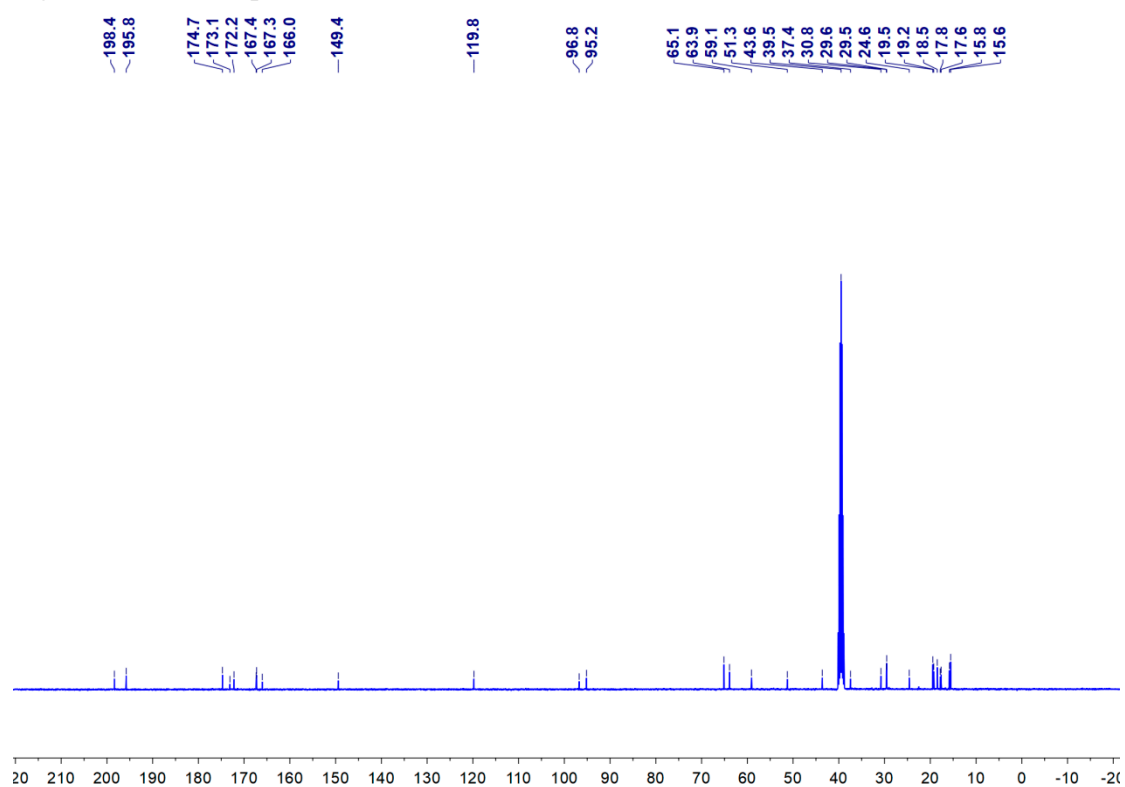


Fig. S46.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO- $d_6$ , 400Hz) of 7

