

## Supplementary information

# Targeted Isolation of Sulfur-containing Metabolites from *Lsr2*-deletion Mutant Strain of *Streptomyces roseosporus*

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## Supplementary Experimental section

Cytotoxicity was evaluated against SGC7901, MDA-MB-231, A549 and HepG 2 cell lines by the MTT colorimetric method.<sup>1</sup> Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2mM L-glutamine, 100 units/mL penicillin, and 100 ug/mL streptomycin at 37 °C in humidified 5% CO<sub>2</sub>. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density (10000 cells per well) to ensure exponential growth for the duration of the assay. After a 24h preincubation growth, the medium was replaced with experimental medium containing the appropriate drug concentration or vehicle controls (0.1% v/v DMSO). After 48 h incubation, cell viability was measured using Alamar Blue reagent according to the manufacturer's instructions. Absorbance was measured at 490 nm. Results were expressed as the mean  $\pm$  standard error for six replicates as a percentage of vehicle control (take as 100%). The percent growth was standardized to controls (0.1% DMSO as negative control) using Microsoft Excel 2013. A statistical analysis including IC<sub>50</sub> determination and graphical output was performed in GraphPad Prism 5 using nonlinear regression variable slope curve fitting.

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Table S1. Sulfur-containing metabolites detected and characterized in  $\Delta Sr/sr2$  strain by UPLC-QTOF-MS and HR-MS/MS analysis

Peaks	$t_R$ (min)	[M+H] <sup>+</sup> or [M+Na] <sup>+</sup>	MS/MS	Molecular Formula	ppm	Tentatively Identification[Ref]	Molecular formula is previous reported in <i>Streptomyces</i> spp.
1	8.67	398.1508	266.1075,248.0944,231.0710,182.0502,160.1115,134.0465	C <sub>24</sub> H <sub>19</sub> N <sub>3</sub> O <sub>3</sub>	-0.2	unknown	no
2	9.57	215.1195	187.1225,170.0967,160.1130,143.0841,132.0812,103.0569	C <sub>13</sub> H <sub>14</sub> N <sub>2</sub> O	5.0	unknown	no
3	10.25	261.1249	233.1293,215.1190,170.0687,120.0818,114.0560	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	3.8	cyclo(L-6-Hyp-L-Phe)[1]	Streptomyces sp. DA18
4	10.64	280.0531 <sup>#</sup>	244.0552,228.0589,214.0445,189.0488,188.0386, 182.0722,157.0761,130.0654	C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> OS	3.5	SF2738D [2-3]	Streptomyces sp.
5	11.04	285.0668 <sup>#</sup>	249.0688,233.0752,219.0598,203.0646,189.0480, 188.0385,157.0765,130.0657	C <sub>13</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S	-2.1	unknown	Streptomyces sp. TPU1236A and other Streptomyces species
6	11.31	285.0669 <sup>#</sup>	247.0915,233.0745,219.0595,203.0647,189.0481, 188.0389,157.0760,130.0655	C <sub>13</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> S	-1.7	SF2738C [2-3]	Streptomyces sp. TPU1236A and other Streptomyces species
7	12.69	292.0751	274.0642,260.0487,244.0541,230.0391,214.0431,189.0479 ,188.0385,182.0712,157.0771,130.0658	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S	1.7	Pyrisulfoxin A [3-4]	Streptomyces californicus
8	13.23	271.0598	243.0619,215.0697,197.0591,169.0630,153.0174, 149.0234,91.0547,65.0384	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	3.0	unknown	Streptomyces sp. AC35 and other Streptomyces species
9	13.75	291.0794	273.0695,259.0539,243.0593,229.0446,204.0485, 188.0537,181.0759,156.0802,130.0660	C <sub>14</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub> S	-3.1	unknown	no
10	14.39	219.1751	203.1433,188.1218,161.1317,147.1147,133.1024,119.0842	C <sub>15</sub> H <sub>22</sub> O	0.9	unknown	no
11	15.01	304.1129	290.0972,262.1018,260.0850,247.0900,233.0741,232.0915 ,203.0647,189.0490,188.0385,157.0770,130.0655	C <sub>15</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub> S	3.3	SF2738E[2-3]	Streptomyces sp.
12	16.41	252.0763 <sup>#</sup>	212.0831,198.0672,182.0717,157.0766,130.0654	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>	4.7	Caerulomycin A [3,5]	Streptomyces caeruleus
13	17.23	261.1613	233.1660,170.1052,130.1229,120.0811	C <sub>15</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>	3.8	Cyclo-(L-phe-L-Leu) [6]	Streptomyces sp. H7372 and other Streptomyces species
14	18.14	274.0644	244.0543,230.0342,214.0435,189.0482,188.0389, 182.0715,157.0762,130.0655	C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub> S	-2.1	Pyrisulfoxin B [3-4]	Streptomyces californicus
15	18.22	273.0694	259.0545,243.0590,229.0441,204.0480,188.0540, 181.0770,156.0810,130.0658	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> S	-1.4	unknown	no
16	19.22	279.0791	263.0850,249.0693,235.0537,219.0597,203.0650,	C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> S	-0.4	unknown	no

			189.0484,157.0763,130.0652					
17	20.31	276.0797	258.0693,244.0537,214.0439,212.0824,189.0487, 182.0712,157.0768,130.0655	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	-3.6	unknown	Streptomyces sp. CS40	
18	20.43	276.0794	231.0583,217.0434,216.0348,189.0479,188.0388, 185.0707,157.0761,130.0652	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	-4.7	unknown	Streptomyces sp. CS40	
19	21.44	276.0795	258.0701,244.0536,214.0441,212.0821,189.0486, 182.0713,157.0765,130.0657	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	-4.3	SF2738A/B [2-3]	Streptomyces sp. CS40	
20a	21.74	284.2235	266.2128,232.1345,212.1166,173.1198,143.0851	C <sub>9</sub> H <sub>29</sub> N <sub>7</sub> OS	0.7	unknown	no	
20b	21.74	284.1387	256.2277,219.1737,194.0954,160.1081,147.1065,133.0856	C <sub>16</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub>	-4.2	unknown	Streptomyces sp. H7372 and other Streptomyces species	
21	22.68	298.2400	280.2274,270.2439,239.1414,184.0747,160.1102,117.0930	C <sub>10</sub> H <sub>31</sub> N <sub>7</sub> OS	4.0	unknown	no	
22	23.48	330.3370	312.3283,298.2385,266.2117,233.1741,209.1686, 175.1346,131.0721	C <sub>20</sub> H <sub>43</sub> NO <sub>2</sub>	-0.6	unknown	no	

# [M+Na]<sup>+</sup>

## References

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- [6] K. Yamamoto, M. Hayashi, Y. Murakami, Y. Araki, Y. Otsuka, T. Kashiwagi, T. Shimamura, H. Ukeda, *Bios. Biotechnol. Biochem.*, 2016, 80, 172-177.

Table S2 Cytotoxic activities of **1-13** isolated from the  $\Delta Srlsr2$  strain of *S roseosporus*

compound	IC <sub>50</sub> (μM)			
	SGC7901	MDA-MB-231	A549	HepG2
<b>1</b>	>40	>40	>40	>40
<b>2</b>	>40	>40	>40	>40
<b>3</b>	>40	>40	>40	>40
<b>4</b>	>40	>40	>40	>40
<b>5</b>	>40	>40	>40	>40
<b>6</b>	>40	>40	>40	>40
<b>7</b>	1.7±0.5	6.3±1.0	14.7±3.2	5.8±0.5
<b>8</b>	>40	>40	>40	>40
<b>9</b>	>40	>40	21.3±2.9	>40
<b>10</b>	>40	>40	>40	>40
<b>11</b>	>40	34.5±5.3	25.8±0.7	7.2±1.1
<b>12</b>	>40	>40	>40	>40
<b>13</b>	>40	>40	>40	>40

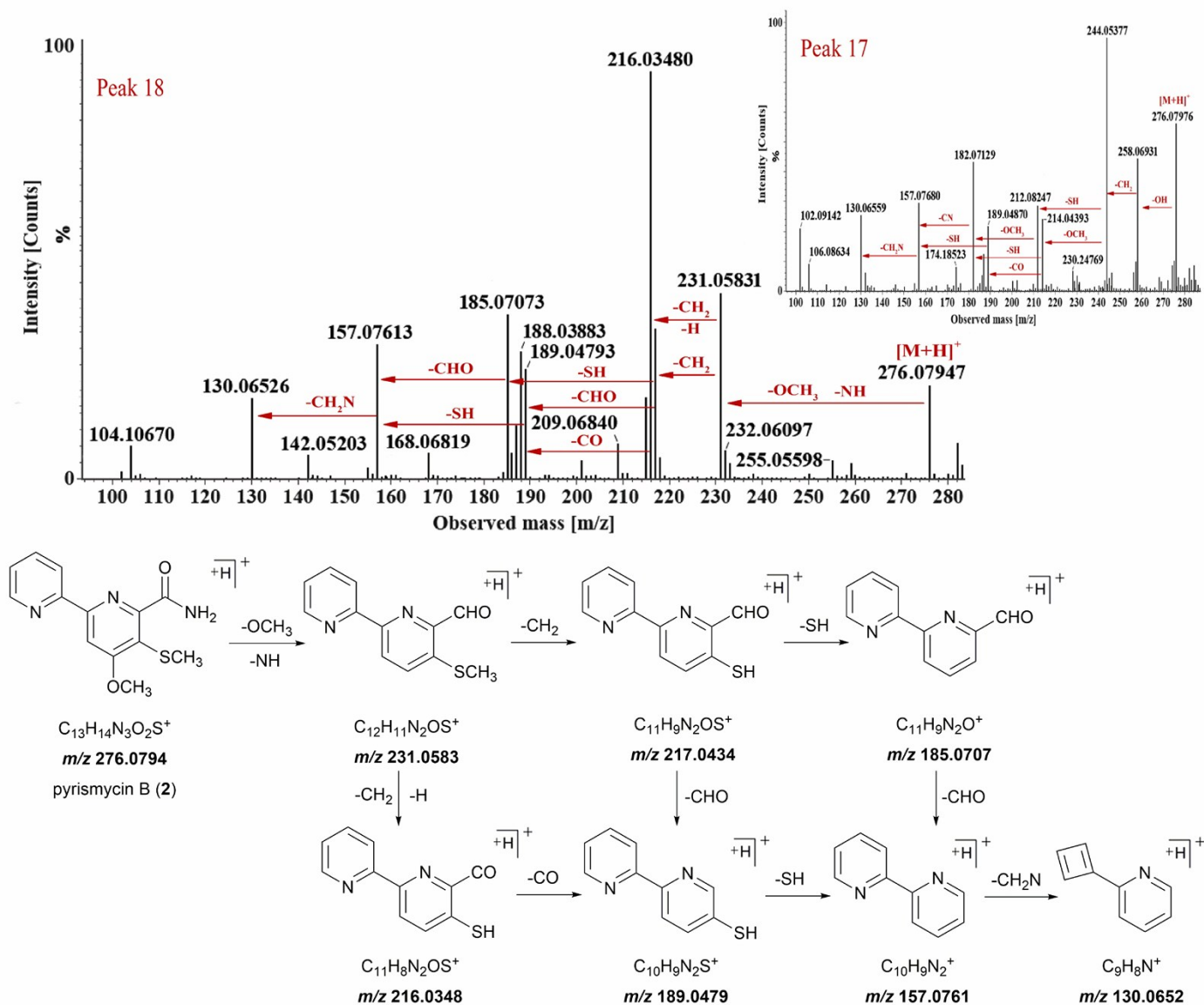


Figure S1 HR-MS/MS spectra and proposed fragmentation pathway of peaks 17 and 18 (**1** and **2**).



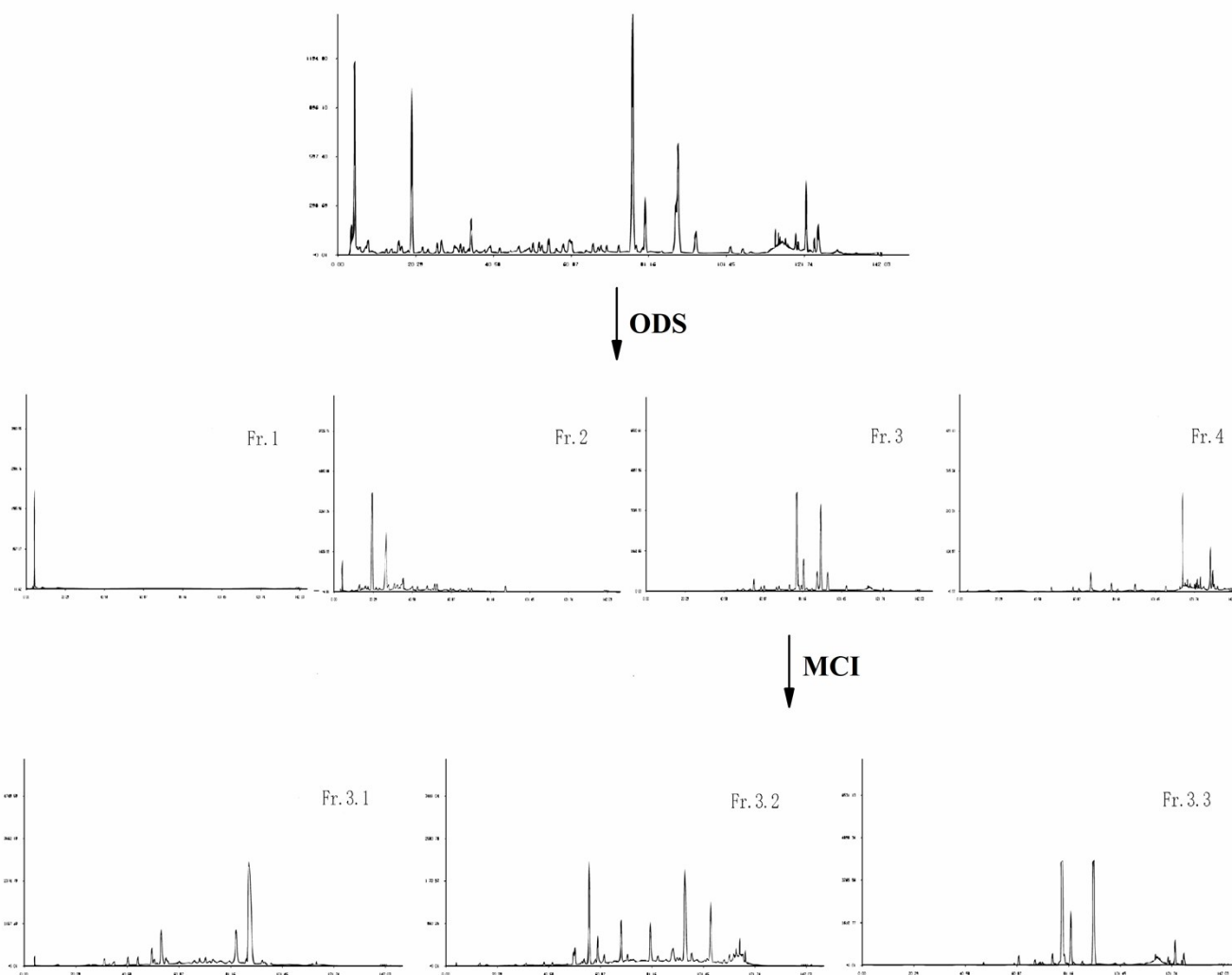


Figure S2. HPLC guided isolation of sulfur-containing metabolites.

