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Electronic Supplementary Information

Quinoline based mono- and bis-(thio)carbohydrazones: synthesis, anticancer activity in 2D and 3D cancer and cancer stem cell models

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Content

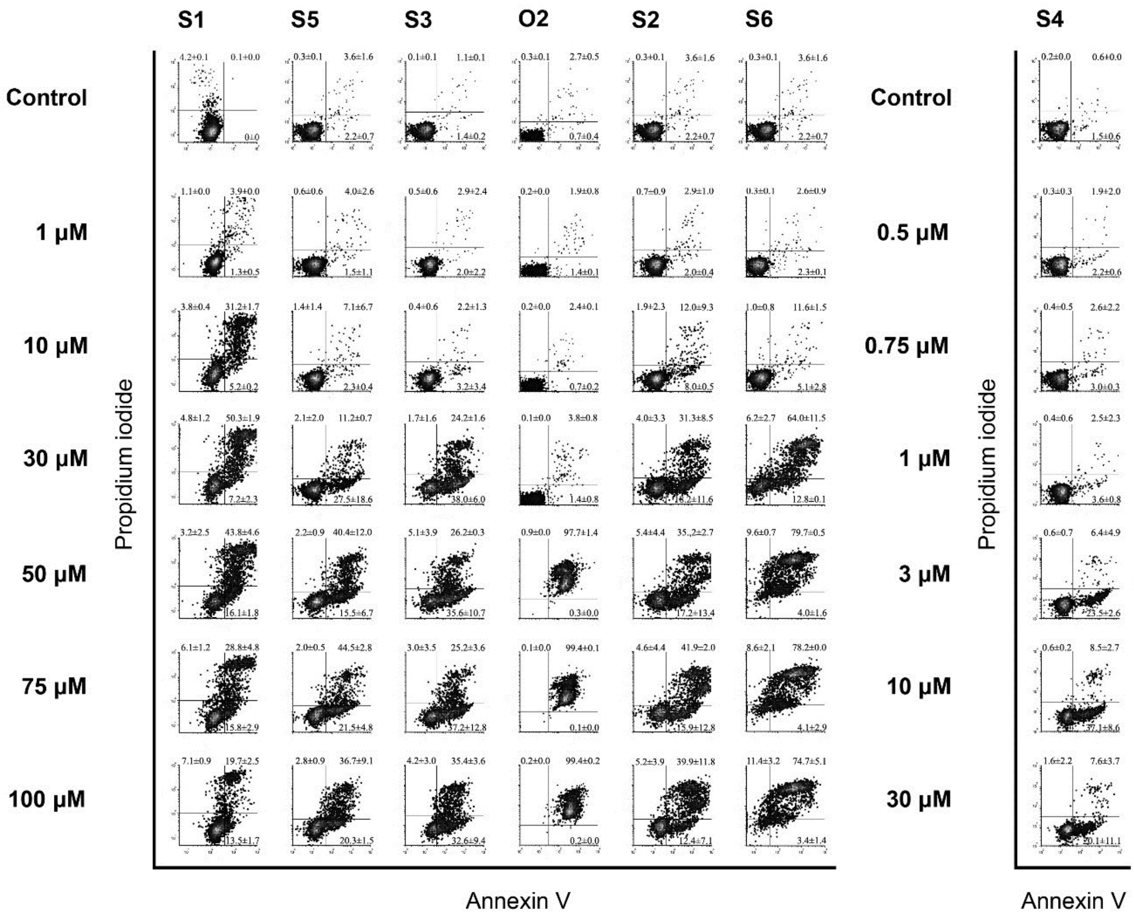


Figure S 1. Cell death response in THP-1 cells determined after 24 h incubation with investigated compounds by means of Annexin V/propidium iodide dual staining method.

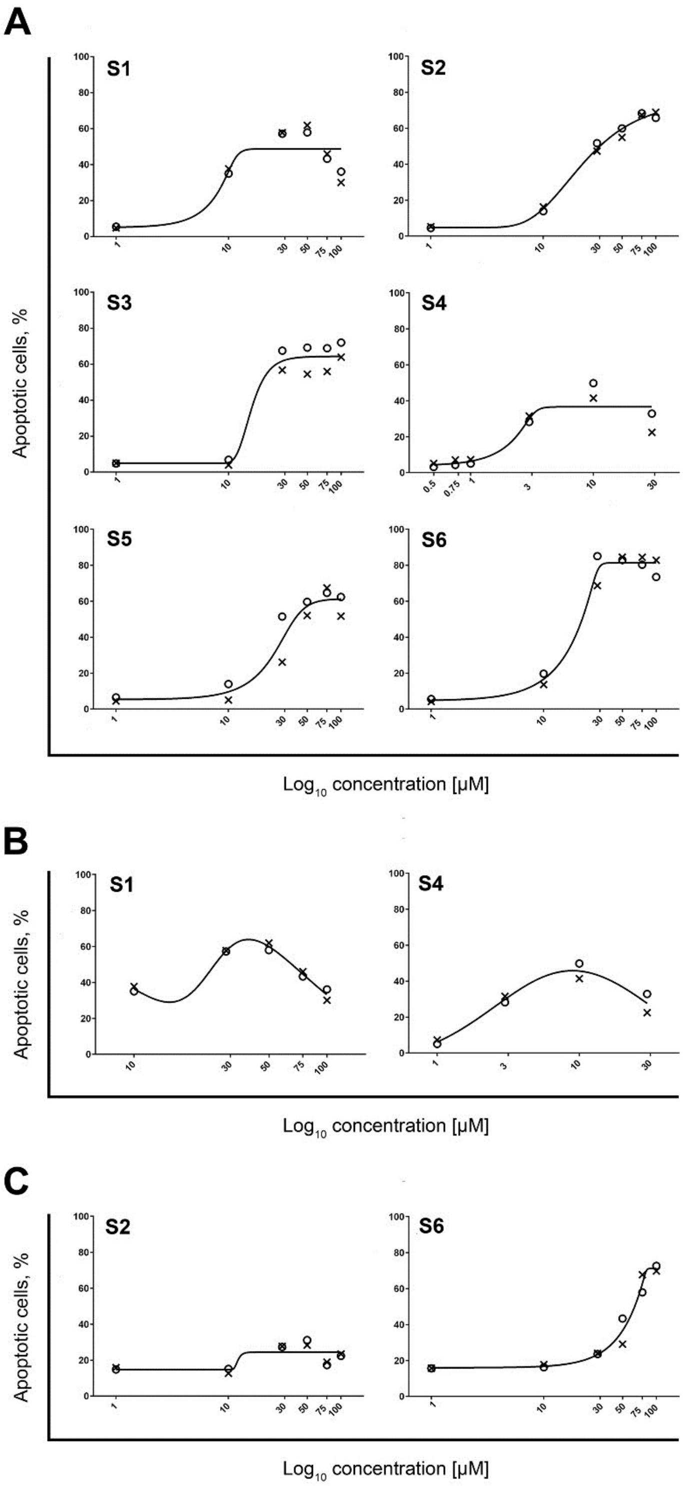


Figure S 2. Concentration-response curves established for 24 h treatment. Standard sigmoidal curves (A) with biphasic curves (B) obtained for THP-1 cells, and sigmoidal (C) curves attained for AsPC-1 cells.

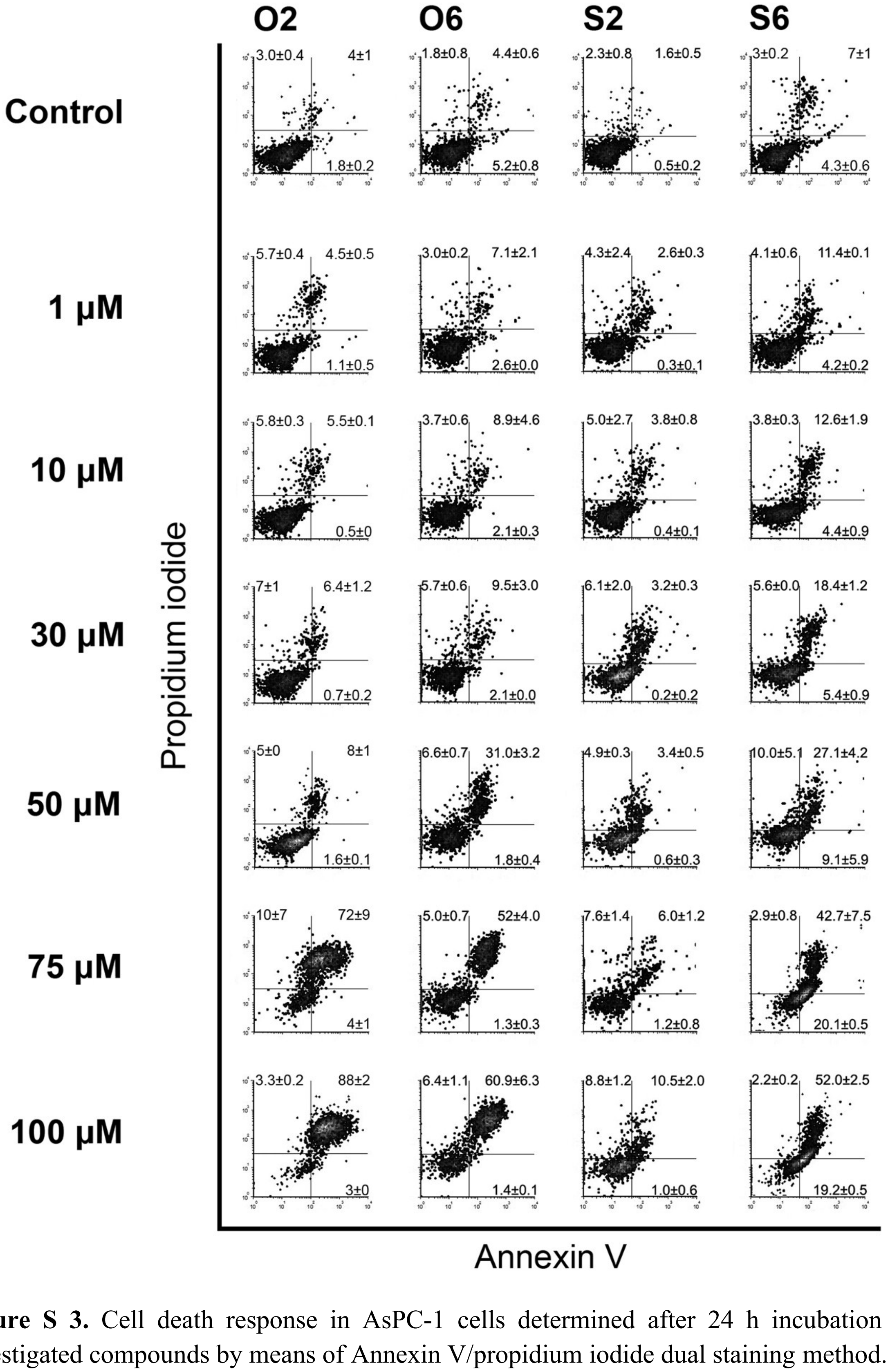


Figure S 3. Cell death response in AsPC-1 cells determined after 24 h incubation with investigated compounds by means of Annexin V/propidium iodide dual staining method.

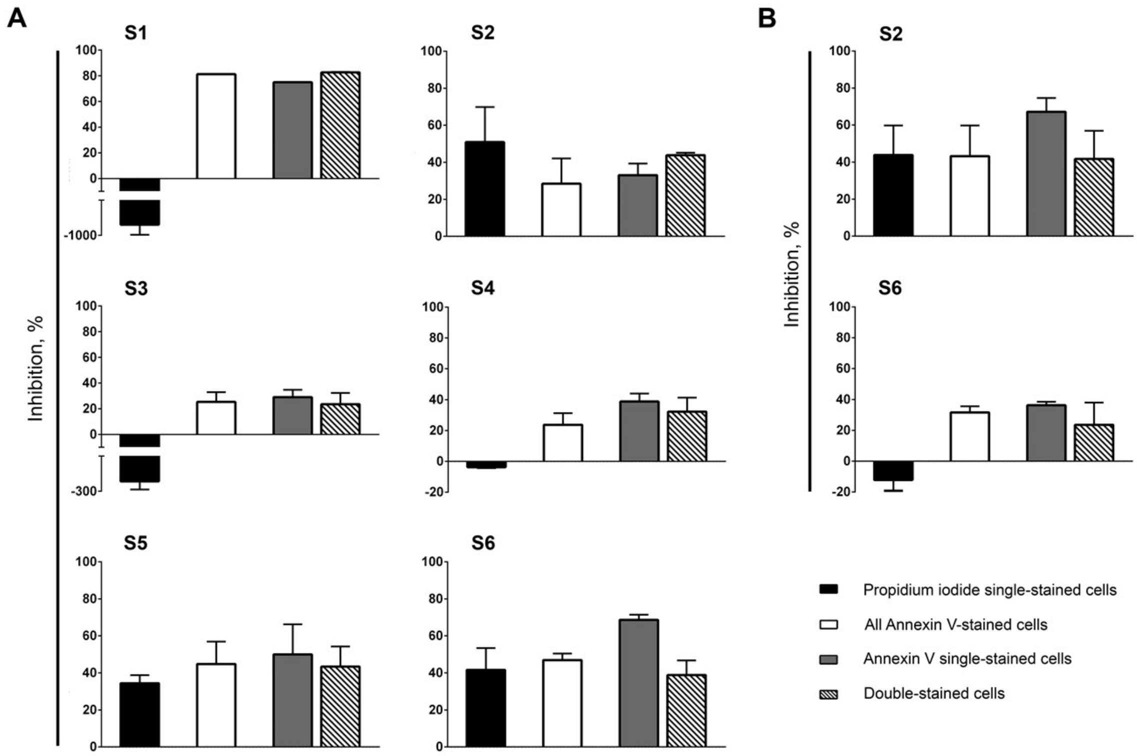


Figure S 4. Role of caspases activation in apoptotic death of THP1 cells (A) and AsPC-1 cells (B).

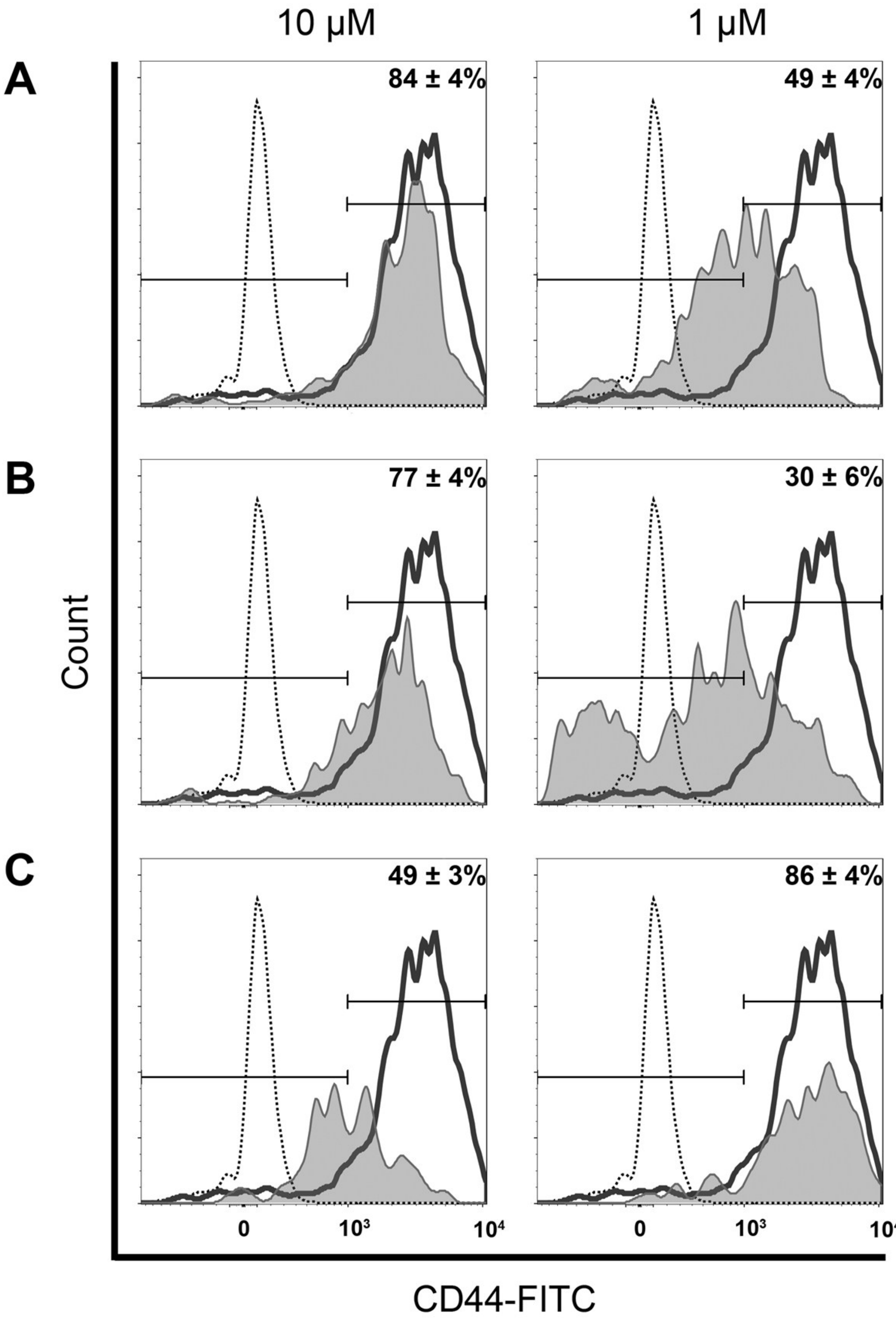
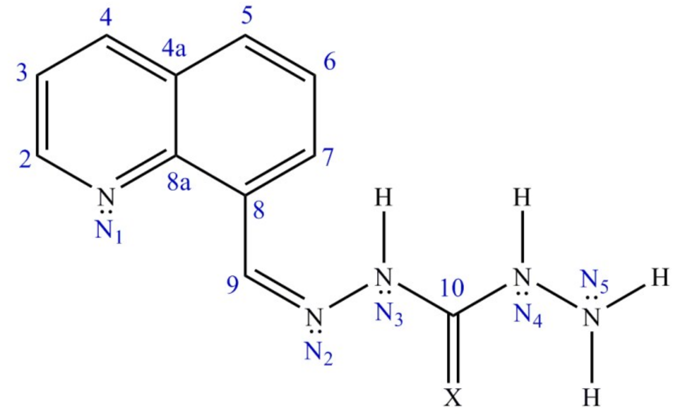
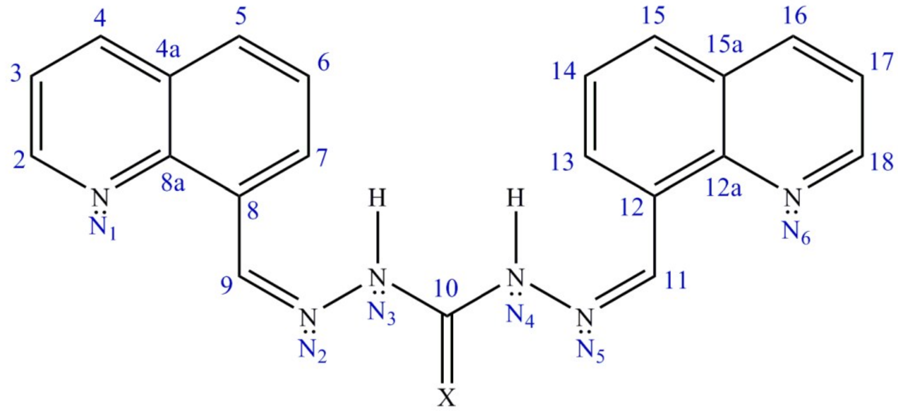
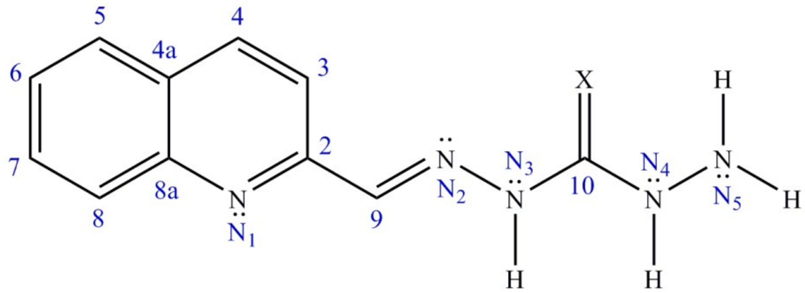
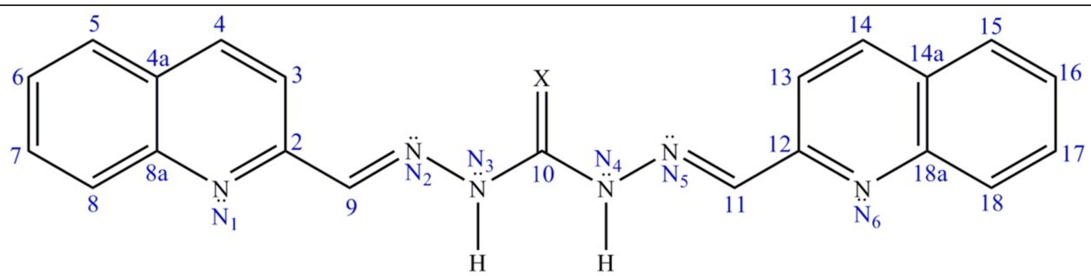


Figure S 5. Changes in expression of CD44 surface marker on AsPC-1 cells assessed after 72 h incubation with S2 (A), S6 (B), and S4 (C).