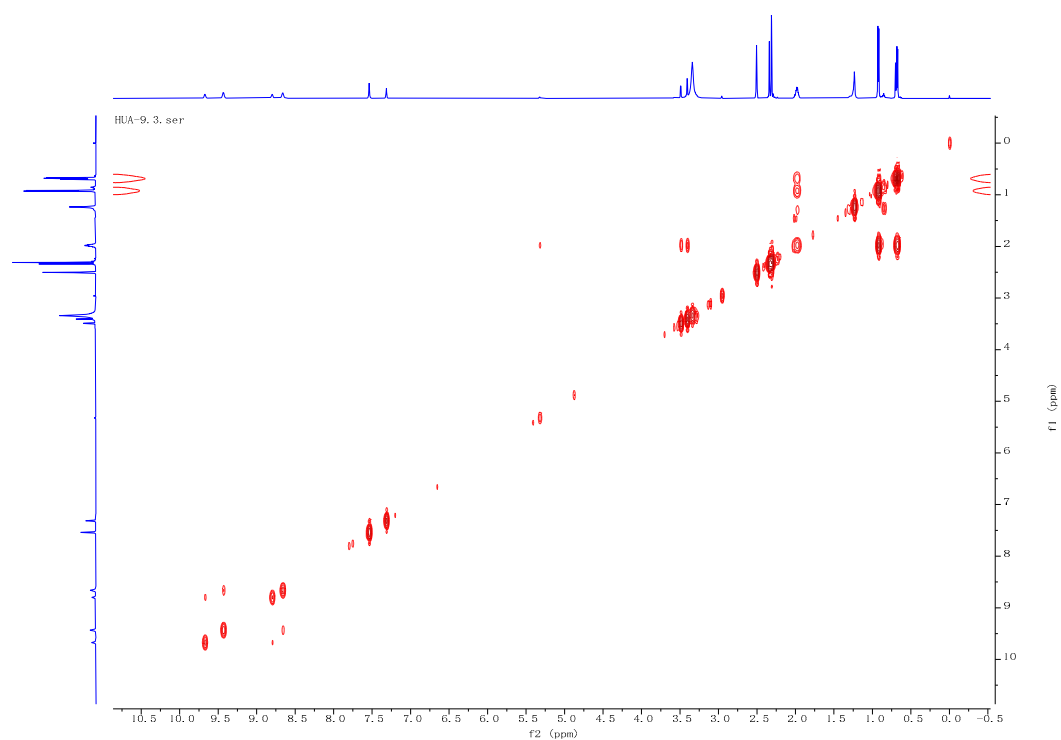


Fig. S47. HMBC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 7

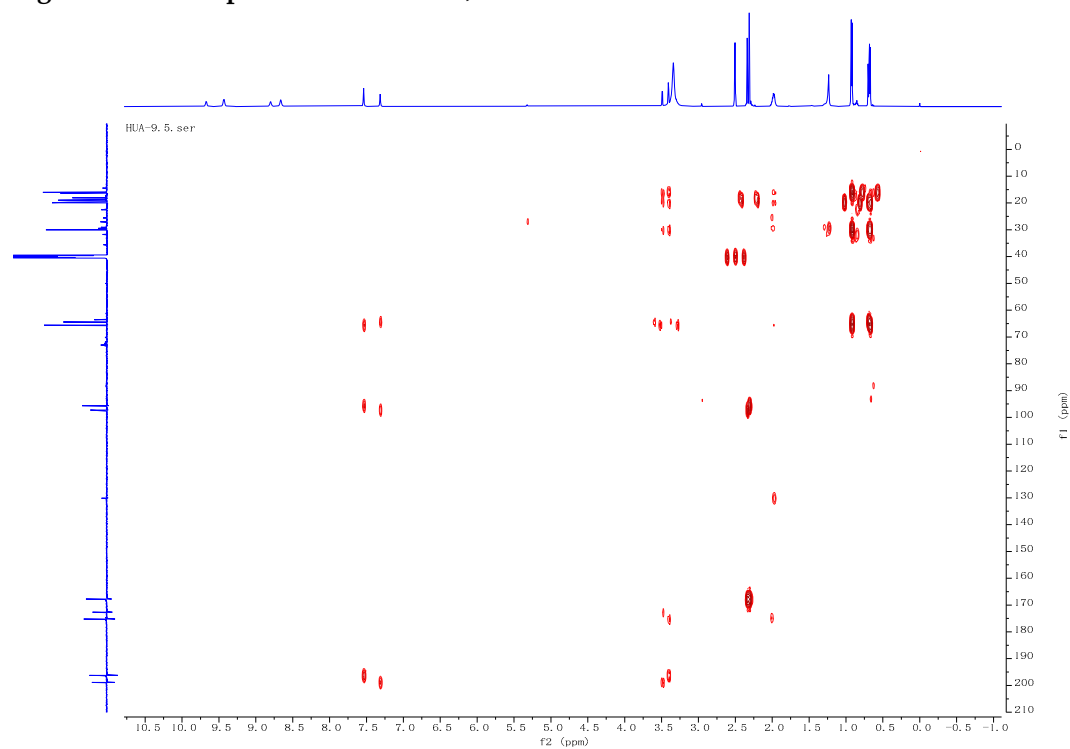