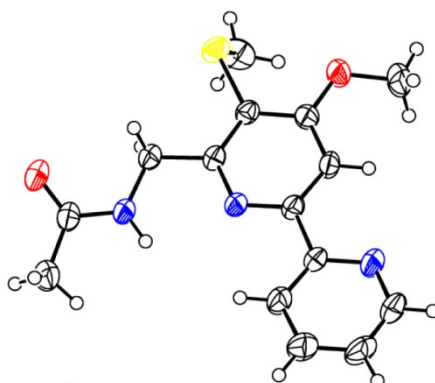
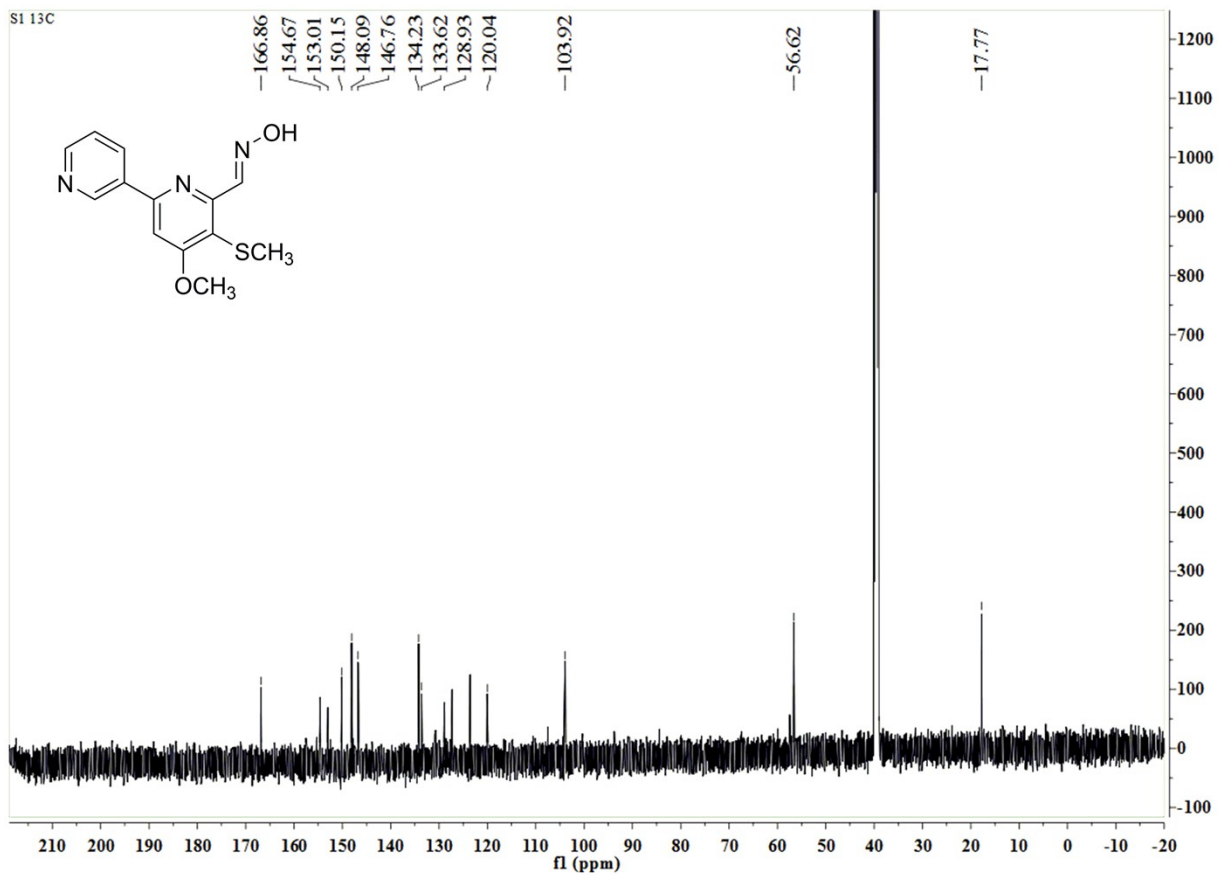
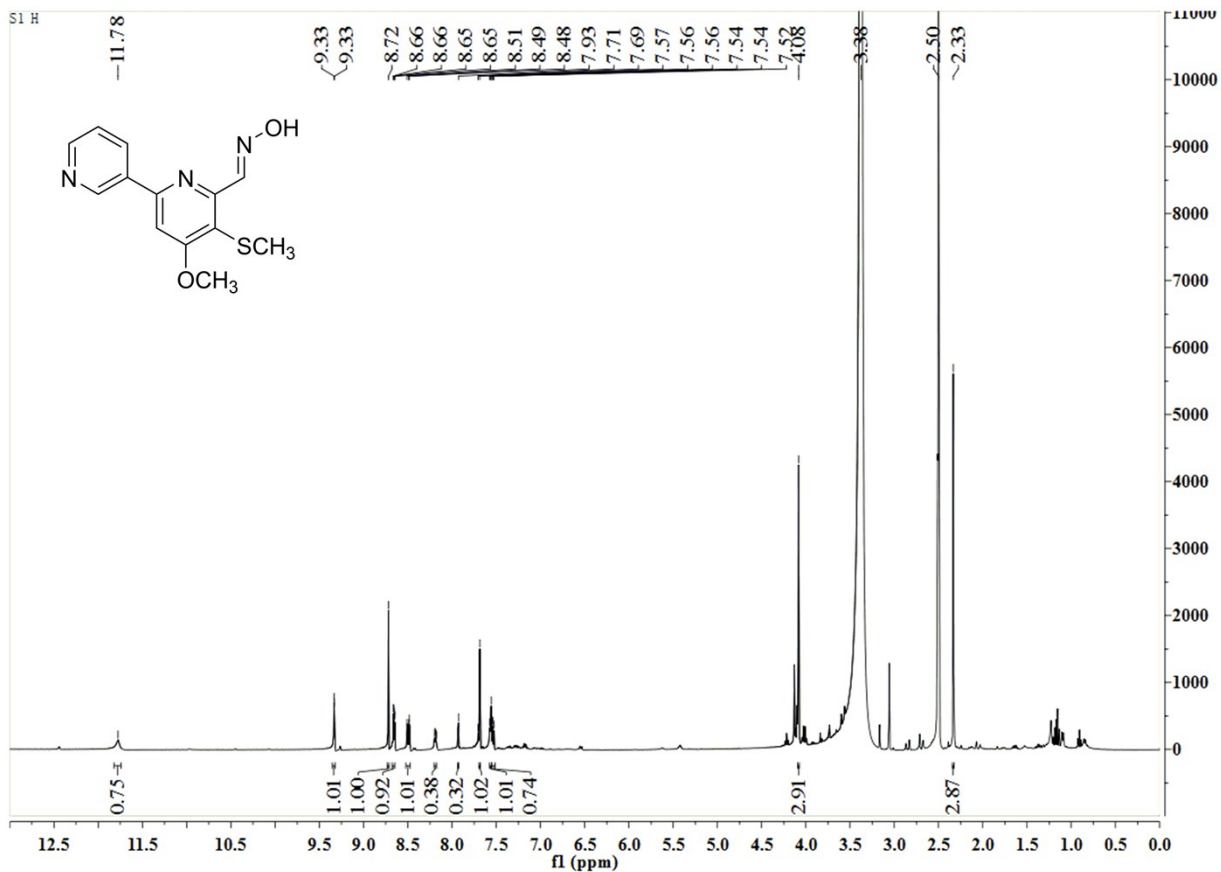


Figure S3. X-ray crystal structure of **10**



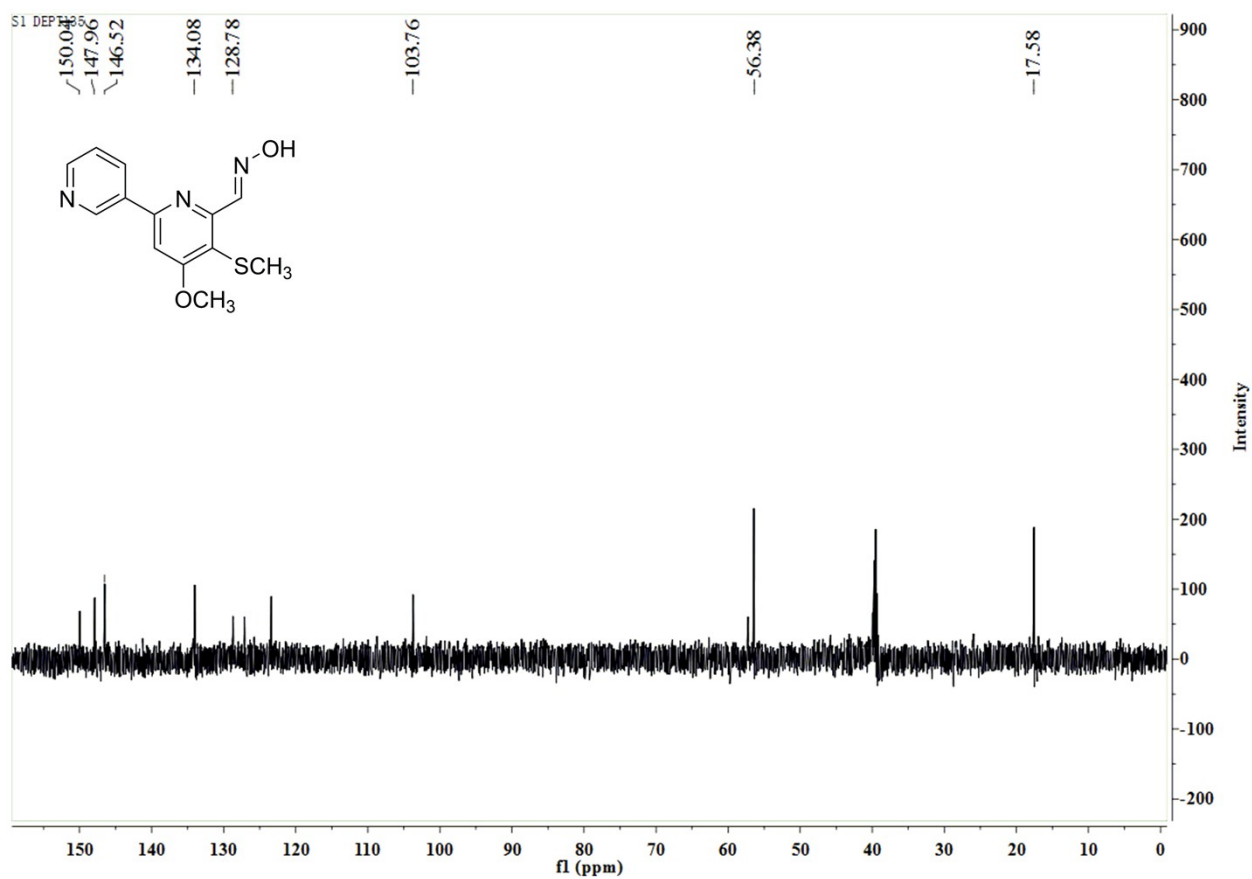


Figure S6. DEPT 135 (100 MHz, DMSO) spectrum of compound 1.

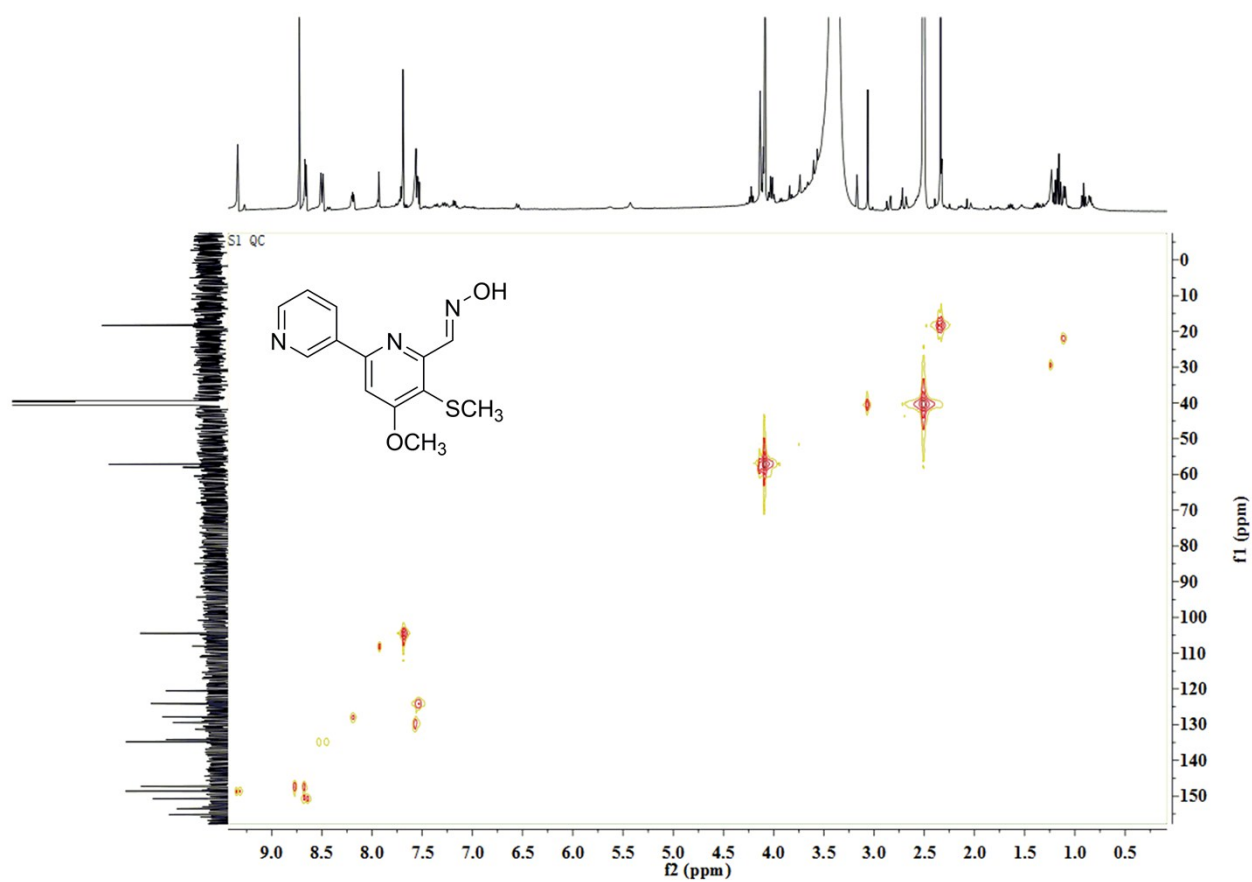


Figure S7. HSQC (400 MHz, DMSO) spectrum of compound 1.

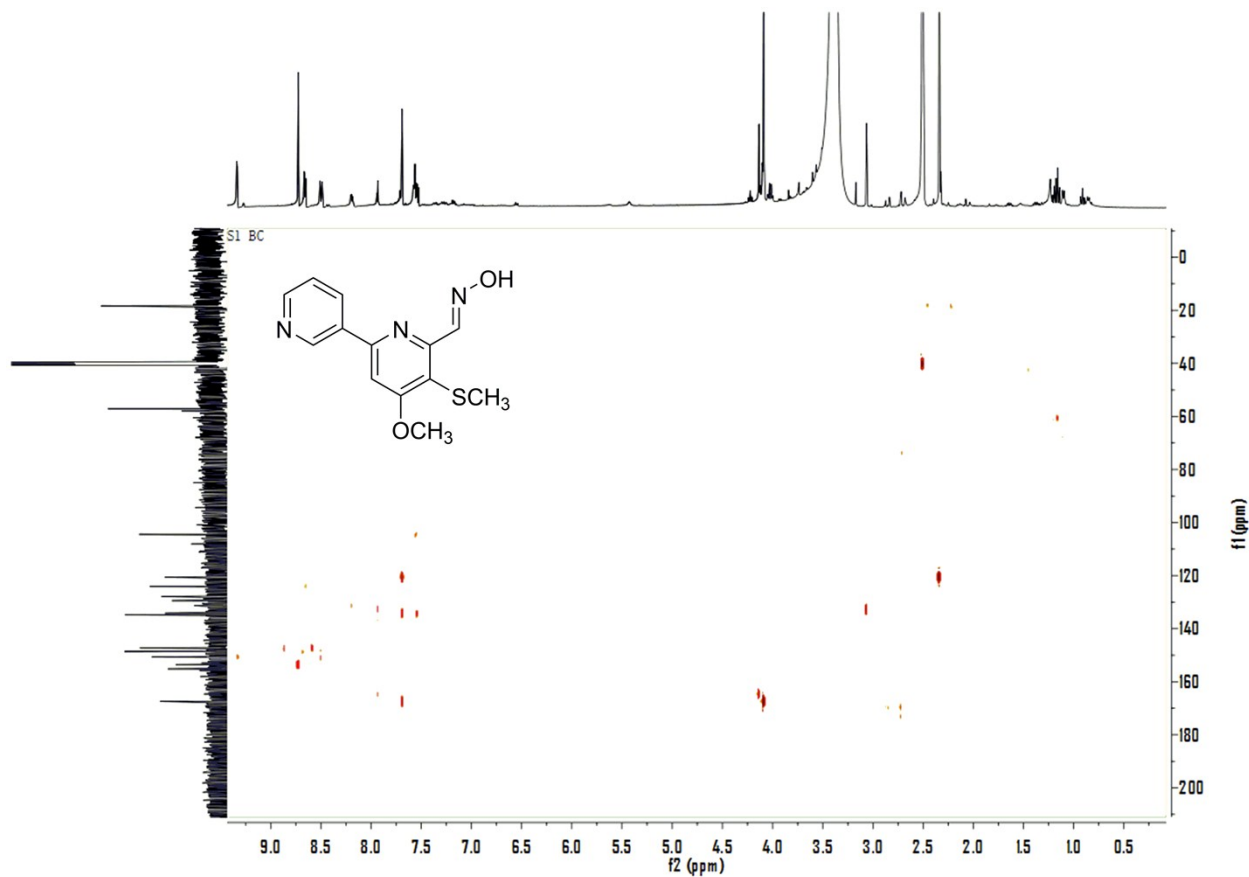


Figure S8. HMBC ( 400 MHz, DMSO) spectrum of compound 1.