Table S 1. Numbering of atoms in carbohydrazone **O1-O6** and thiocarbohydrazone **S1-S6** used in NMR.

O1 (X = O) **S1** (X = S)	
O2 (X = O) **S2** (X = S)	
O3 (X = O) **S3** (X = S)	
o4 (X = O) **s4** (X = S)	

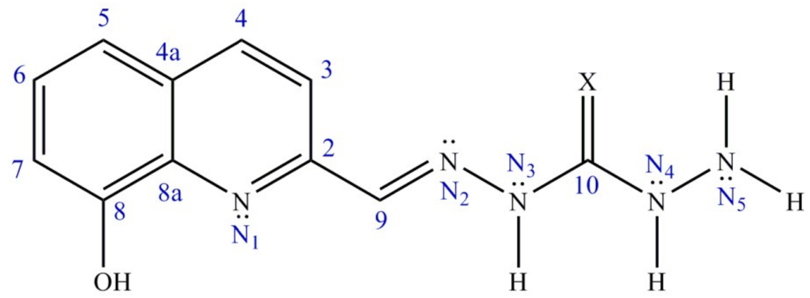
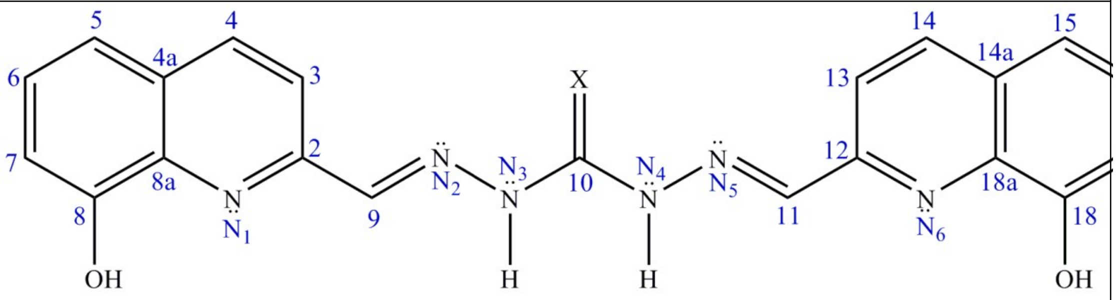
o5 (X = O) **s5** (X = S)	
o6 (X = O) **s6** (X = S)	

Table S 2. Experimental and calculated ^{1}H NMR (500 MHz, DMSO-d_6) data of **O1-O6**

Comp	**O1**		**O2**		**O3**		**O4**		**O5**		**O6**	
δ	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**
2	8.94	9.23	8.99	9.27								
3	7.57	7.76	7.61	7.79	8.34-8.46	8.44	8.38-8.60	8.54	8.30-8.50	8.47	8.17-8.39	8.56
4	8.39	8.63	8.43	8.65	8.27	8.56	8.38-8.60	8.62	8.24	8.53	8.17-8.39	8.59
5	7.98	8.27	8.04	8.31	7.93-7.99	8.25	8.02	8.29	7.36	7.62	7.41	7.65
6	7.63	7.98	7.72	8.03	7.58	7.91	7.63	7.95	7.41	7.82	7.45	7.88
7	8.58	8.89	8.60	8.98	7.74	8.08	7.79	8.12	7.08	7.35	7.12	7.39
8					7.93-7.99	8.35	8.02	8.39	OH 9.71	7.80	OH 9.80	7.83
9	9.14	9.25	9.50	9.40	8.03	7.92	8.31	8.08	8.09	7.93	8.48	8.09
11			9.50	9.40			8.31	8.08			8.48	8.09
12												
13			8.60	8.98			8.38-8.60	8.54			8.17-8.39	8.56
14			7.72	8.03			8.38-8.60	8.62			8.17-8.39	8.59
15			8.04	8.31			8.02	8.29			7.41	7.65
16			8.43	8.65			7.63	7.95			7.45	7.88
17			7.61	7.79			7.79	8.12			7.12	7.39
18			8.99	9.27			8.02	8.39			OH 9.80	7.83
N3	10.65	7.38	11.09	7.67	10.84	7.47	11.31	7.73	10.88	7.50	11.34	7.75
N4	8.16	5.02	11.09	7.67	8.34-8.46	5.13	11.31	7.73	8.30-8.50	5.13	11.34	7.75
N5	4.12	3.23 2.82	8.99		4.15	3.20 2.80			4.14	3.21 2.89		

Table S 3. Experimental and calculated ^{1}H NMR (500 MHz, DMSO-d_6) data of **S1-S6**

Comp	**S1**		**S2**		**S3**		**S4**		**S5**		**S6**	
δ	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**
2	8.96	9.27	9.00	9.36								
3	7.59	7.80	7.62	7.84	8.52	8.38	8,90	8.66	8.82	8.40	8.05-8.41	8.68
4	8.41	8.66	8.35-8.64	8.70	8.34	8.61	8.46-8.63	8.67	8.27	8.57	8.05-8.41	8.64
5	8.03	8.37	8.09	7.43	7.97	8.27	8.06	8.29	7.37	7.64	7.43	7.66
6	7.65	7.99	7.75	8.06	7.59	7.96	7.67	8.00	7.42	7.87	7.43	7.92
7	8.73	8.85	8.88	9.14	7.75	8.12	7.82	8.19	7.09	7.39	7.14	7.45
8					7.97	8.40	8.06	8.46	OH 9.80	7.79	OH 9.89	7.83
9	9.30	9.35	9.91	9.86	8.18	7.95	8.40	8.48	8.23	7.96	8.89	8.48
11			9.48	9.48			8.20	8.05			8.53	8.06
12												
13			8.35-8.64	9.04			8.46-8.63	8.53			8.05-8.41	8.68
14			7.75	8.11			8.46-8.63	8.68			8.05-8.41	8.64
15			8.09	8.41			8.06	8.29			7.43	7.66
16			8.35-8.64	8.66			7.67	7.98			7.43	7.90
17			7.62	7.82			7.82	8.15			7.14	7.41
18			9.00	9.30			8.06	8.46			OH 9.89	7.81
N3	11.67	8.26	12.18	10.37	11.78	8.31	12.51	10.33	11.84	8.31	12.52	10.31
N4	9.95	7.91	12.10	8.38	10.18	7.95	12.16	8.48	10.17	7.92	12.18	8.49
N5	4.91	3.22			4.97	3.22			4.96	3.22		

Table S 4. Experimental and calculated ^{13}C NMR (126 MHz, DMSO-d_6) data of **O1-O6**

	O1		**O2**		**O3**		**O4**		**O5**		**O6**	
δ	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**
2	150.08	150.03	150.16	150.39	154.34	153.38	151.67	152.88	152.25	151.13	151.92	150.23
3	121.67	120.21	121.77	119.98	118.03	115.51	117.78	115.81	118.35	116.76	118.12	117.00
4	136.55	137.01	136.62	137.81	136.19	137.31	136.47	137.41	136.06	137.37	136.40	137.49
4a	127.94	125.93	128.02	125..36	127.66	125.50	127.99	125.83	128.52	126.32	128.75	126.60
5	128.90	129.02	129.28	129.69	127.72	127.43	128.84	127.36	117.74	114.86	117.90	114.86
6	126.45	125.33	126.49	125.26	126.84	126.29	127.79	126.64	127.73	128.56	128.13	128.95
7	125.61	124.17	125.72	124.65	129.82	129.13	127.11	129.20	111.59	106.68	112.13	106.80
8	131.59	130.68	131.65	130.19	128.69	129.02	130	129.20	153.24	152.48	153.37	152.56
8a	145.01	143.34	145.19	143.61	147.26	145.87	147.35	145.84	137.93	134.42	138.13	134.34
9	136.89	135.46	139.89	136.96	140.64	139.93	144.06	142.52	140.50	138.54	144.02	141.31
10	157.21	153.32	152.28	146.03	156.76	152.74	153.99	145.51	156.83	152.52	162.45	145.41
11			139.89	136.96			144.06	142.52			144.02	141.31
12			131.65	130.19			151.67	152.88			151.92	150.23
12a			145.19	143.61								
13			125.72	124.65			117.78	115.81			118.12	117.00
14			126.49	125.26			136.47	137.41			136.40	137.49
14a							127.99	125.83			128.75	126.60

15			128.28	129.69			128.84	127.36			117.90	114.86
15a			128.02	127.66								
16			136.62	137.81			127.79	126.64			128.13	128.95
17			121.77	119.98			127.11	129.20			112.13	106.80
18			150.16	150.39			130	129.20			153.37	152.56
18a							147.35	145.84			138.13	134.34

Table S 5. Experimental and calculated ^{13}C NMR (126 MHz, DMSO-d_6) data of **S1-S6**

	S1		**S2**		**S3**		**S4**		**S5**		**S6**	
δ	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**	**Exp.**	**Calc.**
2	150.29	150.67	150.34	150.999	153.68	151.86	153.96	152.12	151.95	149.01	151.60	149.48
3	121.75	120.65	121.85	120.87	118	115.55	118.72	116.21	118.65	116.73	118.62	117.40
4	136.59	137.69	136.65	137.64	135.89	137.77	136.98	137.61	136.03	137.77	136.48	137.67
4a	127.94	125.87	128.01	125.91	127.46	126.00	128.23	126.03	128.71	126.91	128.90	126.92
5	129.59	131.14	129.99	131.07	127.57	127.45	128.37	127.33	117.71	114.87	117.87	114.75
6	126.40	125.19	126.48	125.21	126.75	127.23	127.75	127.27	127.97	129.71	128.38	129.63
7	126.33	125.44	126.67	125.96	129.57	129.45	130.47	129.73	112	107.03	112.24	107.33
8	131.21	128.45	131.46	129.06	128.40	129.37	129.21	129.42	153.36	152.71	153.44	152.73
8a	145.30	143.83	140.36	144	146.97	145.88	147.70	145.96	138.10	149.01	138.20	134.62
9	138.97	139.68	145.40	144.54	141.98	143.57	149.59	149.27	142.16	142.25	149.17	148.00
10	175.96	182.06	175.21	176.64	175.37	182.93	175.97	177.68	175.72	182.83	175.61	177.76
11			140.48	140.21			144.46	144.71			143.85	143.58
12			130.97	127.99			152.03	151.21			151.60	148.40

12a			140.30	144.16								
13			125.98	125.97			118.41	115.83			118.62	117.00
14			126.48	125.18			136.98	138.02			136.48	138.05
14a							128.23	125.99			128.90	126.99
15			129.99	131.91			128.37	127.59			117.87	115.10
15a			128.01	125.58								
16			136.65	138			127.75	126.97			128.38	129.43
17			121.85	120.27			130.47	129.70			112.24	107.24
18			150.34	150.94			129.21	129.45			153.44	152.68
18a							147.70	145.93			138.20	134.75

Table S 6. Lipinski's pharmacokinetic properties of investigated compounds

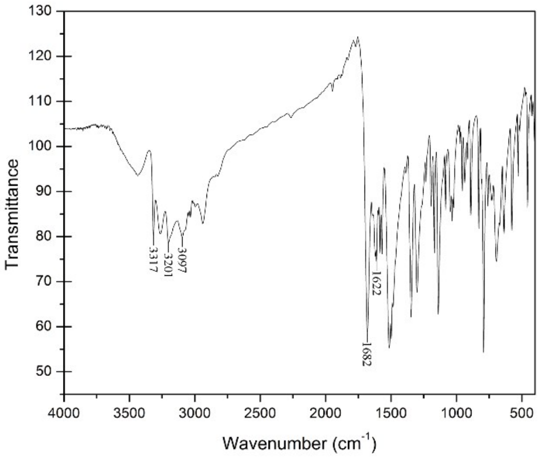
	O1	**S1**	**O2**	**S2**	**O3**	**S3**	**O4**	**S4**	**O5**	**S5**	**O6**	**S6**
MW	229.24	245.30	368.39	384.46	229.24	245.30	368.39	384.46	245.24	261.30	400.39	416.46
log*P*	1.234	1.621	4.115	4.223	1.262	1.720	3.886	4.402	0.825	1.227	3.210	3.526
HBD	4	4	2	2	4	4	2	2	5	5	4	4
HBA	3	2	5	4	3	2	5	4	3	2	5	4

MW – molecular weight

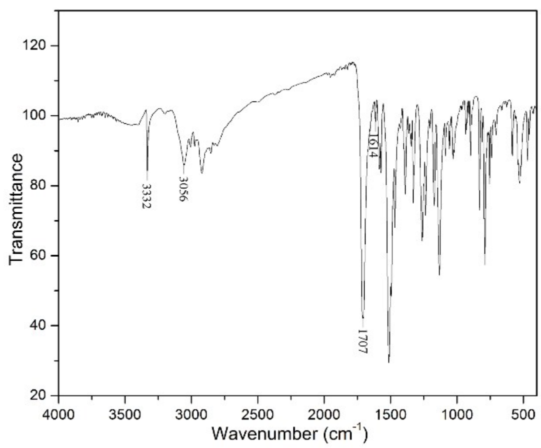
log*P* – predicted octanol/water partition coefficient

HBD – number of hydrogen bond donor atoms per molecule

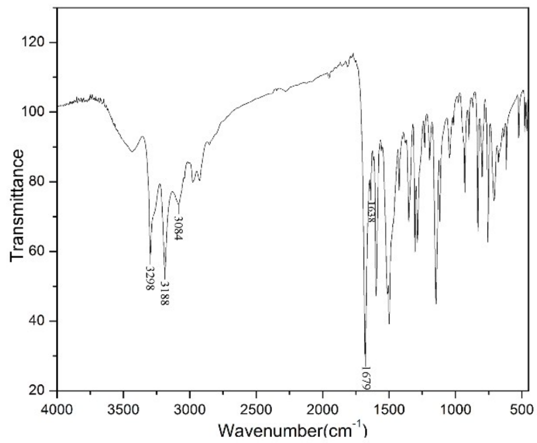
HBA– number of hydrogen bond acceptor atoms per molecule



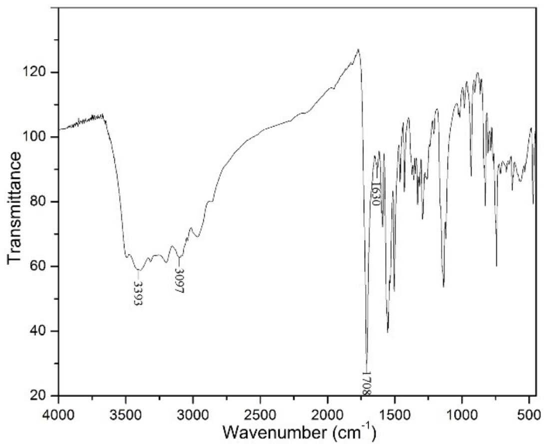
O1



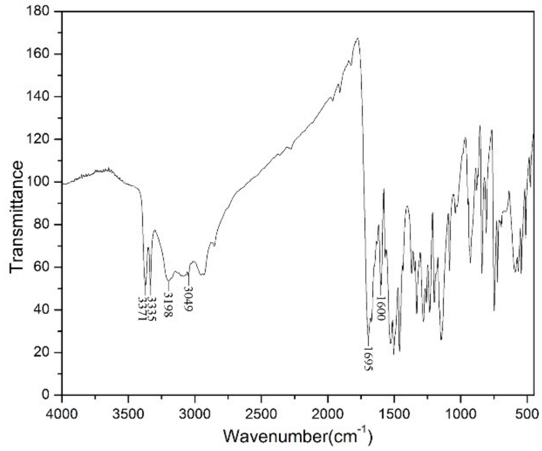
O2



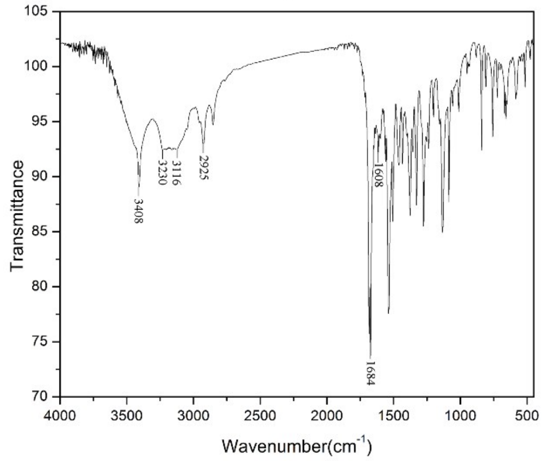
O3



O4

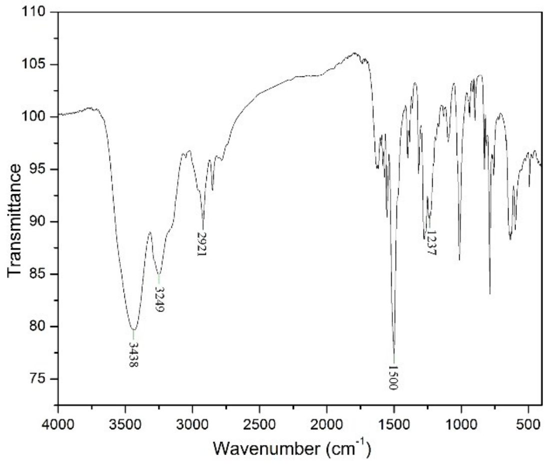


O5

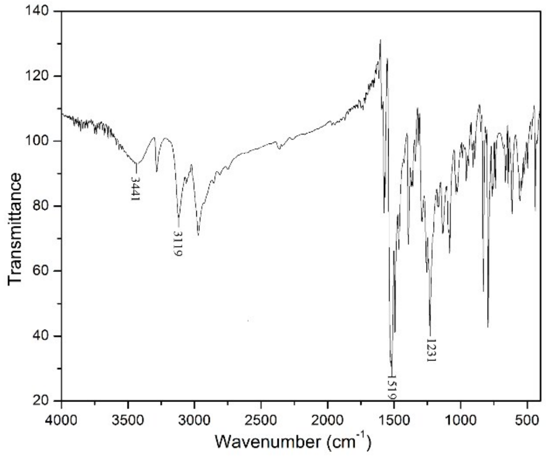


O6

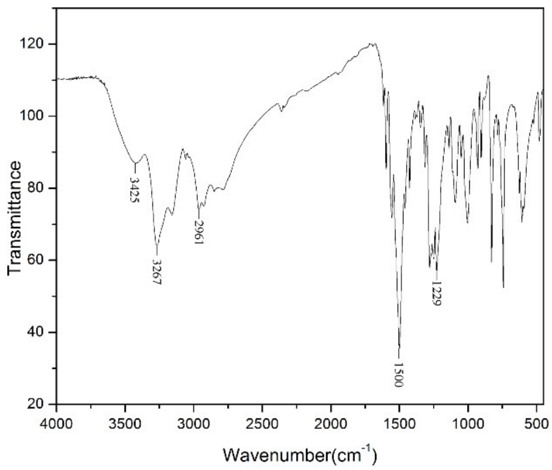
Figure S 6. Experimental FT-IR spectra of compound **O1-O6.**



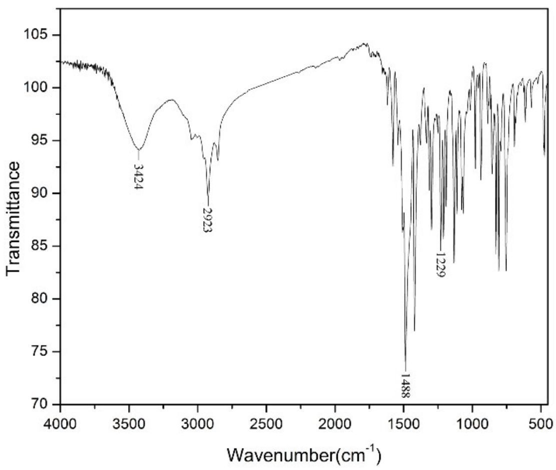
S1



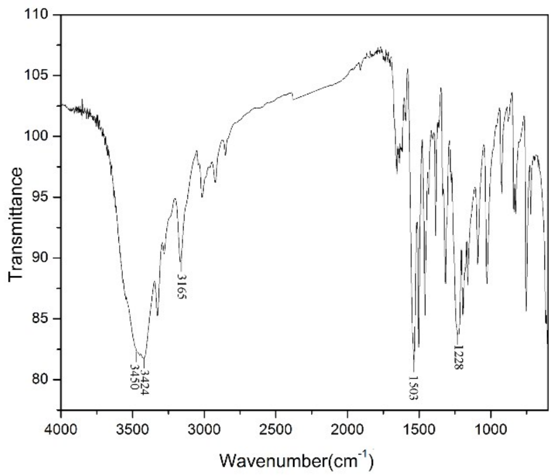
S2



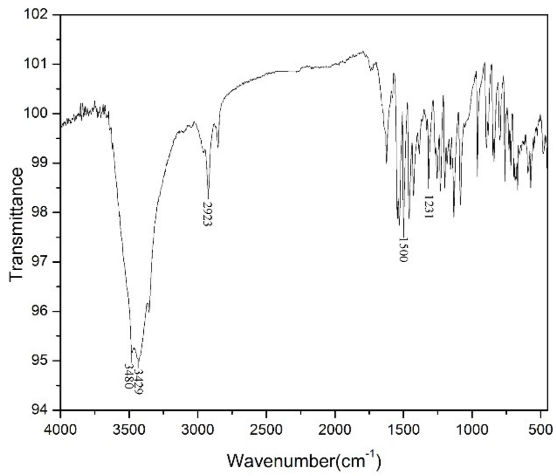
S3



S4



S5



S6

Figure S 7. Experimental FT-IR spectra of compound **S1-S6**.

