Supporting Information

Horseradish Peroxidase (HRP): A Tool for Catalyzing the Formation of Novel Bicoumarins

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1. Structures elucidation of transformed products

Compound **1a** was obtained as a yellow amorphous powder. The molecular formula $C_{22}H_{18}O_{10}$ was determined by HRESIMS *m/z* 443.0969 (calcd for 443.0978 [M+H]+). Four aromatic proton signals δ_H 8.30 (2H, s), 7.12 (2H, s), two methoxyl signals δ_H 3.87, 3.85 (each 3H, s) and two phenolic hydroxyls δ_H 10.02 (2H, brs) were observed in the ^{1}H NMR spectrum of **1a** (Table 1). The ^{13}C NMR spectrum displayed 20 carbon signals, including two singlet methoxyls (Table 2). The spectroscopic data indicated that **1a** was a dimer of **1**. The HSQC and HMBC experiments showed correlations of carbons and protons, which could confirmed the coumarin units. Compared with **1**, the absence of H-3 and singlet of H-4 suggested that **1a** was bicoumarin with connection of C-3 and C-3'. Therefore, on the basis of the natural product isolated from *Chimonanthus praecox*,[1] compound **1a** was identified as 6,8,6',8'-tetramethoxyl-7,7'-dihydroxy-3,3'-bicoumarin. Compound **1b** gave the molecular formula $C_{33}H_{26}O_{15}$ from HRESIMS. Combined with NMR data, **1b** was deduced to be a trimer. The ^{1}H NMR spectrum revealed the signals of two phenolic hydroxyls, H-4, H-5, H-4', H-5', H-4", and H-5", which were assigned by long range correlations observed in the HMBC spectrum. Based on product **1a**, **1b** was determined to be a trimer of **1a** and **1** connected by 7'-OH and C-3".

Compounds **2a** and **2b** possessed the same molecular formula $C_{18}H_{10}O_6$ established by HRESIMS and NMR data. They both were dimer of substrate **2**. The difference was connected positions for coumarin units. The ^{1}H NMR and ^{13}C NMR data of **2a** suggested a symmetrical structure, which suggesting that **2a** was dimer of two coumarin units connected with the same position. The ^{1}H NMR displayed the signals of H-3 (3') δ_H 6.34 (2H, d, J = 9.5 Hz), H-4 (4') δ_H 7.37 (2H, d, J = 9.5 Hz), H-5 (5') δ_H 7.26 (s), H-8 (8') δ_H 7.25 (s) and two phenolic hydroxyls δ_H 9.72 (s), which were further confirmed by the HMBC experiment. The absence of H-8 (8') revealed the connection of C-8 and C-8'. The ^{1}H NMR spectrum of **2b** revealed the signals of H-3 (3'), H-4 (4'), and an aromatic ABX system δ_H 7.11 (d, J = 3.0 Hz), 7.39 (d, J = 8.5 Hz), 7.25 (dd, J = 8.5, 3.0 Hz), an aromatic AB system δ_H 7.29 (d, J = 9.0 Hz), 7.24 (d, J = 9.0 Hz) and one phenolic hydroxyl δ_H 9.99 (s). Compared with 6-hydroxycoumarin (**2**), **2b** was deduced to be dimer with the connection of C-5 and phenolic hydroxyl. The HMBC correlation of H-7 and C-5 confirmed the linkage "C-6-O-C-5'". From the above observations, transformed products of coumarin **2** were established as 6,6'-dihydroxy-7,7'-bicoumarin (**2a**), 6-hydroxy-5-(6-coumarinyloxy)coumarin (**2b**) respectively.

On the basis of physical and spectroscopic data, compounds **3a** and **3b** were both deduced to be dimeric structures of substrate (**3**). The structure of **3** displayed H-3, an ABX spin system (H-5, H-7, H-8), a 4-methyl and 6-OH. Analysis of the ^{1}H NMR spectrum of **3a** showed the signals of H-3 (3'), an aromatic AB spin system [δ_H 7.20 (d, J = 8.5 Hz), 7.31 (d, J = 8.5 Hz)], two isolated aromatic protons δ_H 7.13 (s), 7.14 (s), and two phenolic hydroxyls δ_H 9.70 (s), 9.47 (s). The absence of H-7 and H-5' indicated the C-7-C-5' linkage between two coumarin unites. The NMR data were accurately assigned on the basis

of HSQC and HMBC spectra. Compound **3b**, similar to **3a**, also consisted of two 6-hydroxy-4-methylcoumarin units. Compared with substrate, the ^{1}H NMR spectrum revealed the absence of H-5' and one phenolic hydroxyl group. Therefore, combined with HRESIMS, **3b** was deduced to be a dimeric-coumarin with linkage of C-5'-O-C-6. Both coumarin units were established by HSQC and HMBC experiments. Accordingly, products **3a** and **3b** were elucidated as 6,6'-dihydroxy- 4,4'-dimethyl-7,5'-bicoumarin, 6-hydroxy-4-methyl-5-(4-methyl-6-coumarinyloxy) oumarin, respectively.

Compound **4a**, a yellow amorphous powder, had the molecular formula $C_{18}H_{10}O_6$ on the basis of HRESIMS and NMR data, which deduced **4a** as a dimer of 7-hydroxycoumarin. The ^{1}H NMR displayed the proton signals of coumarin units, except for H-3' and H-8. Combined with 2D-NMR, **4a** was proposed to be 7,7'-dihydroxy-3,8'-bicoumarin. The structure of **4a** was further confirmed by comparison with natural product obtained from *Gnidia socotrana*.[2]

Products **5a** and **5b** were assigned the same molecular formula $C_{20}H_{14}O_6$ by HRESIMS. 14 protons were observed from the ^{1}H NMR spectra of **5a** and **5b** respectively, including all of the phenolic hydroxyls. Thus, **5a** and **5b** were both bicoumarins derived from 7-hydroxy-4-methylcoumarin (**5**). From their physical data upon comparisons with values reported in the literature, **5a** and **5b** were identified as 7,7'-dihydroxy-4,4'-dimethyl-3,8'-bicoumarin, 7,7'-dihydroxy-4,4'-dimethyl-3,6'-bicoumarin, respectively.

Table S1 ^{1}H NMR spectroscopic data of compounds (500 MHz, DMSO-d_6; δ_H in ppm, J in Hz).[a]

No.	1a	1b	2a	2b	3a	3b	4a	5a	5b
3			6.34 d (9.5)	6.47 d (9.5)	6.24 s	6.42 s	6.23 d (9.5)		
4	8.30 s	8.35 s	7.37 d (9.5)	7.98 d (9.5)			8.00d (9.5)		
5	7.12 s	7.16 s	7.26 s	7.11 d (3.0)	7.14 s	7.21 d (3.0)	7.58 d (8.5)	7.68 d (8.5)	7.69 d (8.0)
6							6.95 d (8.5)	6.846 dd (8.5, 2.0)	6.87 dd (8.0, 2.5)
7				7.25 dd (8.5, 3.0)		7.02 dd (9.0, 3.0)			
8			7.25 s	7.39 d (8.5)	7.13 s	7.34 d (9.0)		6.76 d (2.0)	6.79 d (2.5)
3'			6.34 d (9.5)	6.44 d (9.5)	6.40 s	6.32 s		6.17 s	6.15 s
4'	8.30 s	8.44 s	7.37 d (9.5)	7.89 d (9.5)			7.97 s		
5'	7.12 s	7.40 s	7.26 s				7.59 d (8.5)	7.53 s	7.70 d (8.5)
6'							6.84 dd (8.5, 2.5)	6.85 s	6.98 d (8.5)
7'				7.29 d (9.0)	7.20 d (8.5)	7.24 d (8.5)			
8'			7.25 s	7.24 d (9.0)	7.31 d (8.5)	7.30 d (9.0)	6.81 d (2.5)		
4"		7.11 s							
5"		6.93 s							

[a] The assignments of substituents were list in Characters of transformed products section.

Table S2 ^{13}C NMR spectroscopic data of compounds (125 MHz, DMSO-d_6, δ_C in ppm).[a]

No.	1a	1b	2a	2b	3a	3b	4a	5a
2	159.3	159.1	159.8	159.8	159.4	159.6	160.1	159.1
3	110.2	109.5	116.2	116.80	116.2	115.0	111.237	112.0
4	143.4	144.1	117.1	143.7	153.8	152.5	144.77	151.0
5	104.7	104.8	119.9	112.7	109.2	110.3	129.7	126.1
6	145.9	146.1	151.4	154.2	152.0	153.6	112.8	113.1
7	144.4	144.8	117.5	119.8	129.8	118.6	159.21[a]	160.0
8	134.5	134.5	142.3	117.6	119.3	117.6	110.2	102.0
9	142.5	138.3	147.3	148.7	145.9	148.0	153.2	153.8
10	117.4	121.3	118.6	119.3	119.0	120.4	111.194	110.1
2'	159.3	158.6	159.8	159.6	160.0	159.4	159.16[a]	161.1
3'	110.2	109.9	116.2	116.77	114.5	116.2	114.9	109.50
4'	143.4	142.7	117.1	137.9	152.5	151.8	144.81	154.2
5'	104.7	105.8	119.9	136.1	120.9	146.6	129.2	127.0
6'	145.9	148.8	151.4	146.0	151.7	137.2	113.3	112.5
7'	144.4	142.3	117.5	121.2	119.3	114.2	161.4	158.9
8'	134.5	139.8	142.3	113.8	117.2	120.5	102.0	113.9
9'	142.5	141.2	147.3	146.8	147.0	146.7	155.2	152.6
10'	117.4	116.6	118.6	114.1	119.8	114.9	111.5	112.1
2"		155.9						
3"		139.6						
4"		119.3						
5"		103.6						
6"		146.0						
7"		141.8						
8"		134.7						
9"		139.0						
10"		116.8						

[a] Assignments are interchangeable within column.

References

1 L.J. Shi, S. X. Yang, J. L. Bi, G. F. Yin and Y. H. Wang, *Nat. Prod. Res. Dev.*, 2012, 24, 1335.

2 K. Franke, A. Porzel and J. Schmidt, *Phytochemistry*, 2002, 61, 873.

Characterization of transformed products

6,8,6',8'-tetramethoxyl-7,7'-dihydroxy-3,3'-bicoumarin (**1a**): Yellow amorphous powder; ^1H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 3.85 (6H, s, 6-OCH_3, 6'-OCH_3), 3.87(6H, s, 8-OCH_3, 8'-OCH_3), 10.02 (2H, s, 10-OH, 10'-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2 and δ_C 56.2 (6-OCH_3, 6'-OCH_3), 60.8 (8-OCH_3, 8'-OCH_3); HRESIMS *m/z* 443.0969 (calcd for 443.0978, $C_{22}H_{19}O_{10}$ $[M+H]^+$).

6,8,6',8'-tetramethoxyl-7-hydroxy-7'-O-(6,8-dimethoxyl-7-hydroxy-3-coumarinyl)-3,3'-bicoumarin (**1b**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 3.74 (3H, s, 6-OCH_3), 3.86 (3H, s, 8-OCH_3), 3.84 (3H, s, 6'-OCH_3), 3.93 (3H, s, 8'-OCH_3), 3.87 (3H, s, 6"-OCH_3), 3.89 (3H, s, 8"-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2 and δ_C 44.1 (6-OCH_3), 56.2 (8-OCH_3), 55.9 (6'-OCH_3), 61.6 (8'-OCH_3), 56.6 (6"-OCH_3), 60.7 (8"-OCH_3); HRESIMS *m/z* 663.1340 (calcd for 663.1350, $C_{33}H_{27}O_{15}$ $[M+H]^+$).

6,6'-dihydroxy-7,7'-bicoumarin (**2a**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 9.72 (2H, s, 6-OH, 6'-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see TableS2; HRESIMS *m/z* 323.0546 (calcd for 323.0556, $C_{18}H_{11}O_{15}$ $[M+H]^+$).

6-hydroxy-5-(6-coumarinyloxy)-coumarin (**2b**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 9.99 (1H, s, 6'-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2; HRESIMS *m/z* 323.0546 (calcd for 323.0556, $C_{18}H_{11}O_{15}$ $[M+H]^+$).

6,6'-dihydroxy-4,4'-dimethyl-7,5'-bicoumarin (**3a**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 1.80 (3H, s, 4-Me), 2.42 (3H, s, 4'-Me),9.70 (6-OH), 9.47 (6'-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2 and δ_C 22.2 (4-Me), 18.0 (4'-Me); HRESIMS *m/z* 351.0858 (calcd for 351.0858, $C_{20}H_{15}O_6$ $[M+H]^+$).

6-hydroxy-4-methyl-5-(4-methyl-6-coumarinyloxy)coumarin (**3b**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 2.36 (3H, s, 4-Me), 2.40 (3H, s, 4'-Me), 9.95 (brs, 6'-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2 and δ_C 18.0 (4-Me), 22.3 (4'-Me); HRESIMS *m/z* 373.0677 (calcd for 373.0688, $C_{20}H_{14}NaO_6$ $[M+Na]^+$).

7,7'-dihydroxy-3,8'-bicoumarin (**4a**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 10.65 (1H, s, 7'-OH), 10.63 (1H, s, 7-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2; HRESIMS *m/z* 345.0363 (calcd for 345.0375, $C_{18}H_{11}O_6$ $[M+H]^+$).

7,7'-dihydroxy-4,4'-dimethyl-3,8'-bicoumarin (**5a**): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 2.13 (3H, s, 4-CH_3), 2.42 (3H, s, 4'-CH_3), 10.59 (1H, s, 6-OH), 10.50 (1H, s, 6'-OH); ^{13}C NMR (DMSO-d_6, 125 MHz) see Table S2 and δ_C 16.0 (4-CH_3), 18.2 (4'-CH_3); HRESIMS *m/z* 351.0863 (calcd for 351.0869, $C_{20}H_{15}O_6$ $[M+H]^+$).

7,7'-dihydroxy-4,4'-dimethyl-3,6'-bicoumarin (5b): Yellow amorphous powder; ^{1}H NMR (DMSO-d_6, 500 MHz) see Table S1 and δ_H 2.16 (3H, s, 4-CH_3), 2.35 (3H, s, 4'-CH_3), 10.52 (1H, s, 6-OH), 10.53 (1H, s, 6'-OH); HRESIMS *m/z* 373.0679 (calcd for 373.0688, $C_{20}H_{14}NaO_6$ [M+ Na]$^+$).