Fig. S48. HSQC spectrum (DMSO-*d*<sub>6</sub>, 400Hz) of 7

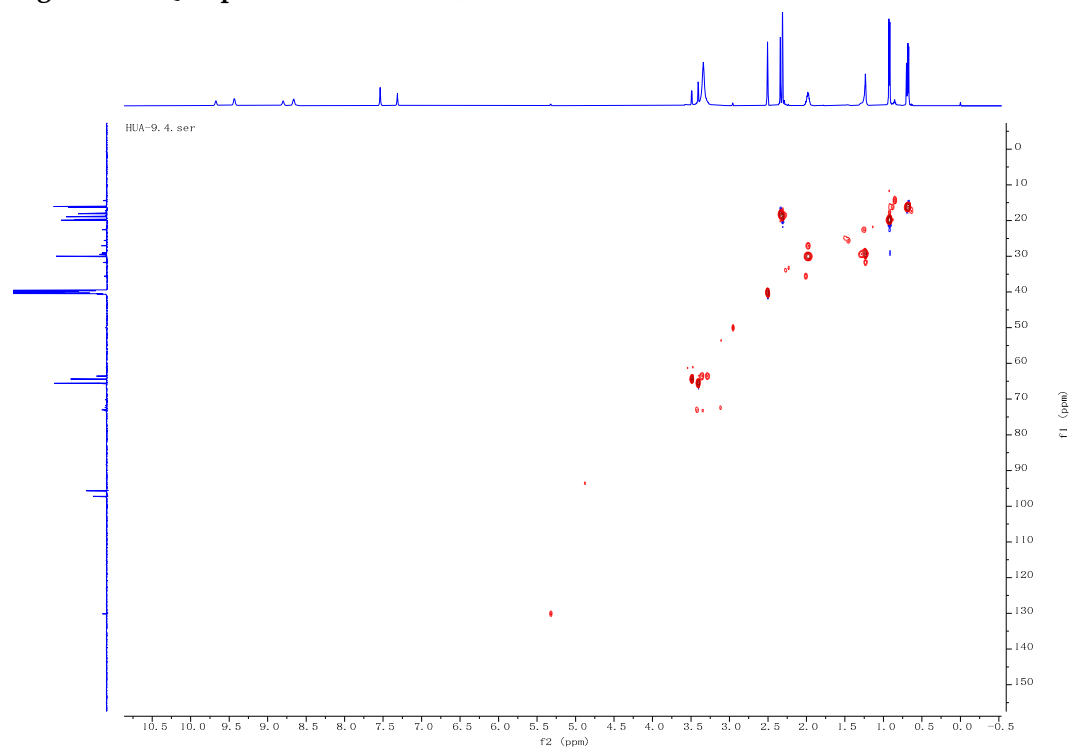




Fig. S49. UV spectrum (MeOH) of 7

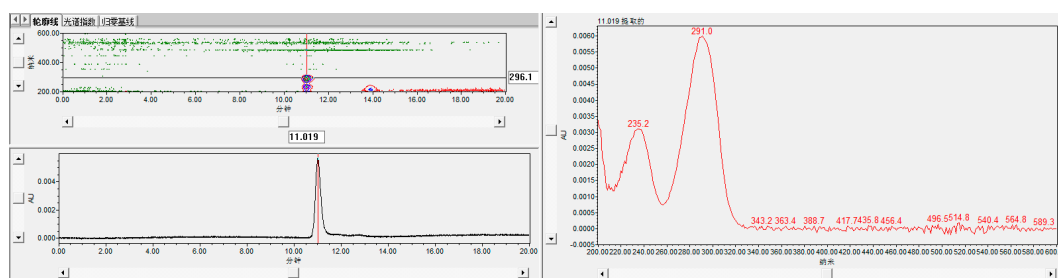