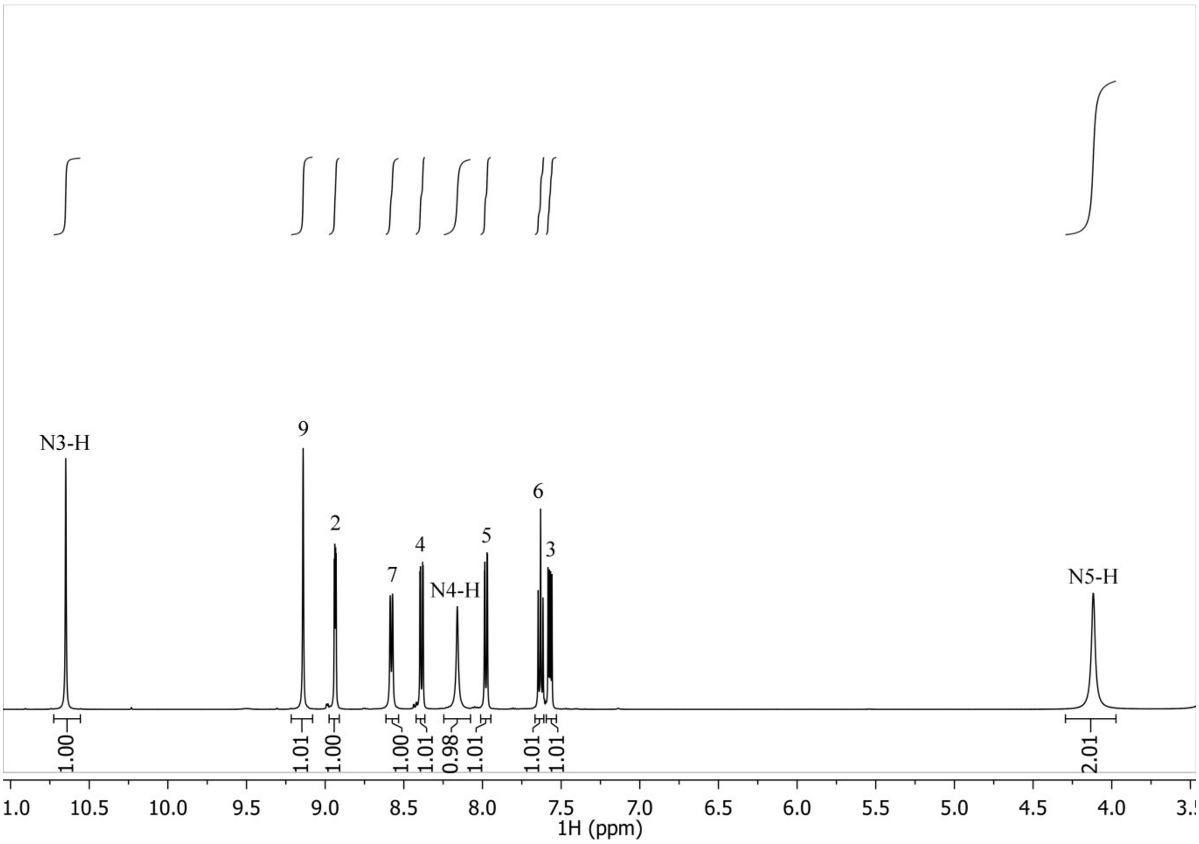


Figure S 8. ^{1}H NMR spectrum of **O1** in DMSO-d_6.

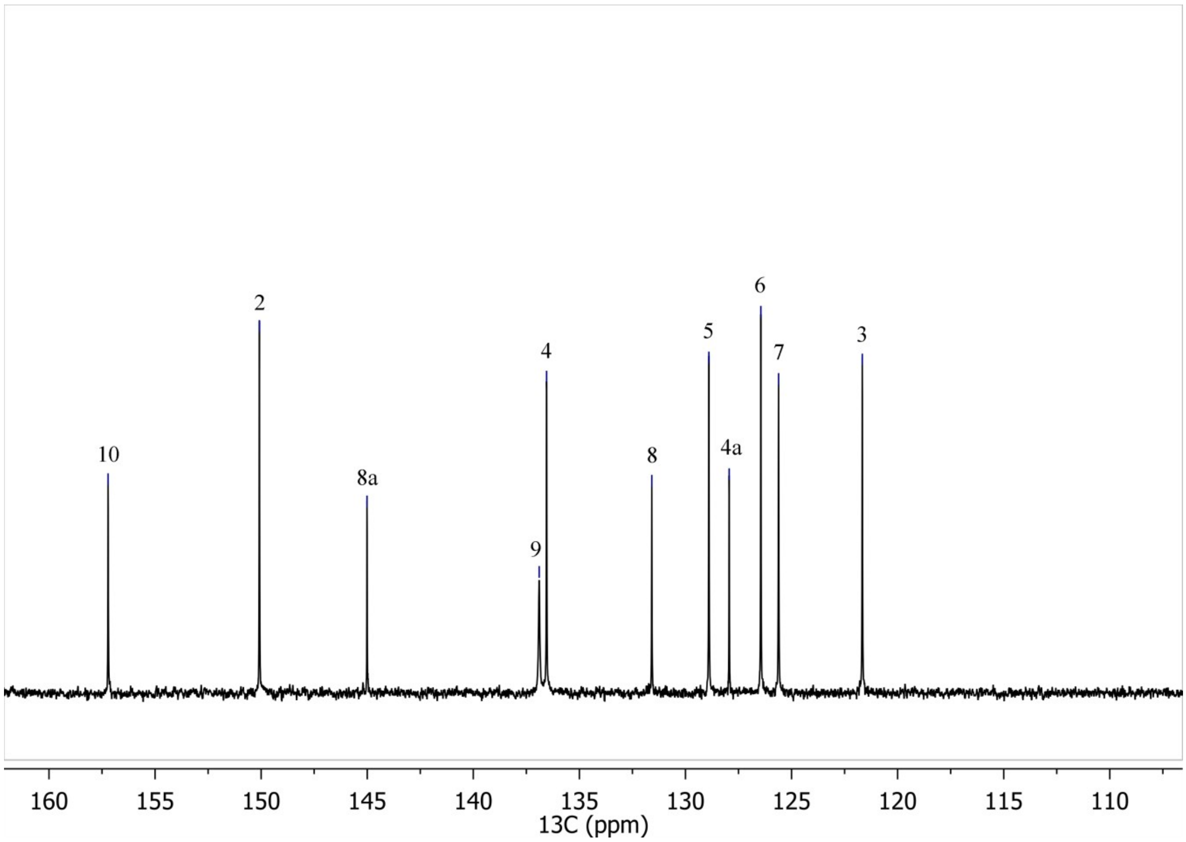


Figure S 9. ^{13}C NMR spectrum of **O1** in DMSO-d_6.

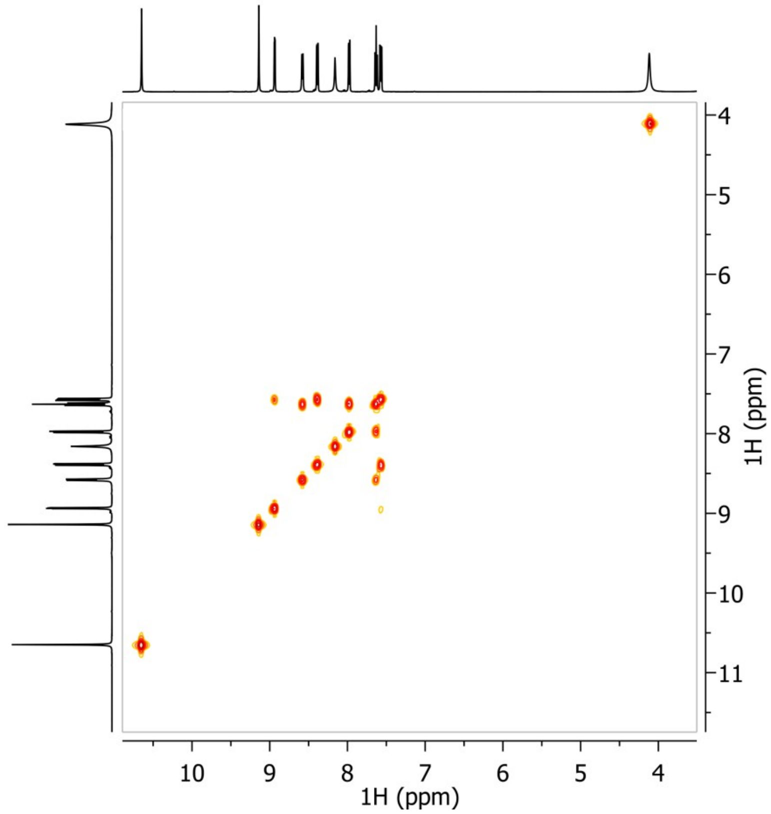


Figure S 10. COSY spectrum of **O1**.

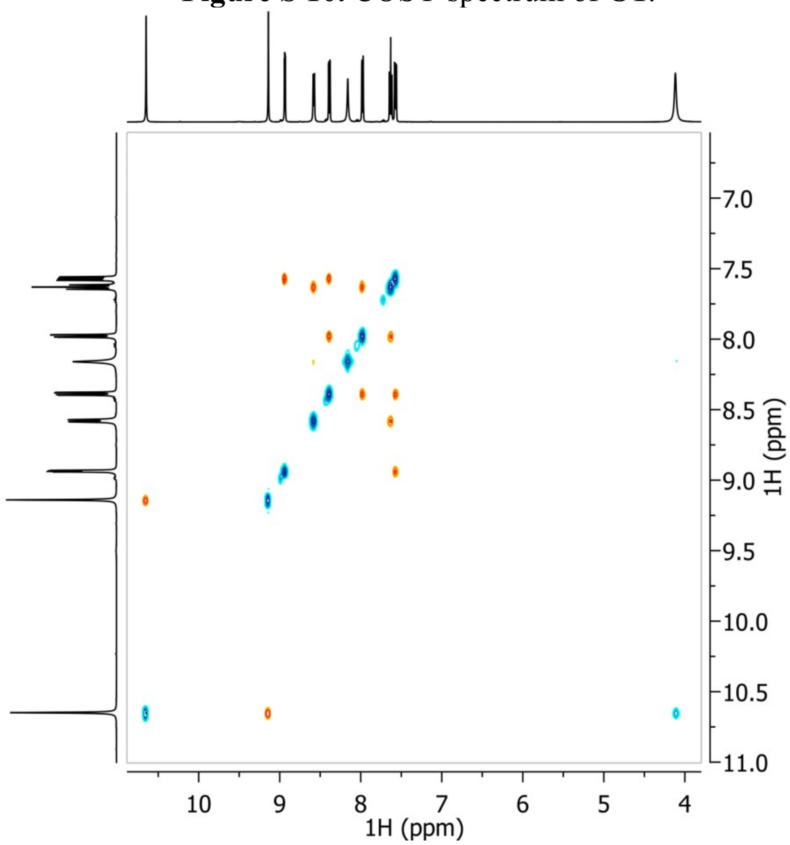


Figure S 11. NOESY spectrum of **O1**.

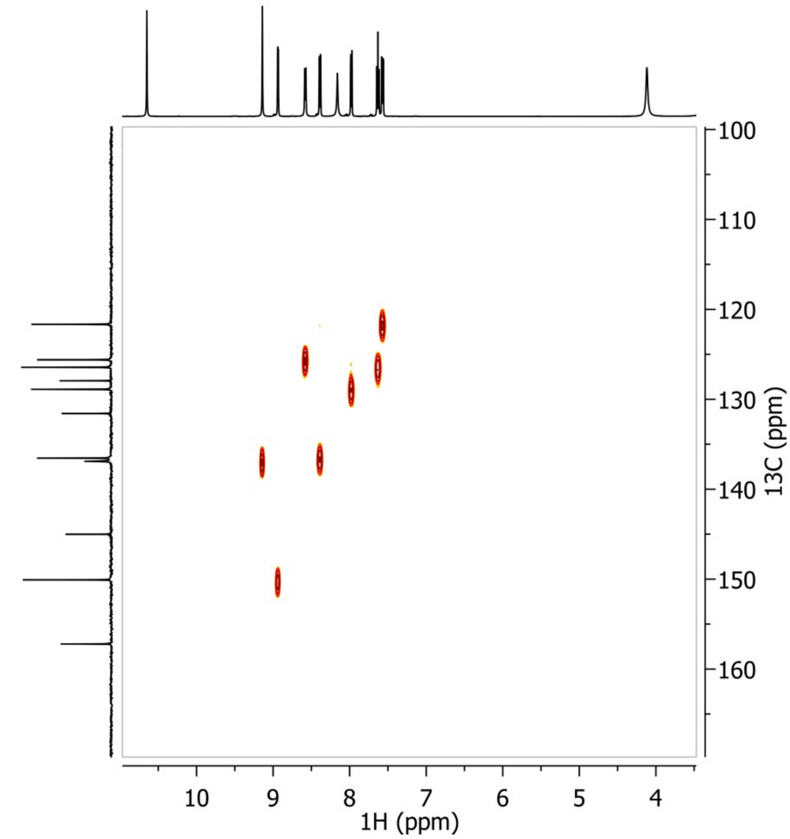


Figure S 12. $^{1}H–^{13}C$ HSQC spectrum of **O1**.

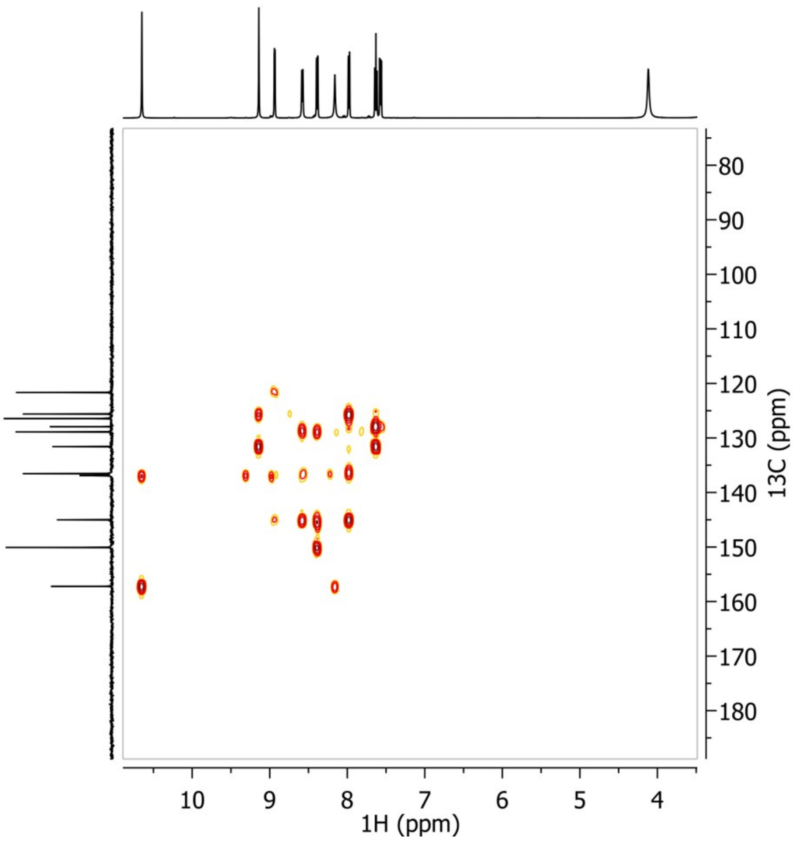


Figure S 13. $^{1}H–^{13}C$ HMBC spectrum of O1.

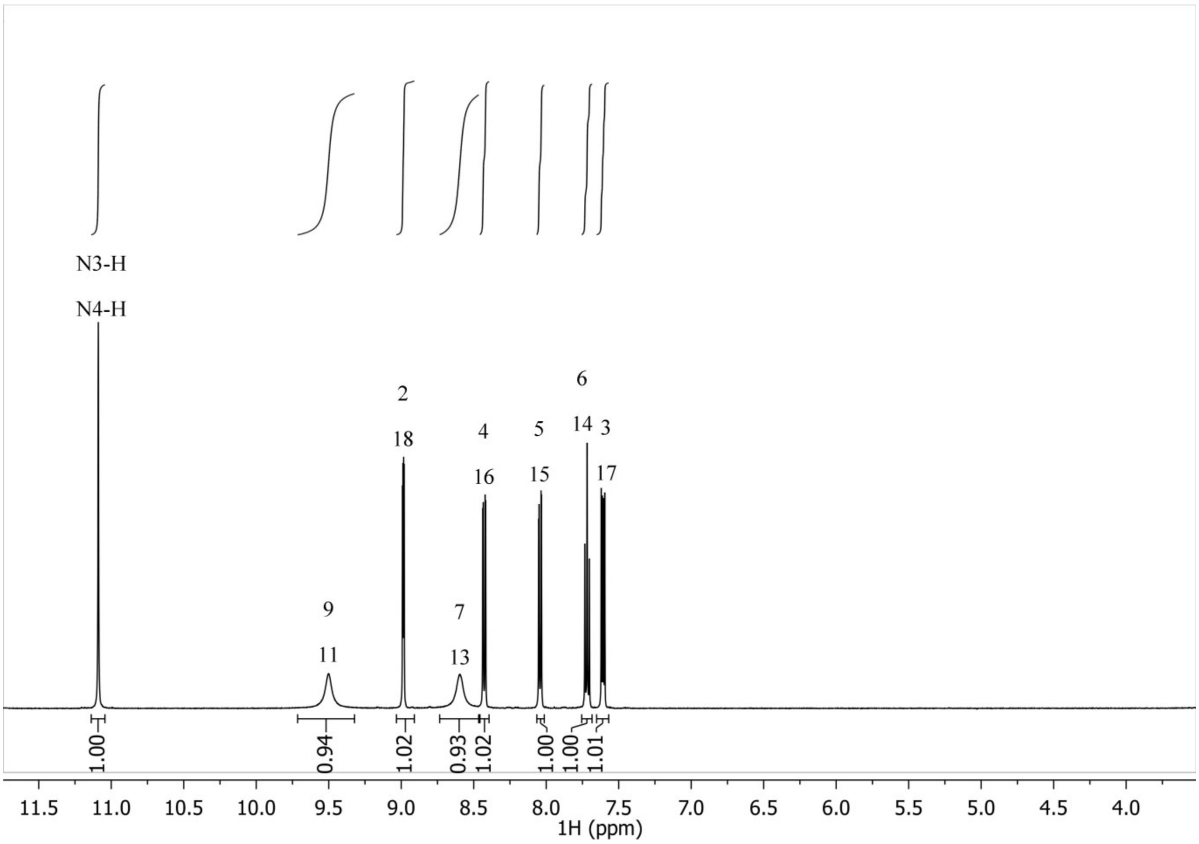


Figure S 14. ^{1}H NMR spectrum of **O2** in DMSO-d_6.