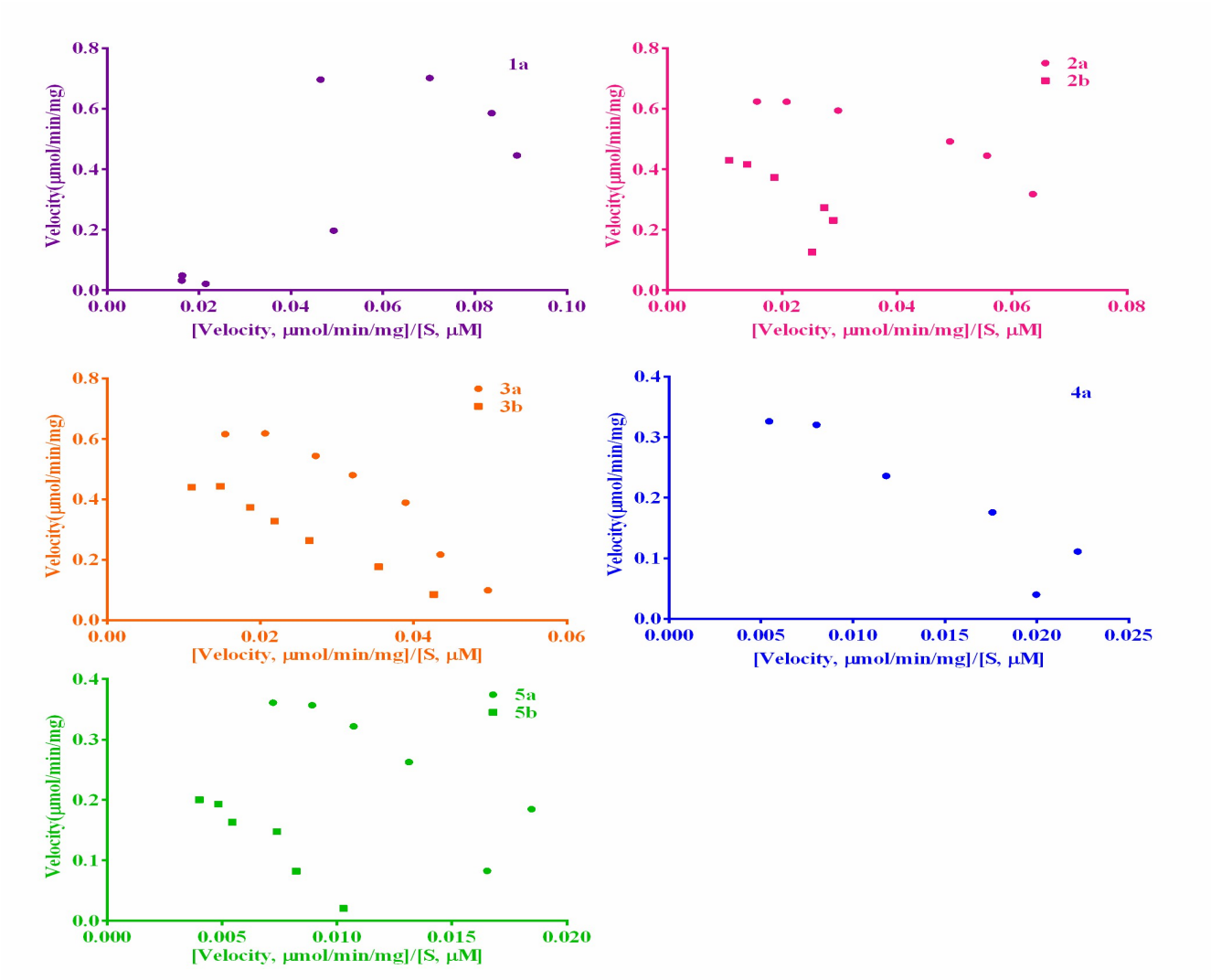
2. α-glucosidase inhibitory effects of the transformed products

Table S3 α-glucosidase inhibitory effects of coumarins.

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	>200	**3b**	111.6
1a	>200	**4**	116.11
1b	>200	**4a**	36.99
2	>200	**5**	151.09
2a	>200	**5a**	56.32

3. Kinetic analysis of the formation of bicoumarins in HRP catalyzed reactions

Fig. S1 Eadie–Hofstee plots of the kinetic analysis for various biocoumarins



4. Spectra of bicoumarins.

For compound **1a**:

SI-1. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **1a**
SI-2. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **1a**
SI-3. The HSQC spectrum of compound **1a**
SI-4. The HMBC spectrum of compound **1a**
SI-5. The NOESY spectrum of compound **1a**
SI-6. The HRESIMS spectrum of compound **1a**

For compound **1b**:

SI-7. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **1b**
SI-8. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **1b**
SI-9. The HMQC spectrum of compound **1b**
SI-10. The HMBC spectrum of compound **1b**
SI-11. The HRESIMS spectrum of compound **1b**

For compound **2a**:

SI-12. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **2a**
SI-13. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **2a**
SI-14. The HMQC spectrum of compound **2a**
SI-15. The HMBC spectrum of compound **2a**
SI-16. The HRESIMS spectrum of compound **2a**

For compound **2b**:

SI-17. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **2b**
SI-18. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **2b**
SI-19. The HMQC spectrum of compound **2b**
SI-20. The HMBC spectrum of compound **2b**
SI-21. The HRESIMS spectrum of compound **2b**

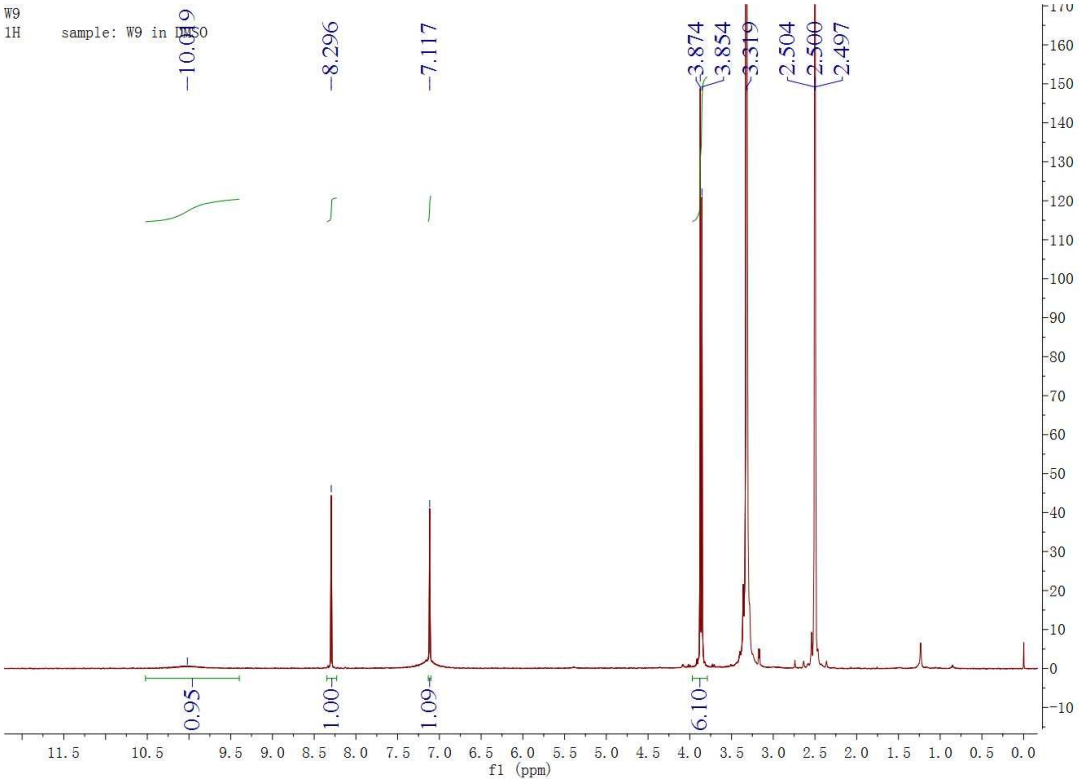
For compound **3a**:

SI-22. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3a**
SI-23. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3a**
SI-24. The HMQC spectrum of compound **3a**
SI-25. The HMBC spectrum of compound **3a**
SI-26. The HRESIMS spectrum of compound **3a**

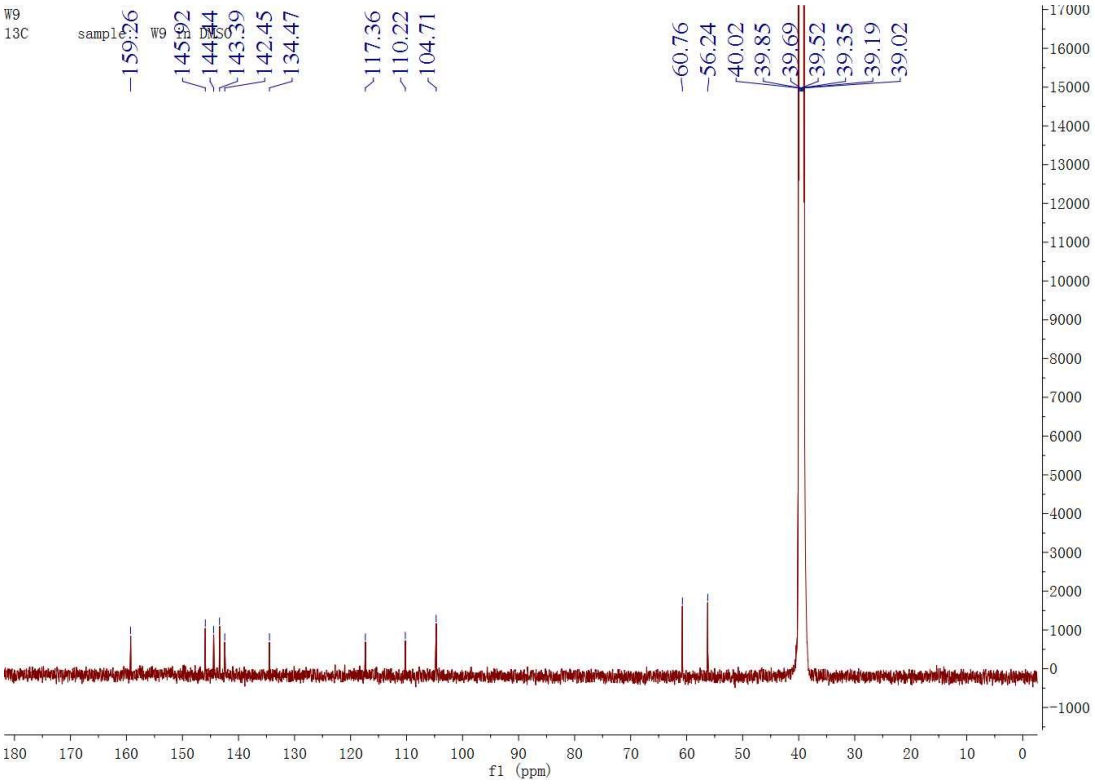
For compound **3b**:

SI-27. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3b**
SI-28. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3b**
SI-29. The HMQC spectrum of compound **3b**
SI-30. The HMBC spectrum of compound **3b**
SI-31. The HRESIMS spectrum of compound **3b**

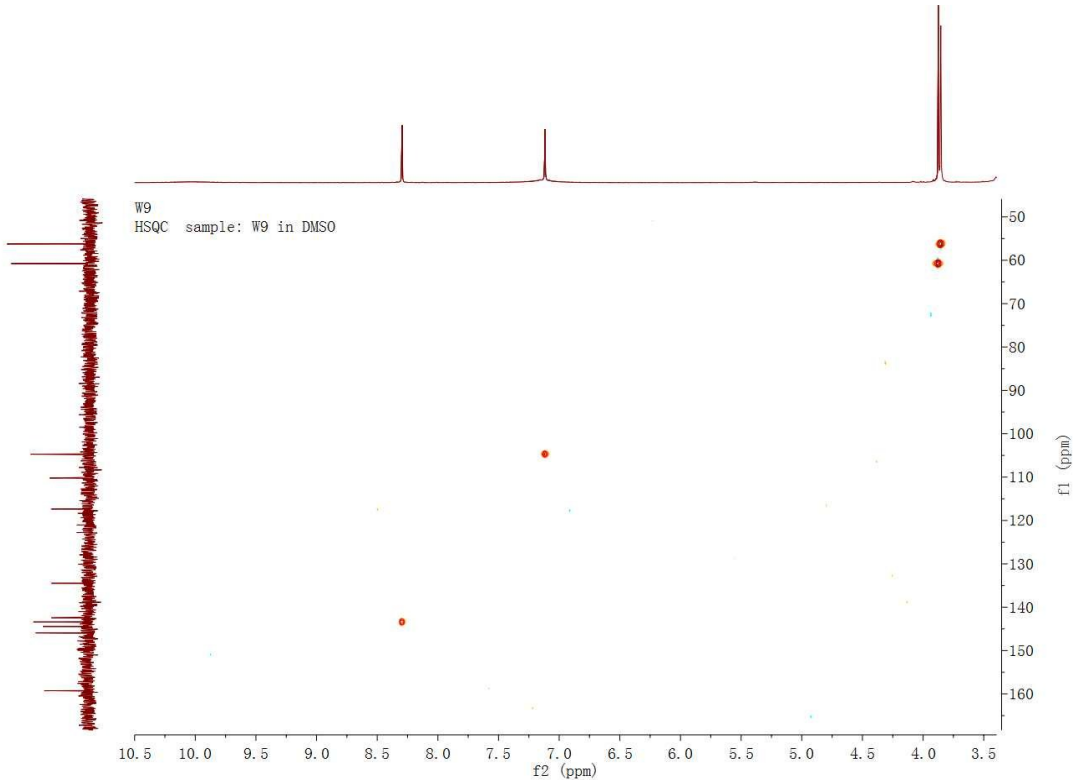
SI-1. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **1a**



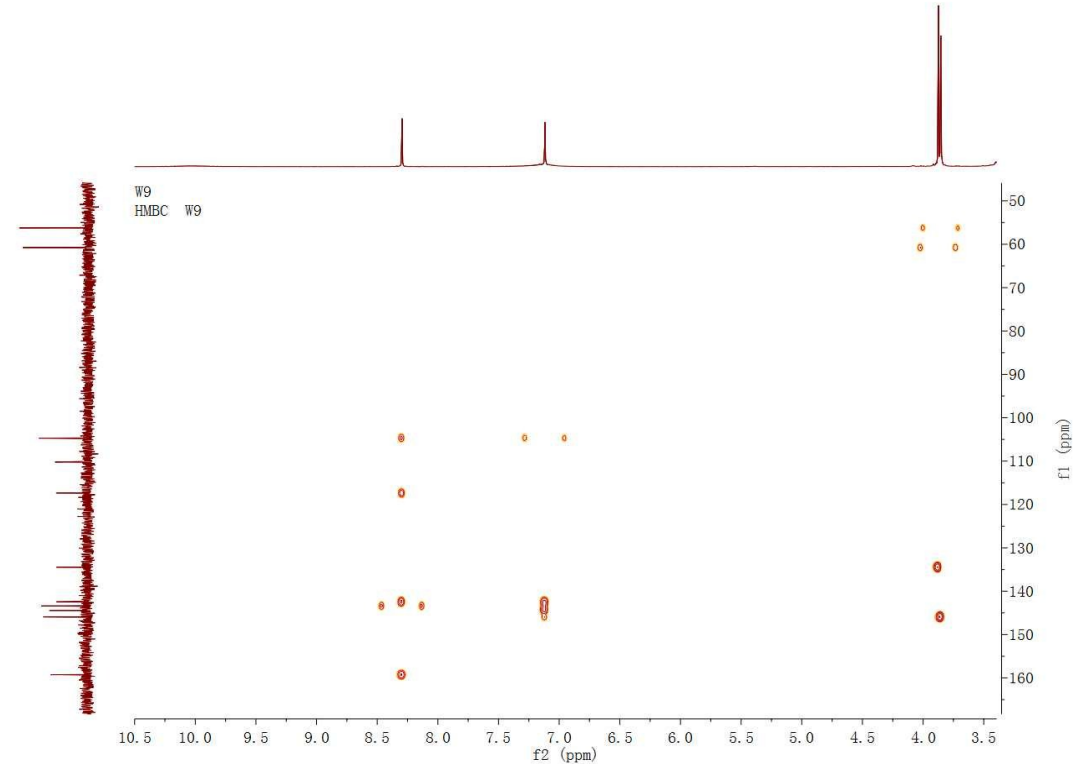
SI-2. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **1a**