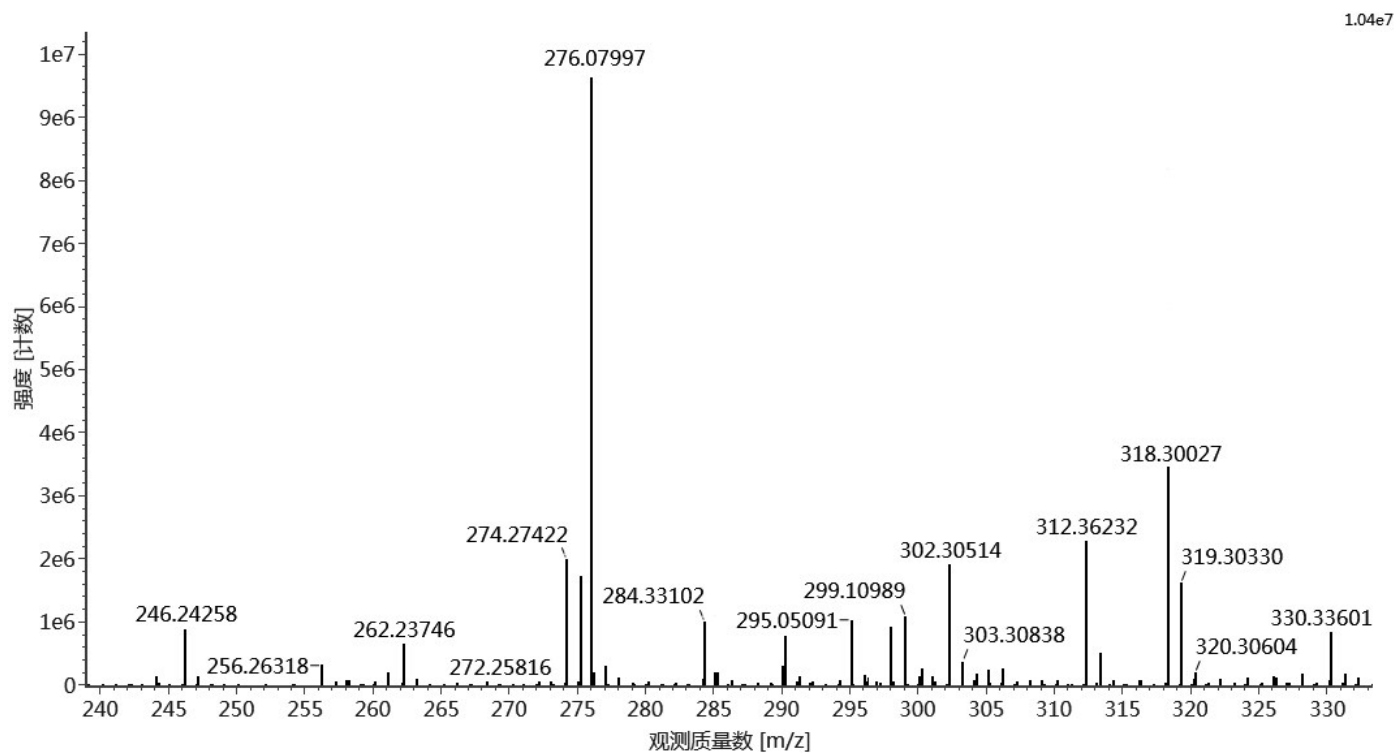


Figure S9. HRESIMS spectrum of compound 1.

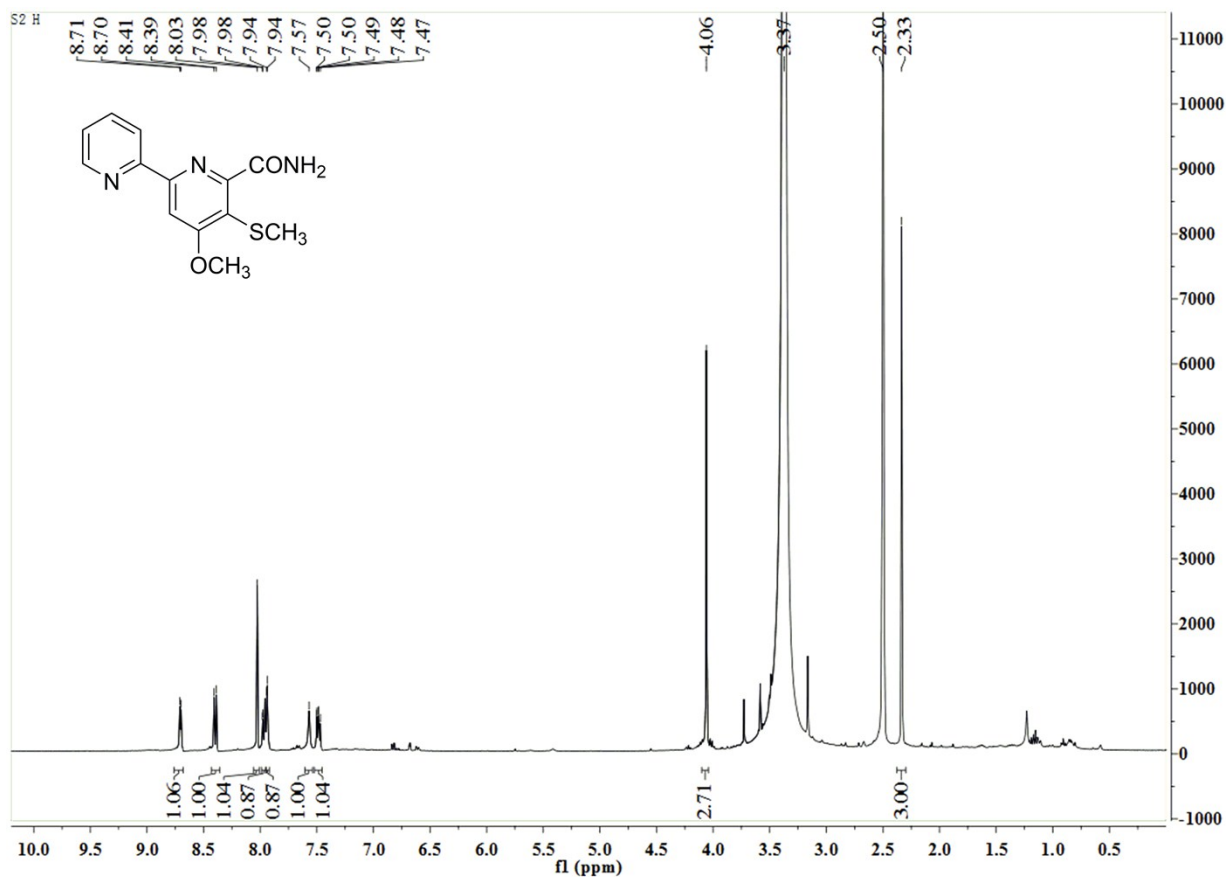


Figure S10.  $^1\text{H}$  NMR ( 400 MHz, DMSO) spectrum of compound 2.

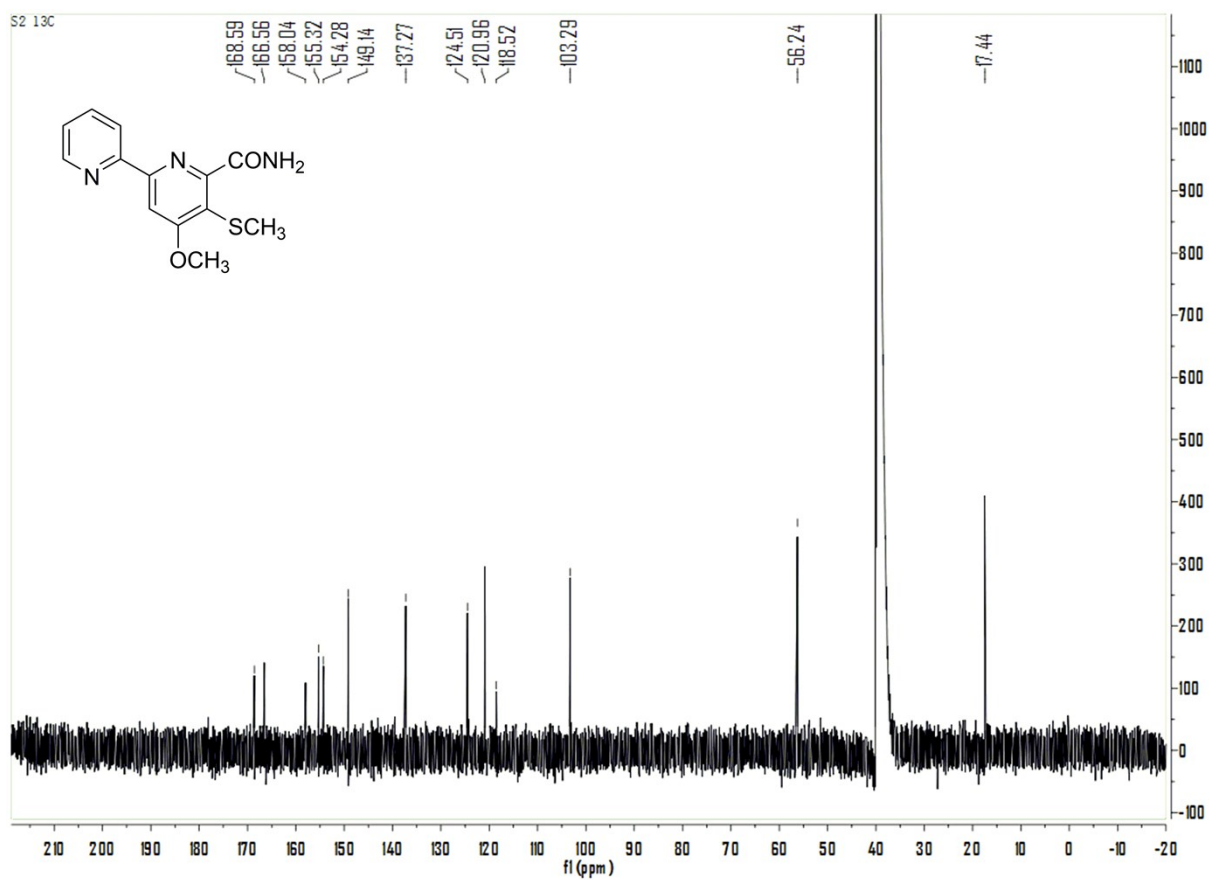


Figure S11.  $^{13}\text{C}$  NMR ( 100 MHz, DMSO) spectrum of compound 2.

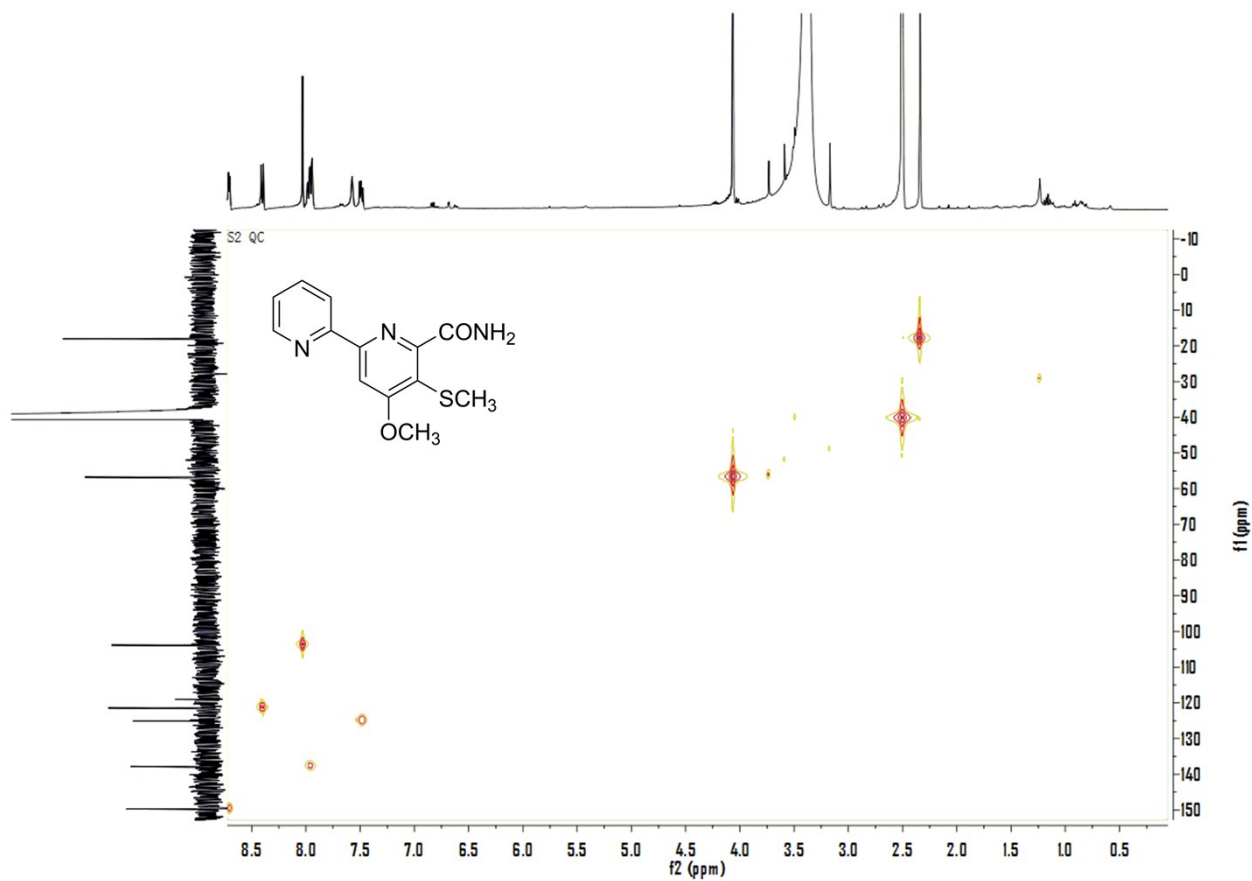


Figure S12. HSQC ( 400 MHz, DMSO) spectrum of compound 2.

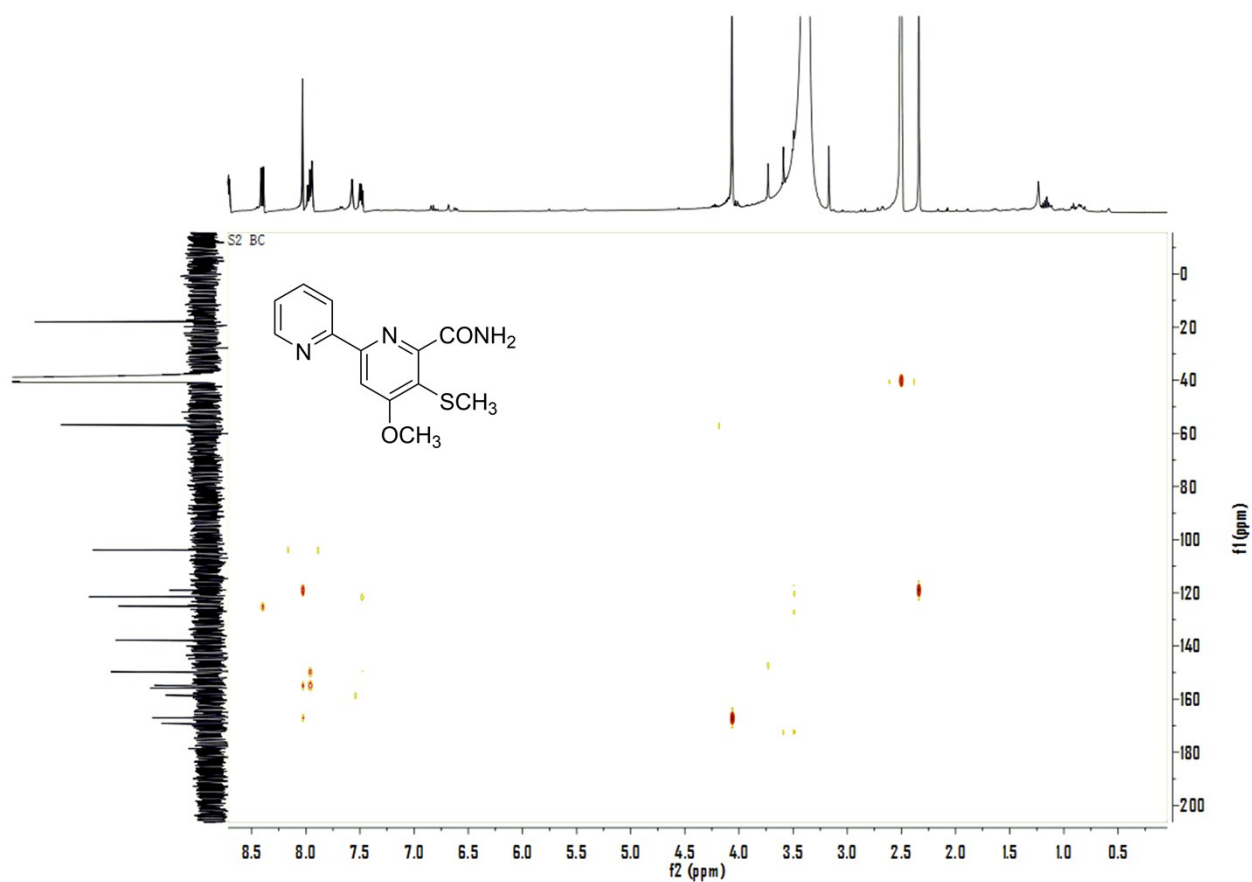


Figure S13. HMBC ( 400 MHz, DMSO) spectrum of compound 2.