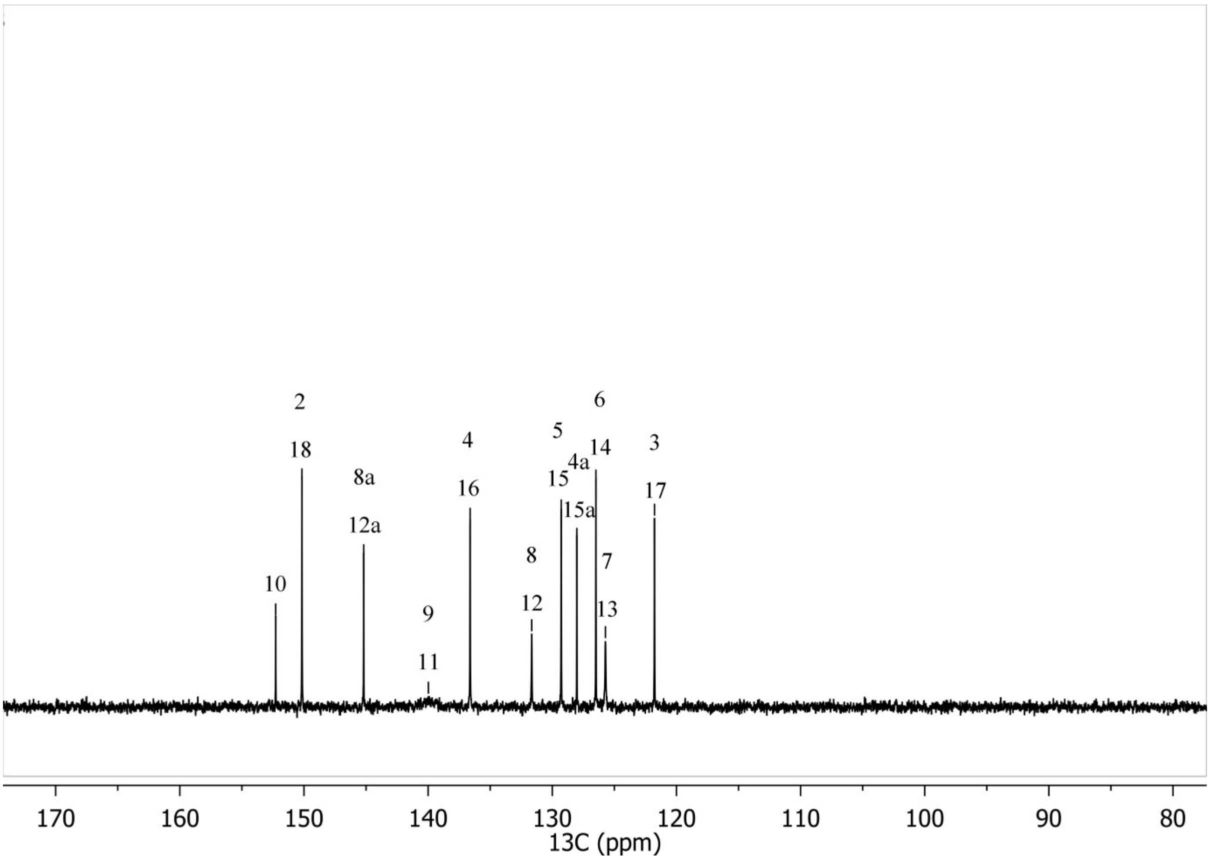


Figure S 15. ^{13}C NMR spectrum of **O2** in DMSO-d_6.

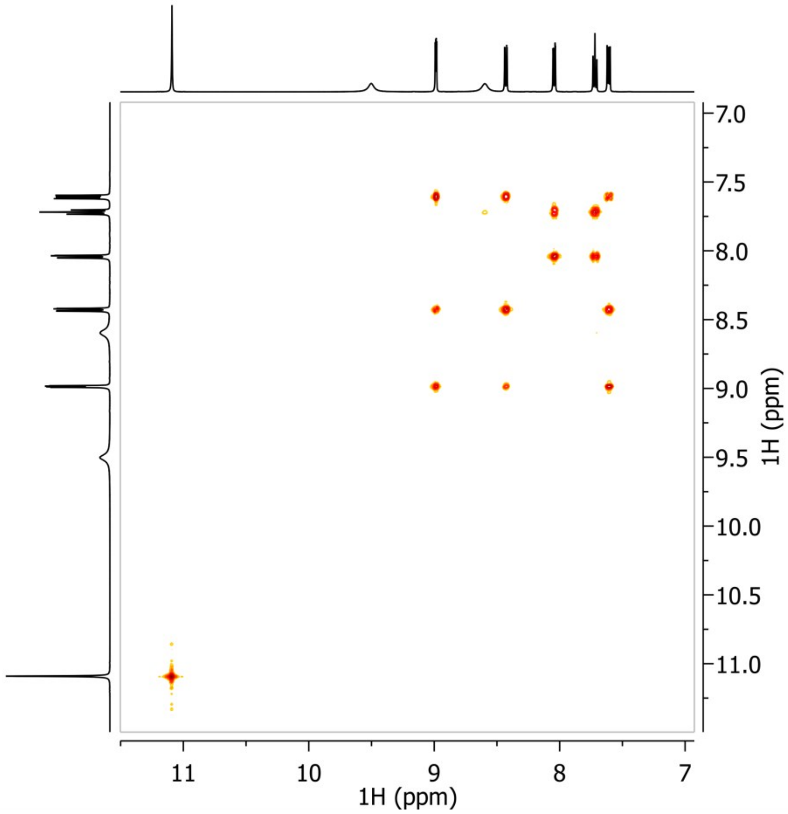


Figure S 16. COSY spectrum of **O2**.

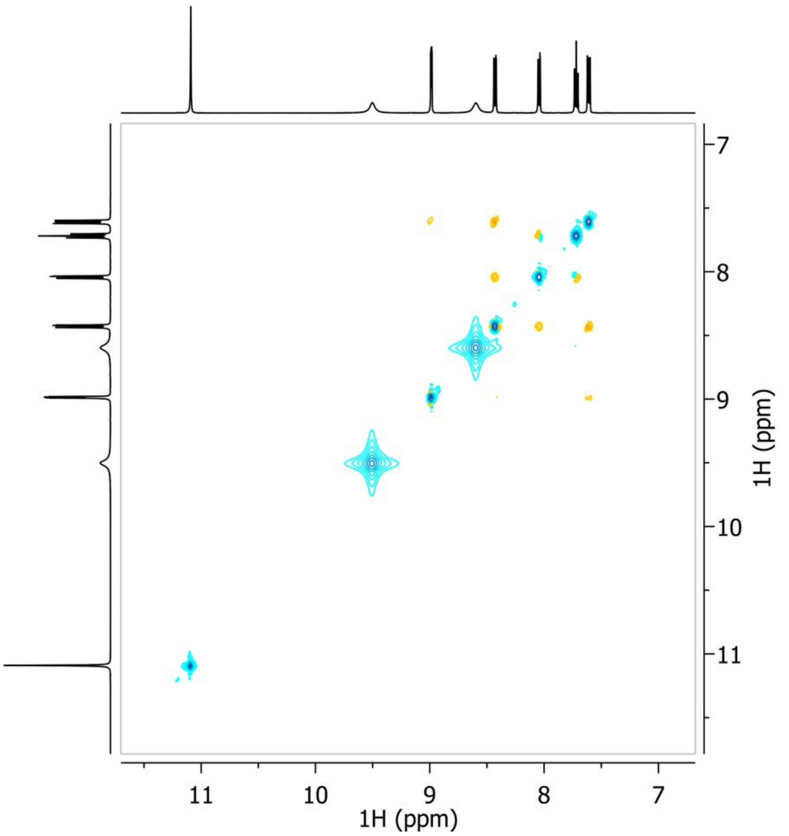


Figure S 17. NOESY spectrum of **O2**.

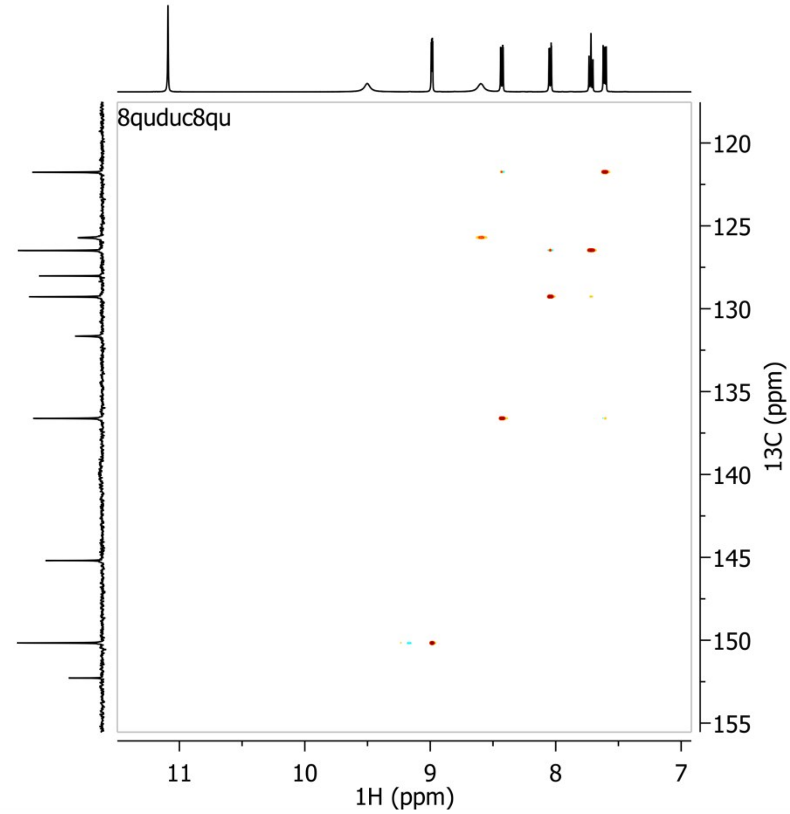


Figure S 18. ^{1}H–^{13}C HSQC spectrum of **O2**.

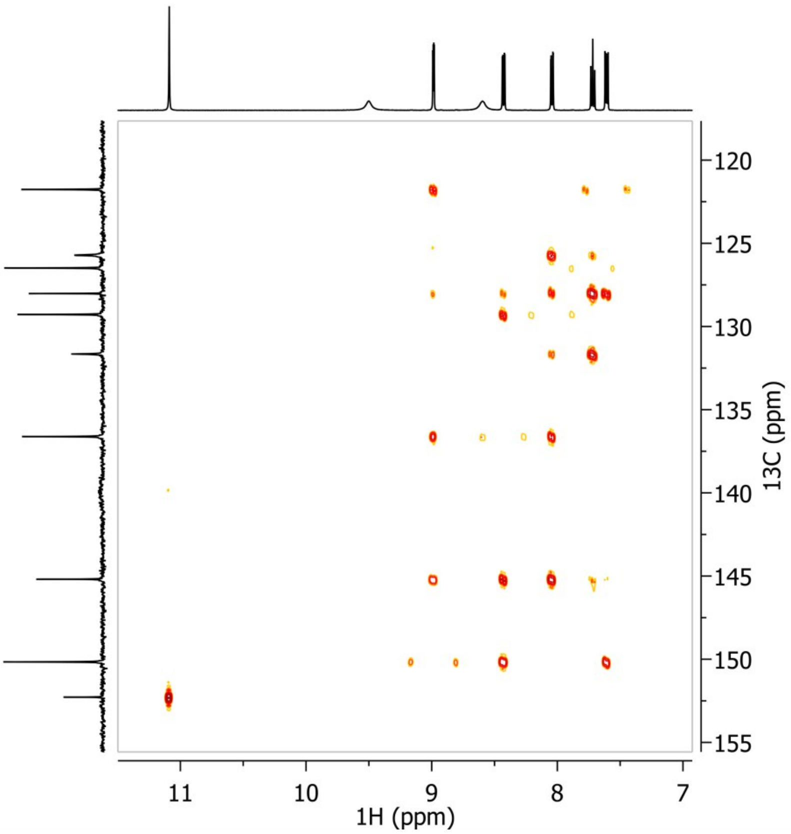


Figure S 19. ^{1}H–^{13}C HMBC spectrum of **O2**.

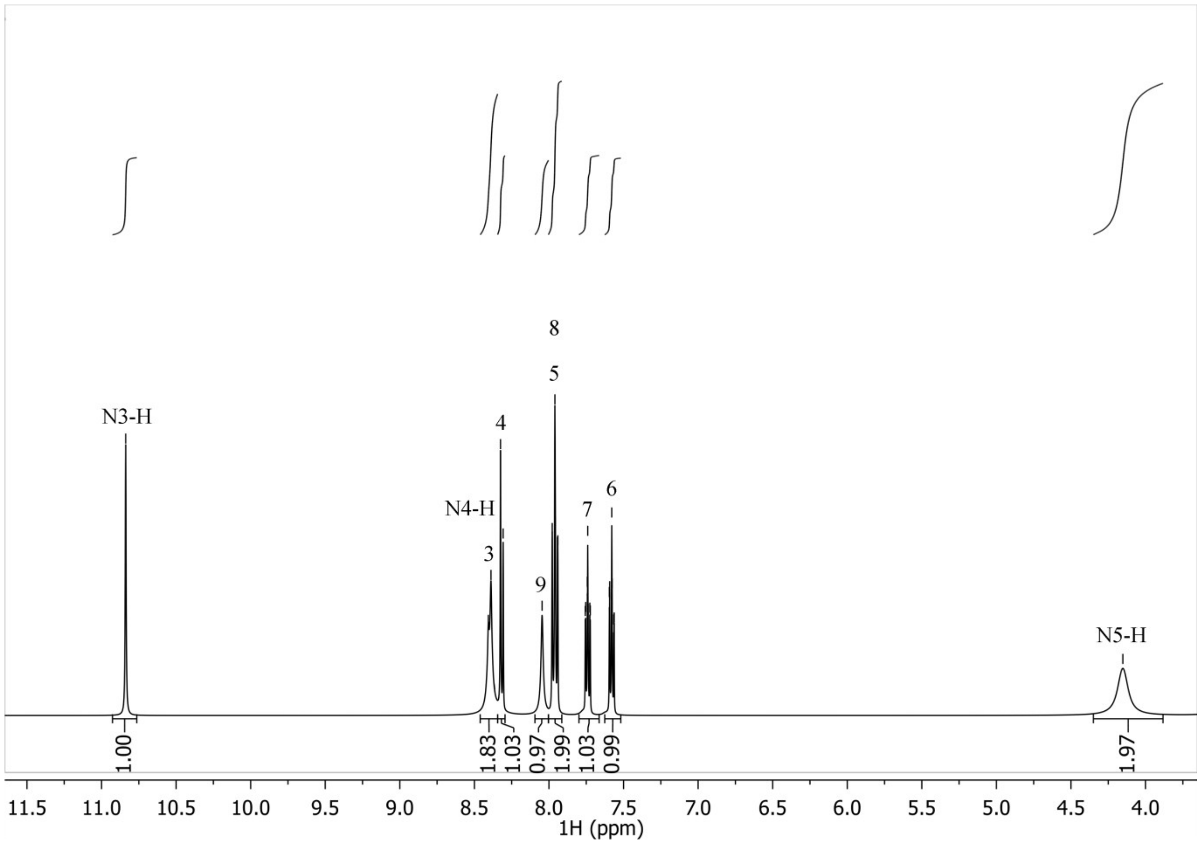


Figure S 20. ^{1}H NMR spectrum of **O3** in DMSO-d_6.

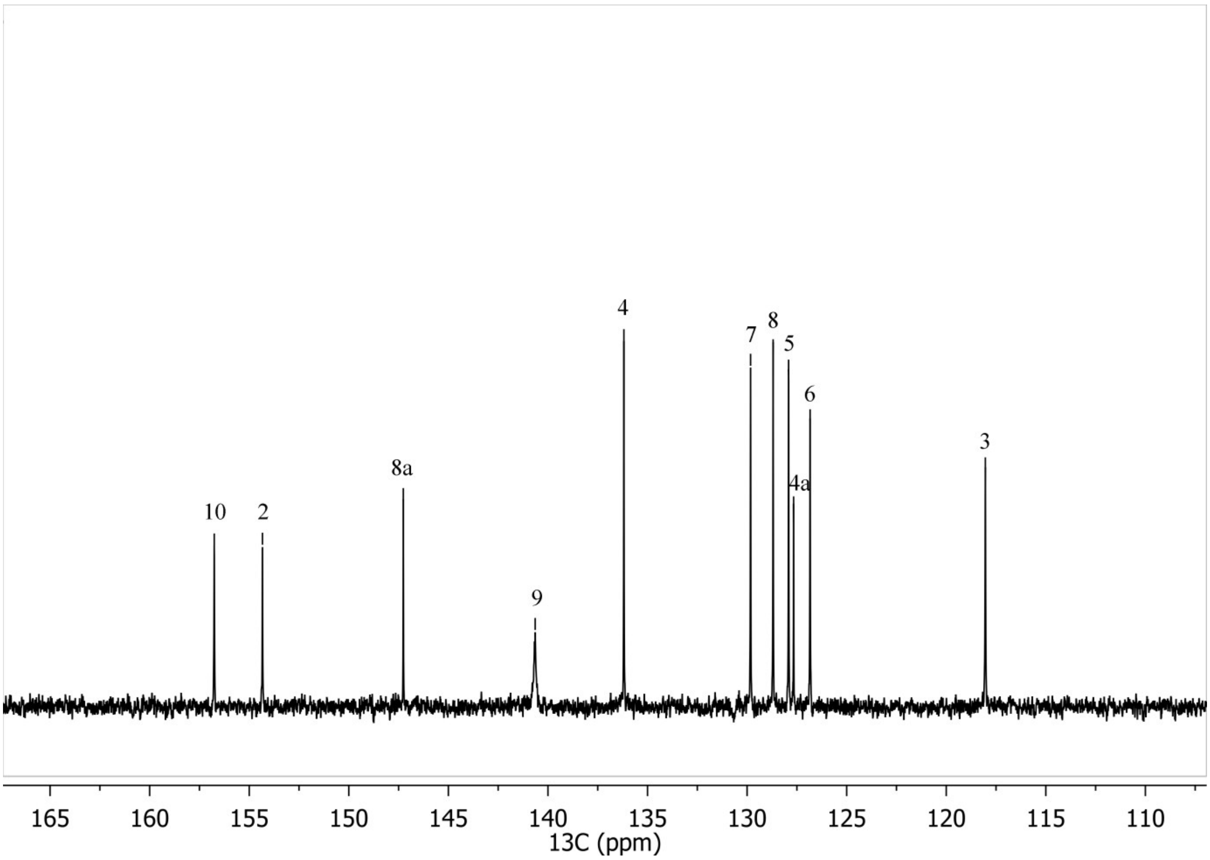


Figure S 21. ^{13}C NMR spectrum of **O3** in DMSO-d_6.