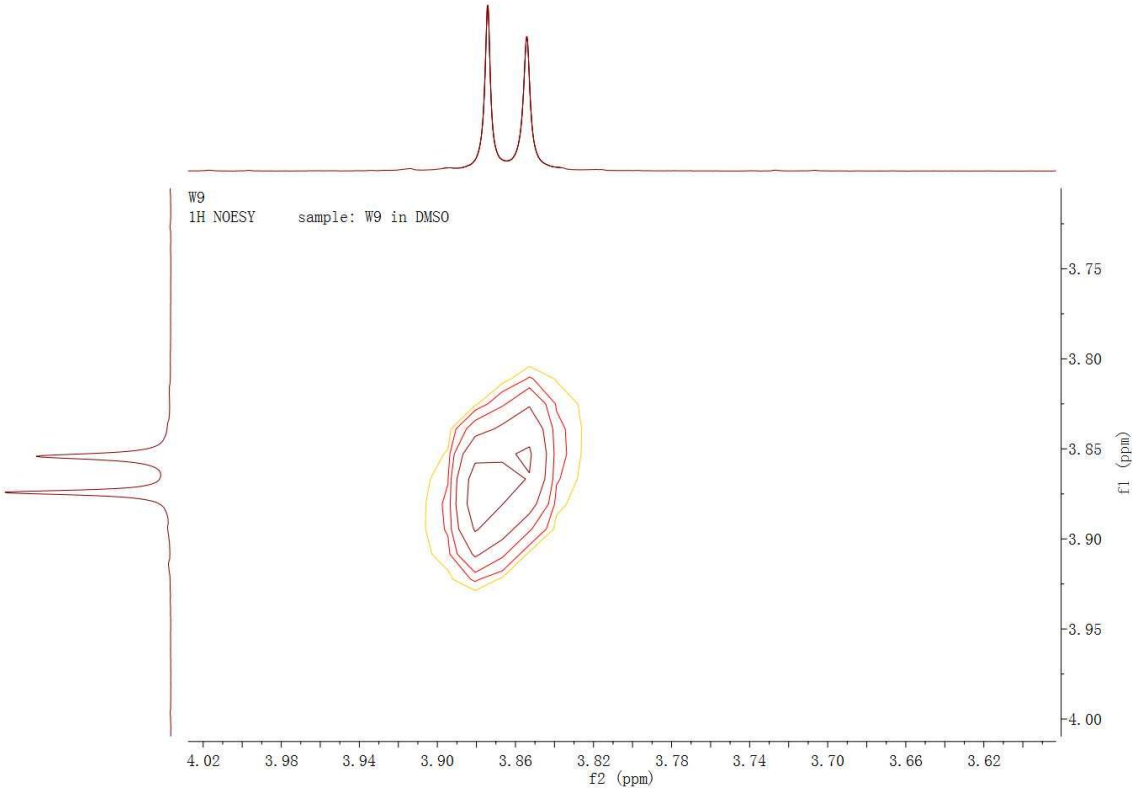
SI-3. The HSQC spectrum of compound **1a**



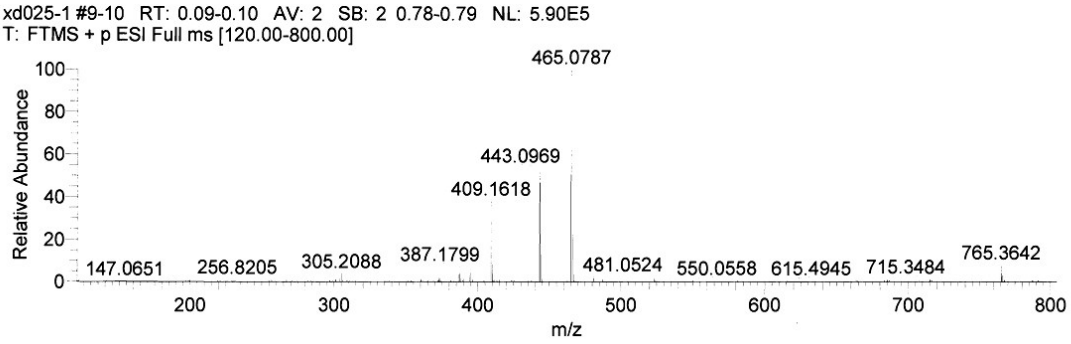
SI-4. The HMBC spectrum of compound **1a**



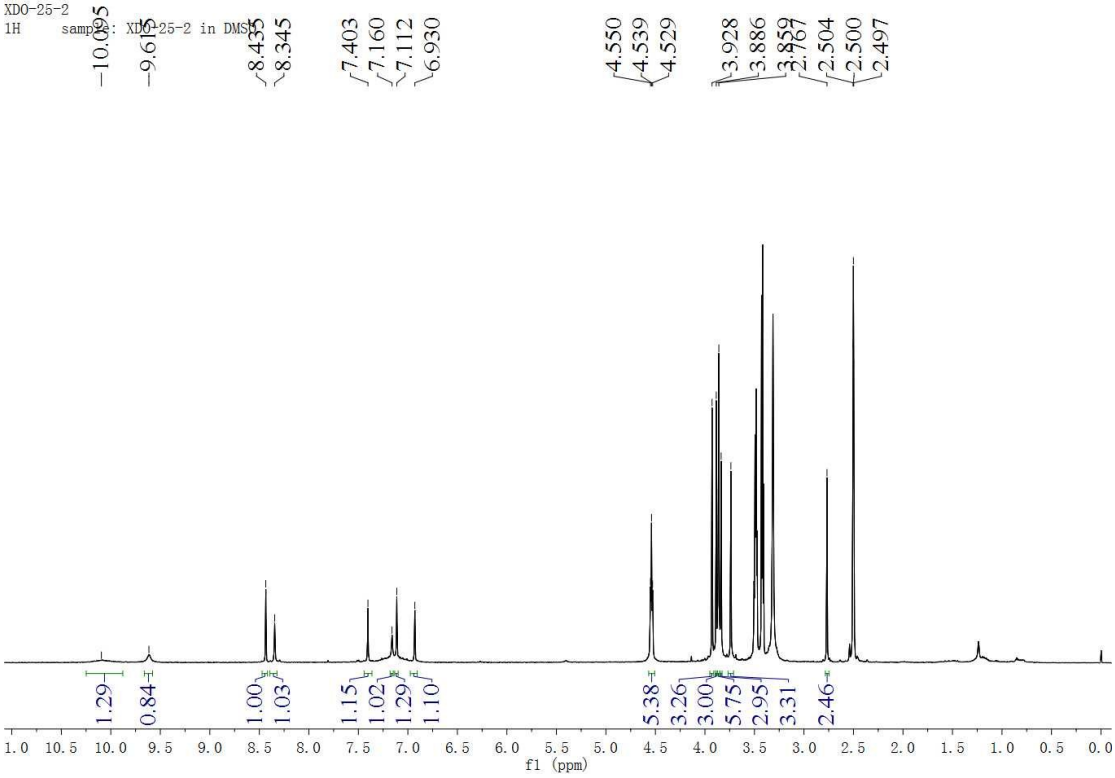
SI-5. The NOESY spectrum of compound **1a**



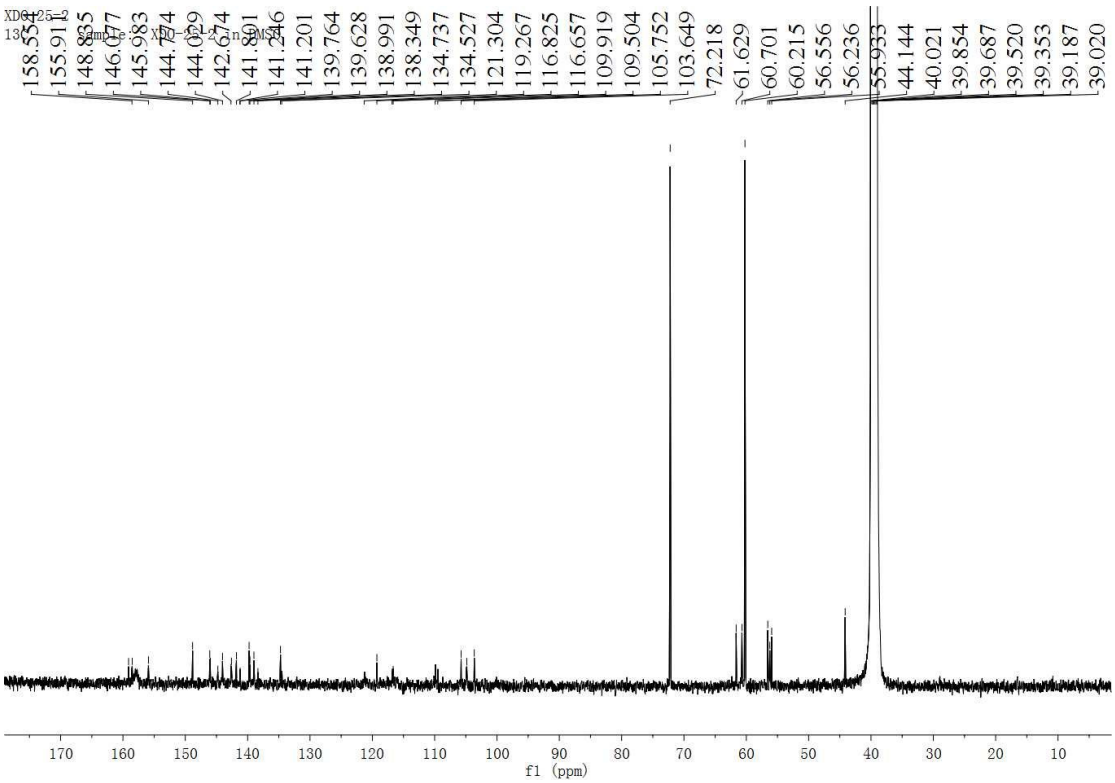
SI-6. The HRESIMS spectrum of compound **1a**



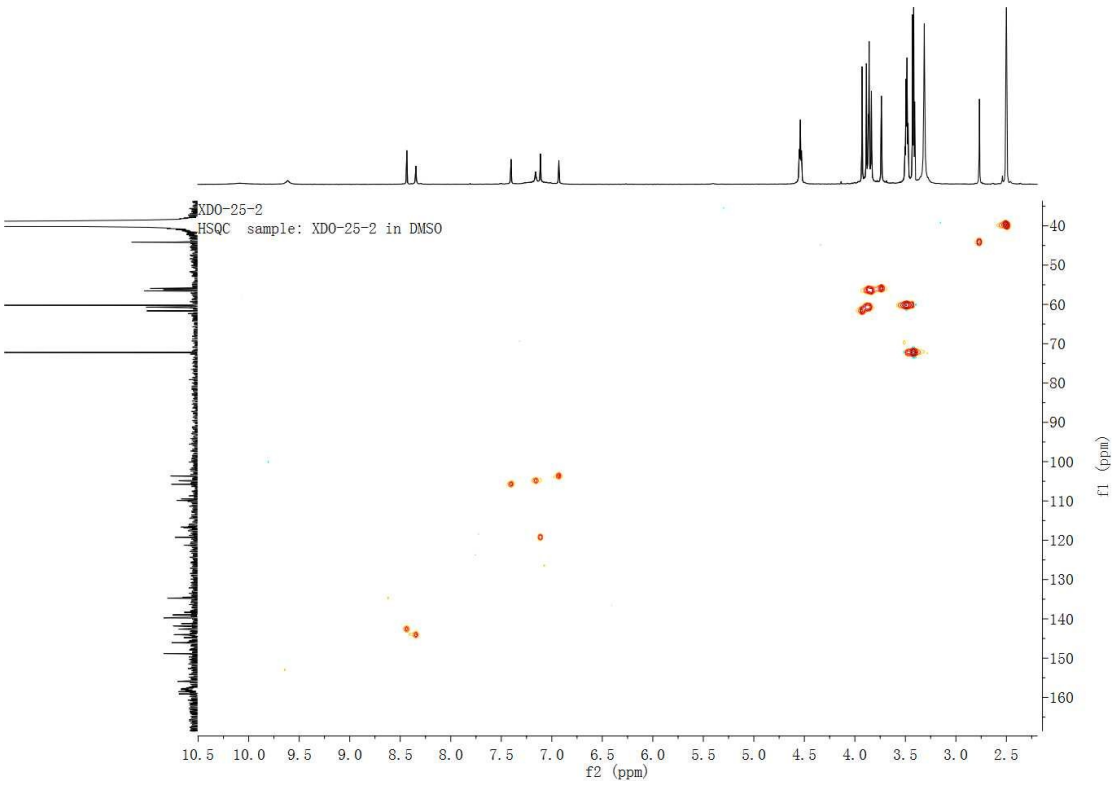
SI-7. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **1b**



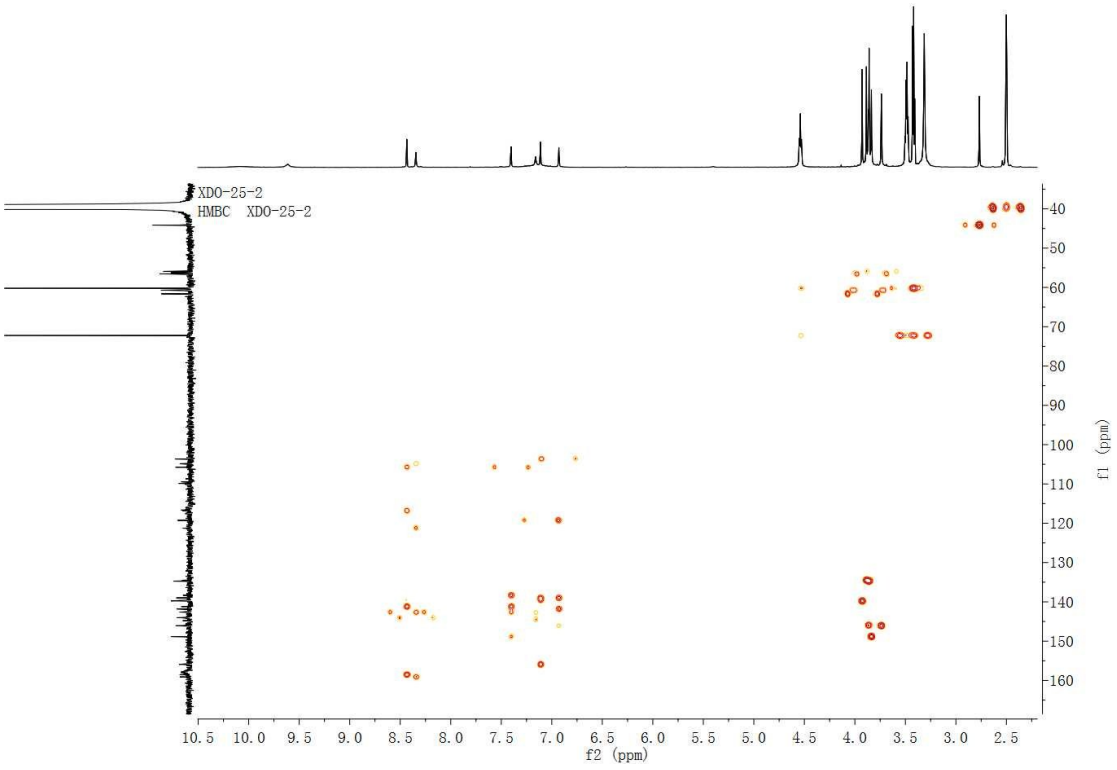
SI-8. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **1b**