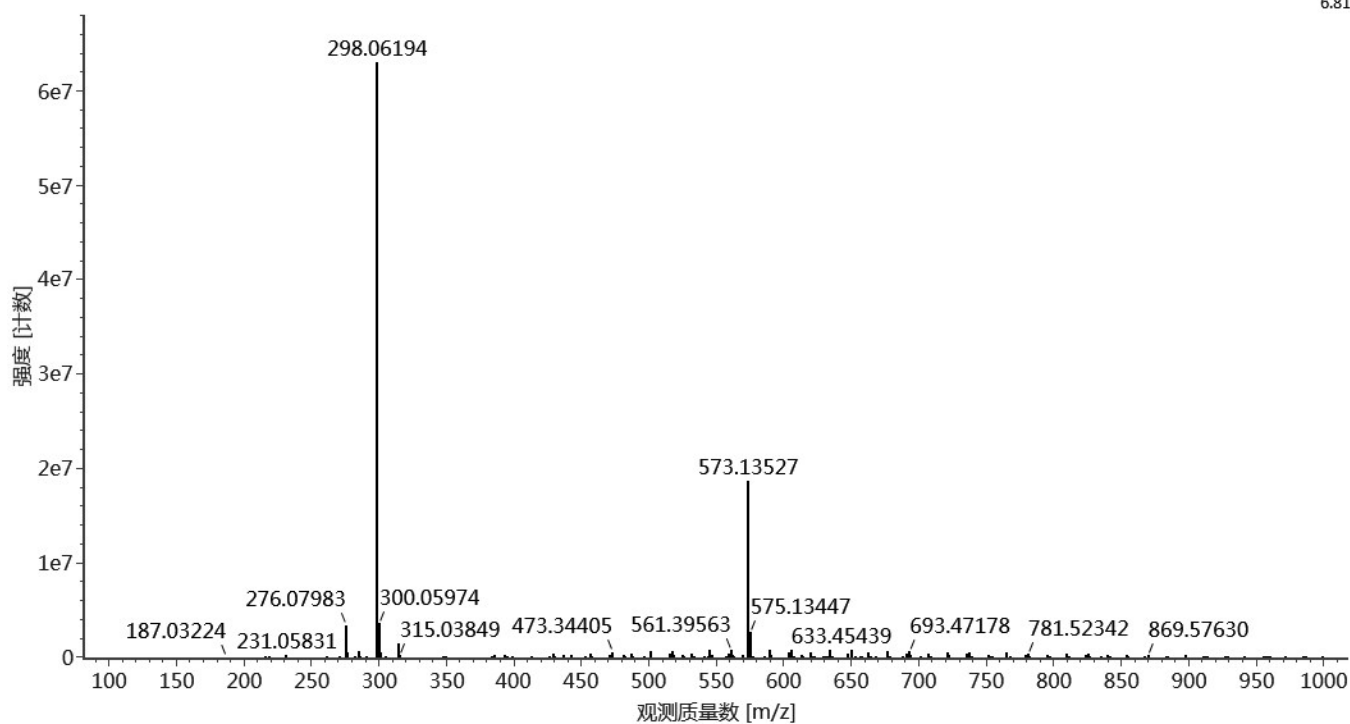
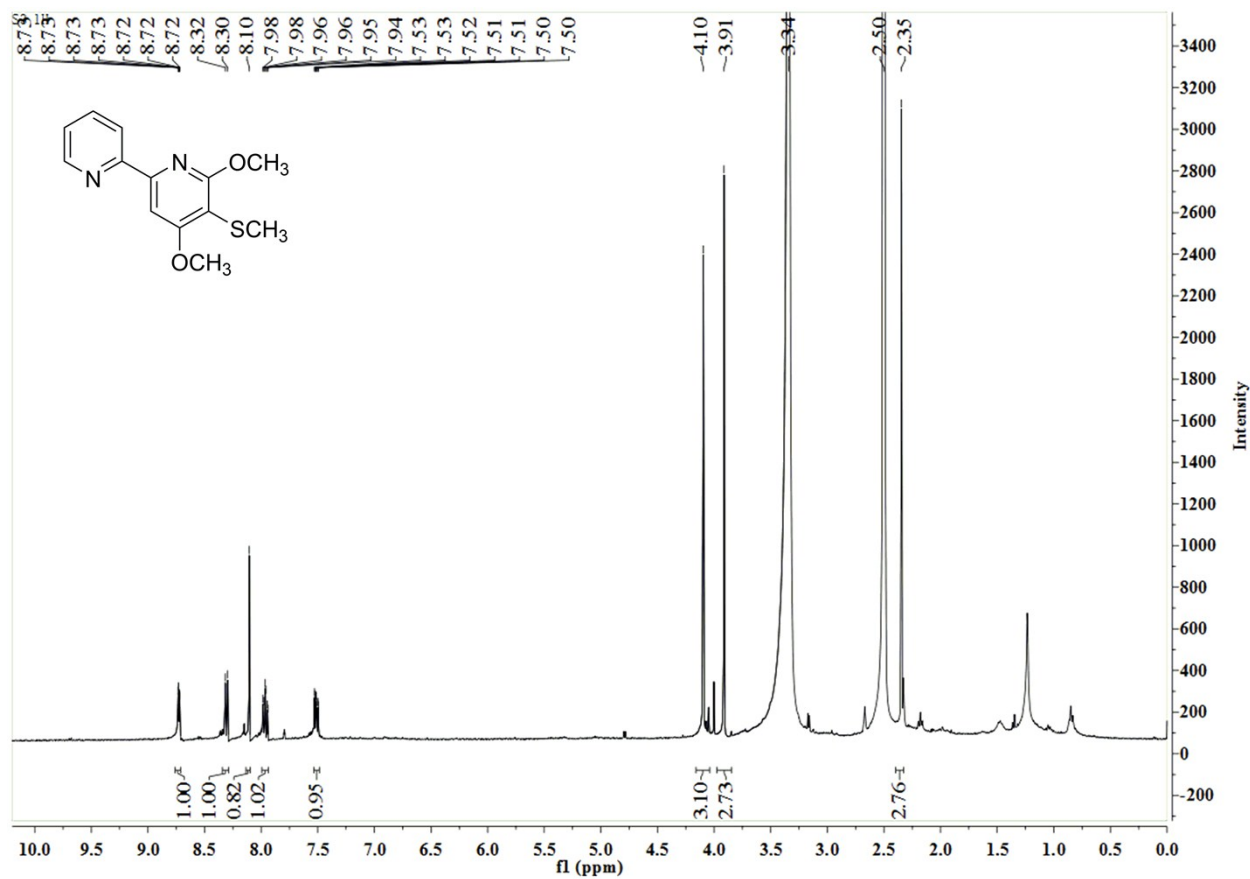


Figure S14. HRESIMS spectrum of compound 2.

Figure S15. <sup>1</sup>H NMR (400 MHz, DMSO) spectrum of compound 3.

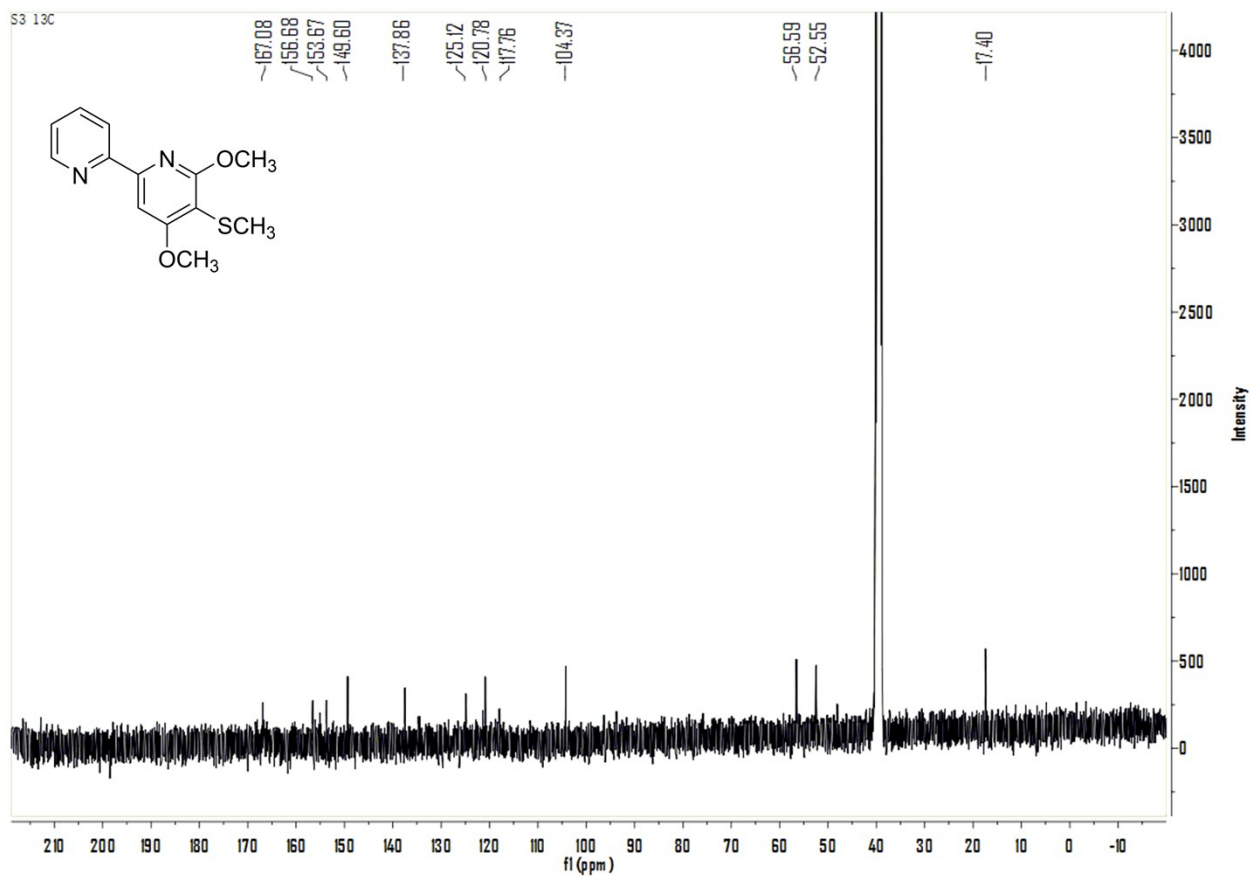


Figure S16.  $^{13}\text{C}$  NMR ( 100 MHz, DMSO) spectrum of compound 3.

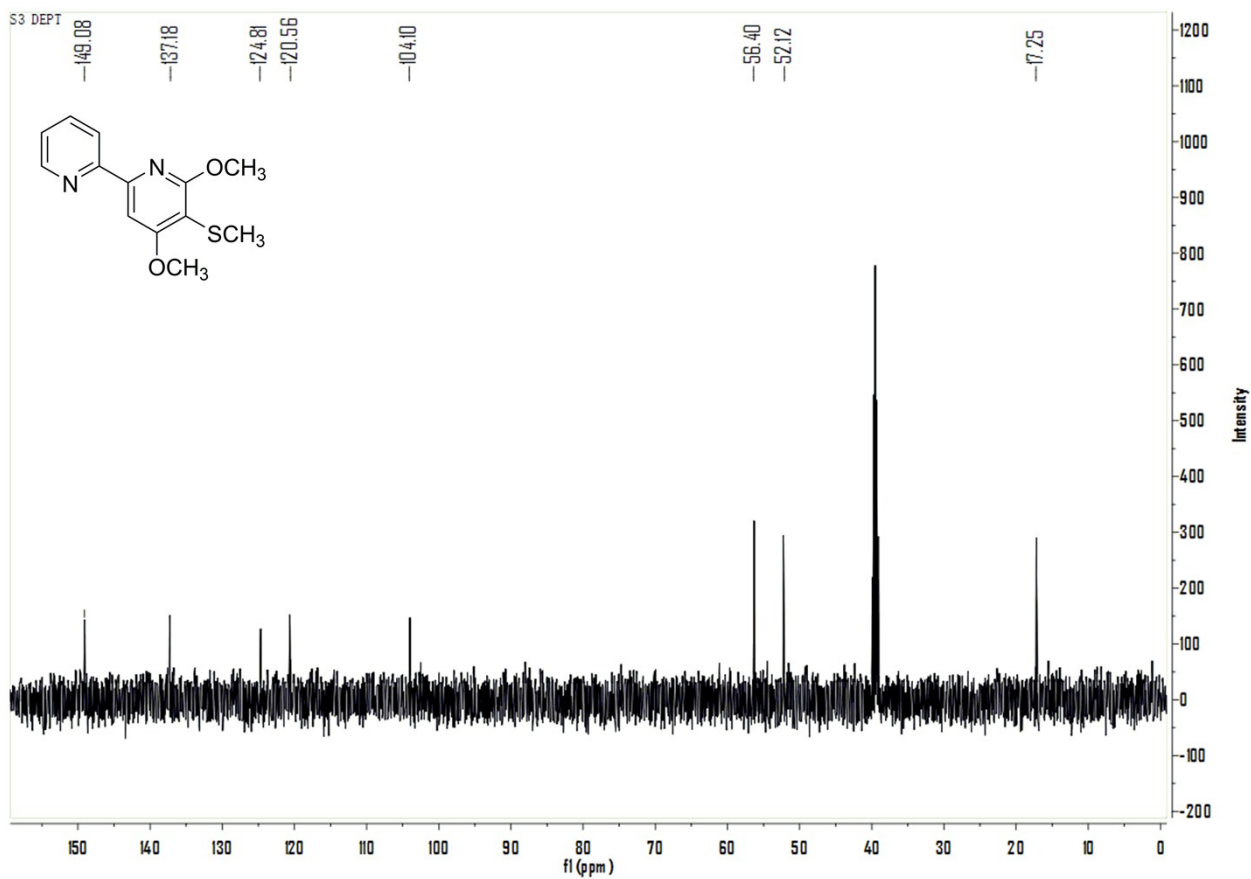


Figure S17. DEPT 135 ( 100 MHz, DMSO) spectrum of compound 3.



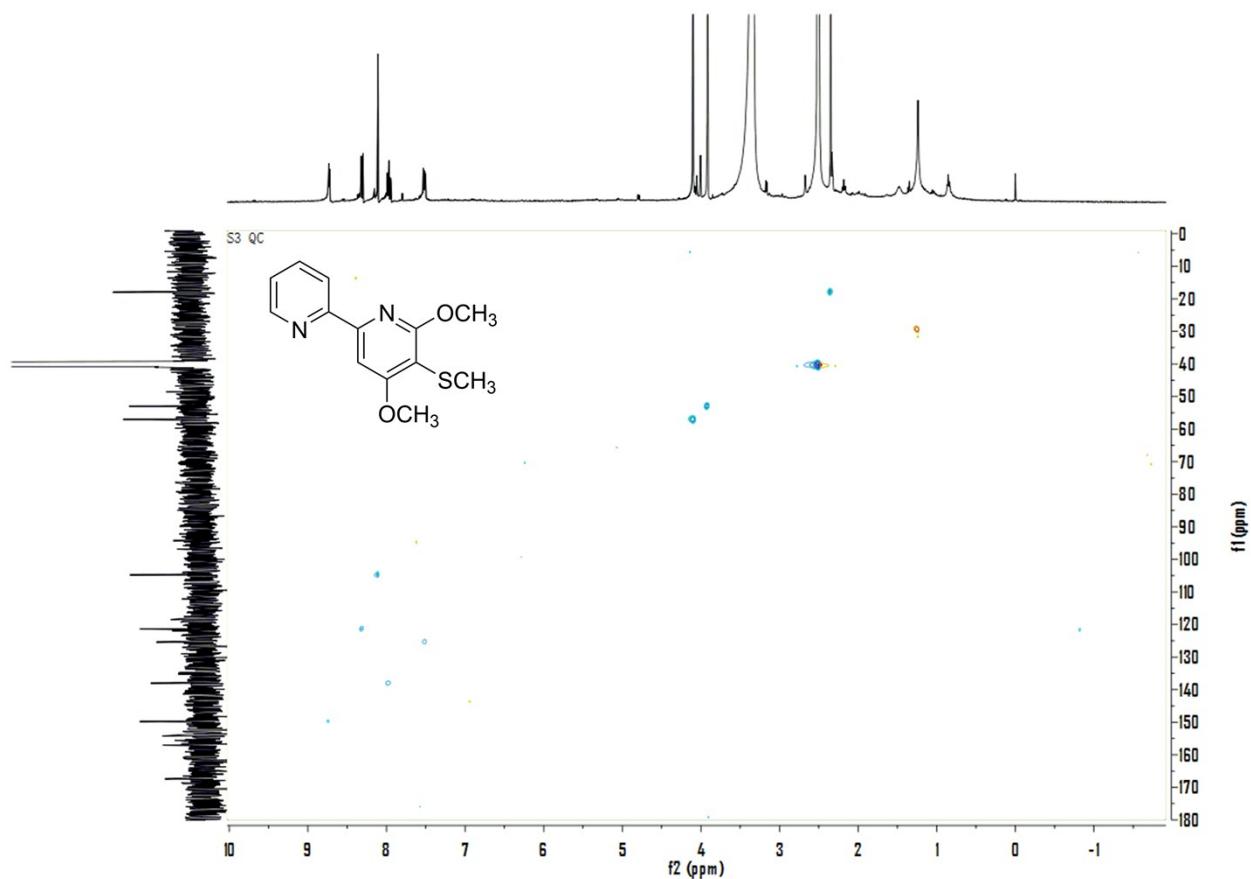


Figure S18. HSQC( 400 MHz, DMSO) spectrum of compound **3**.