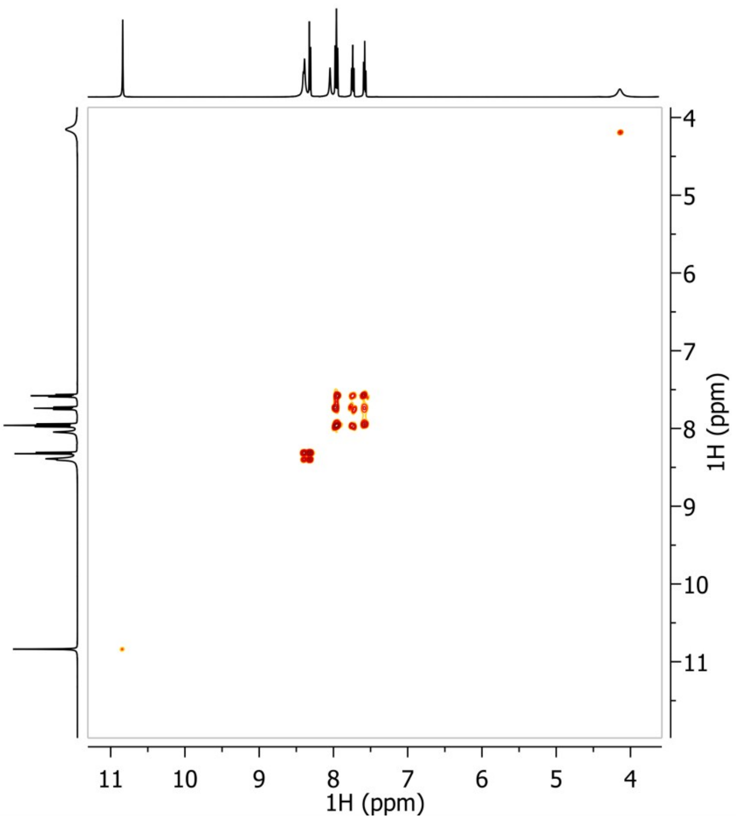


Figure S 22. COSY spectrum of **O3**.

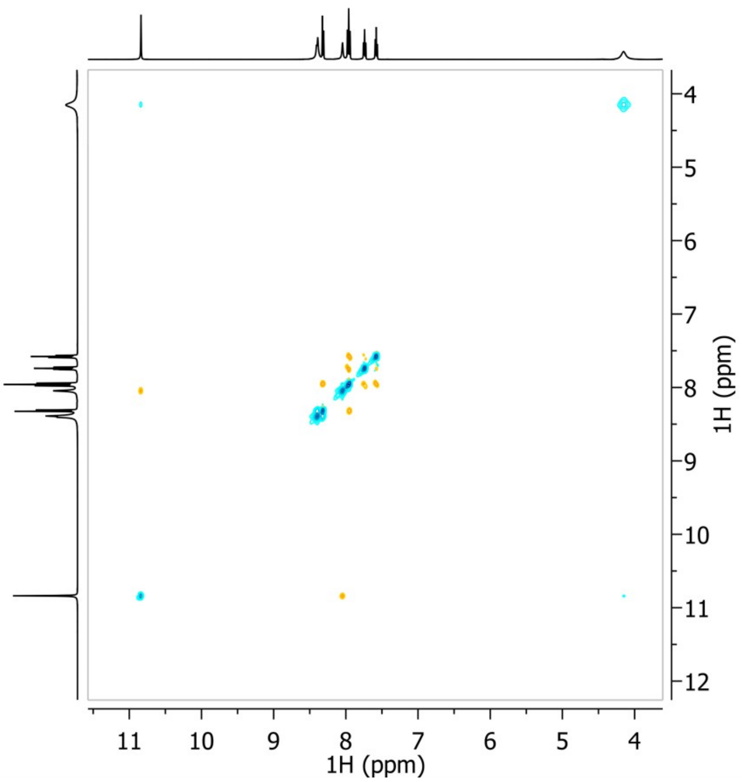


Figure S 23. NOESY spectrum of **O3**.

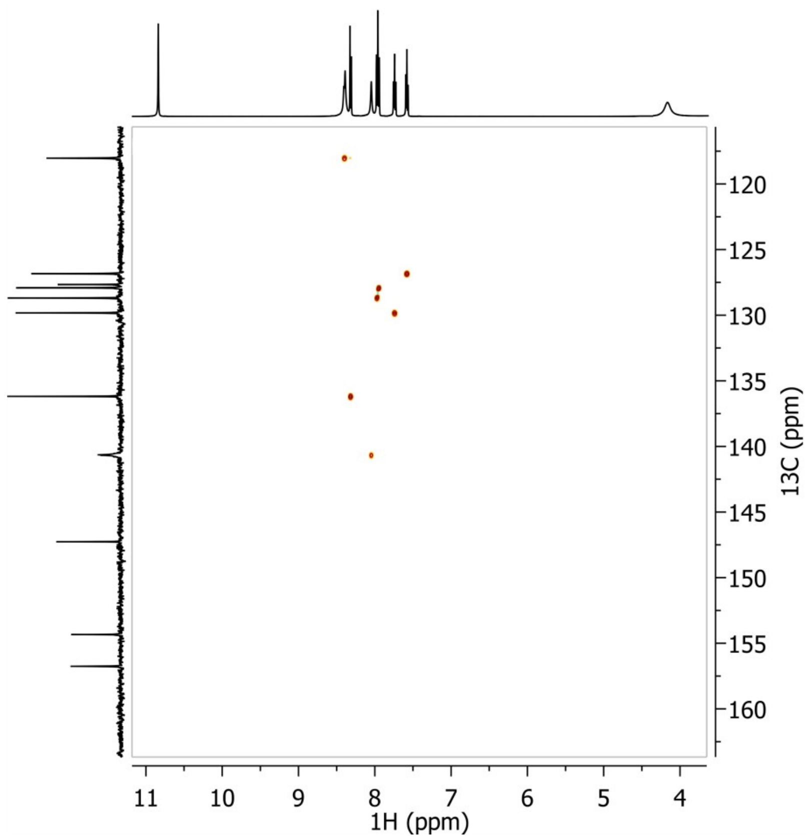


Figure S 24. ^{1}H–^{13}C HSQC spectrum of **O3**.

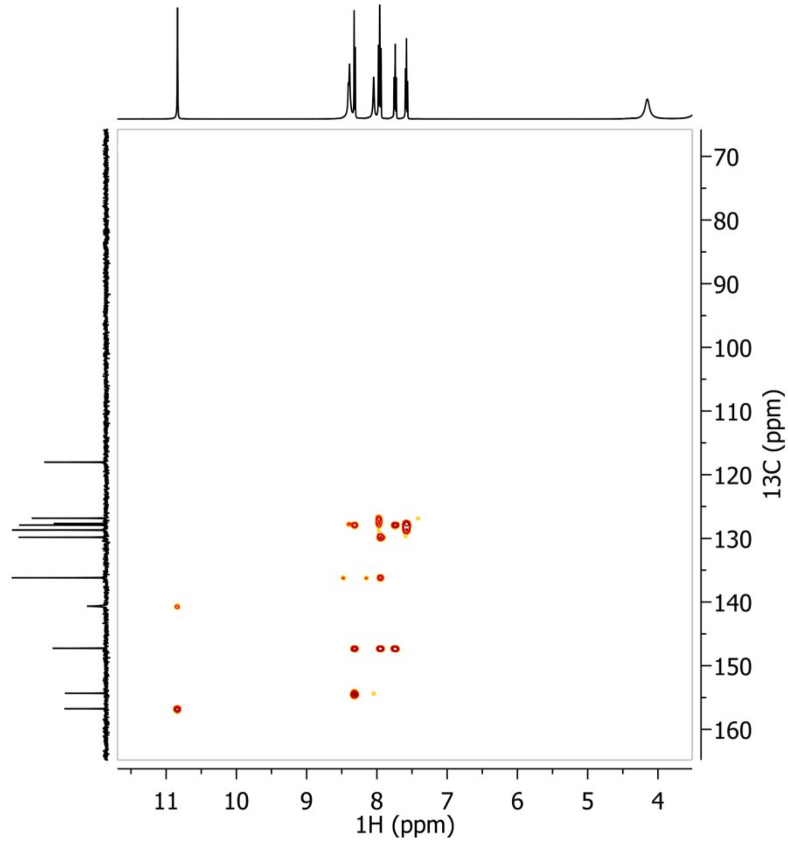


Figure S 25. ^{1}H–^{13}C HMBC spectrum of **O3**.

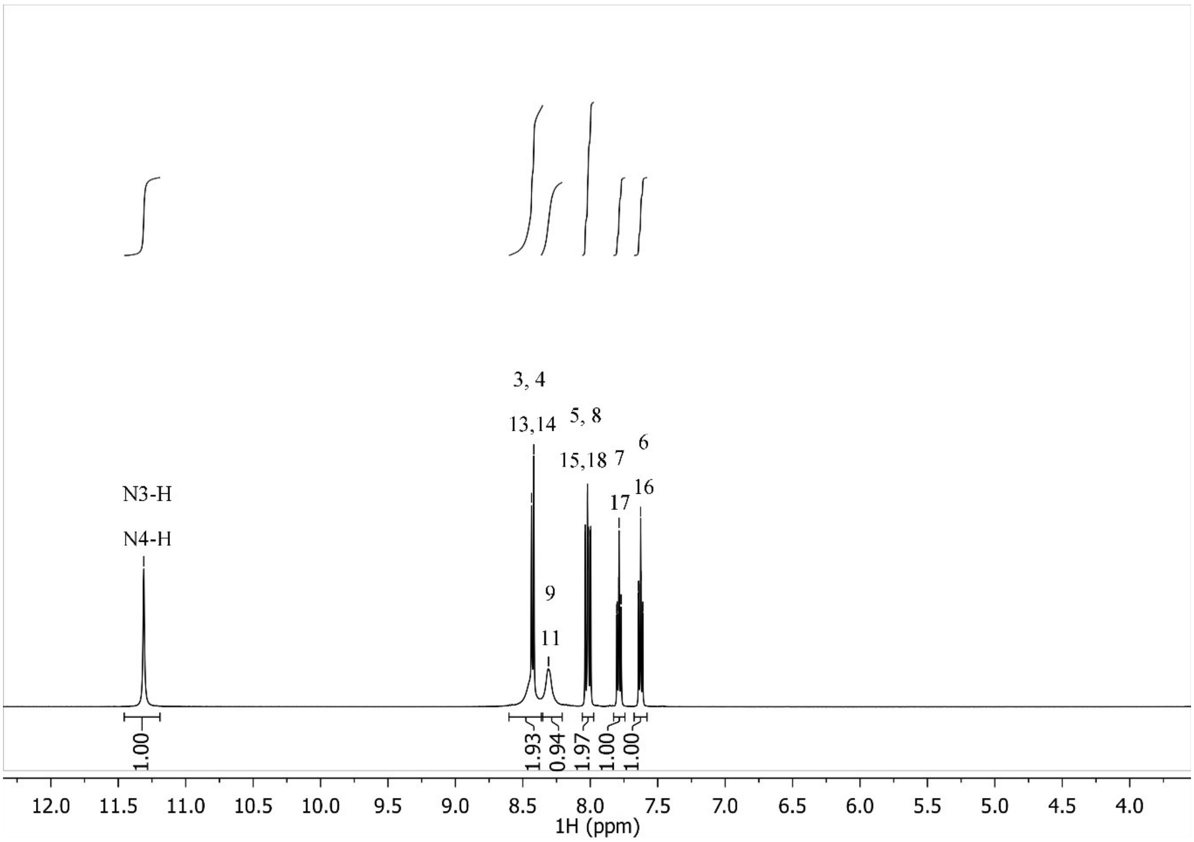


Figure S 26. ^{1}H NMR spectrum of **O4** in DMSO-d_6.

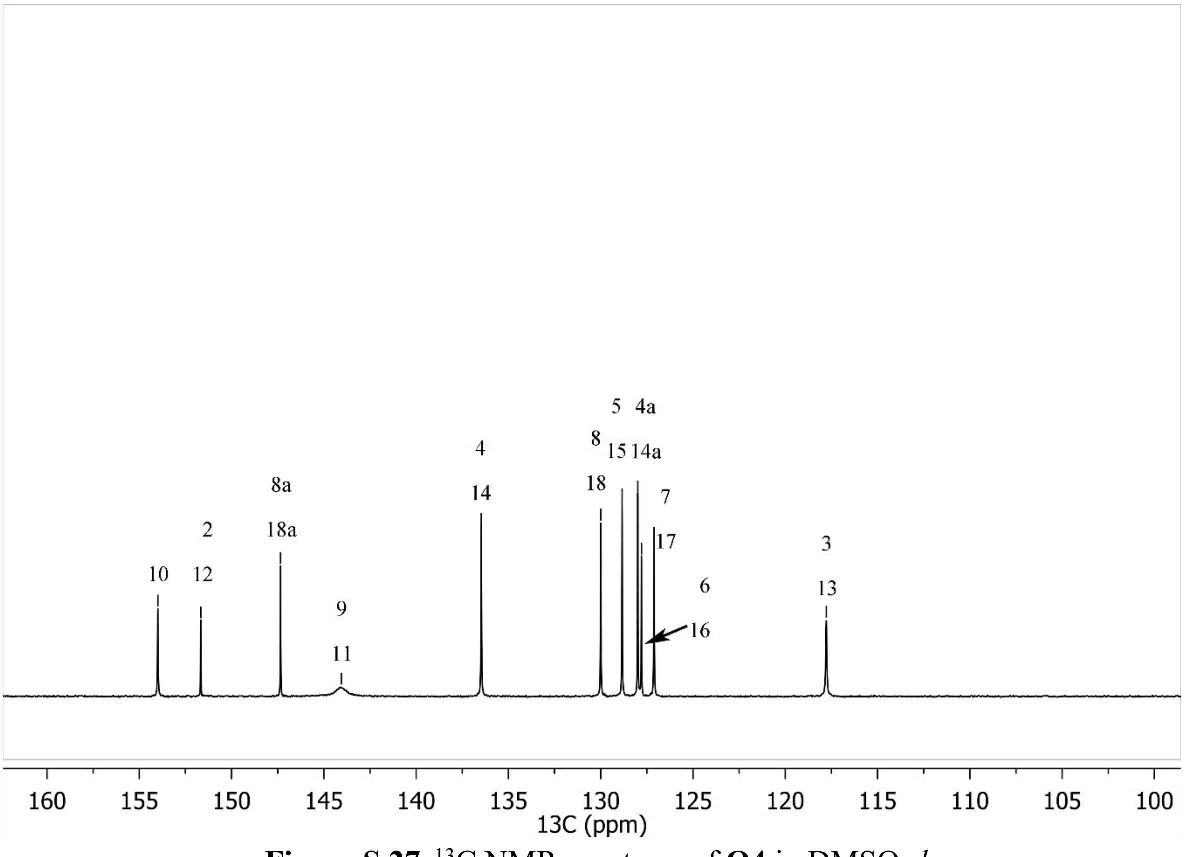


Figure S 27. ^{13}C NMR spectrum of **O4** in DMSO-d_6.

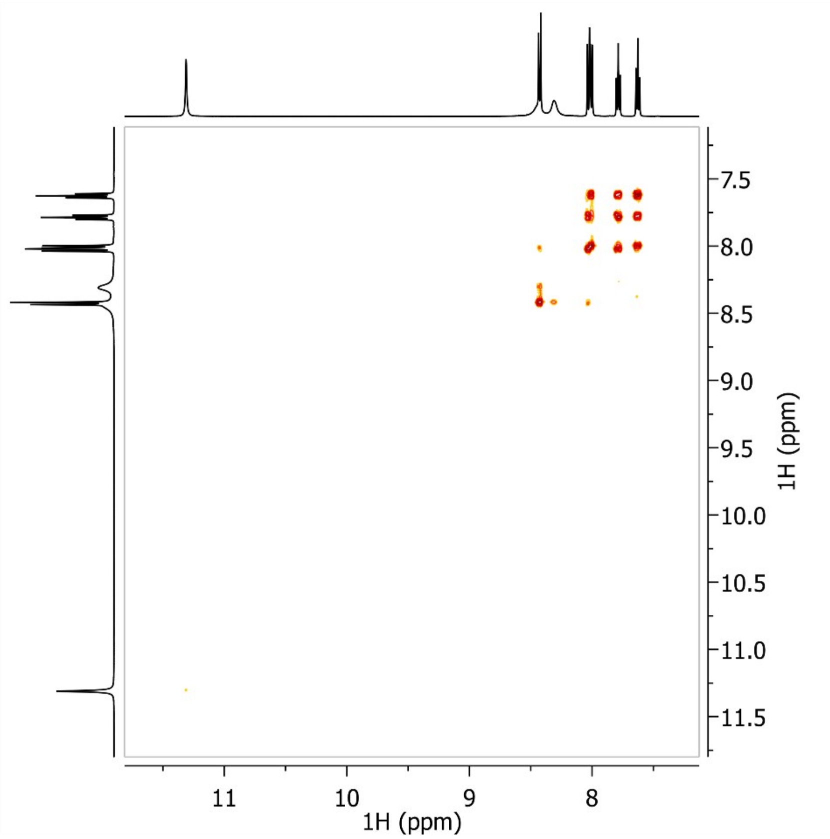


Figure S 28. COSY spectrum of **O4**.

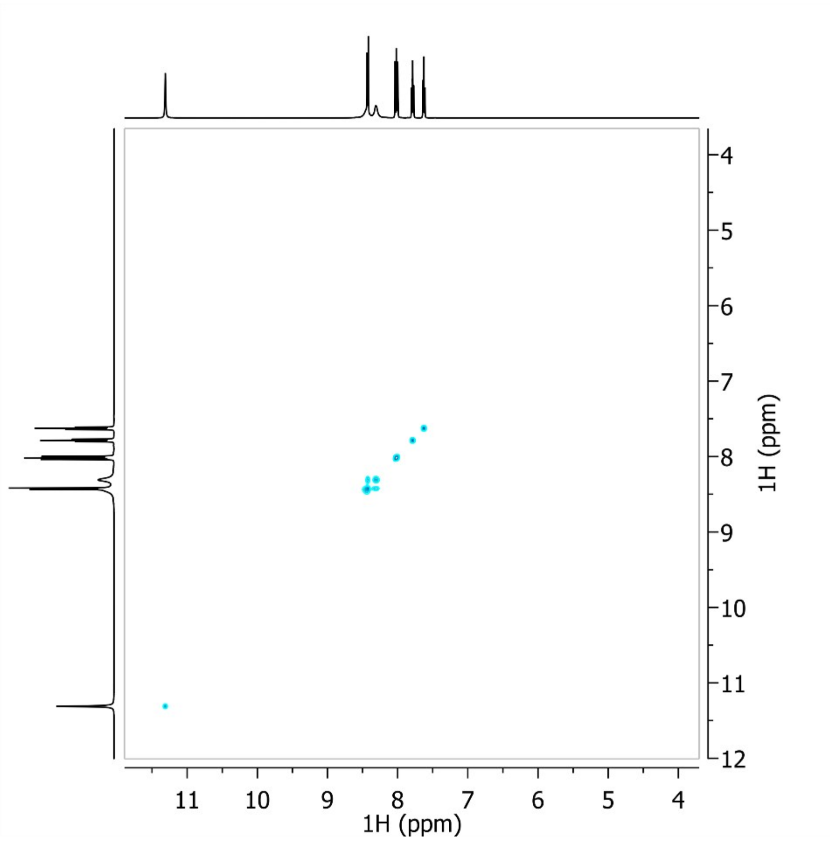


Figure S 29. NOESY spectrum of **O4**.