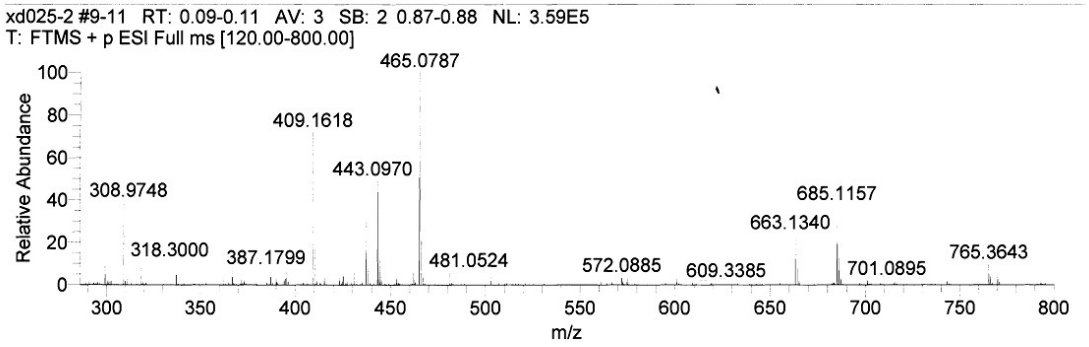
SI-9. The HMQC spectrum of compound **1b**



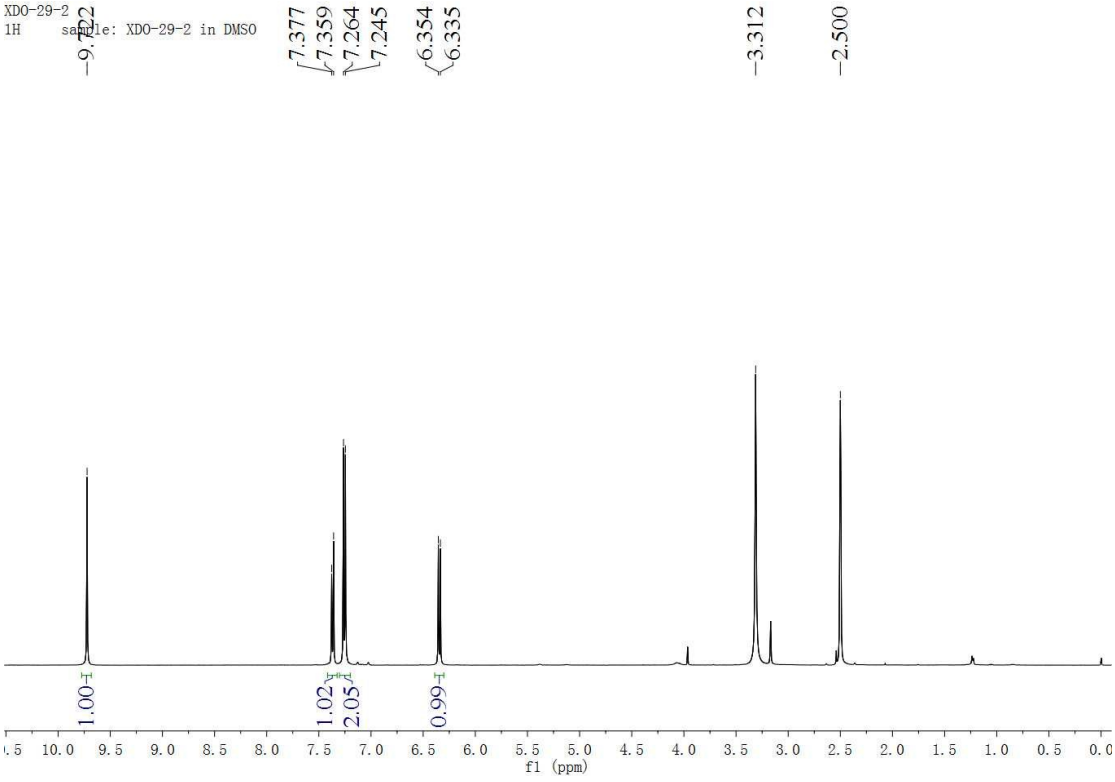
SI-10. The HMBC spectrum of compound **1b**



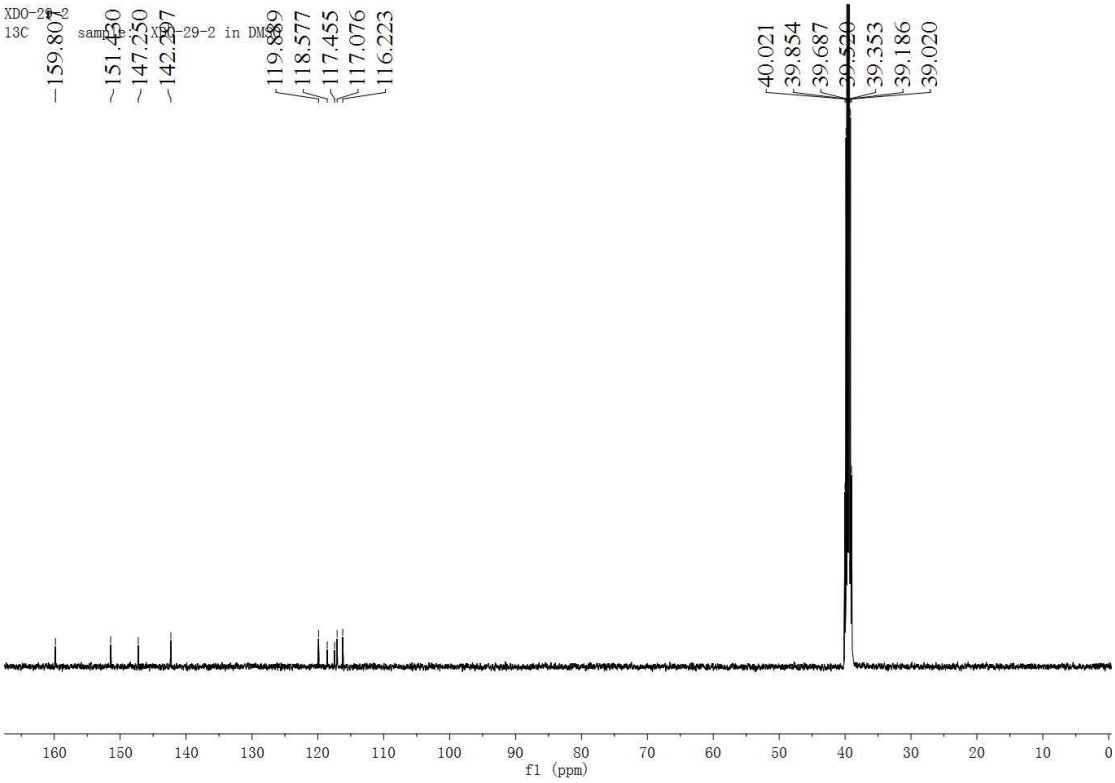
SI-11. The HRESIMS spectrum of compound **1b**



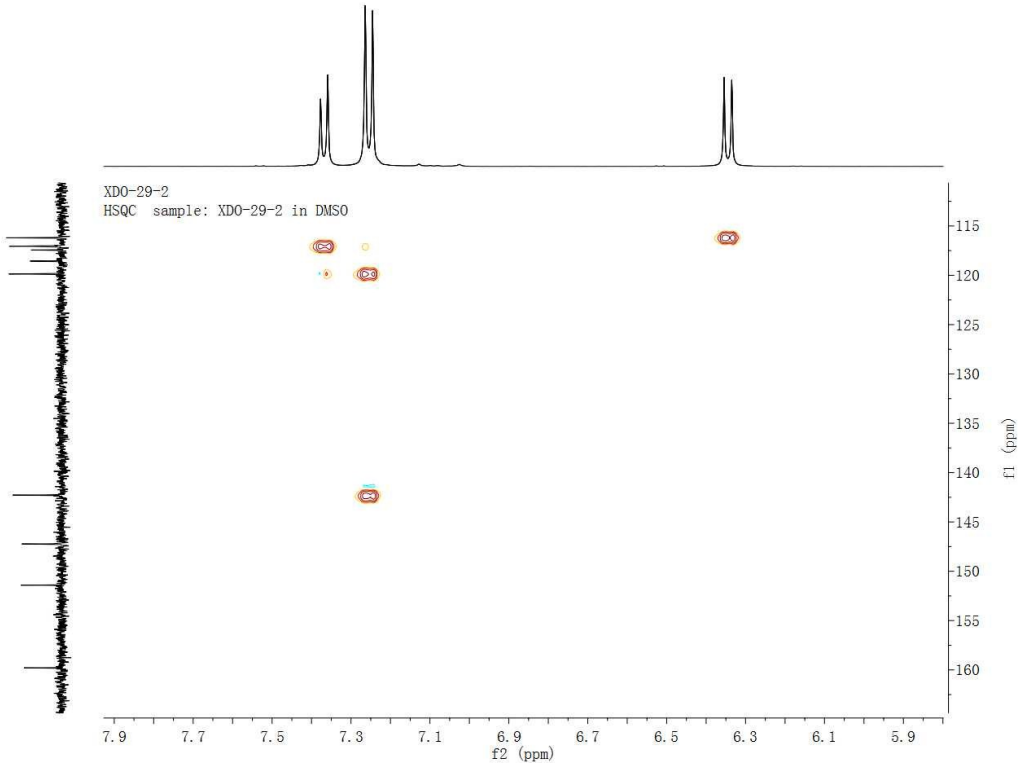
SI-12. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **2a**



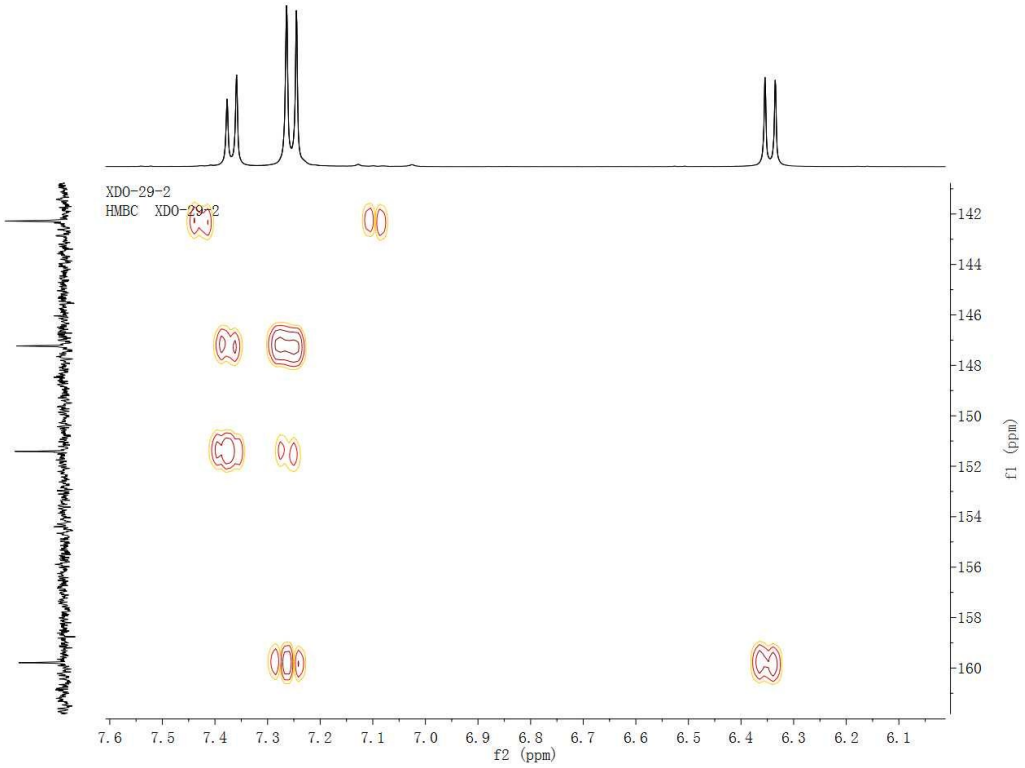
SI-13. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **2a**



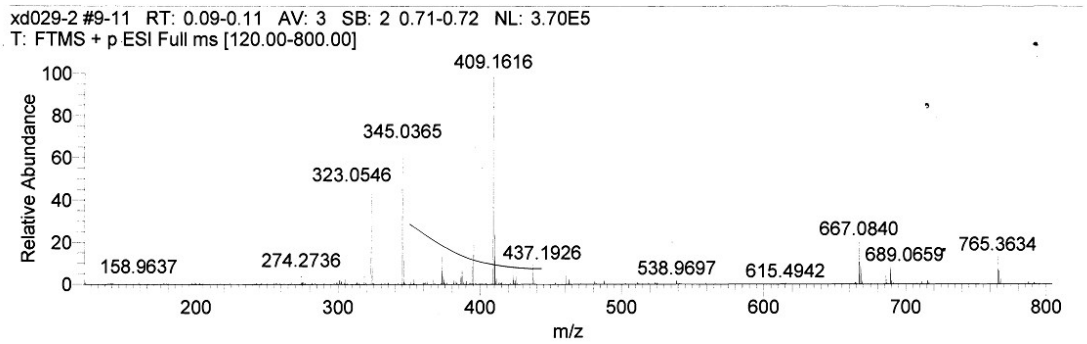
SI-14. The HMQC spectrum of compound **2a**



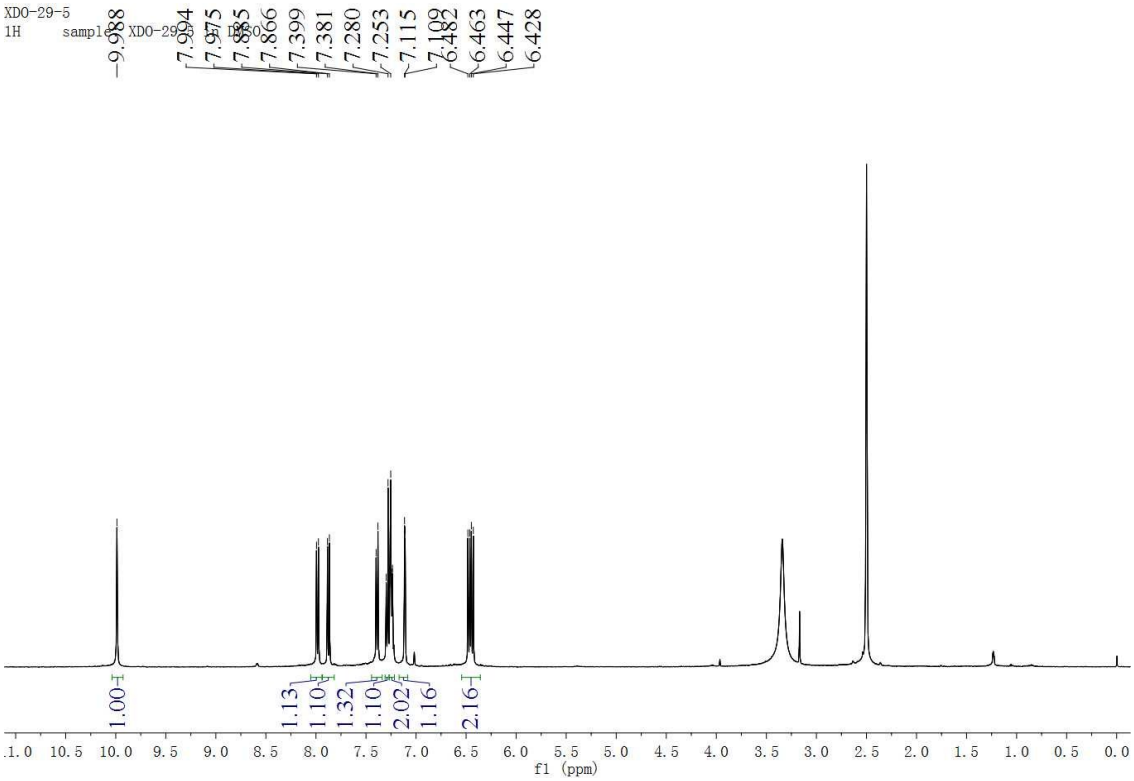
SI-15. The HMBC spectrum of compound **2a**