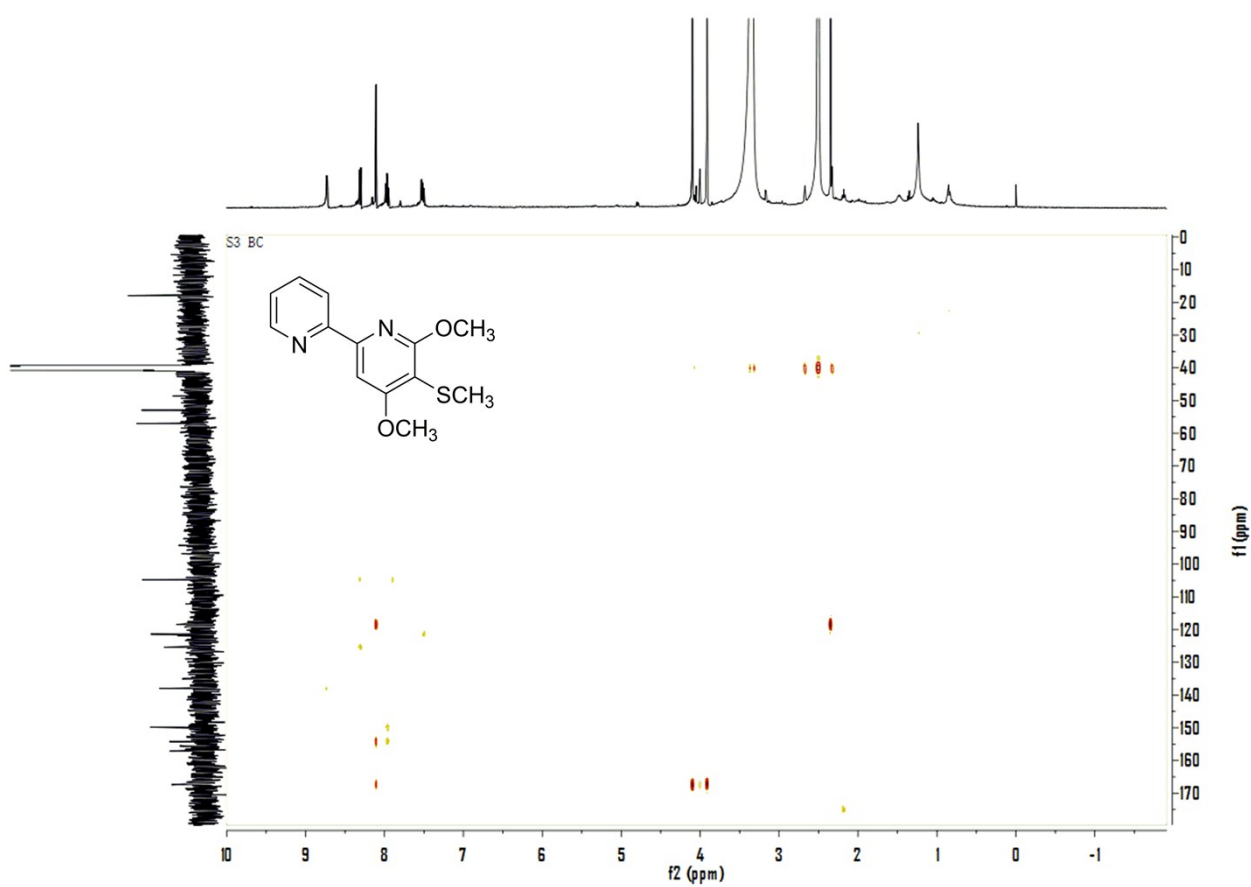


Figure S19. HMBC ( 400 MHz, DMSO) spectrum of compound **3**.

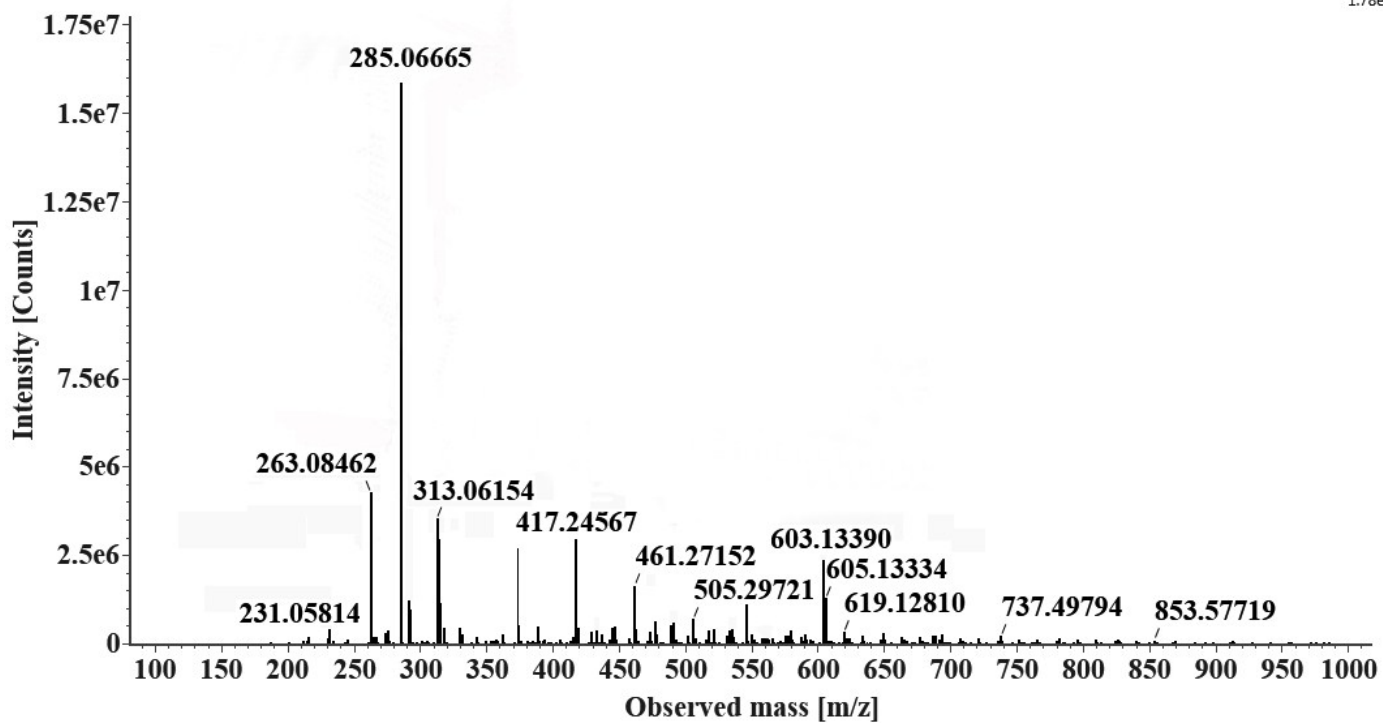
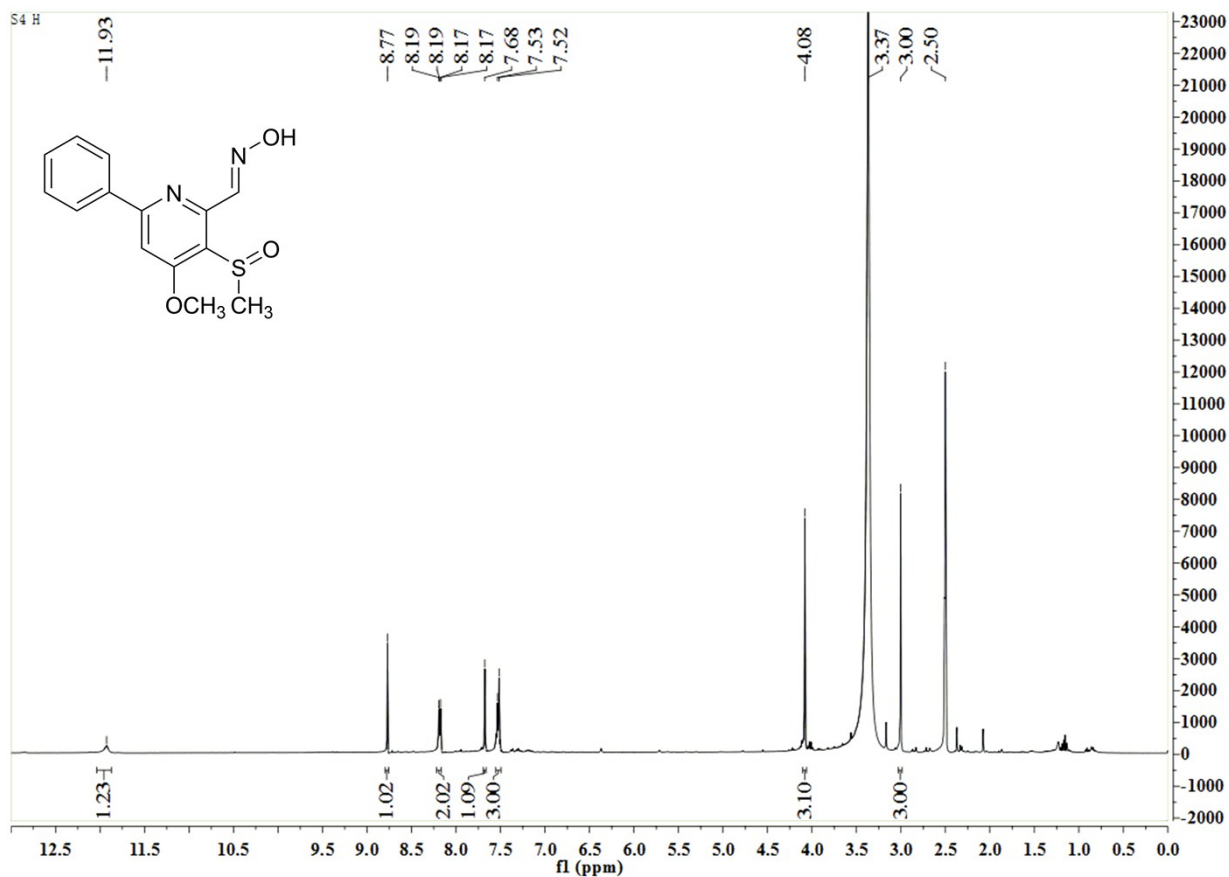
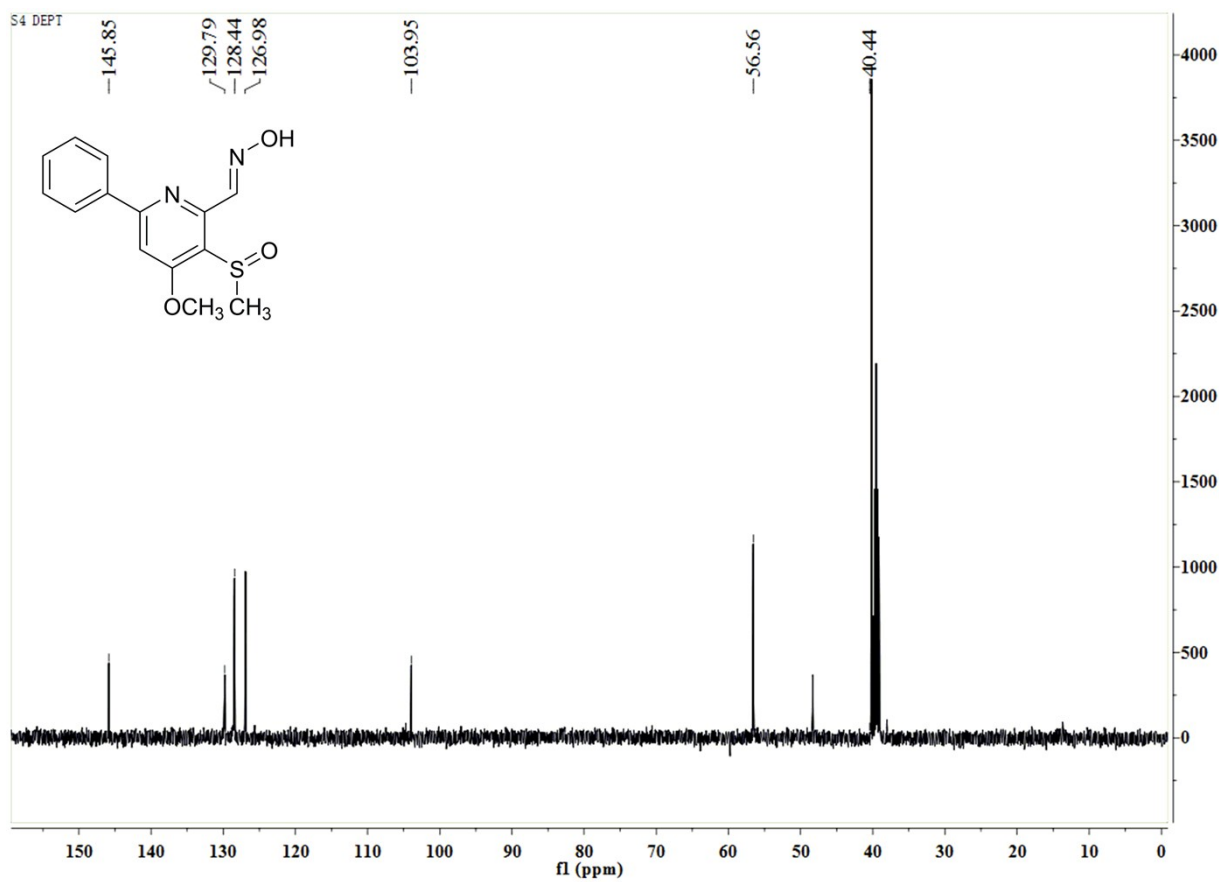
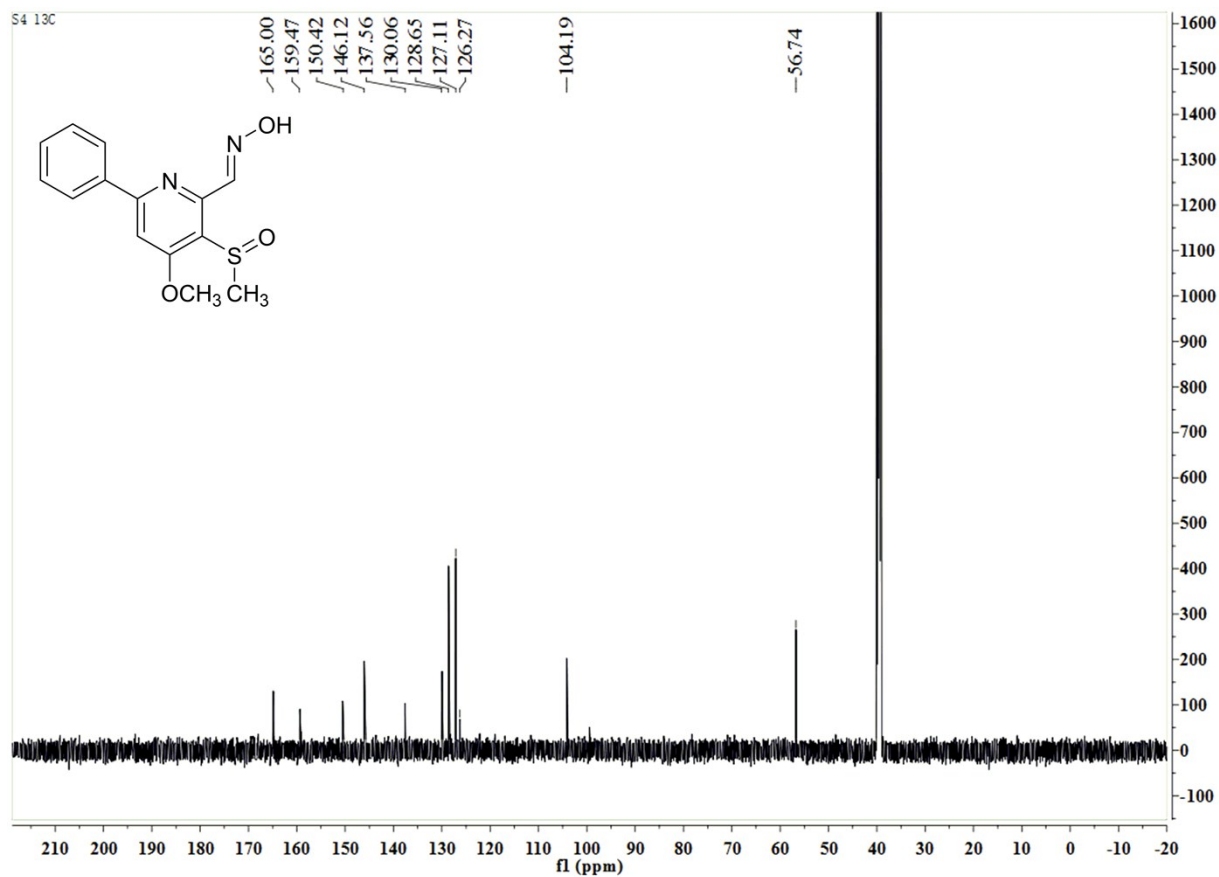


Figure S20. HRESIMS spectrum of compound 3

Figure S21. <sup>1</sup>H NMR (400 MHz, DMSO) spectrum of compound 4.