Fig. S50 (+)HR-ESI-MS of compound 7

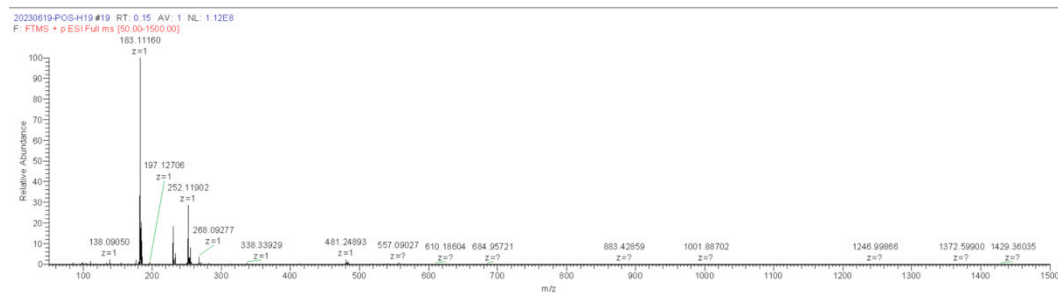
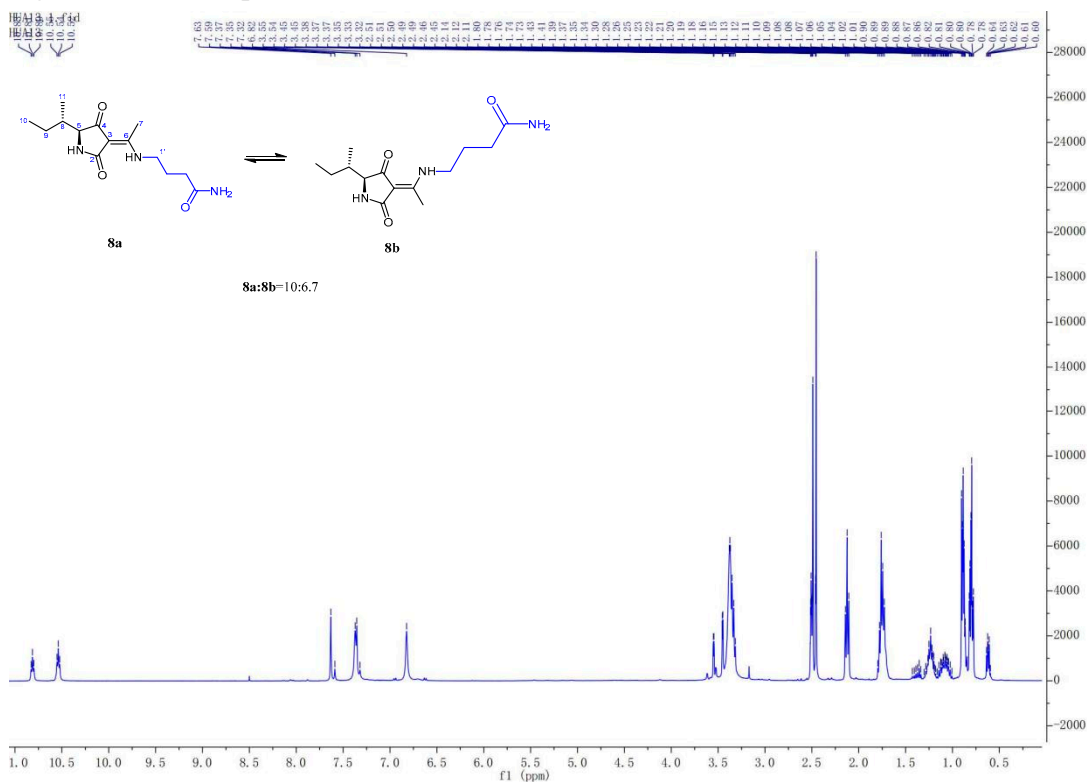
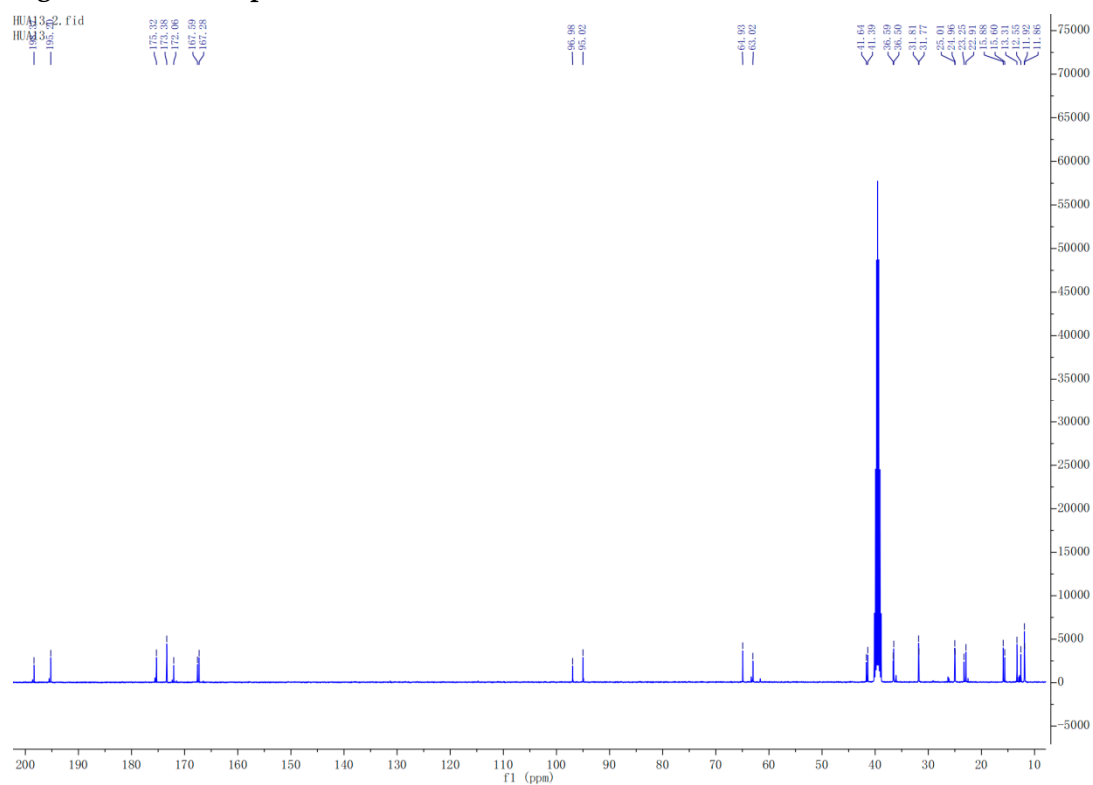