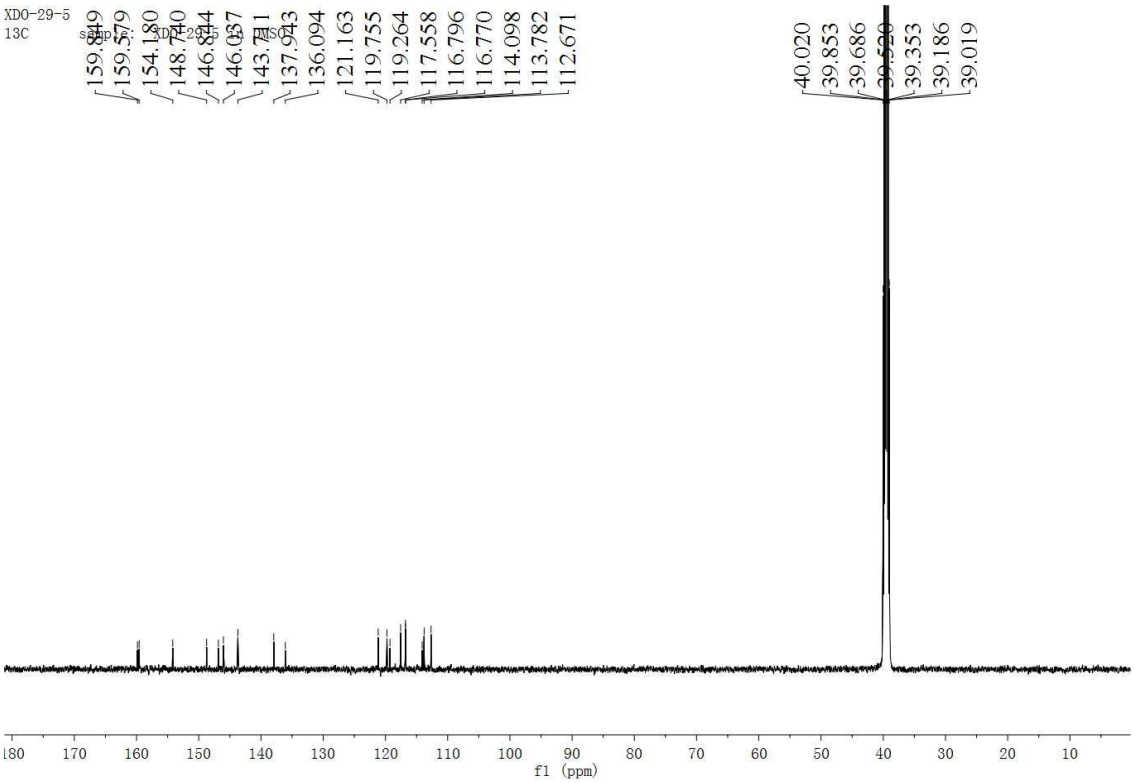
SI-16. The HRESIMS spectrum of compound **2a**



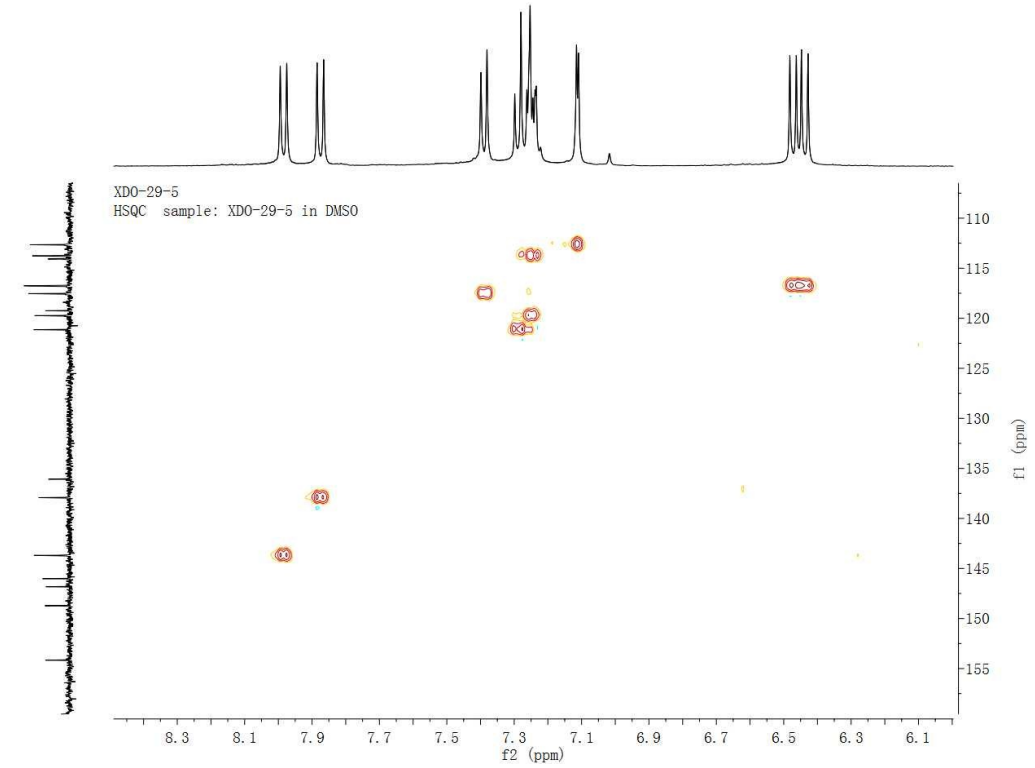
SI-17. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **2b**



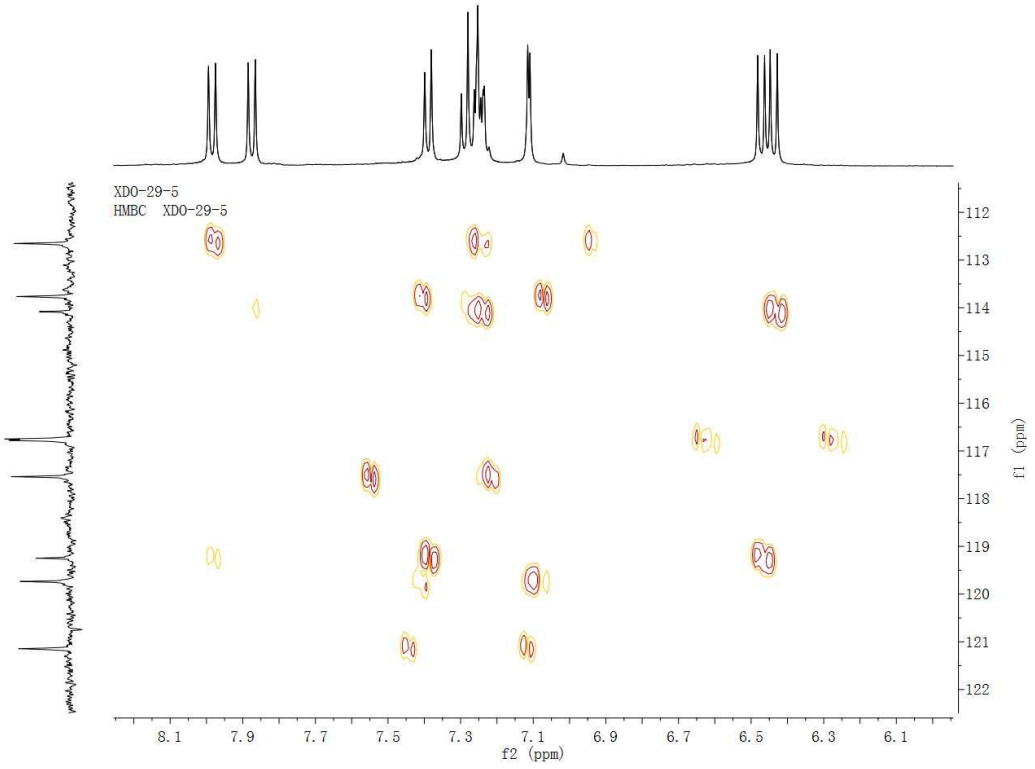
SI-18. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **2b**



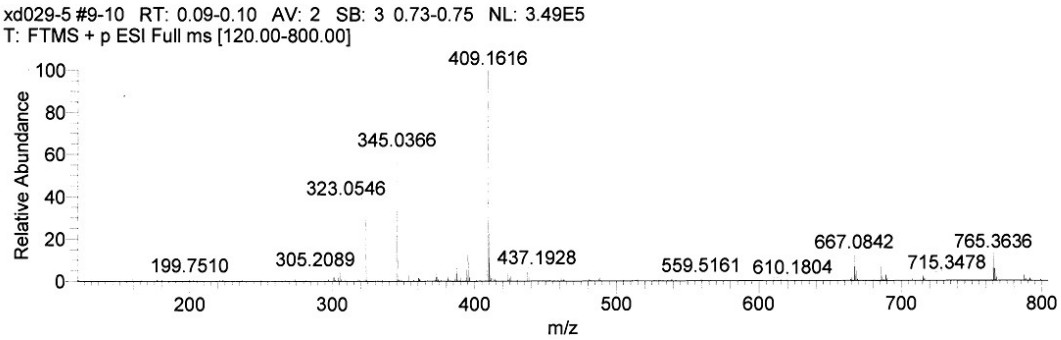
SI-19. The HMQC spectrum of compound **2b**



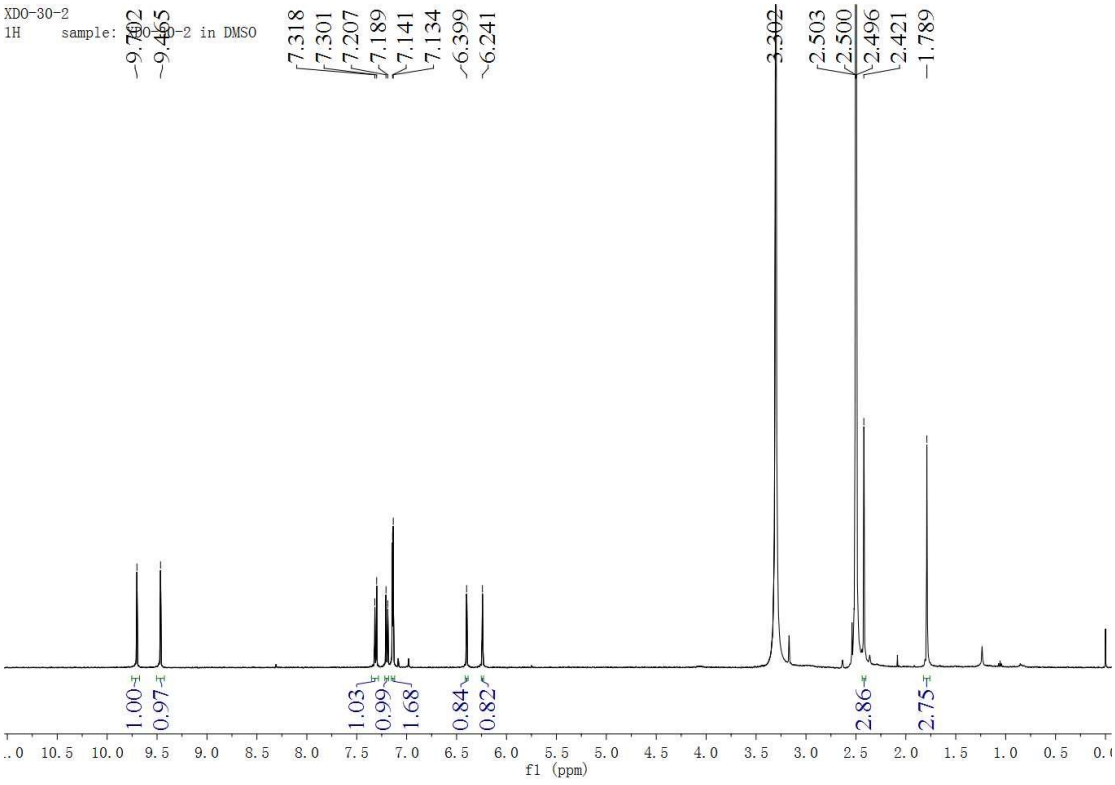
SI-20. The HMBC spectrum of compound **2b**



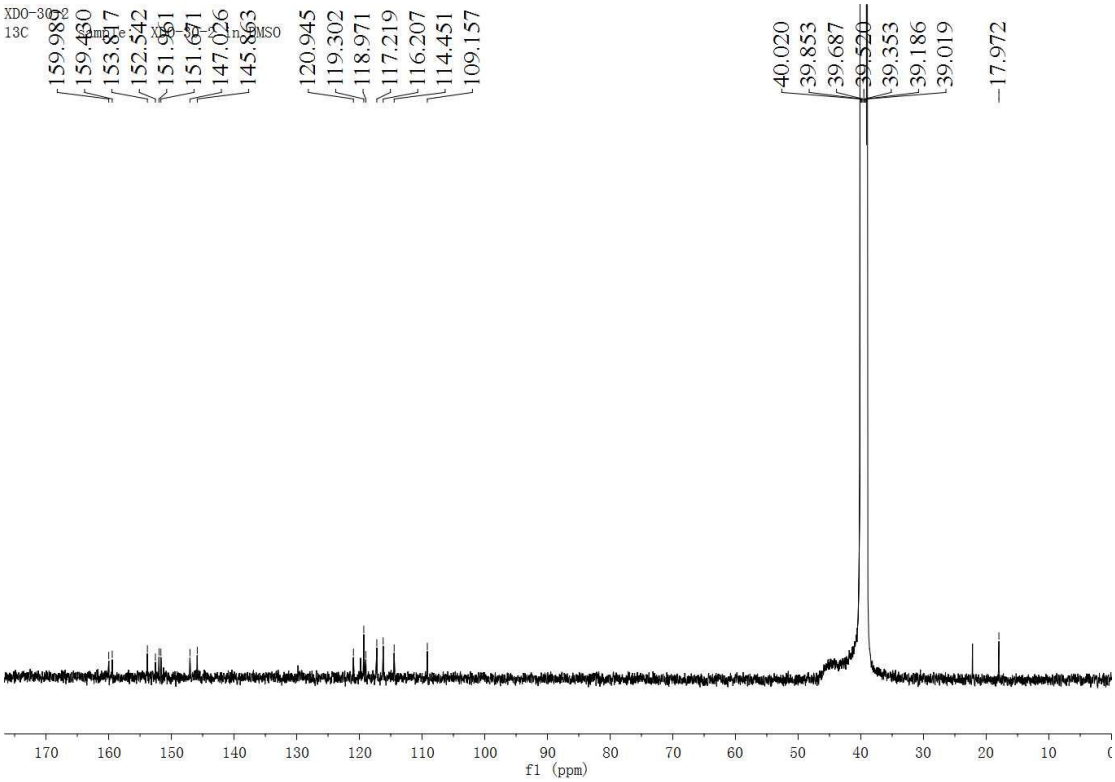
SI-21. The HRESIMS spectrum of compound **2b**