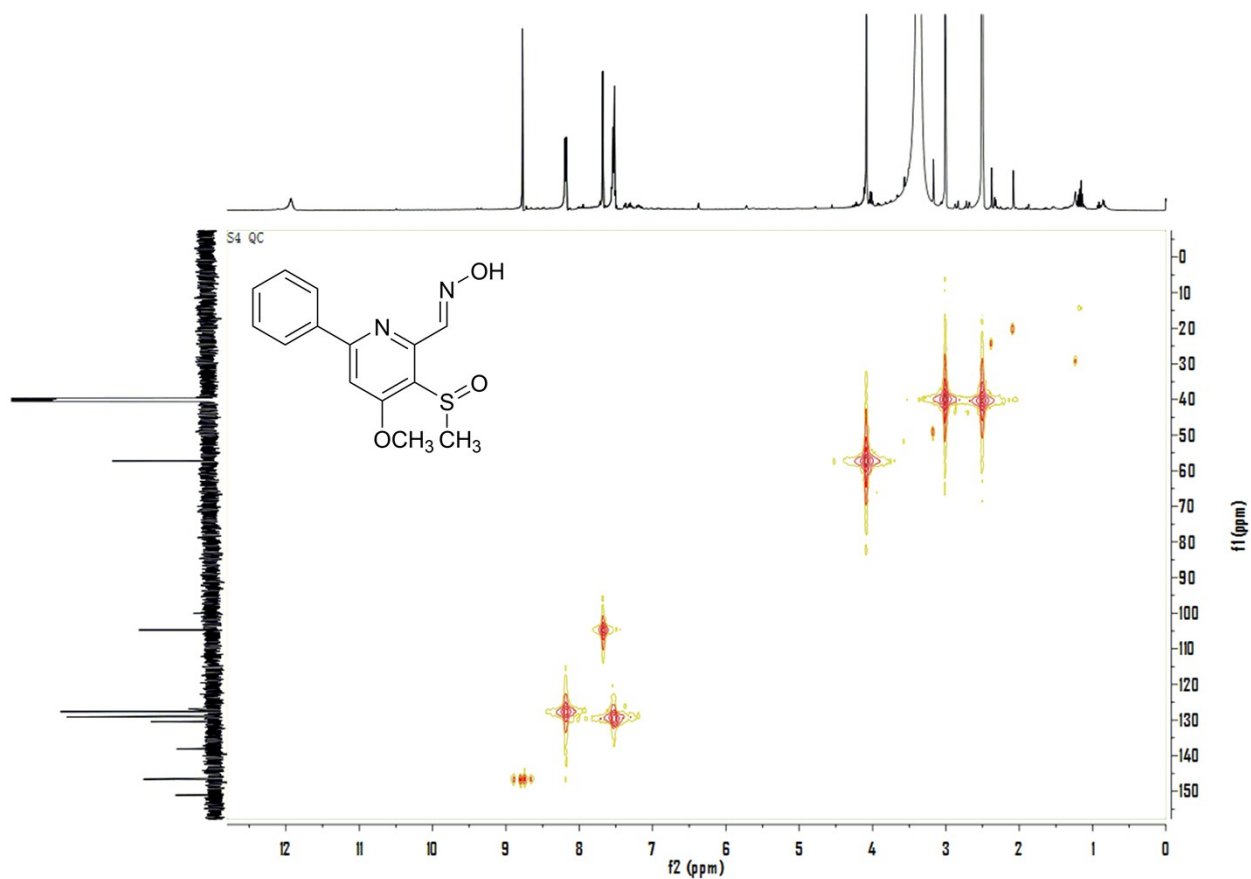


Figure S24. HSQC ( 400 MHz, DMSO) spectrum of compound 4.

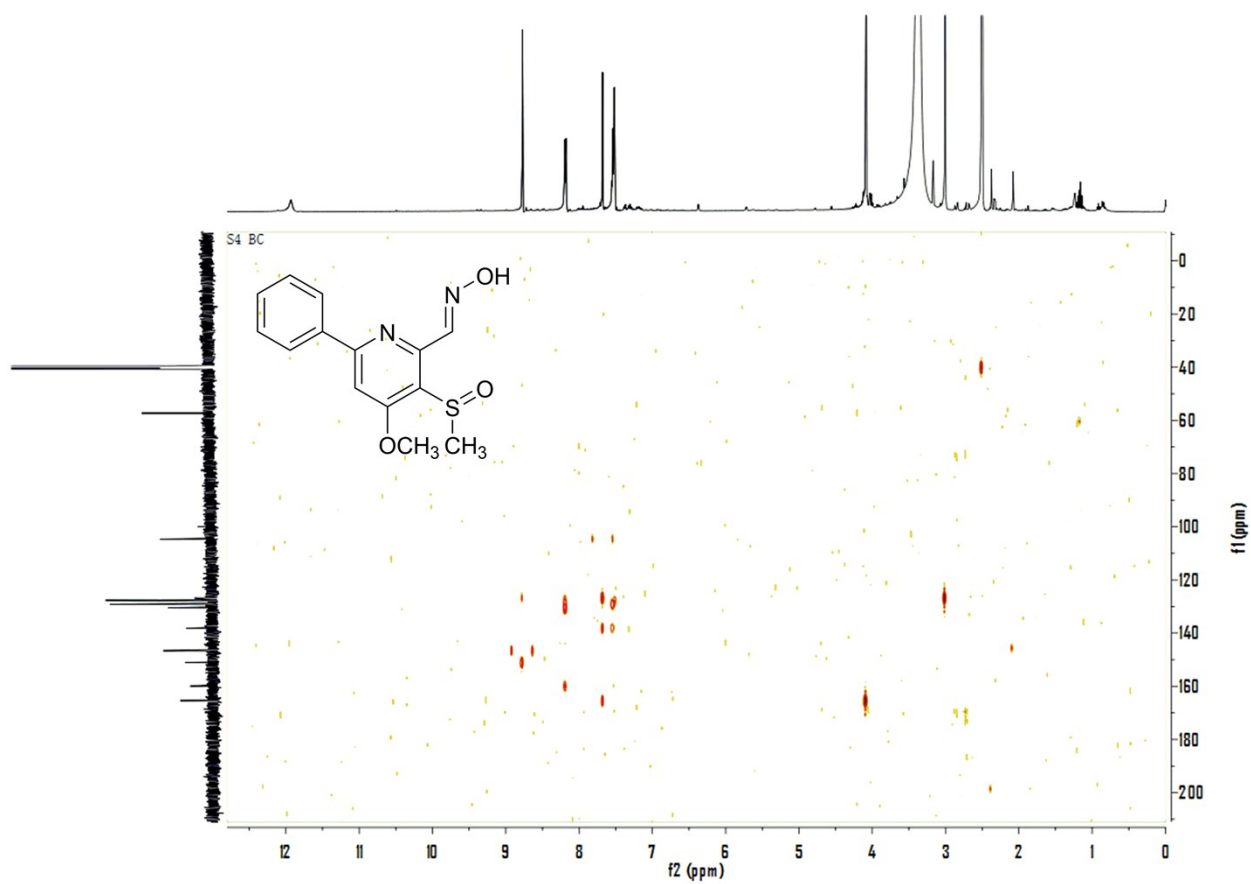


Figure S25. HMBC ( 400 MHz, DMSO) spectrum of compound 4.

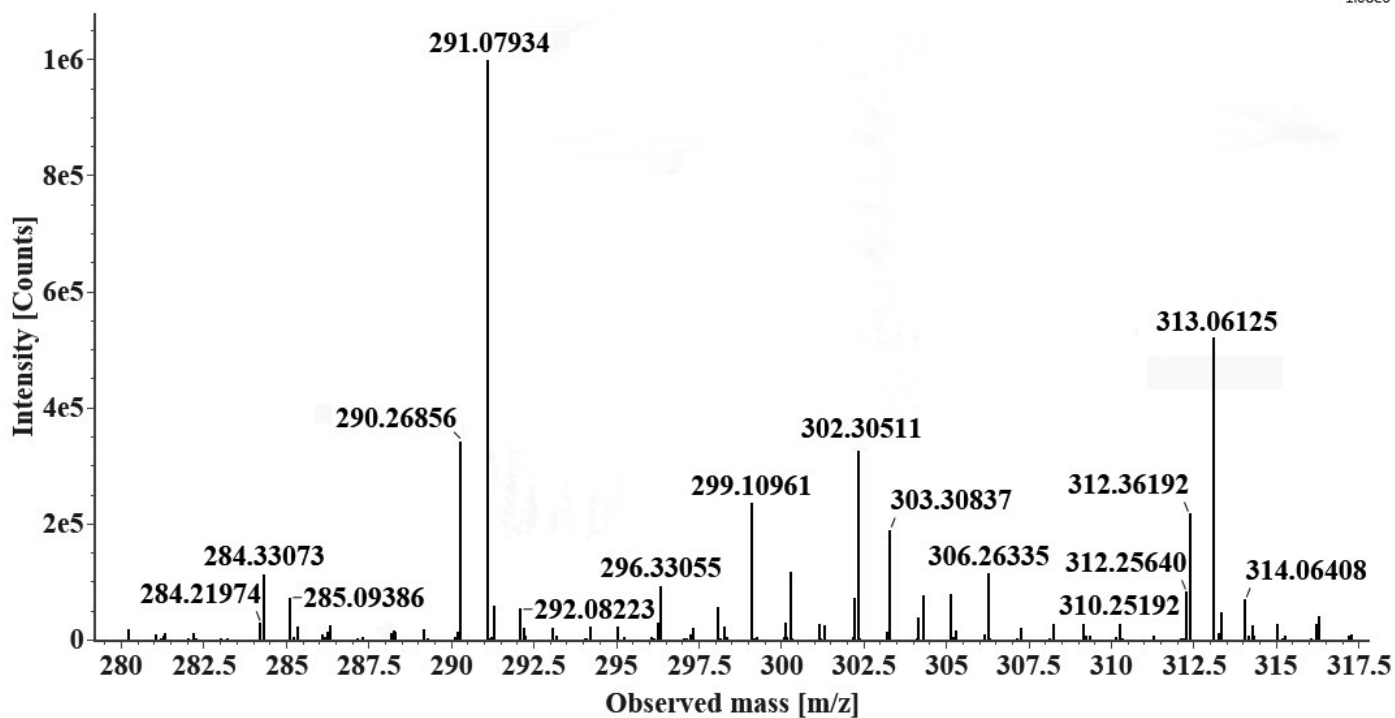
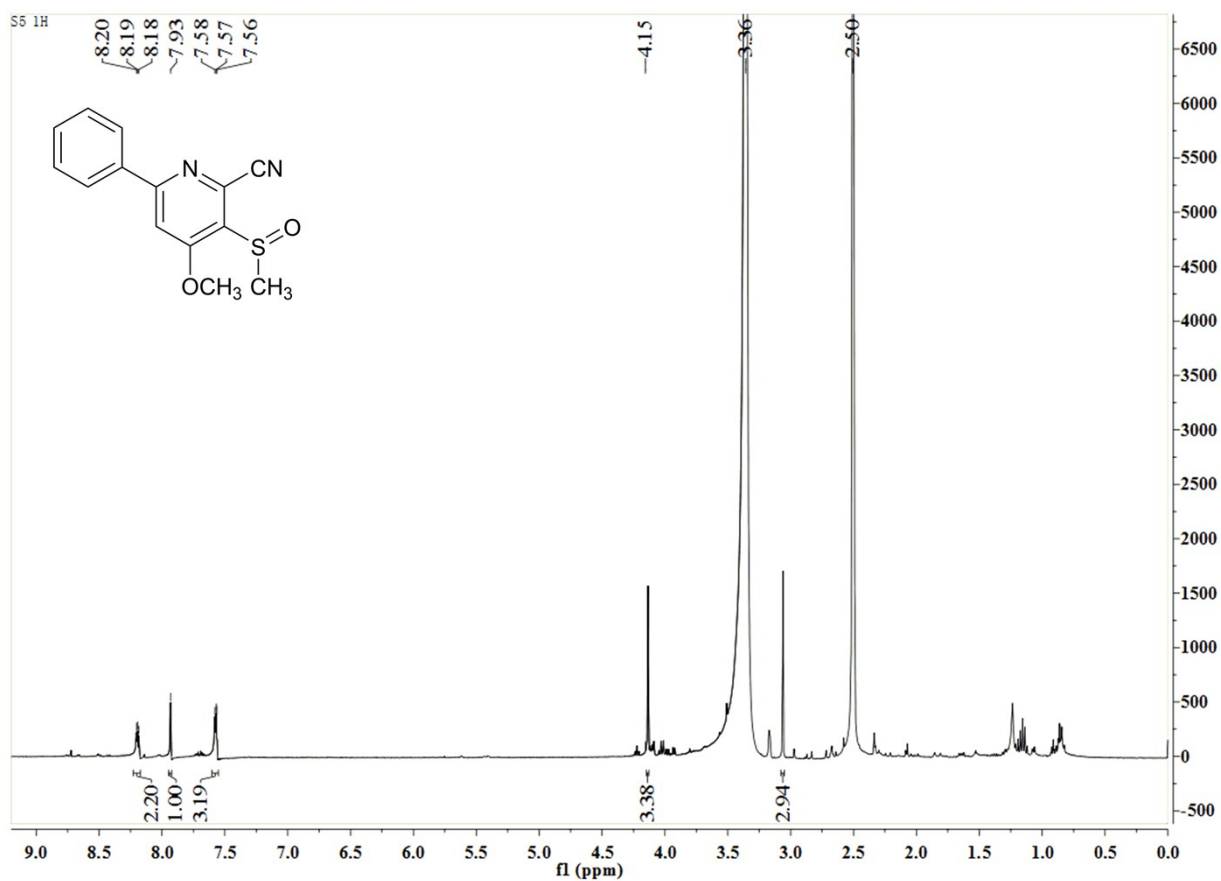


Figure S26. HRESIMS spectrum of compound 4.

Figure S27. <sup>1</sup>H NMR (400 MHz, DMSO) spectrum of compound 5.