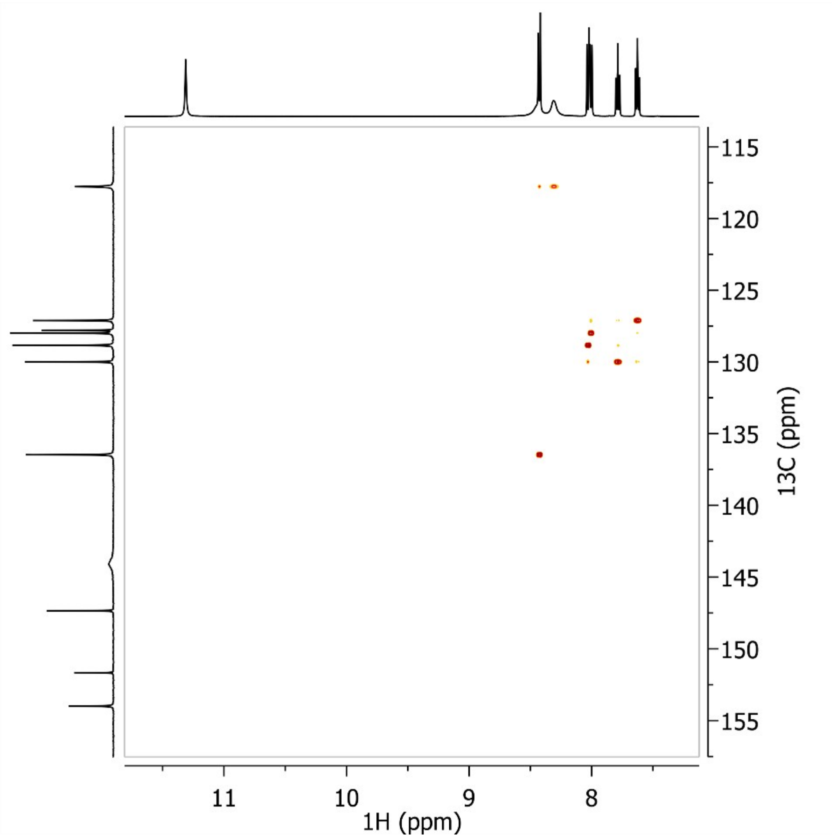


Figure S 30. ^{1}H–^{13}C HSQC spectrum of **O4**.

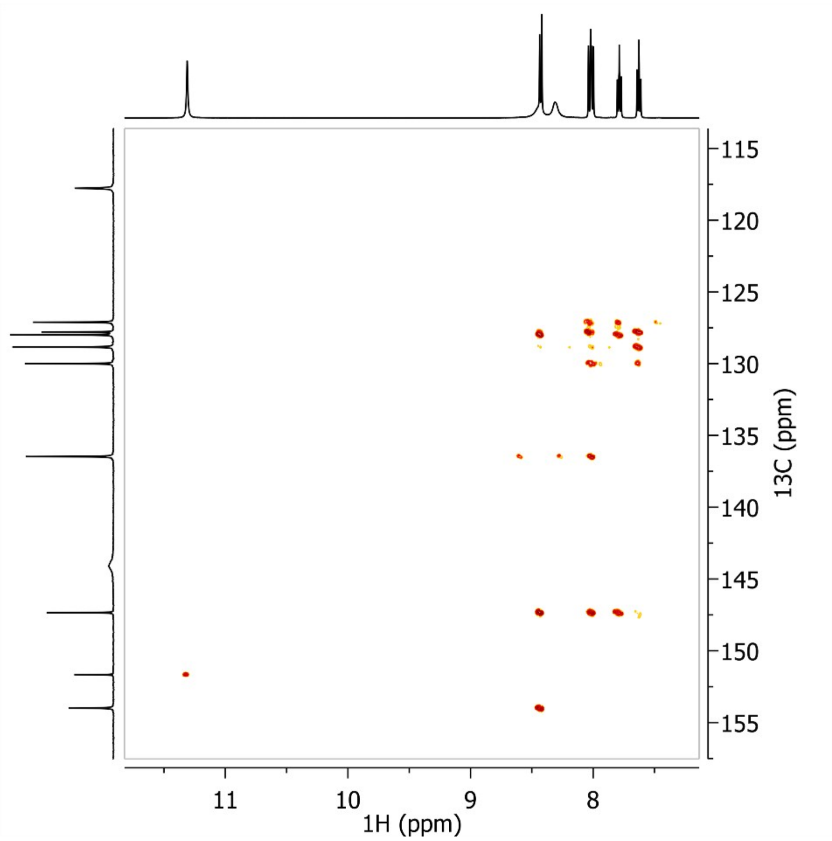


Figure S 31. ^{1}H–^{13}C HMBC spectrum of **O4**.

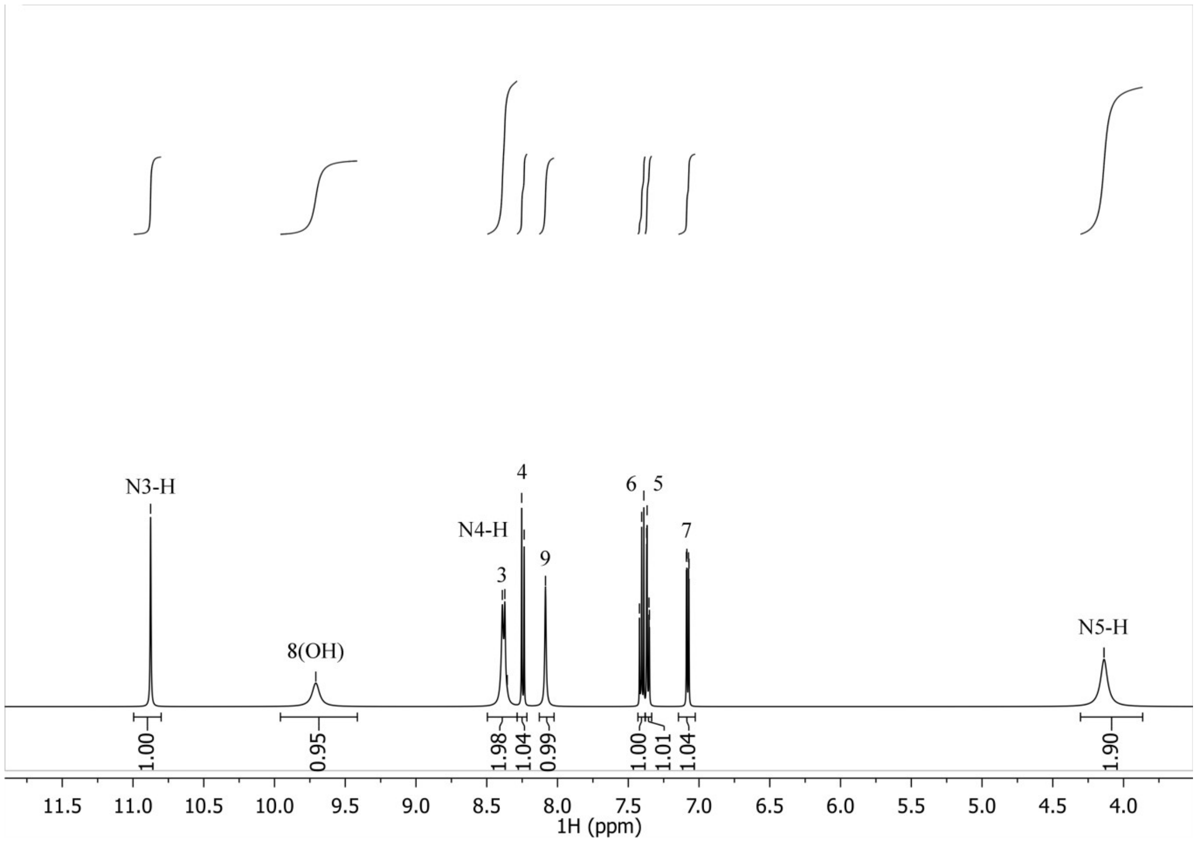


Figure S 32. ^{1}H NMR spectrum of **O5** in DMSO-d_6.

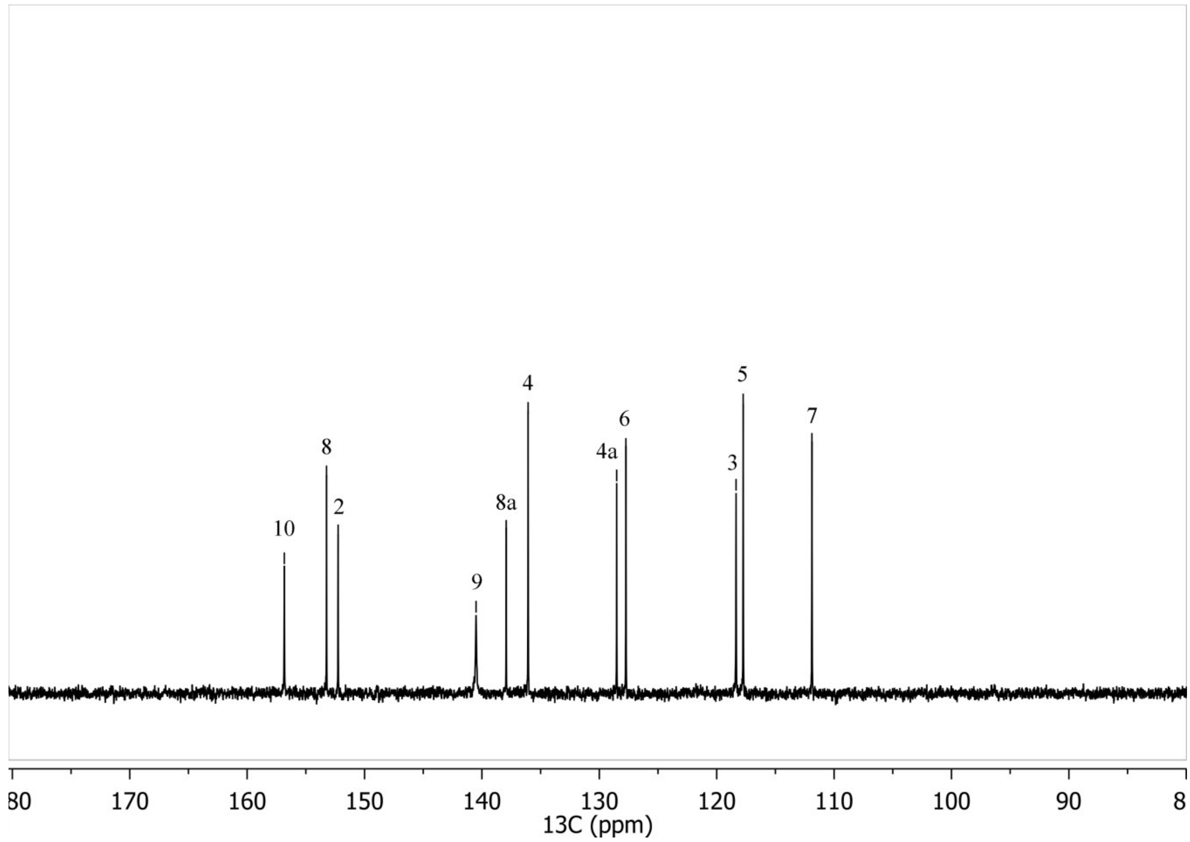


Figure S 33. ^{13}C NMR spectrum of **O5** in DMSO-d_6.

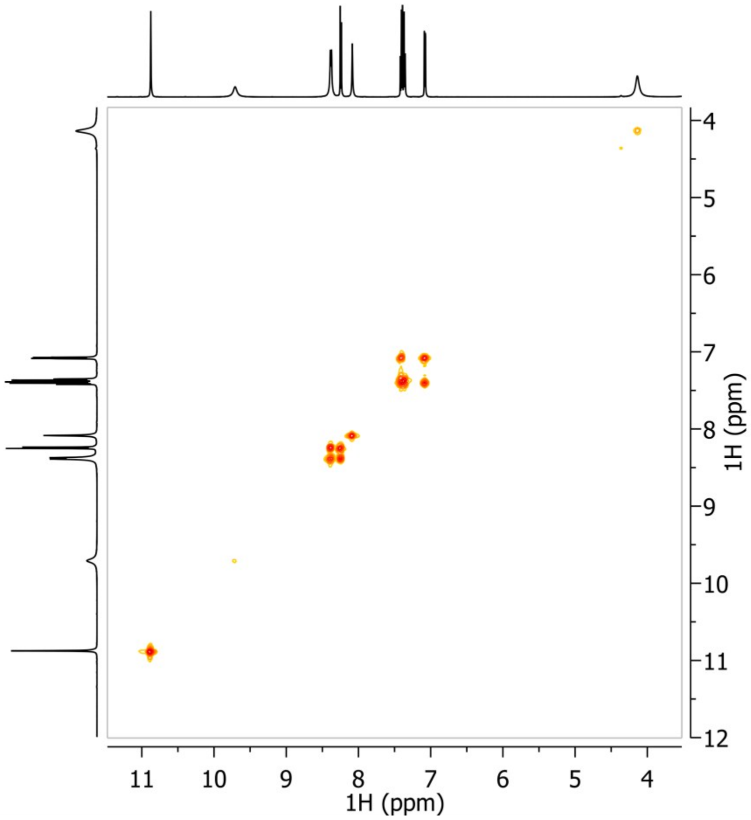


Figure S 34. COSY spectrum of **O5**.

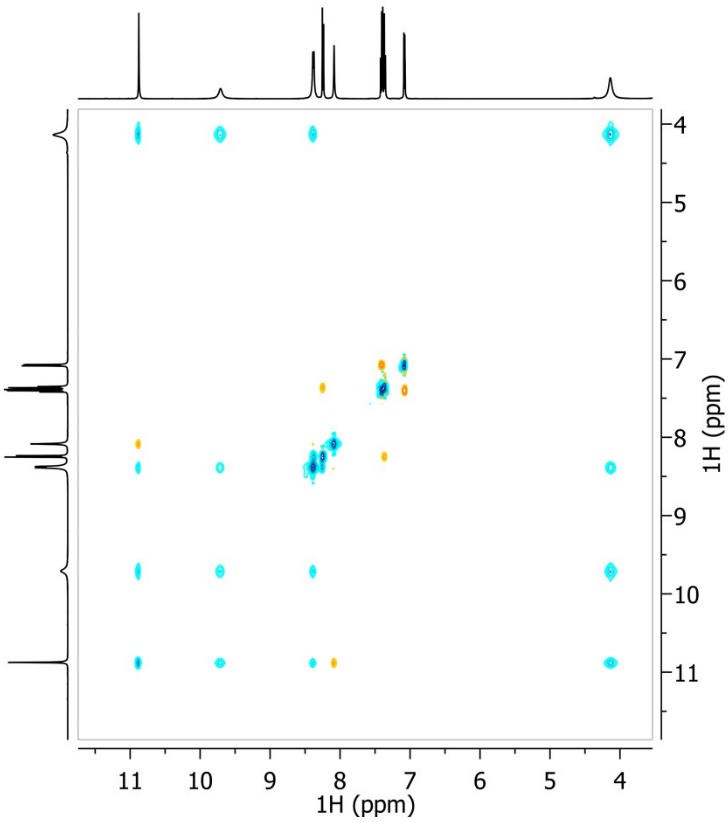


Figure S 35. NOESY spectrum of **O5**.

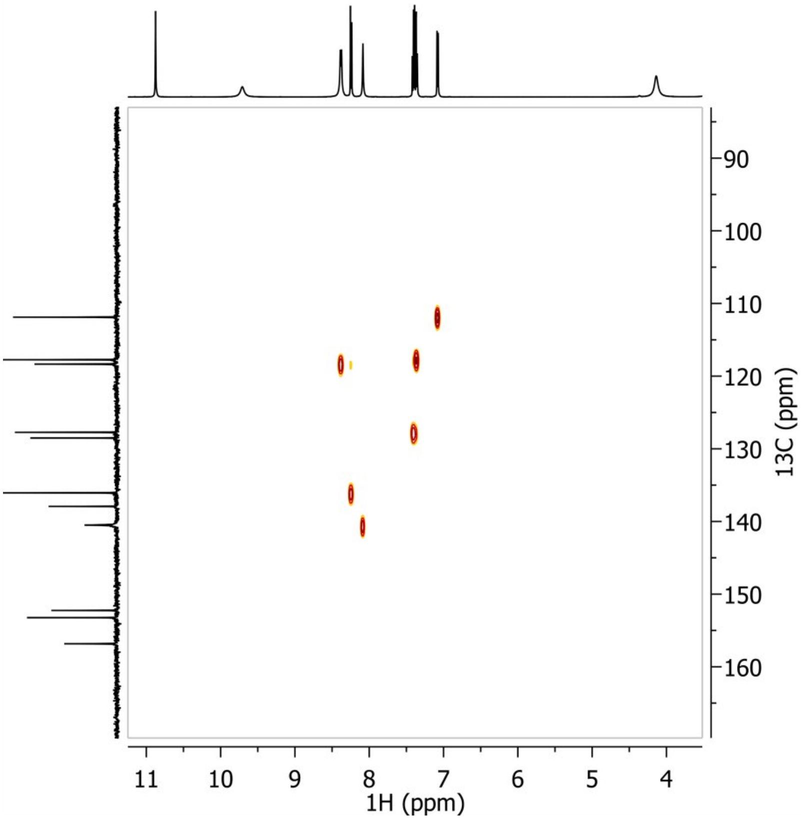


Figure S 36. ^{1}H–^{13}C HSQC spectrum of **O5**.

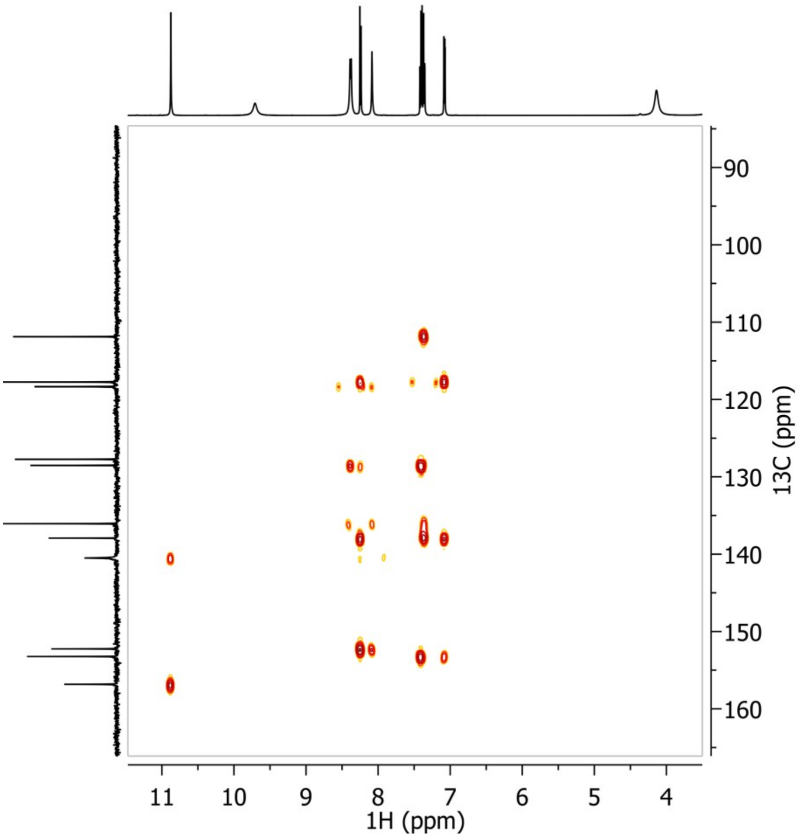


Figure S 37. ^{1}H–^{13}C HMBC spectrum of **O5**.