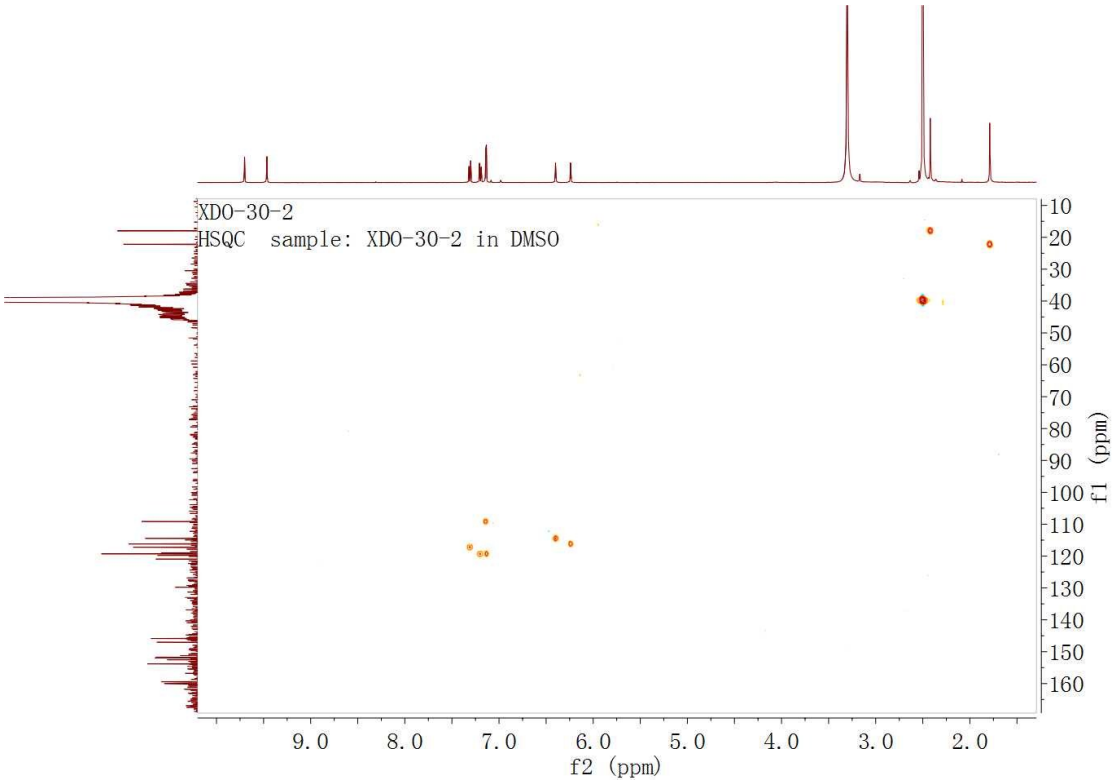
SI-22. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3a**



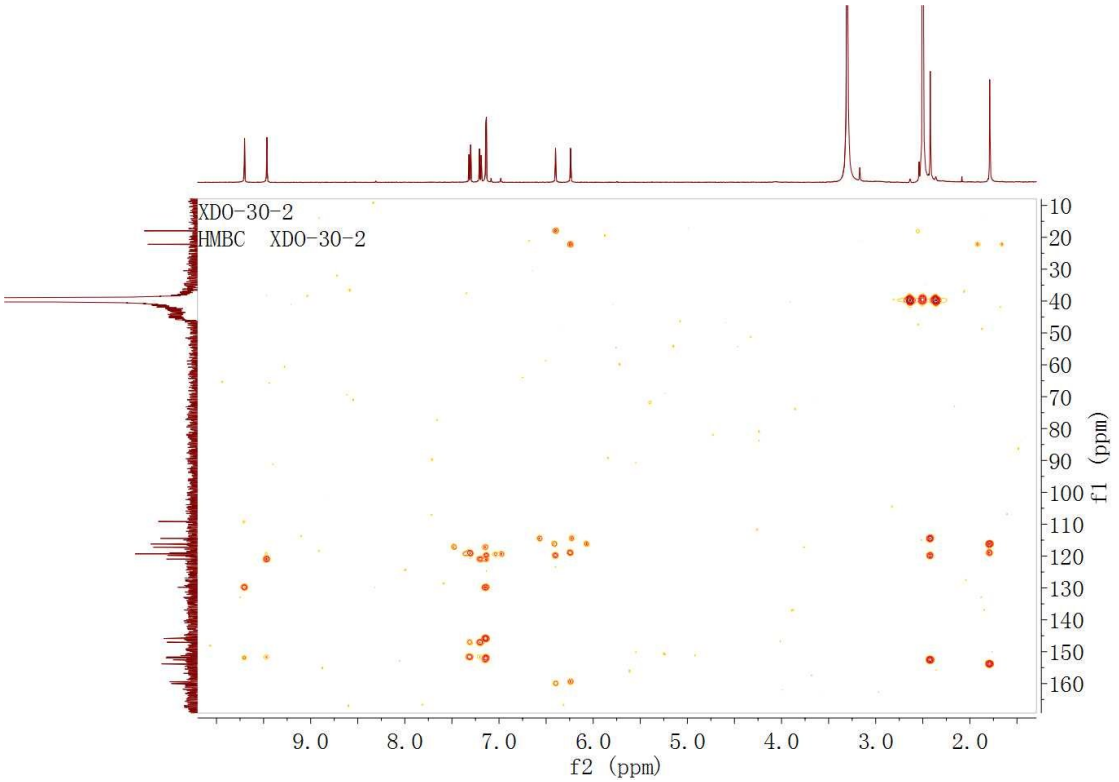
SI-23. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3a**



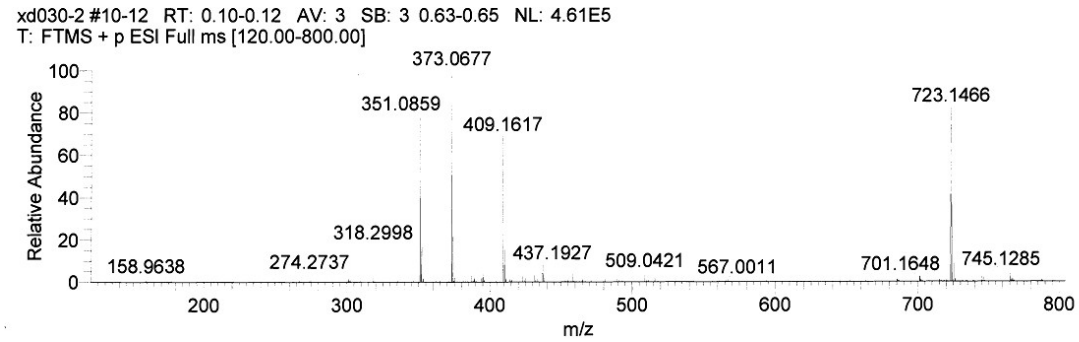
SI-24. The HMQC spectrum of compound **3a**



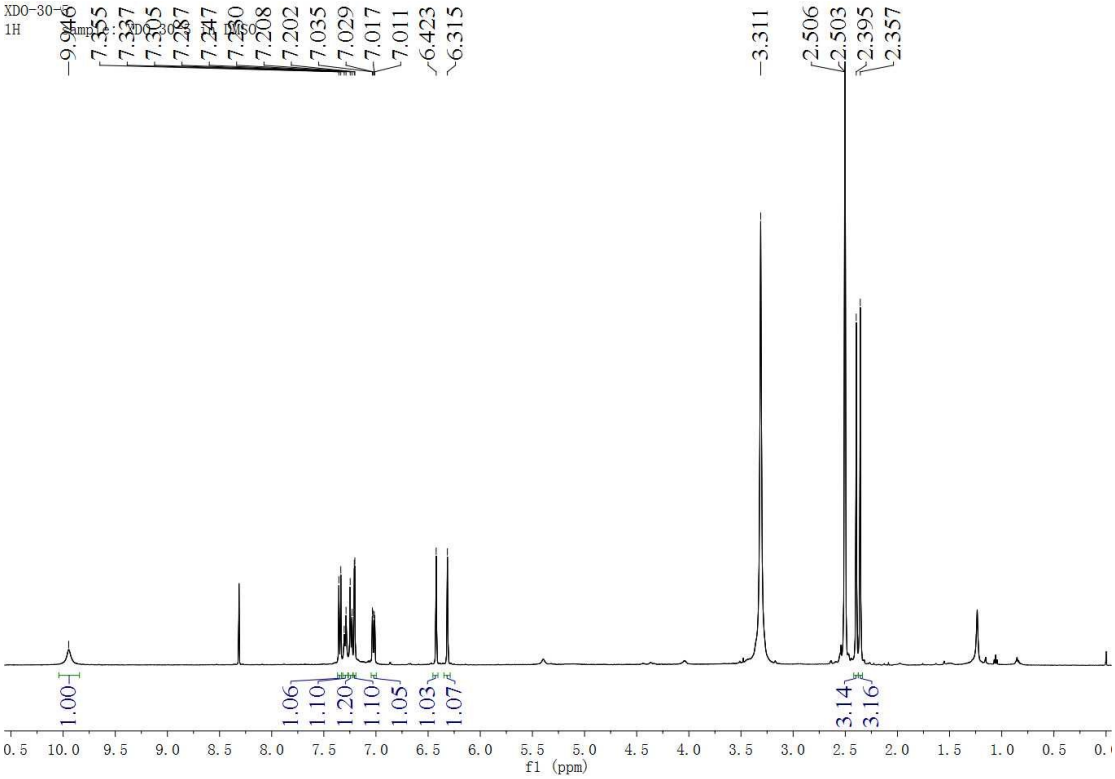
SI-25. The HMBC spectrum of compound **3a**



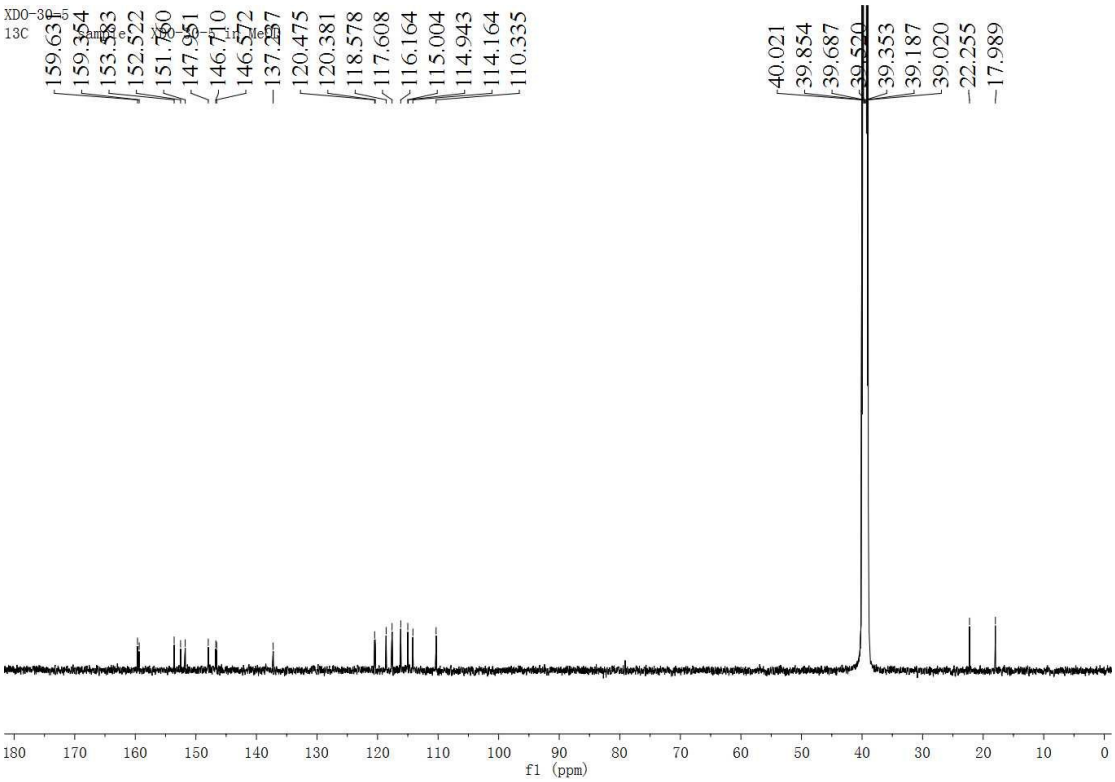
SI-26. The HRESIMS spectrum of compound **3a**



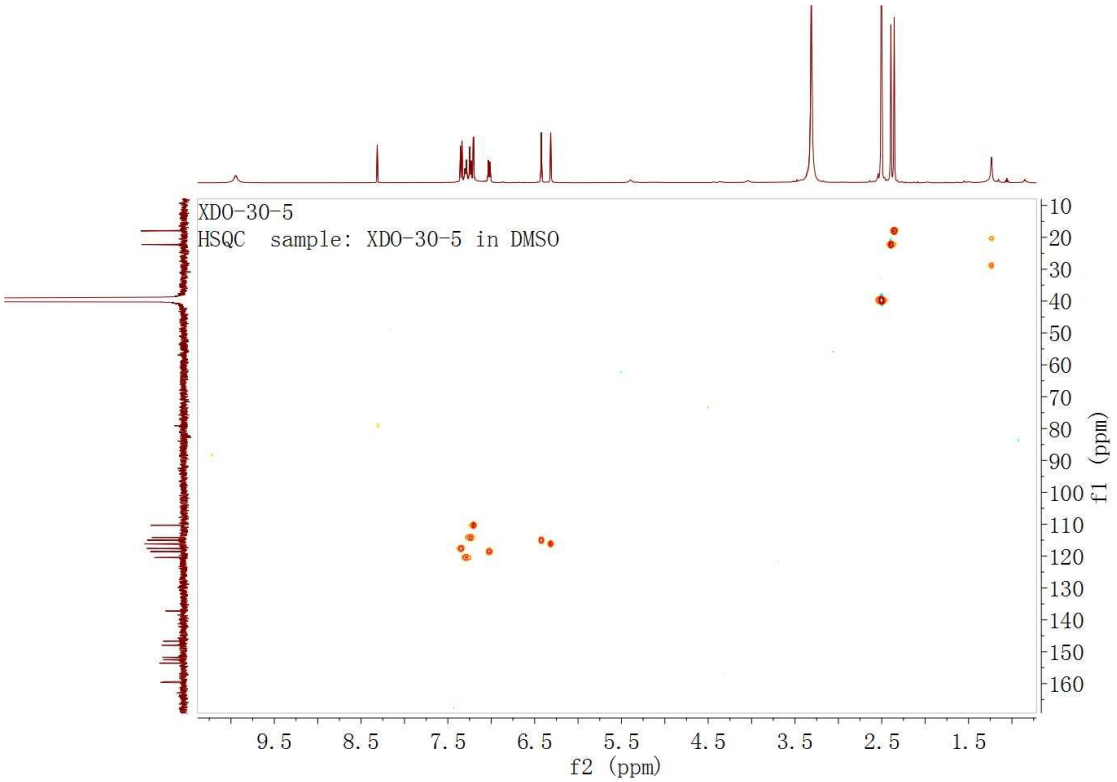
SI-27. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3b**