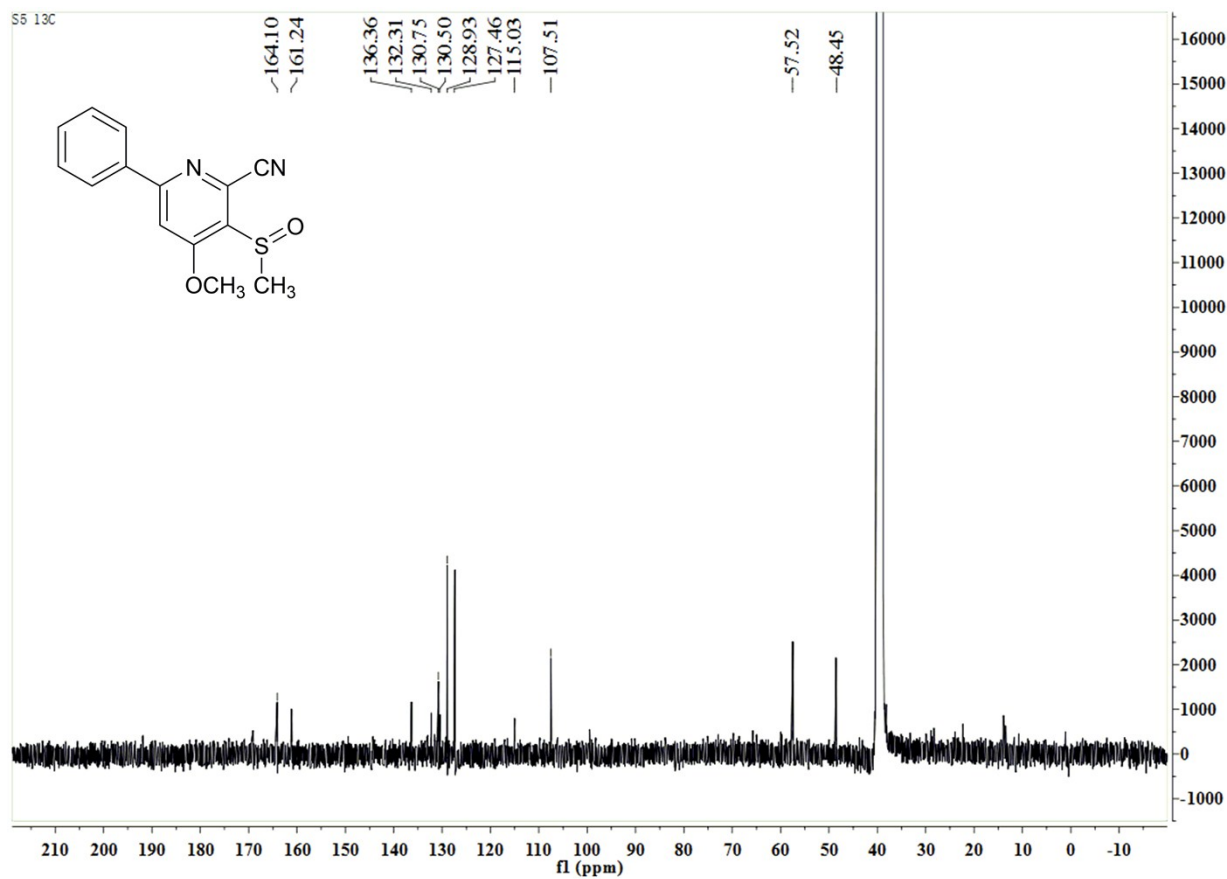


Figure S28. <sup>13</sup>C NMR ( 100 MHz, DMSO) spectrum of compound 5.

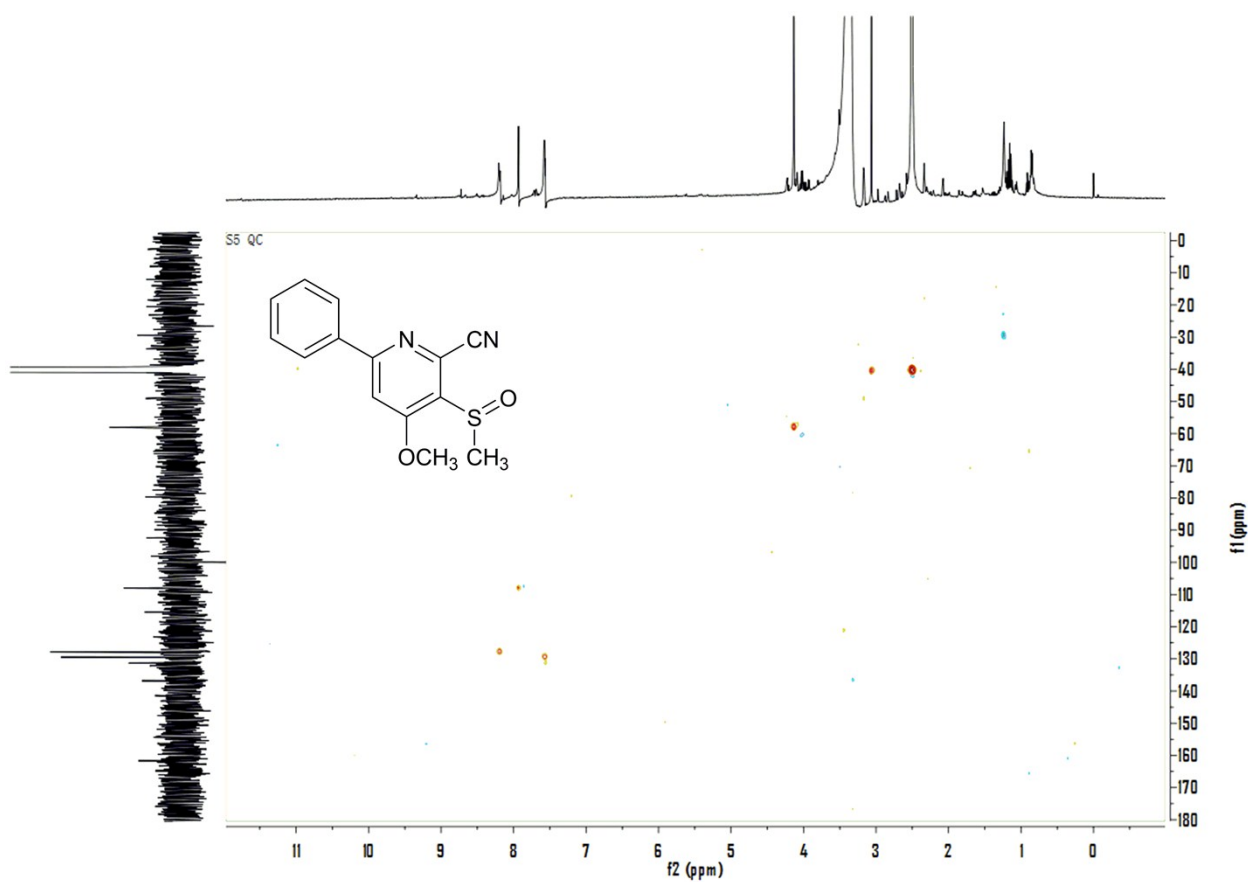


Figure S29. HSQC ( 400 MHz, DMSO) spectrum of compound 5.

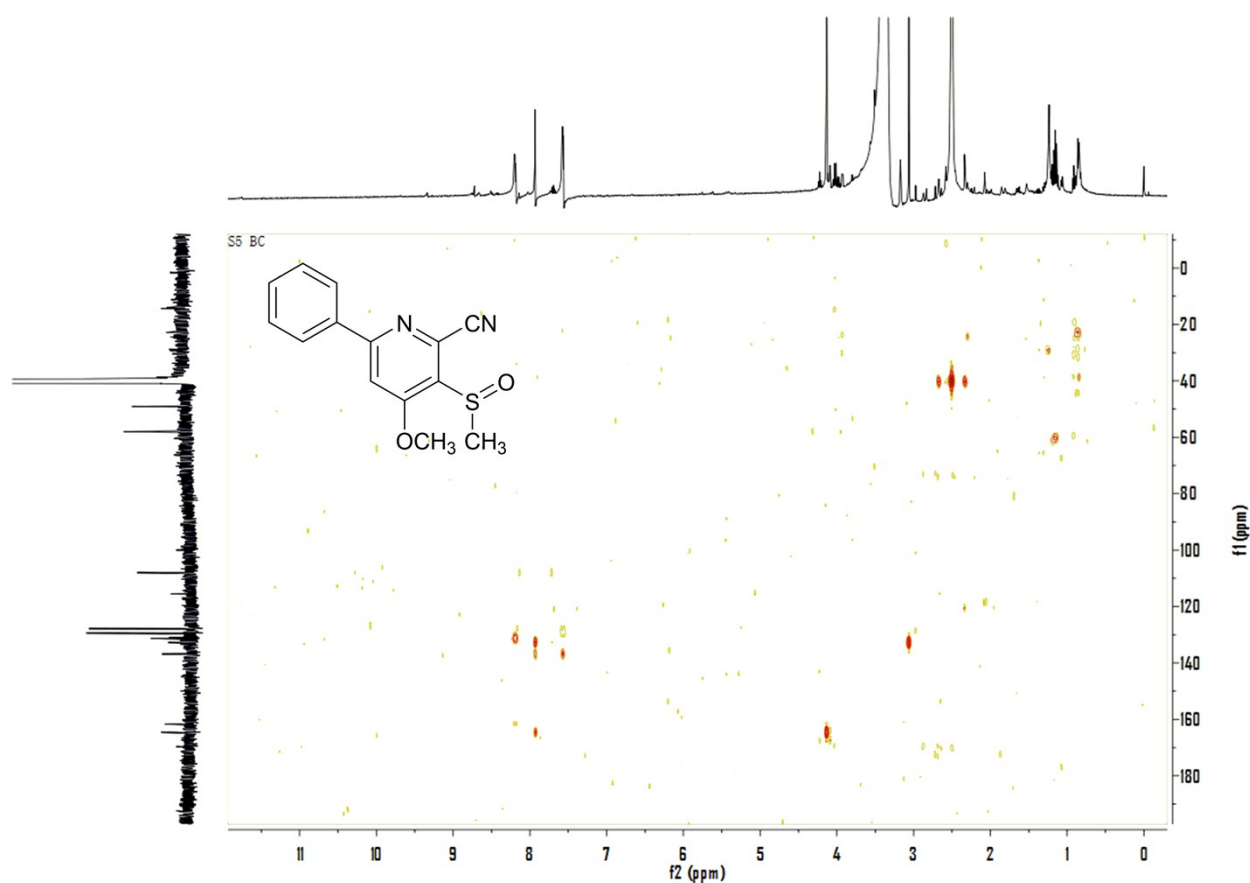


Figure S30. HMBC ( 400 MHz, DMSO) spectrum of compound **5**.

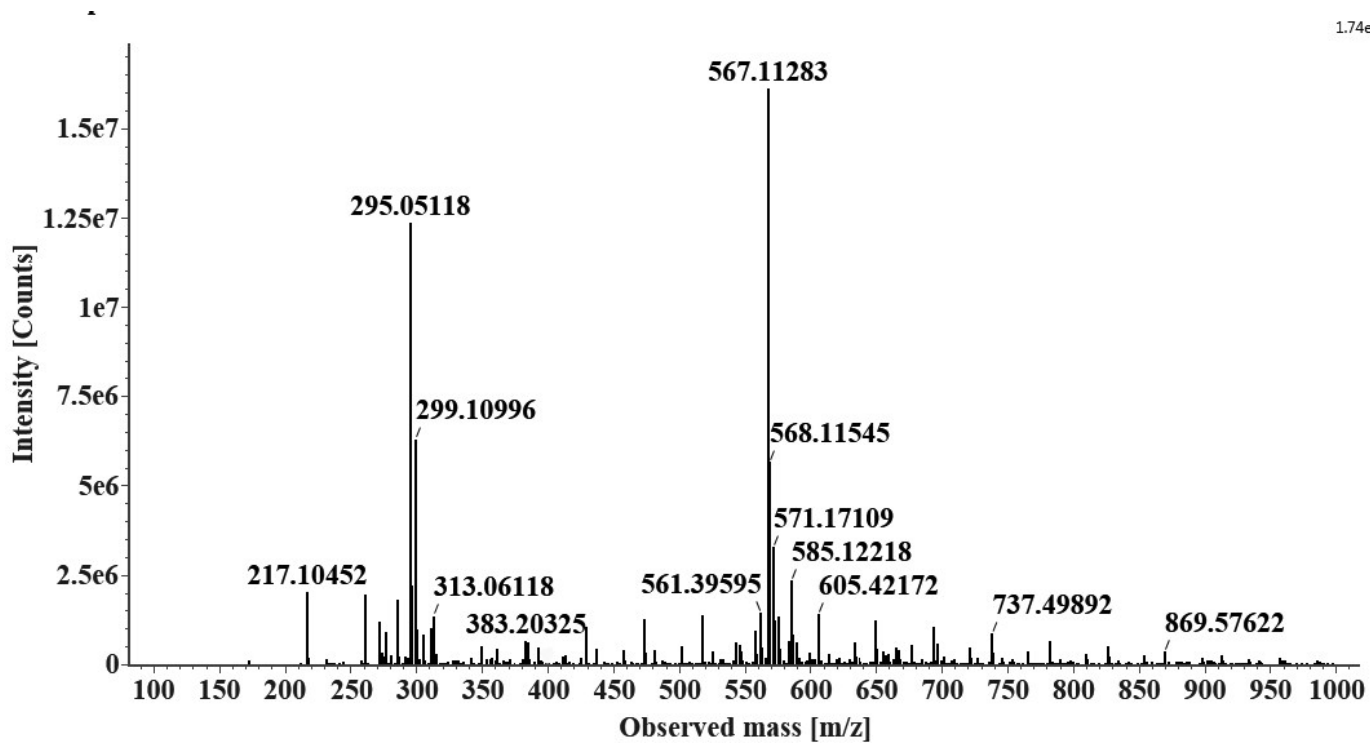


Figure S31. HRESIMS spectrum of compound **5**

1.74e7



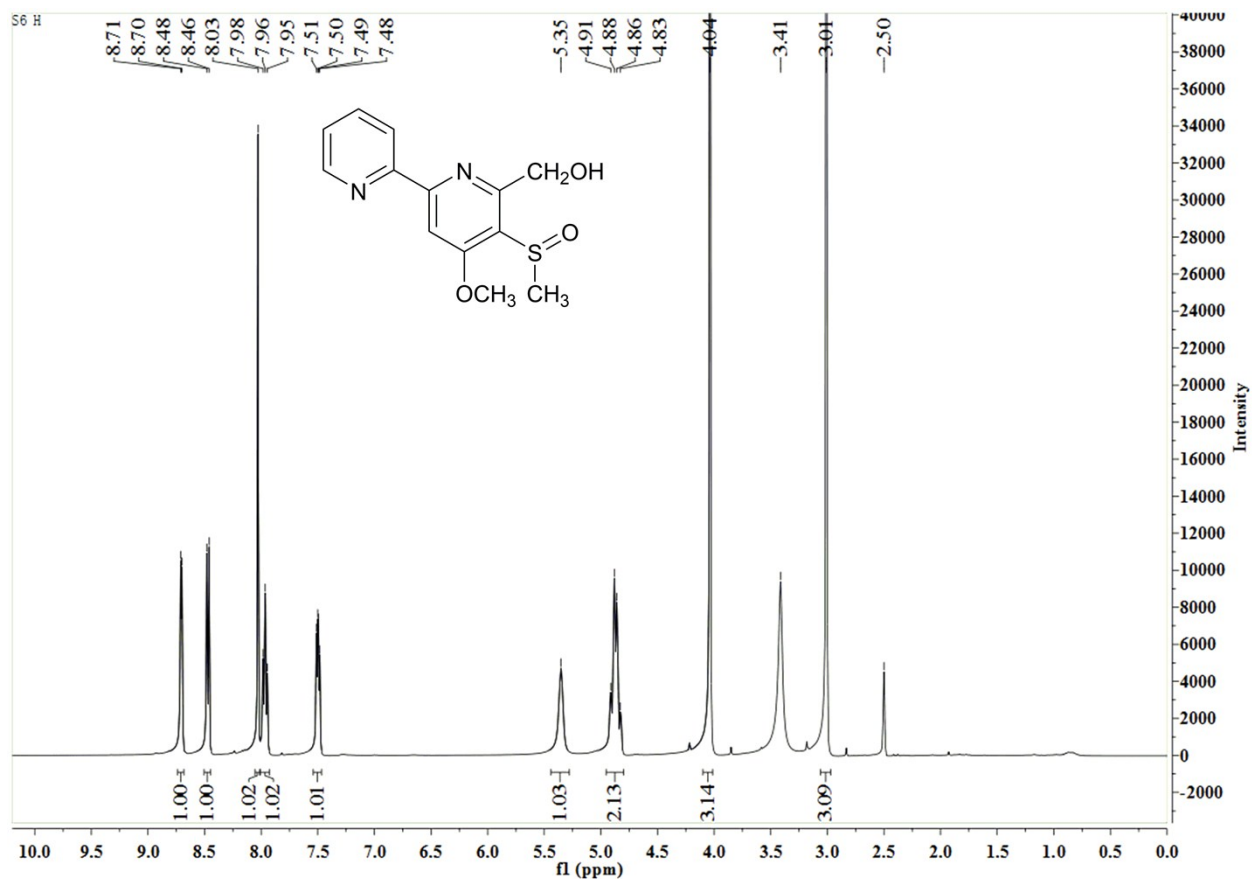


Figure S32.  $^1\text{H}$  NMR ( 400 MHz, DMSO) spectrum of compound 6.