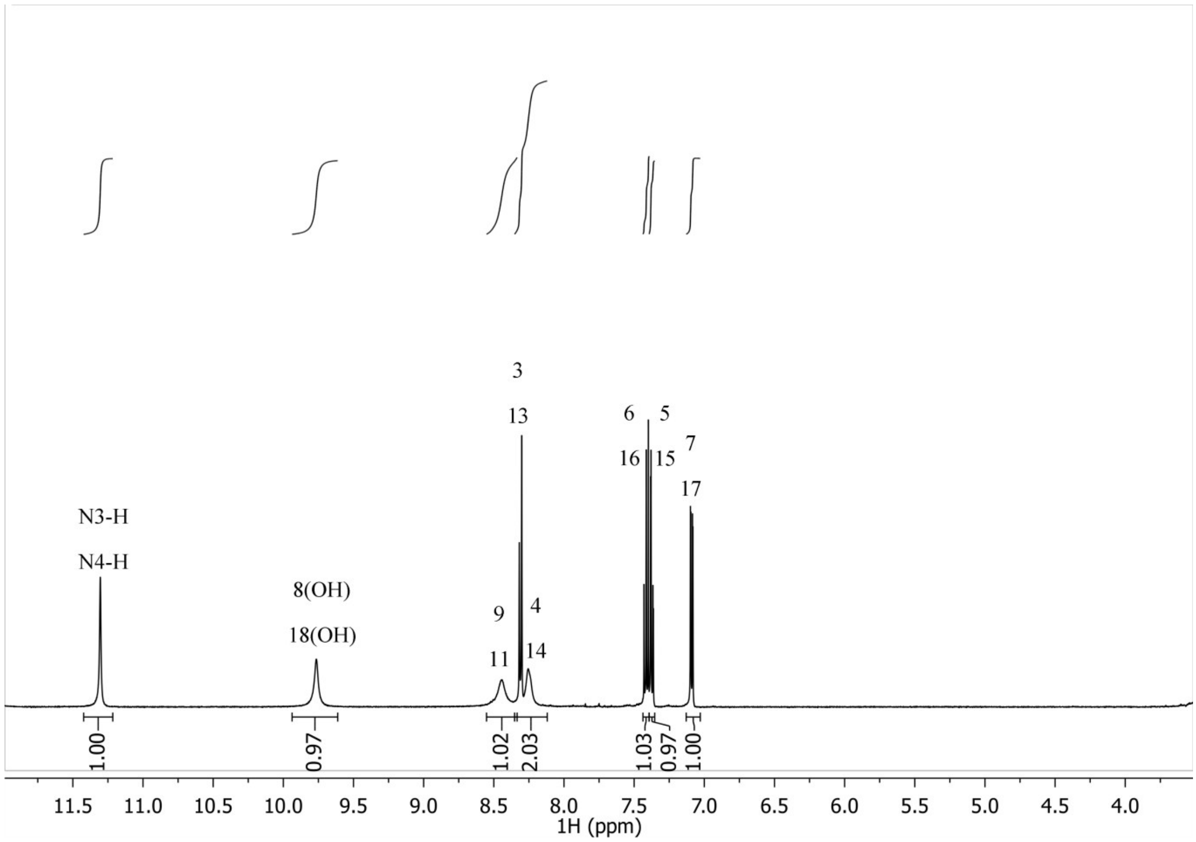


Figure S 38. ^{1}H NMR spectrum of **O6** in DMSO-d_6.

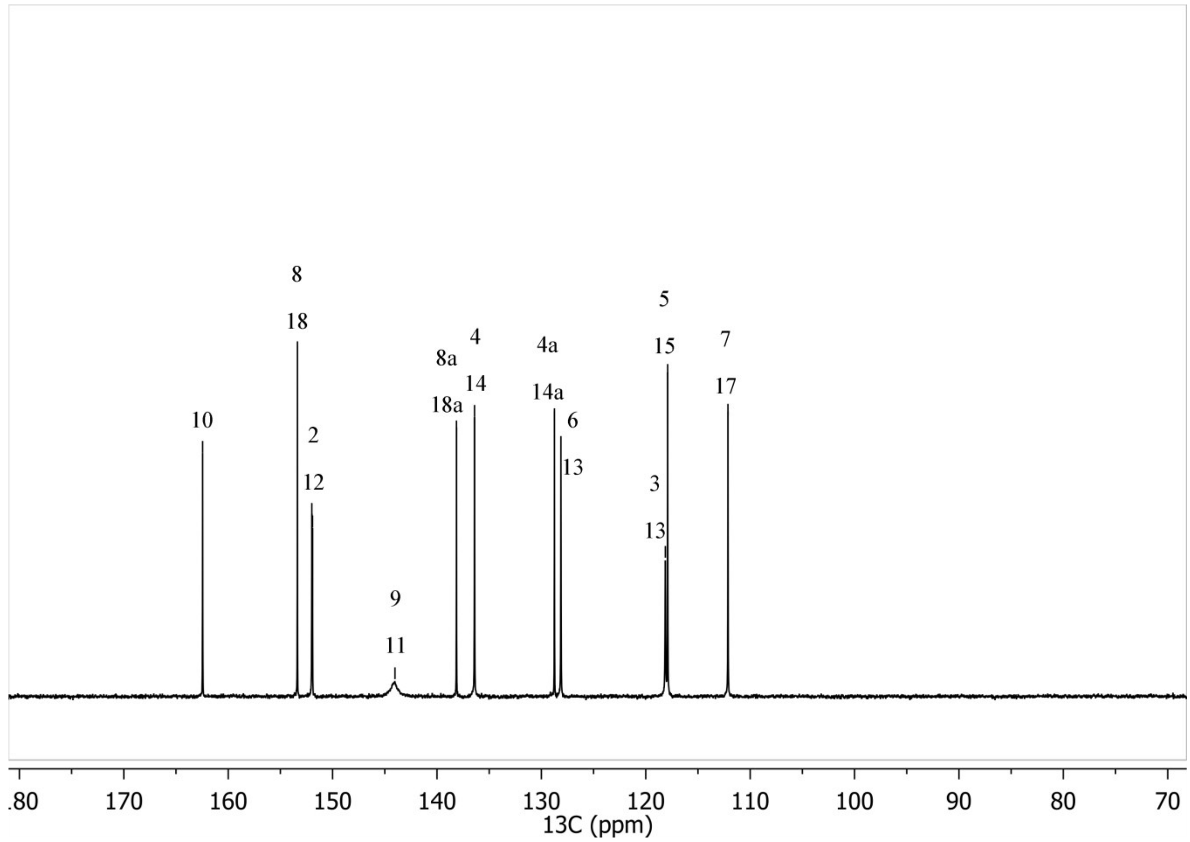


Figure S 39. ^{13}C NMR spectrum of **O6** in DMSO-d_6.

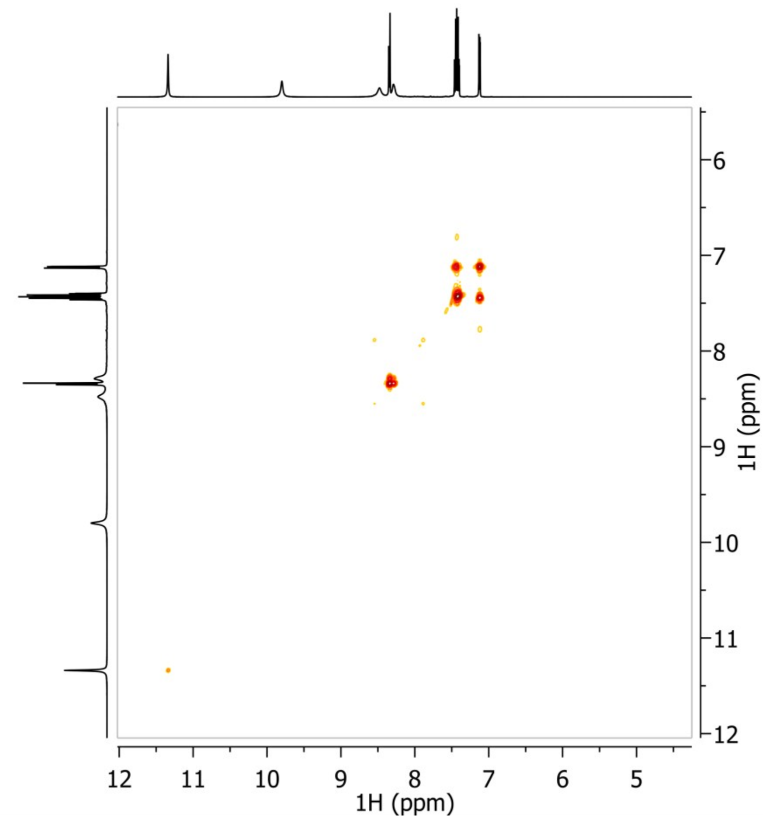


Figure S 40. COSY spectrum of **O6**.

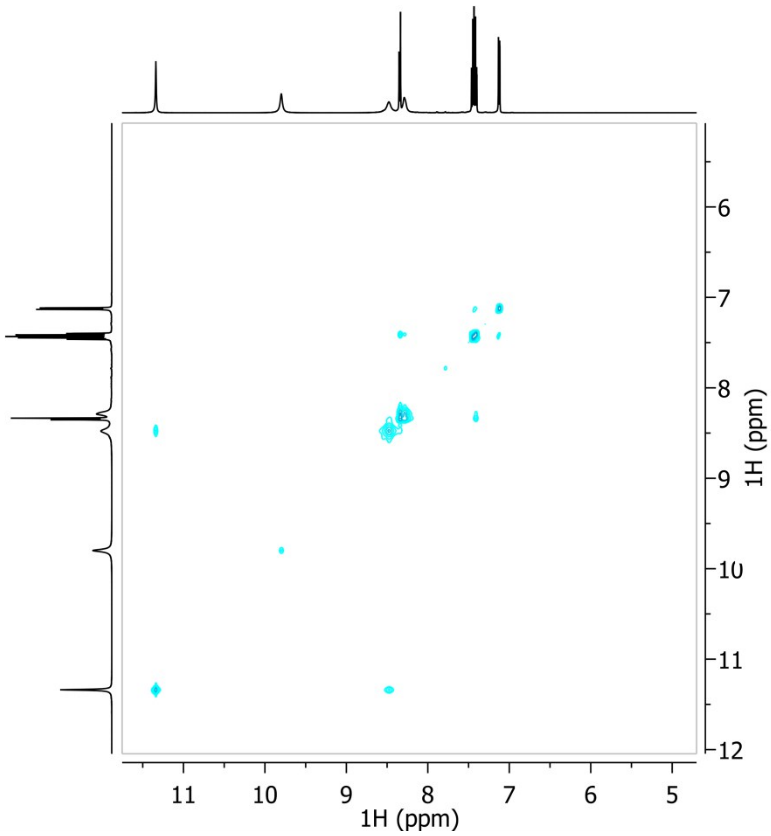


Figure S 41. NOESY spectrum of **O6**.

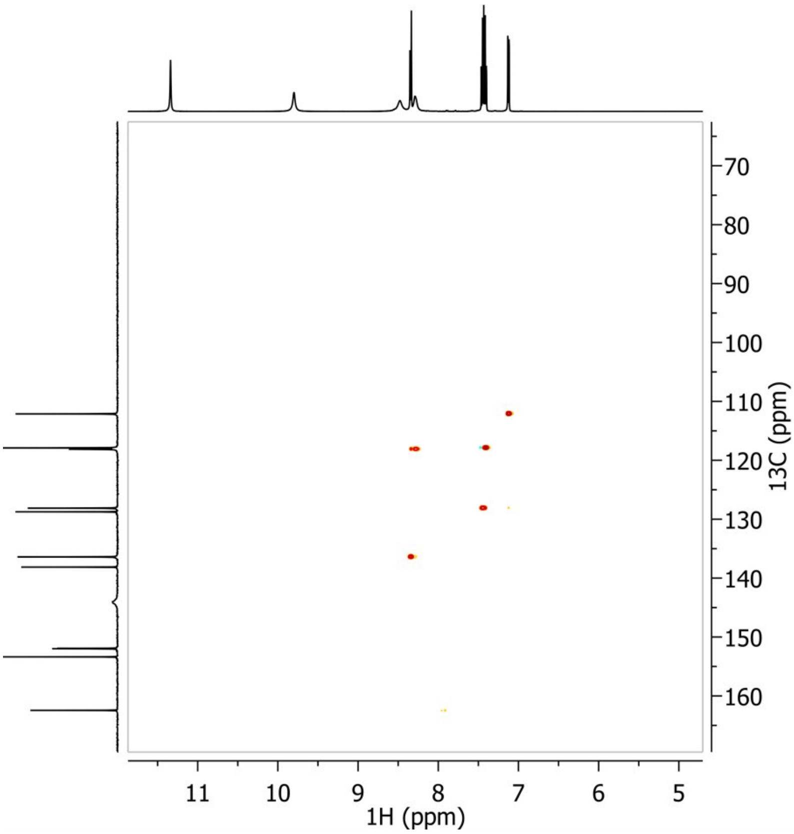


Figure S 42. ^{1}H–^{13}C HSQC spectrum of **O6**.

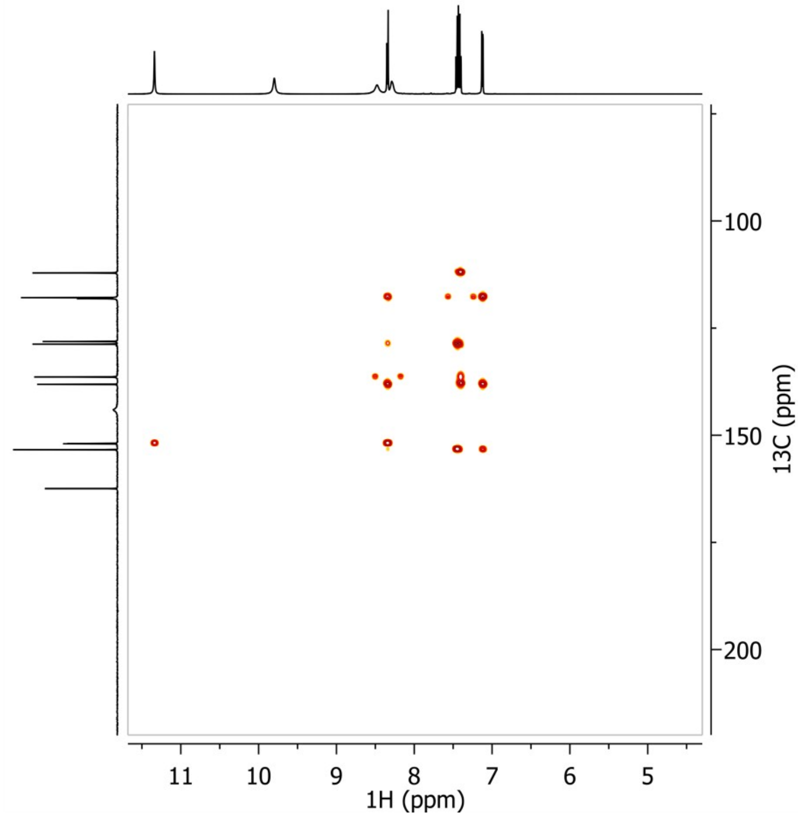


Figure S 43. ^{1}H–^{13}C HMBC spectrum of **O6**.

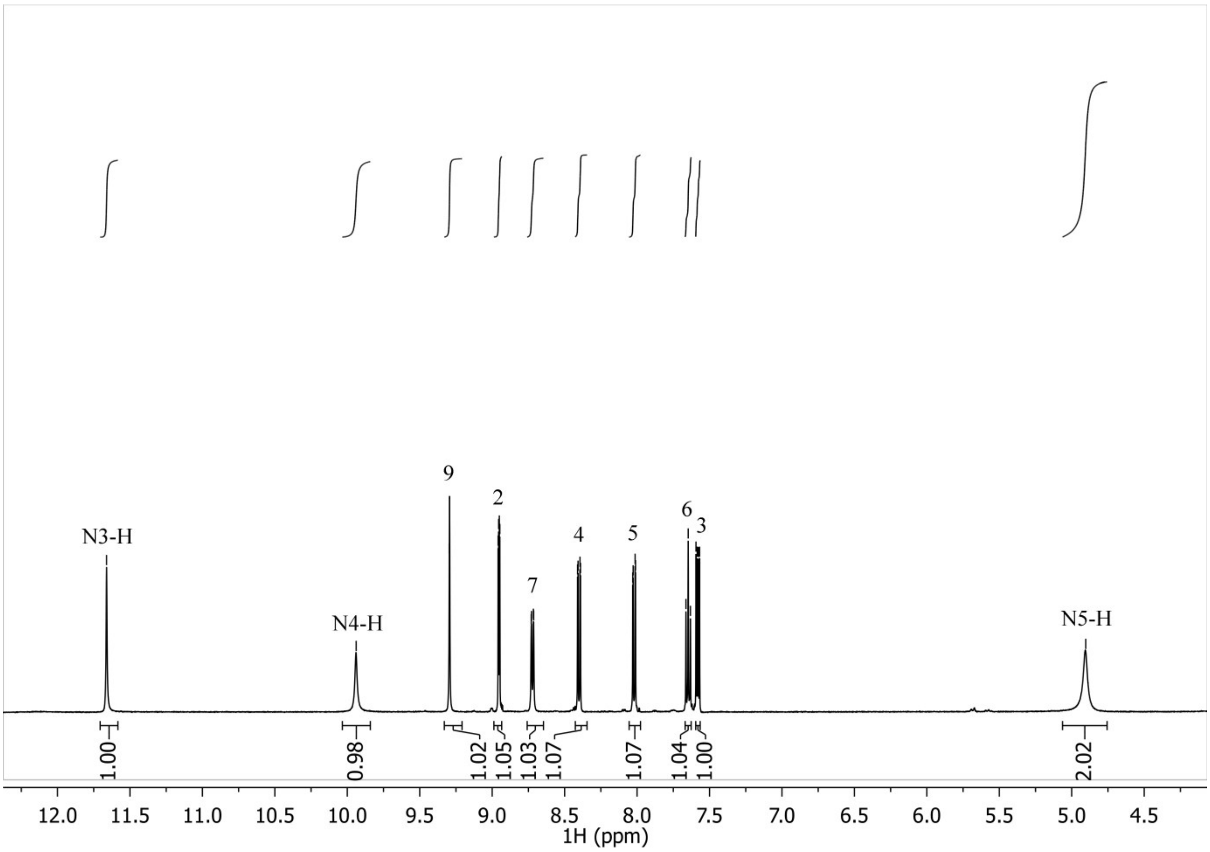


Figure S 44. ^{1}H NMR spectrum of **S1** in DMSO-d_6.

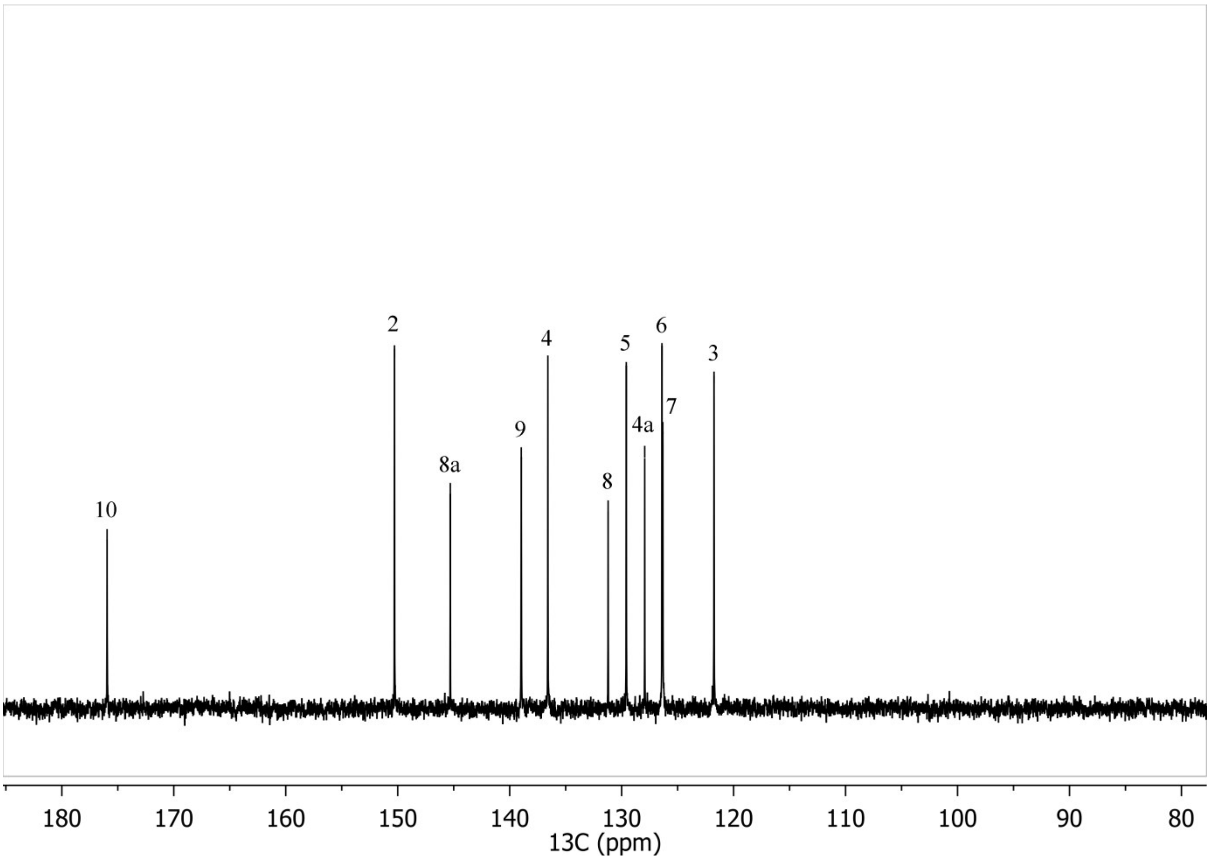


Figure S 45. ^{13}C NMR spectrum of **S1** in DMSO-d_6.