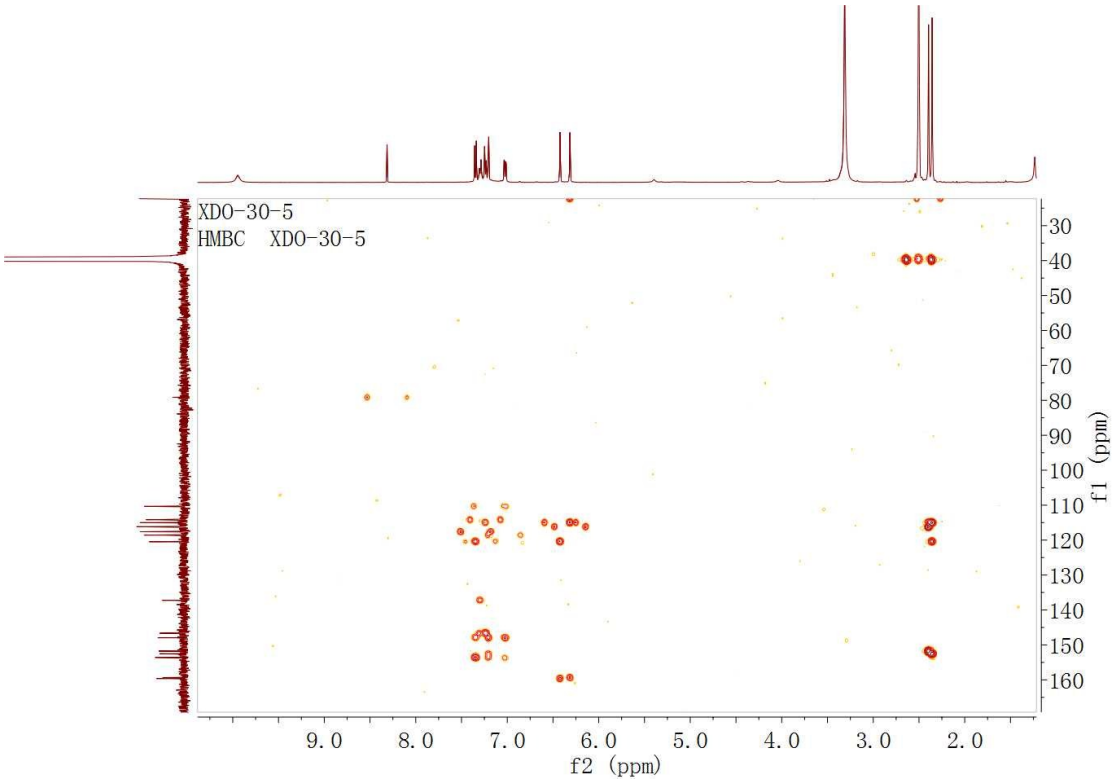
SI-28. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3b**



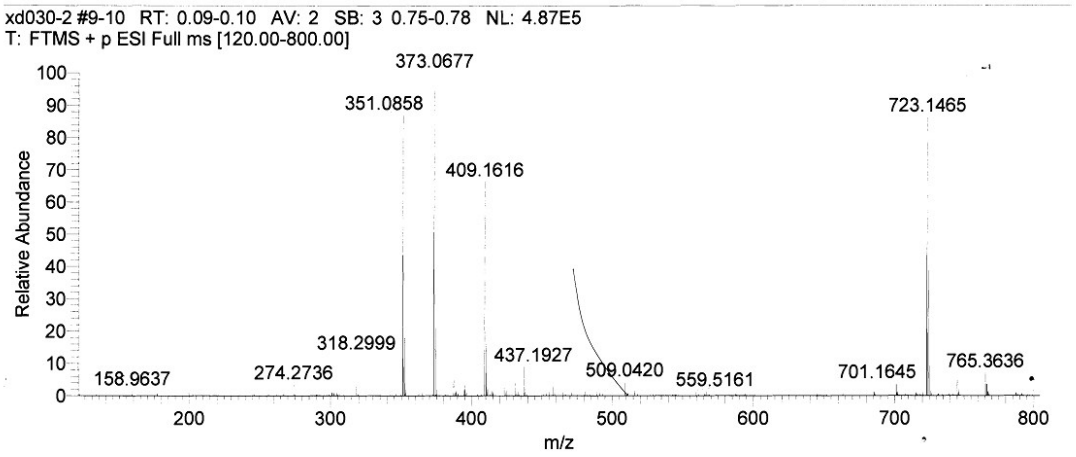
SI-29. The HMQC spectrum of compound **3b**



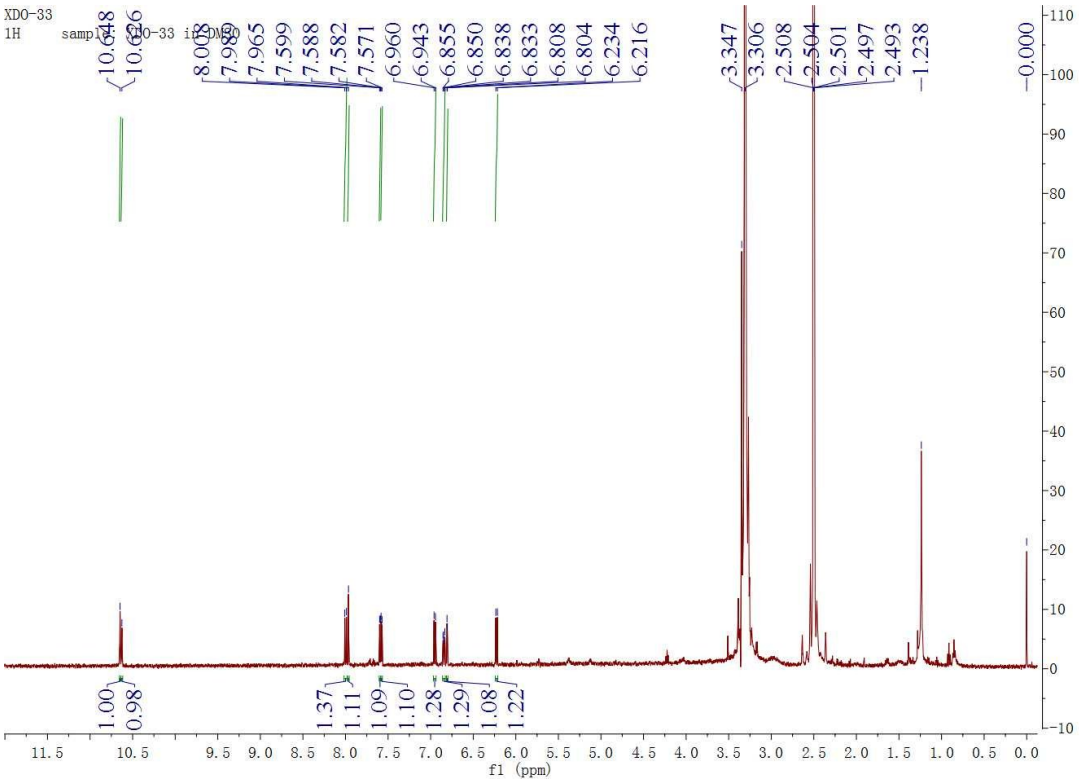
SI-30. The HMBC spectrum of compound **3b**



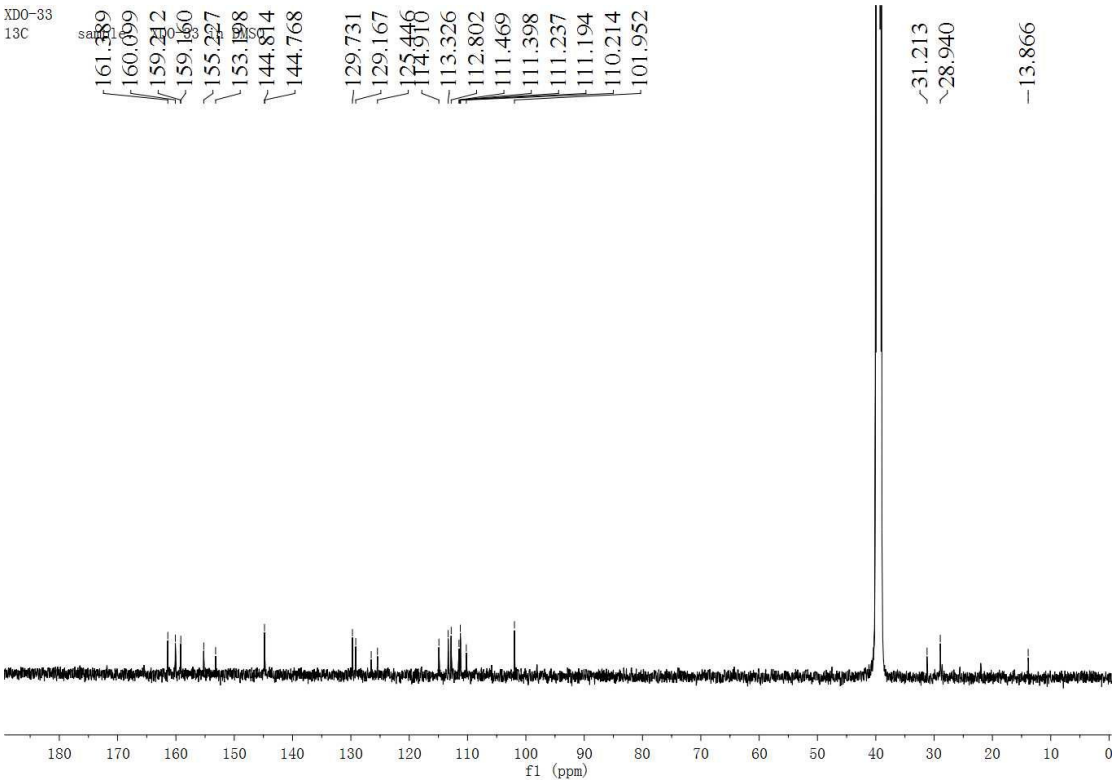
SI-31. The HRESIMS spectrum of compound **3b**



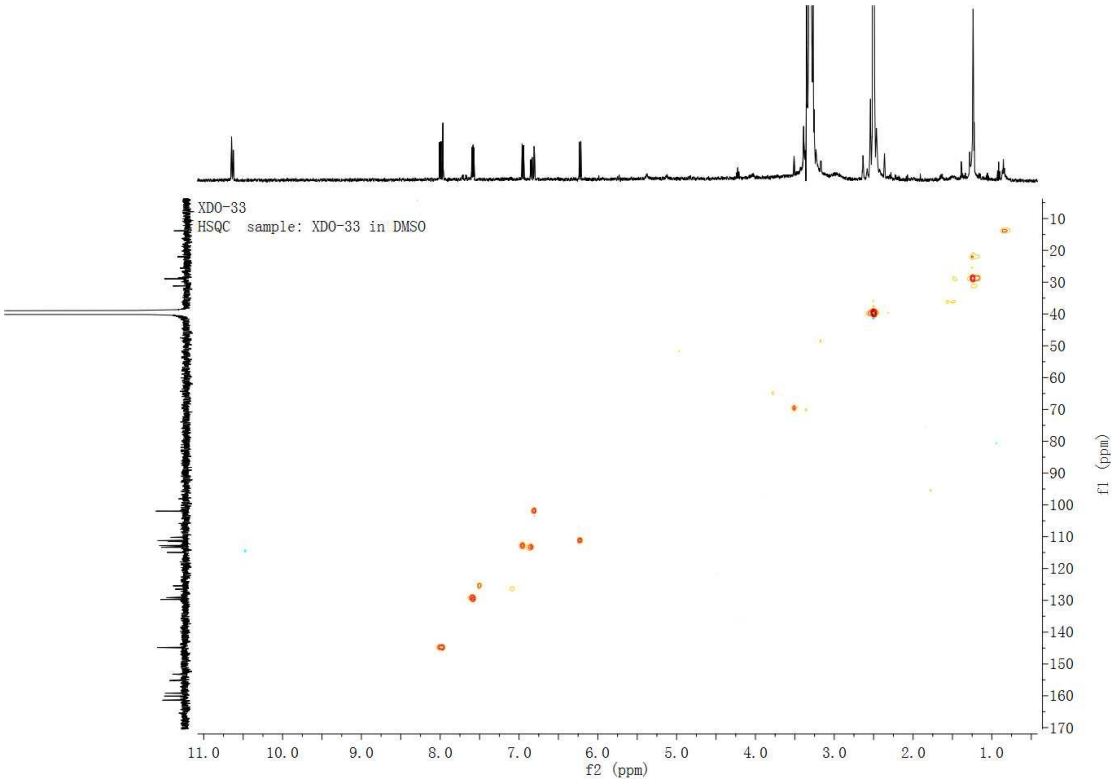
SI-32. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **4a**



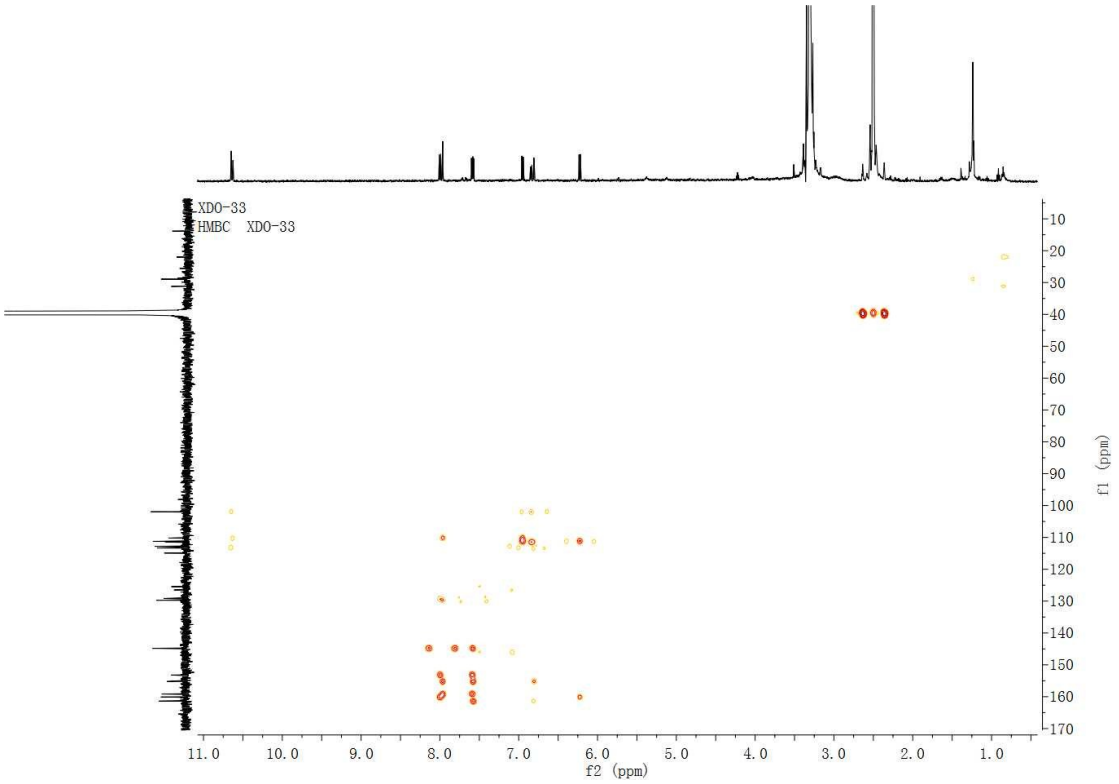
SI-33. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **4a**