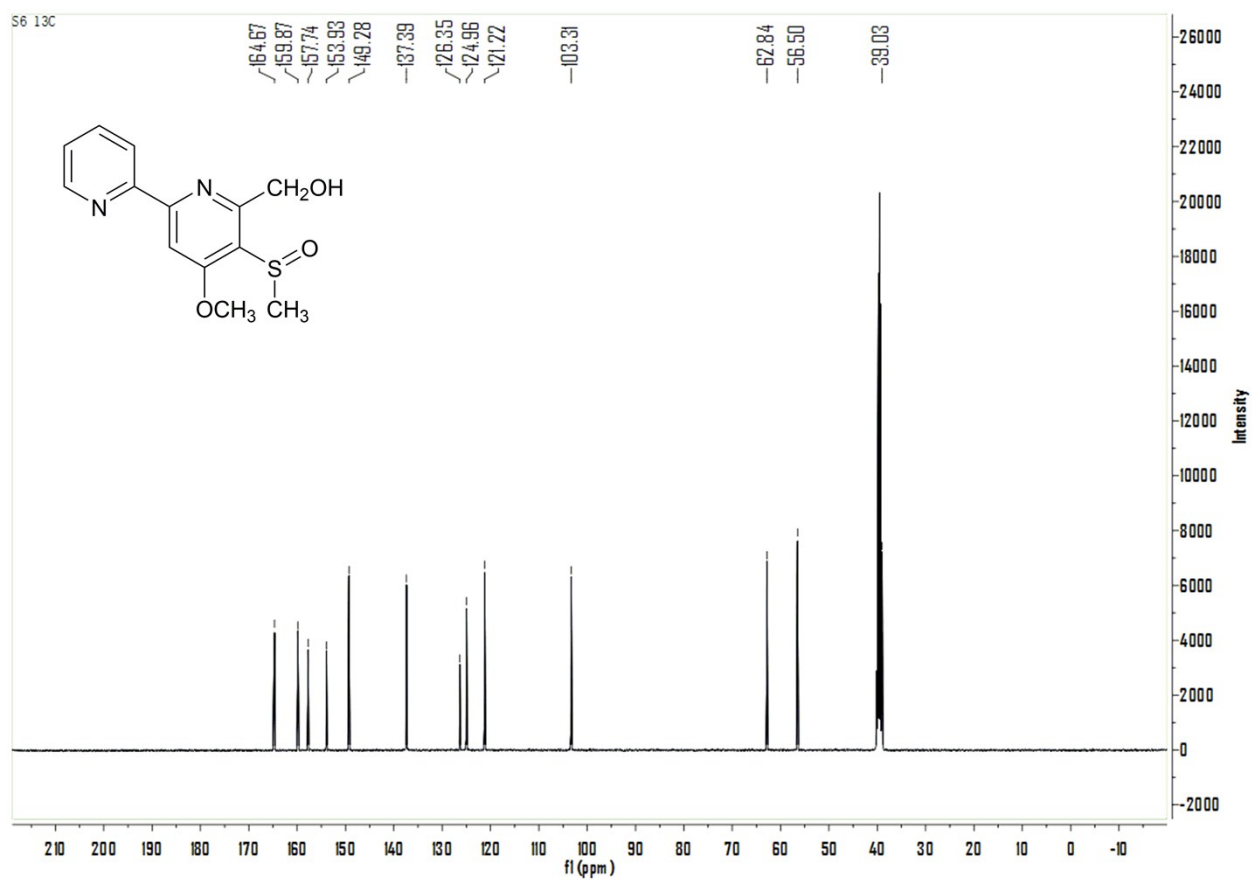


Figure S33.  $^{13}\text{C}$  NMR ( 100 MHz, DMSO) spectrum of compound 6.



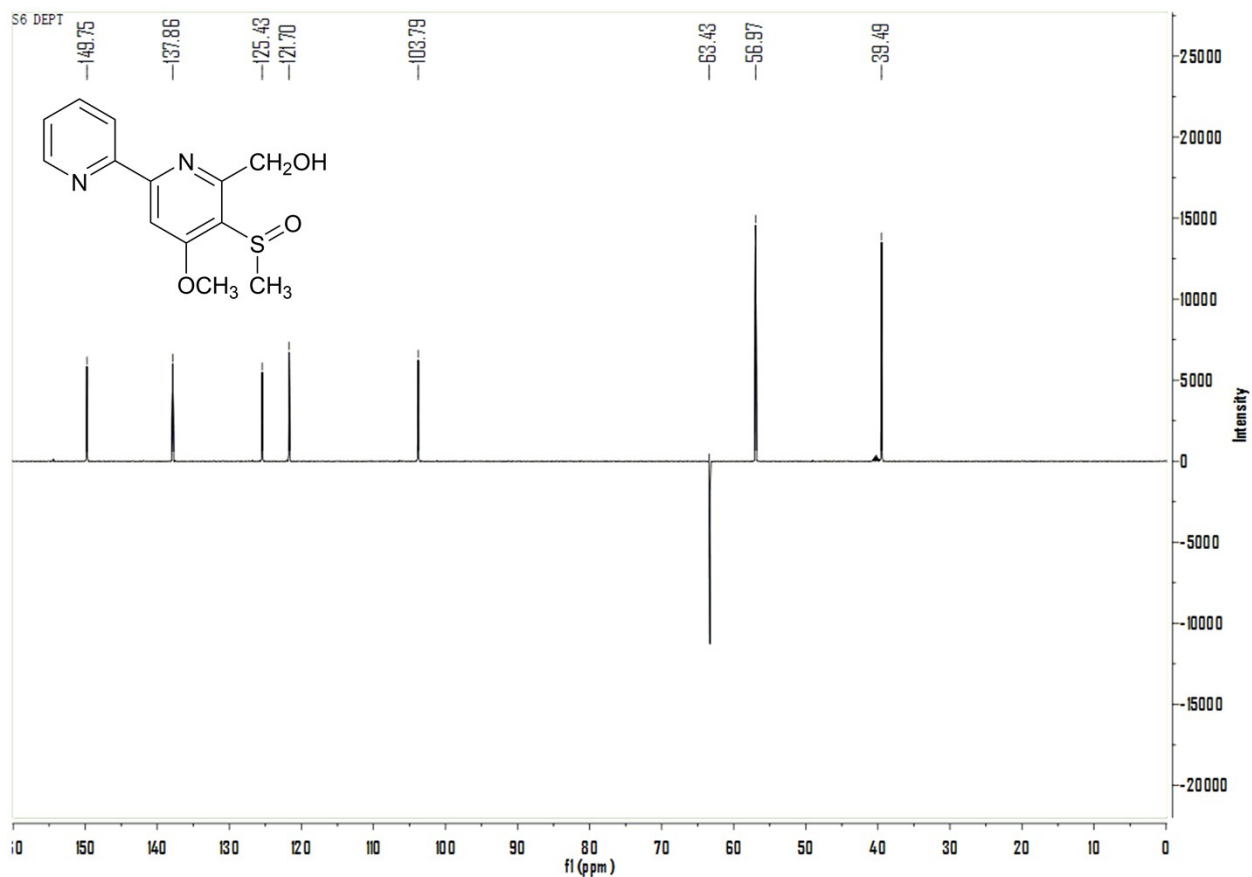


Figure S34. DEPT 135 ( 100 MHz, DMSO) spectrum of compound 6.

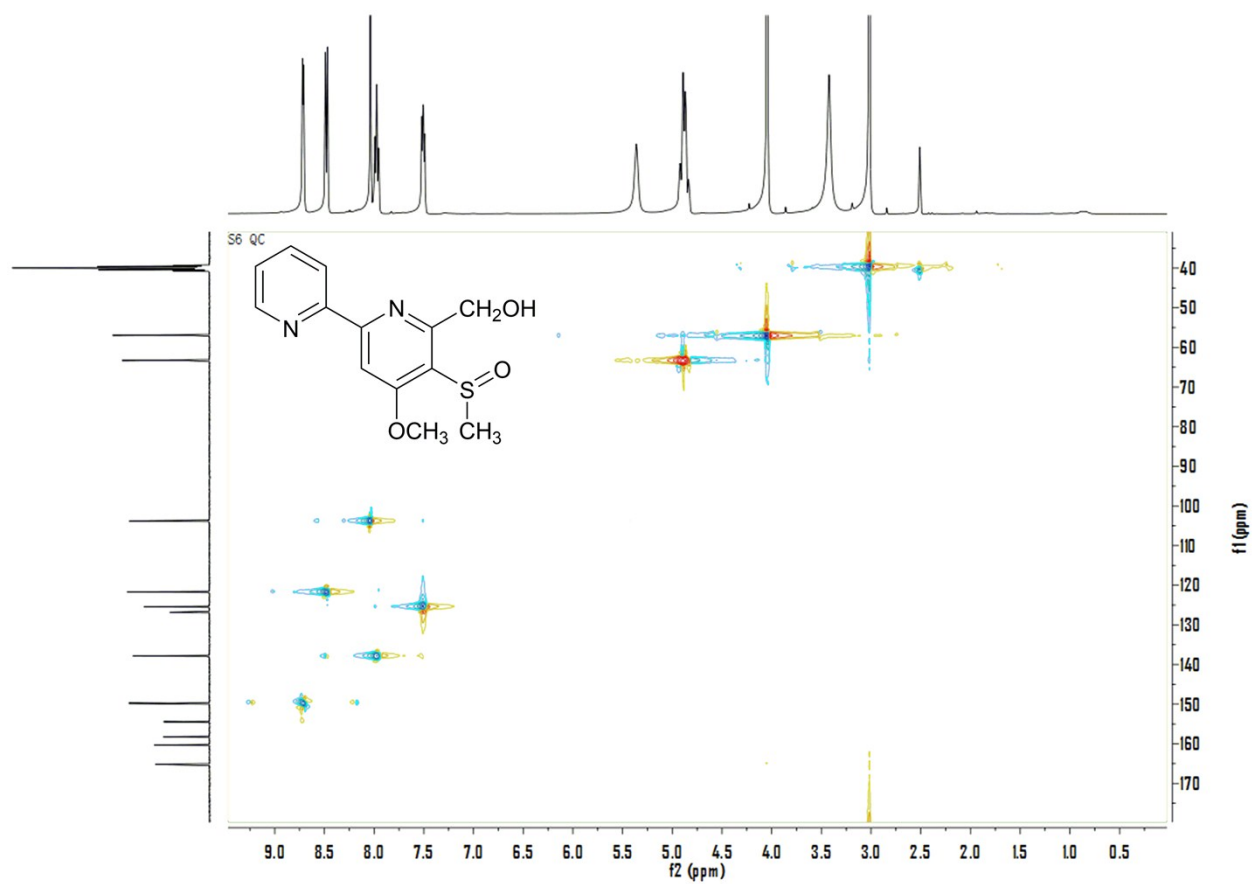


Figure S35. HSQC ( 400 MHz, DMSO) spectrum of compound 6.

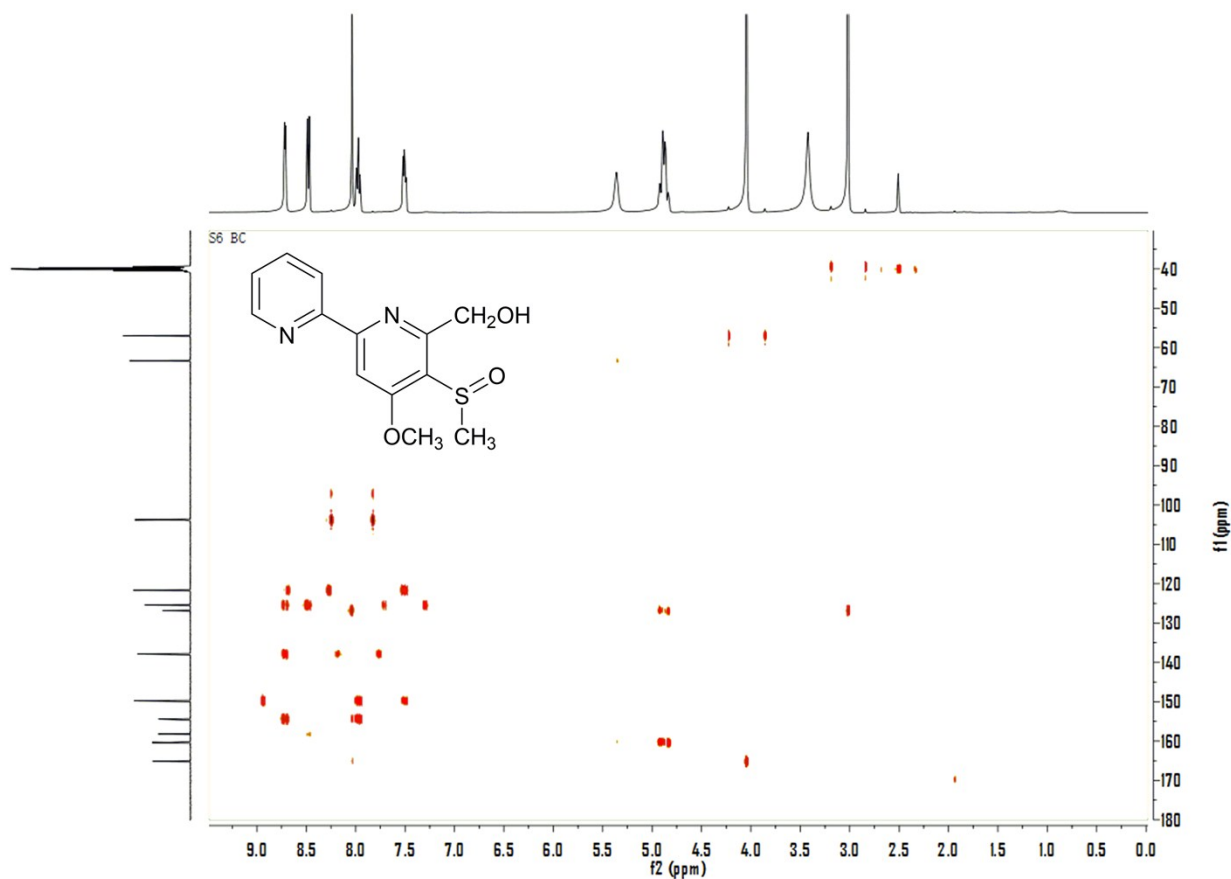


Figure S36. HMBC ( 400 MHz, DMSO) spectrum of compound 6.

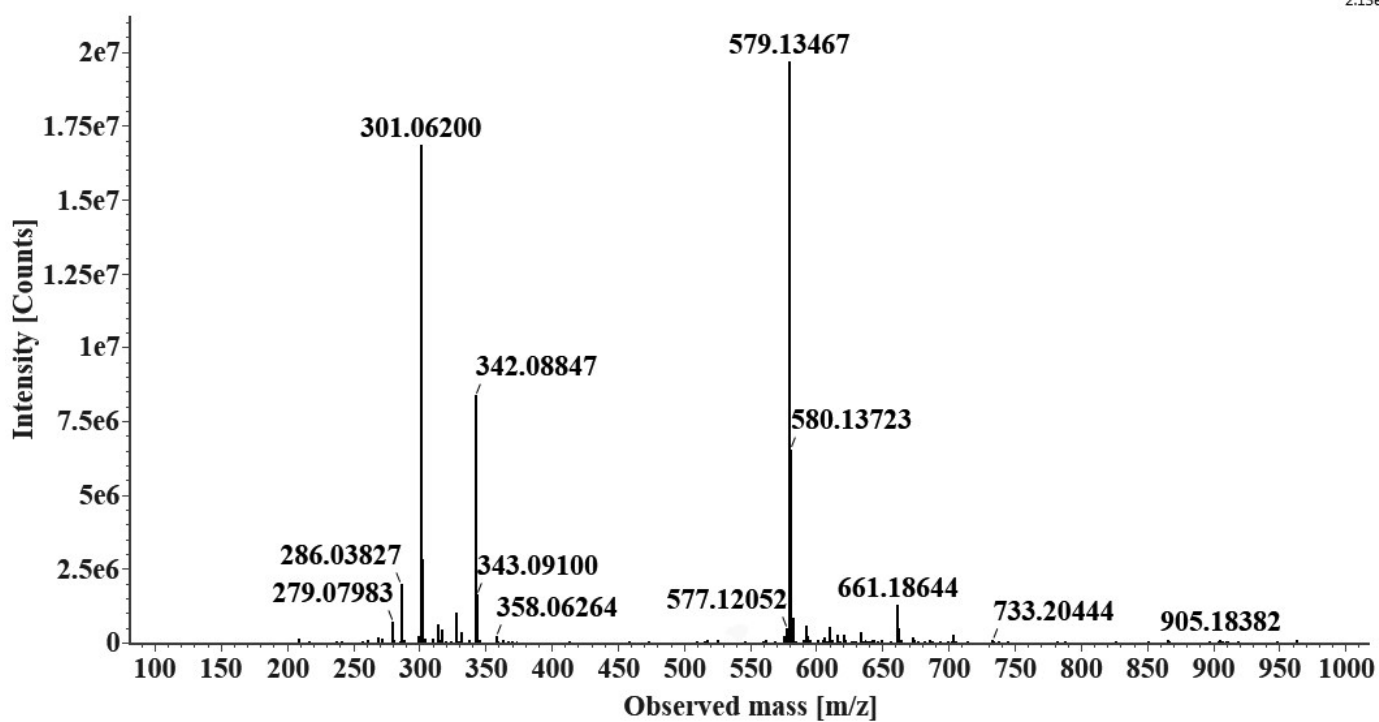


Figure S37. HRESIMS spectrum of compound 6.

2.13e7