Fig. S51.  $^1\text{H}$  NMR spectrum (DMSO- $d_6$ , 600Hz) of 8



**Fig. S52.**  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ , 600Hz) of **8**



**Fig. S53.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (DMSO- $d_6$ , 600Hz) of **8**

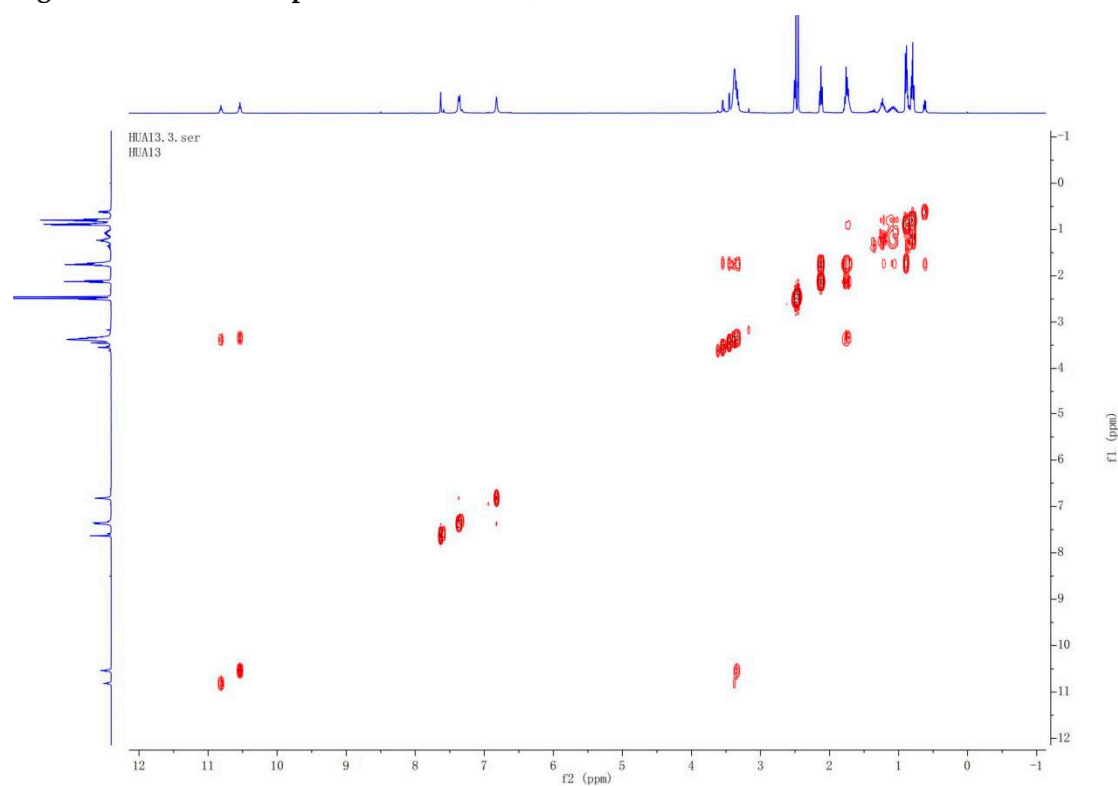


Fig. S54. HMBC spectrum (DMSO-*d*<sub>6</sub>, 600Hz) of 8

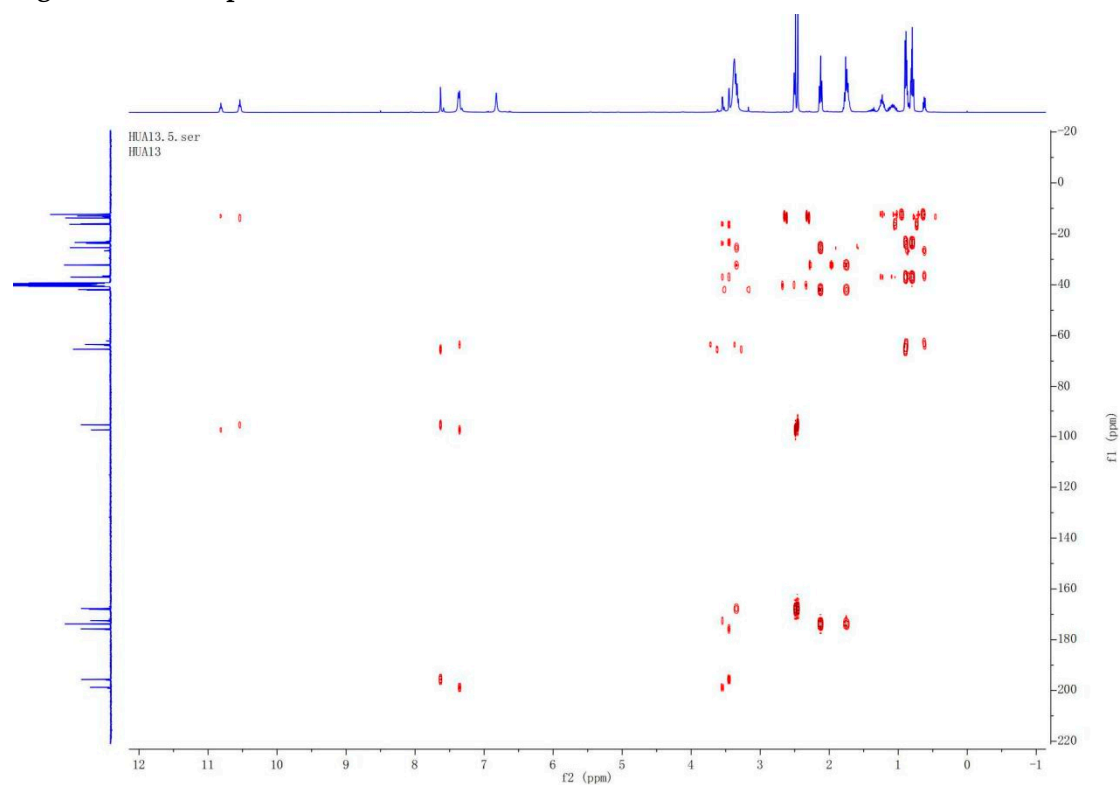
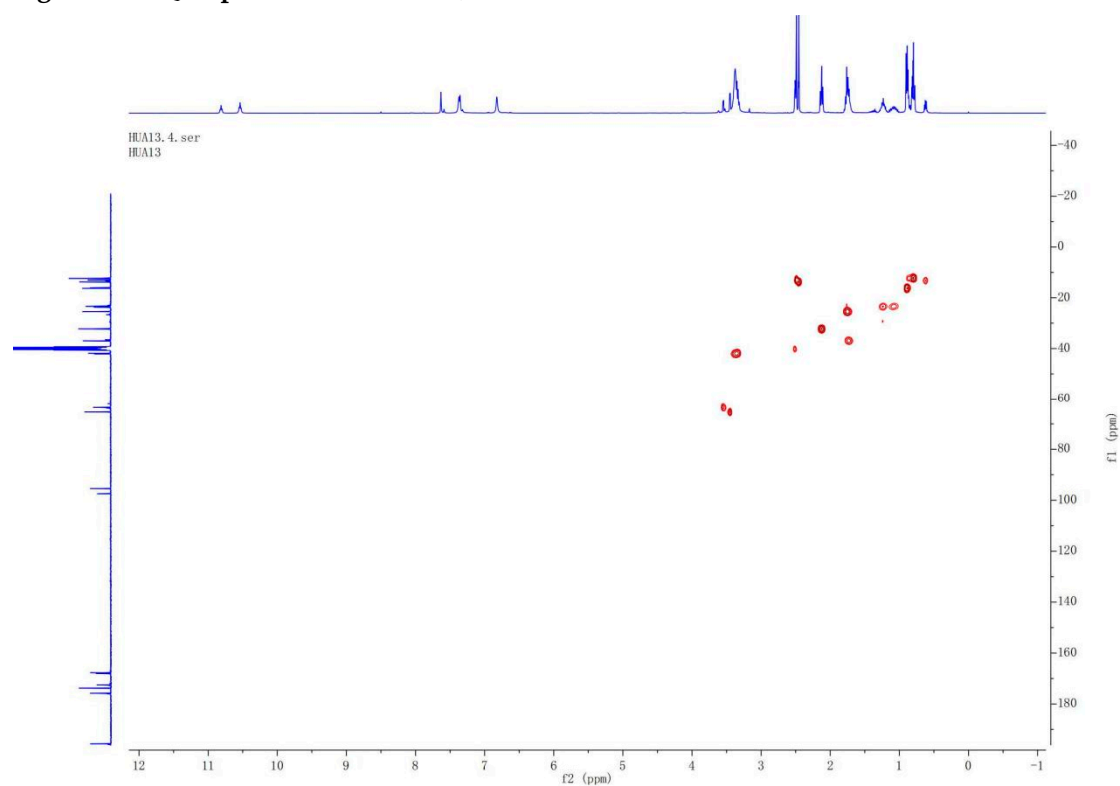