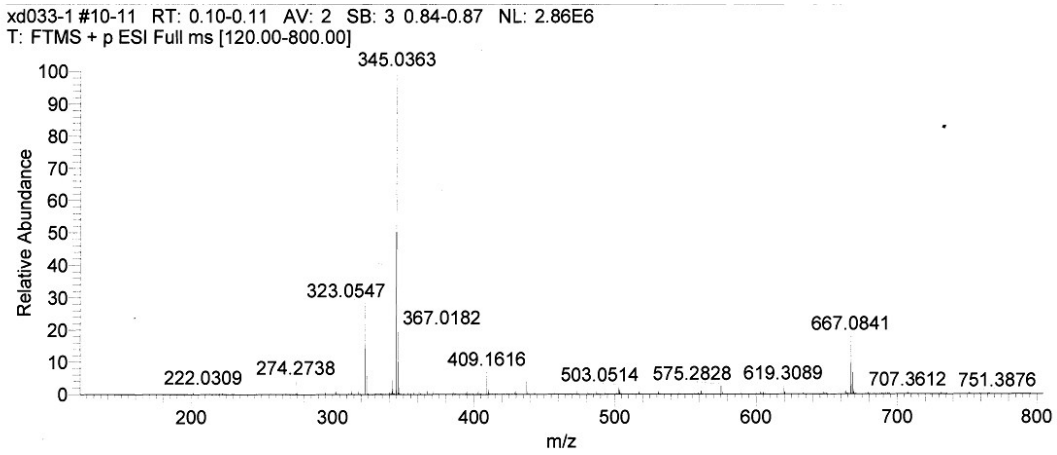
SI-34. The HMQC spectrum of compound **4a**



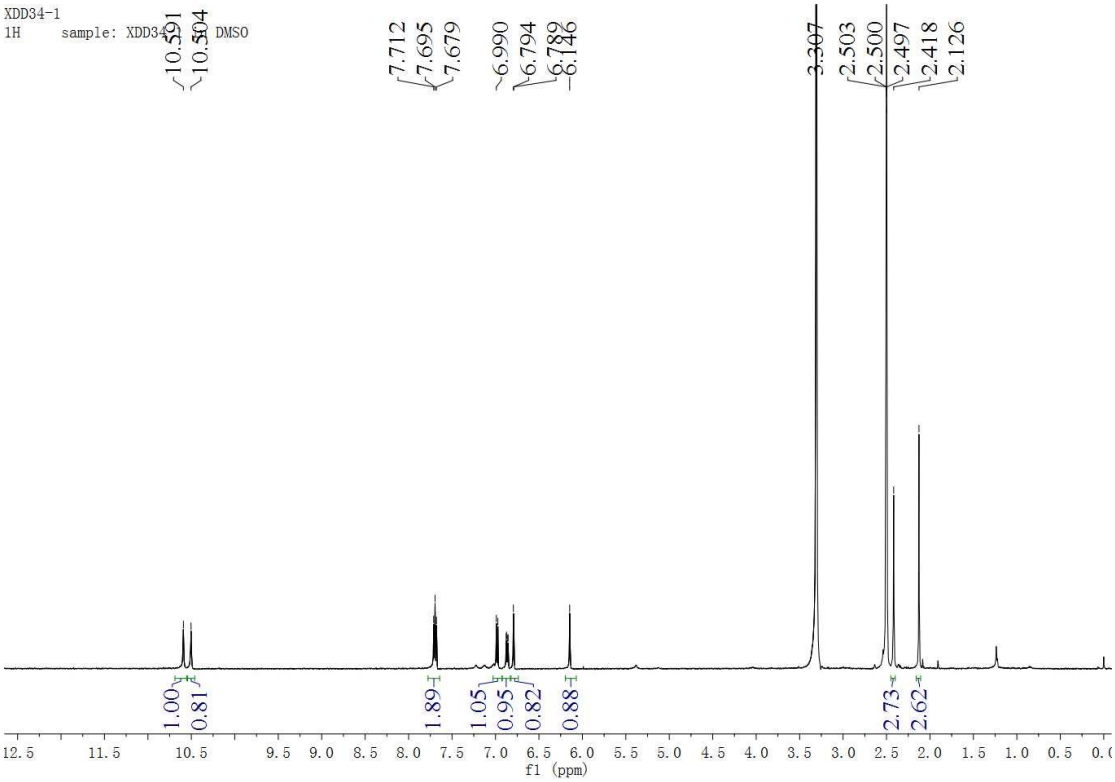
SI-35. The HMBC spectrum of compound **4a**



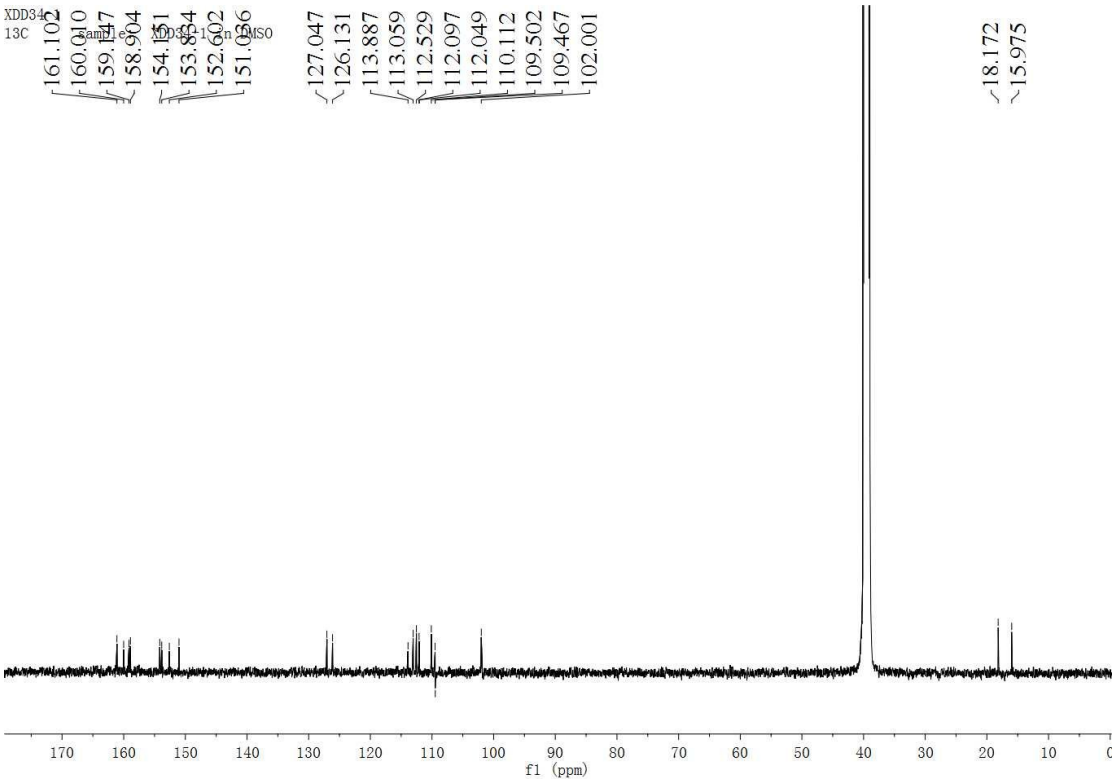
SI-36. The HRESIMS spectrum of compound **4a**



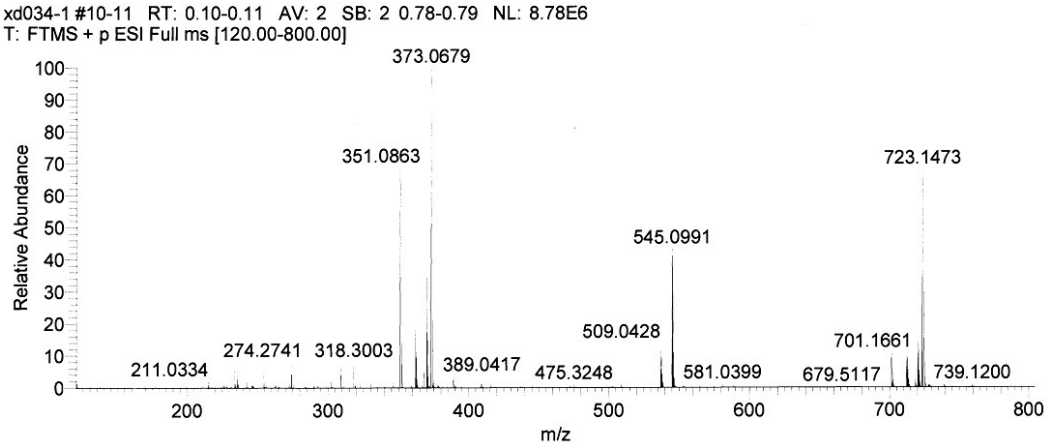
SI-37. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **5a**



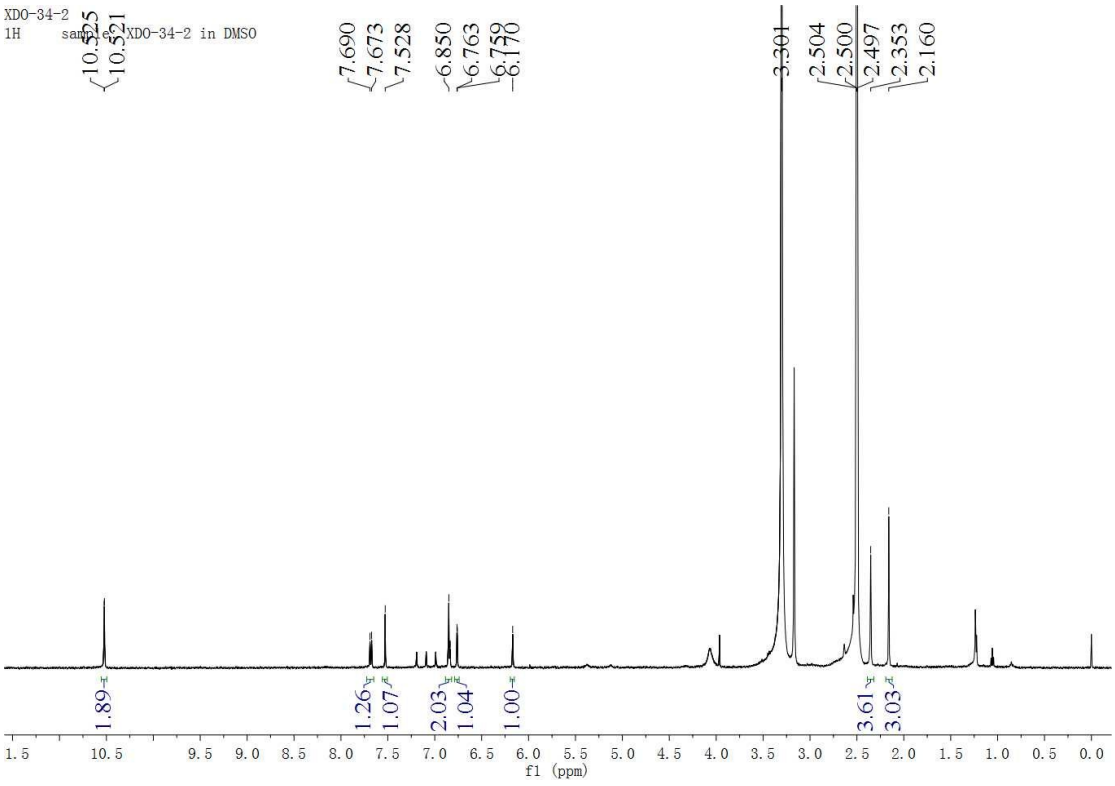
SI-38. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **5a**



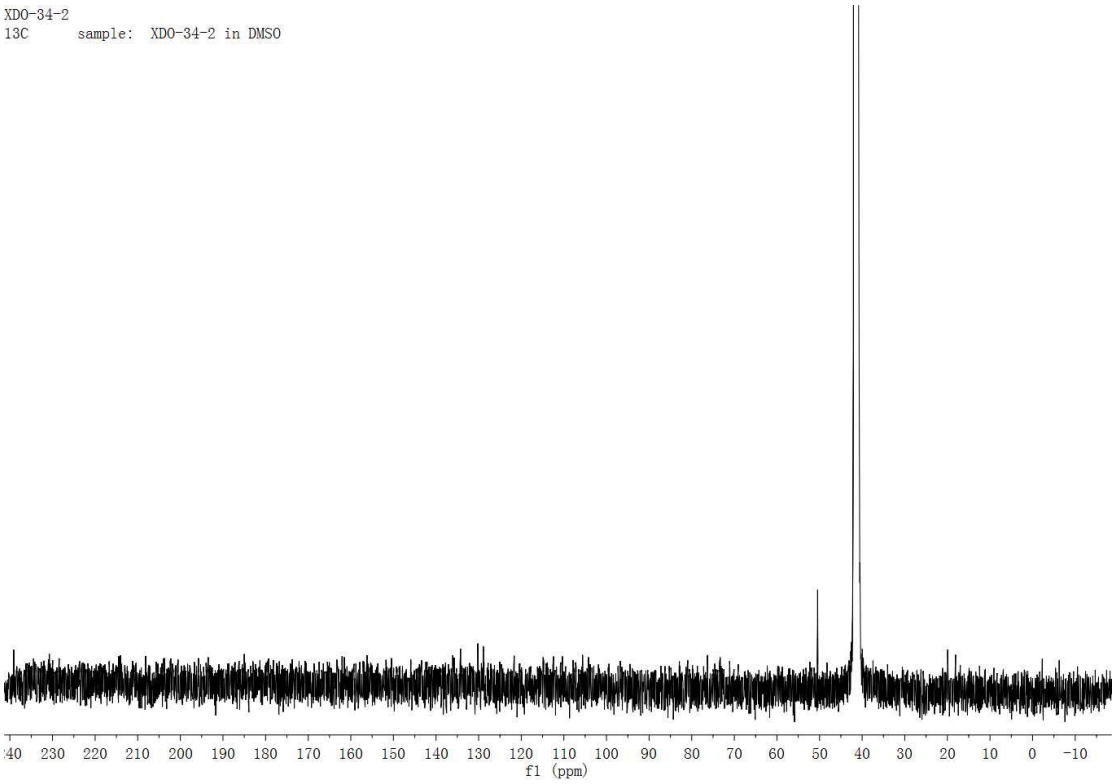
SI-39. The HRESIMS spectrum of compound **5a**



SI-40. The ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **5b**



SI-41. The ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **5b**



SI-42. The HRESIMS spectrum of compound **5b**