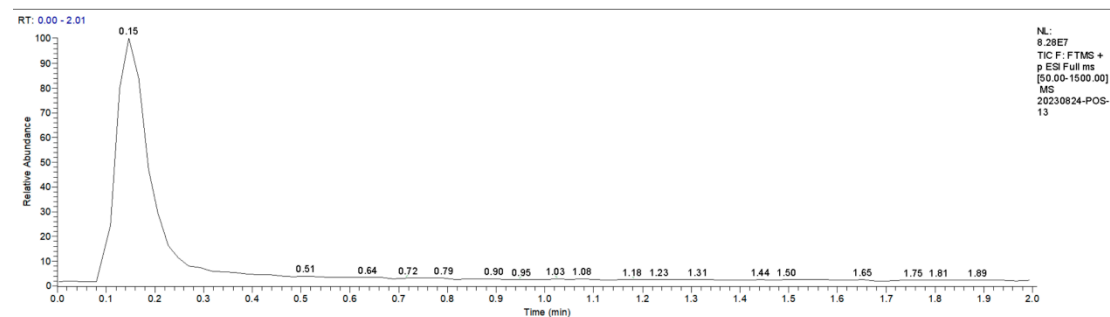
Fig. S55. HSQC spectrum (DMSO-*d*<sub>6</sub>, 600Hz) of 8



**Fig. S56. (+)-HR-ESI-MS of compound 8**

D:\RAWDATA\20230824\20230824-POS-13

8/24/2023 9:17:16



20230824-POS-13 #22 RT: 0.17 AV: 1 NL: 2.52E7

F: FTMS +p ESI Full ms [50.00-1500.00]