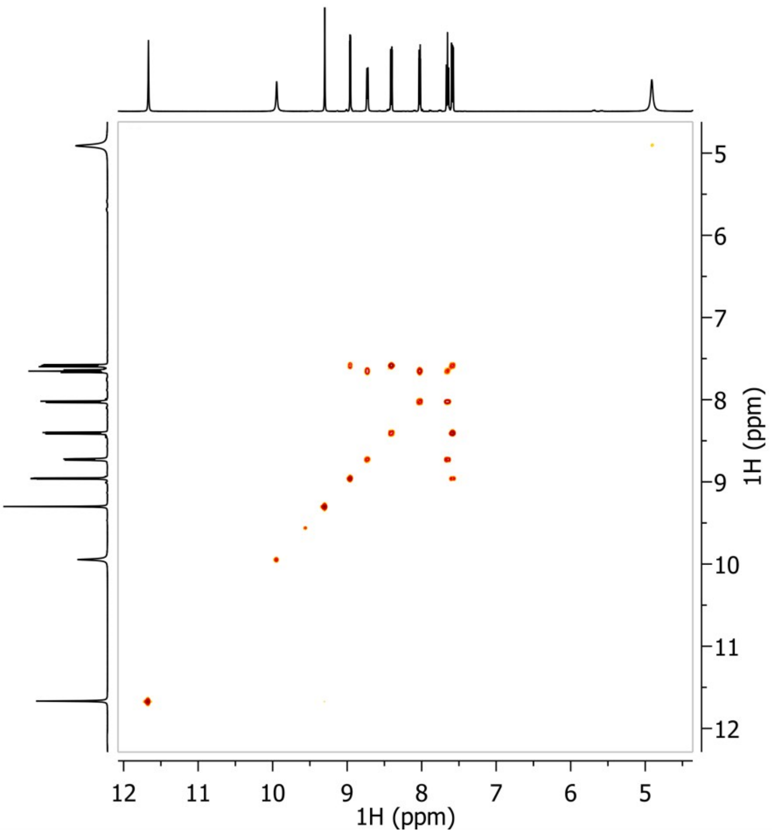


Figure S 46. COSY spectrum of **S1**.

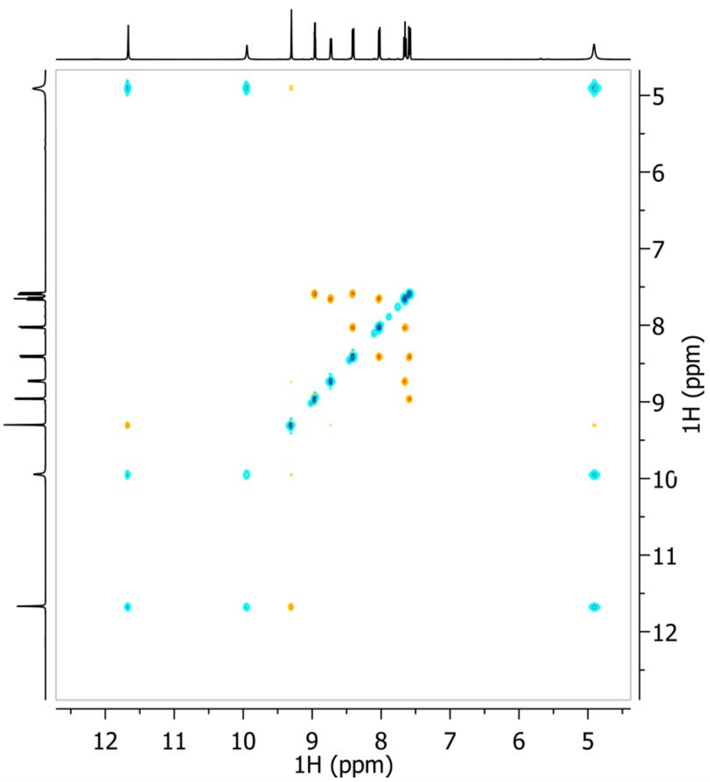


Figure S 47. NOESY spectrum of **S1**.

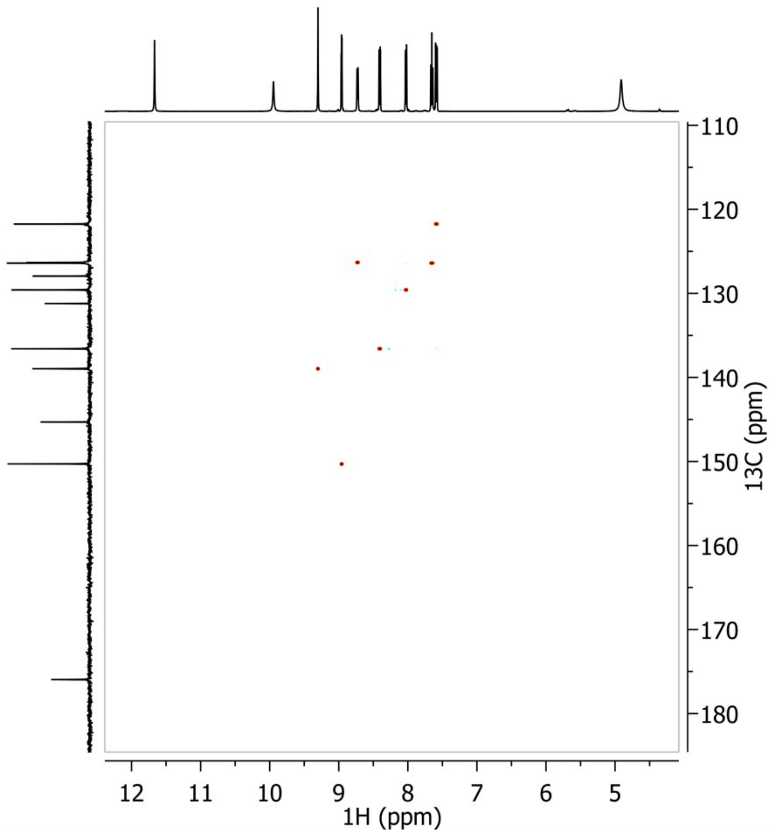


Figure S 48. ^{1}H–^{13}C HSQC spectrum of **S1**.

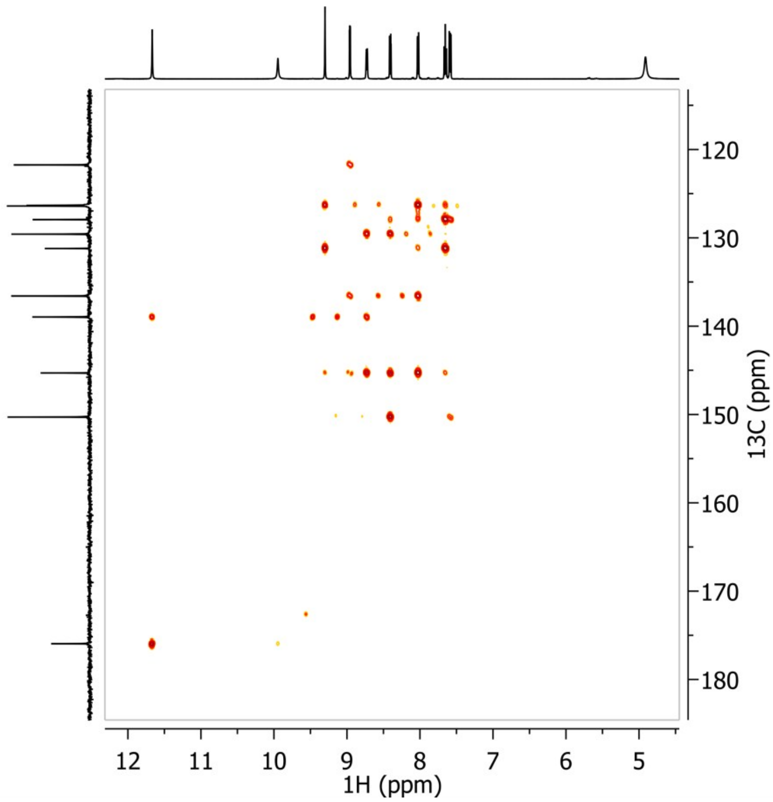


Figure S 49. ^{1}H–^{13}C HMBC spectrum of **S1**.

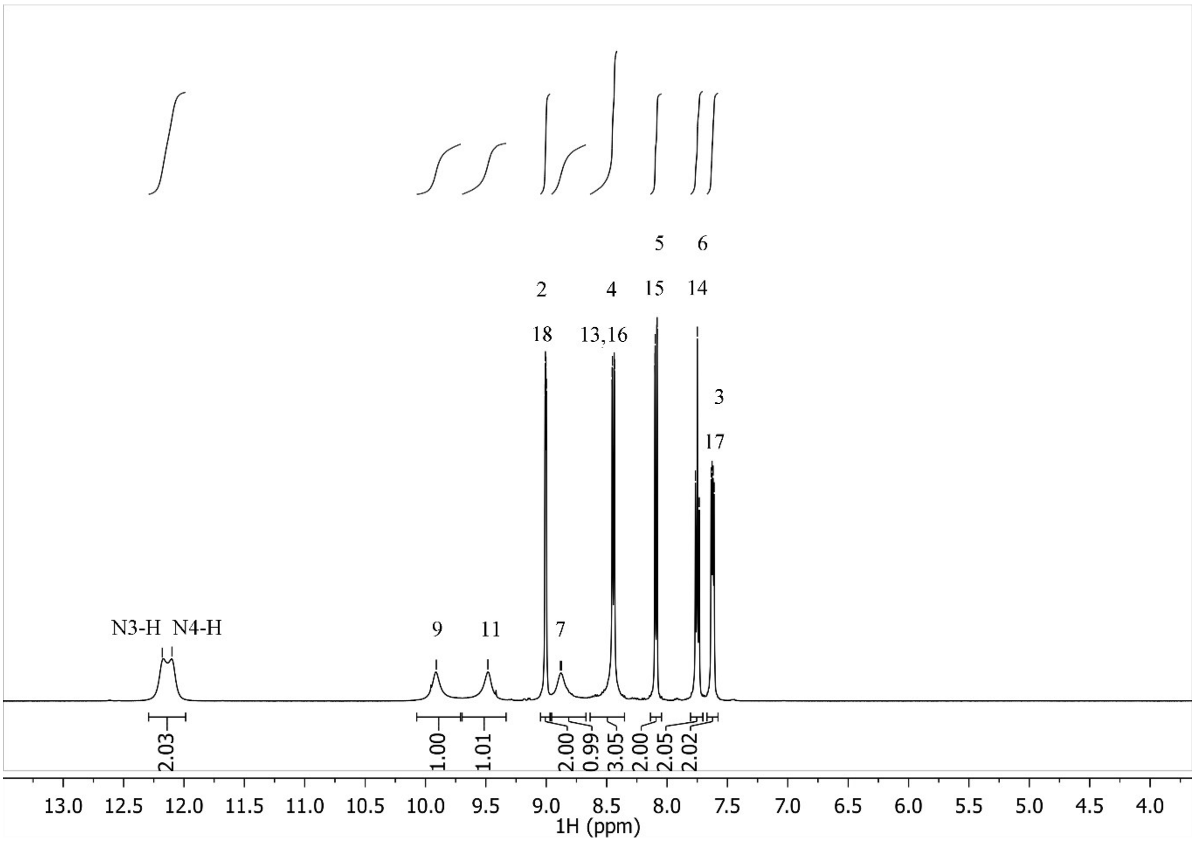


Figure S 50. ^{1}H NMR spectrum of **S2** in DMSO-d_6.

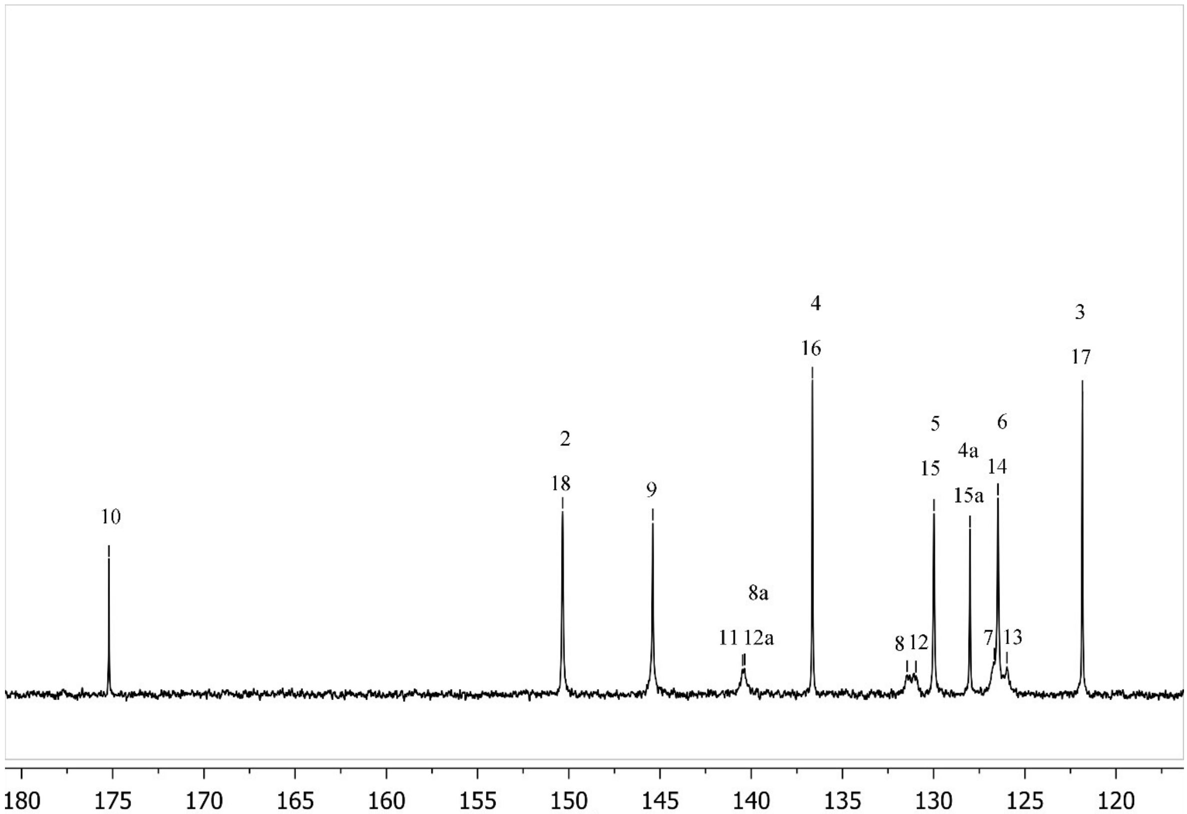


Figure S 51. ^{13}C NMR spectrum of **S2** in DMSO-d_6.

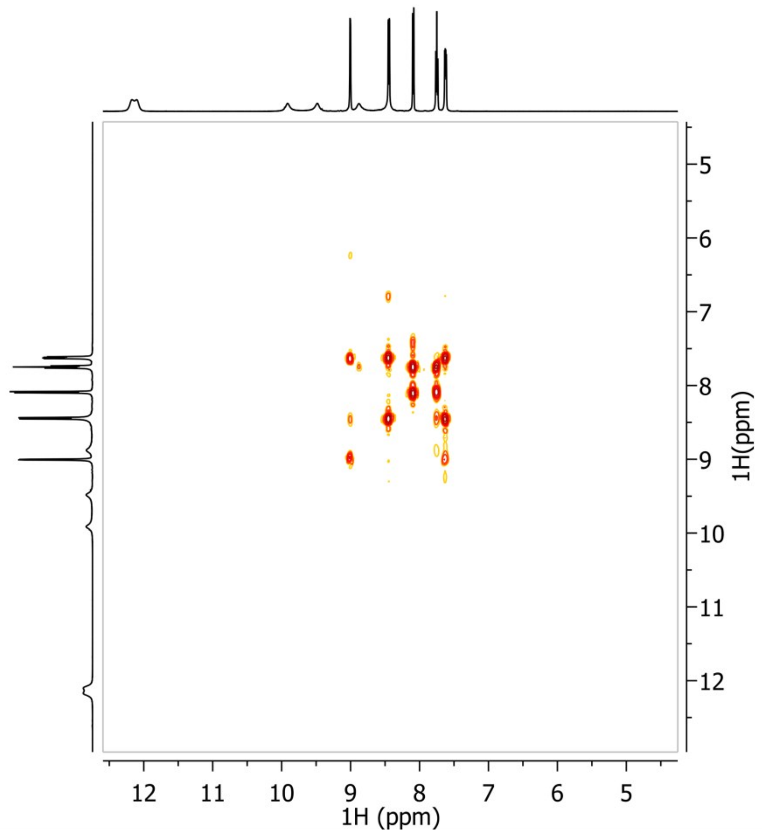


Figure S 52. COSY spectrum of **S2**.

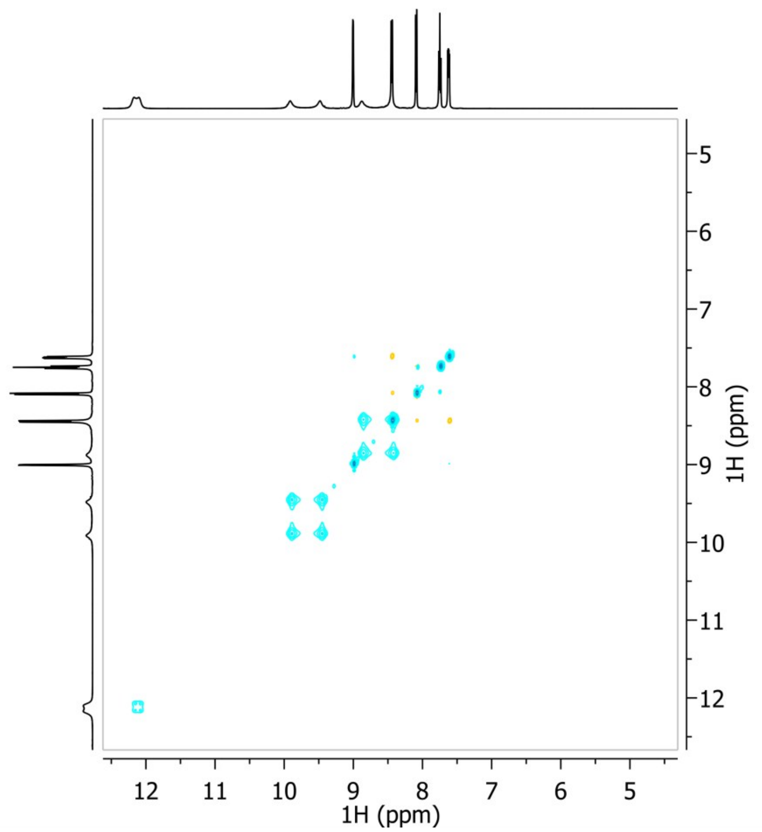


Figure S 53. NOESY spectrum of **S2**.

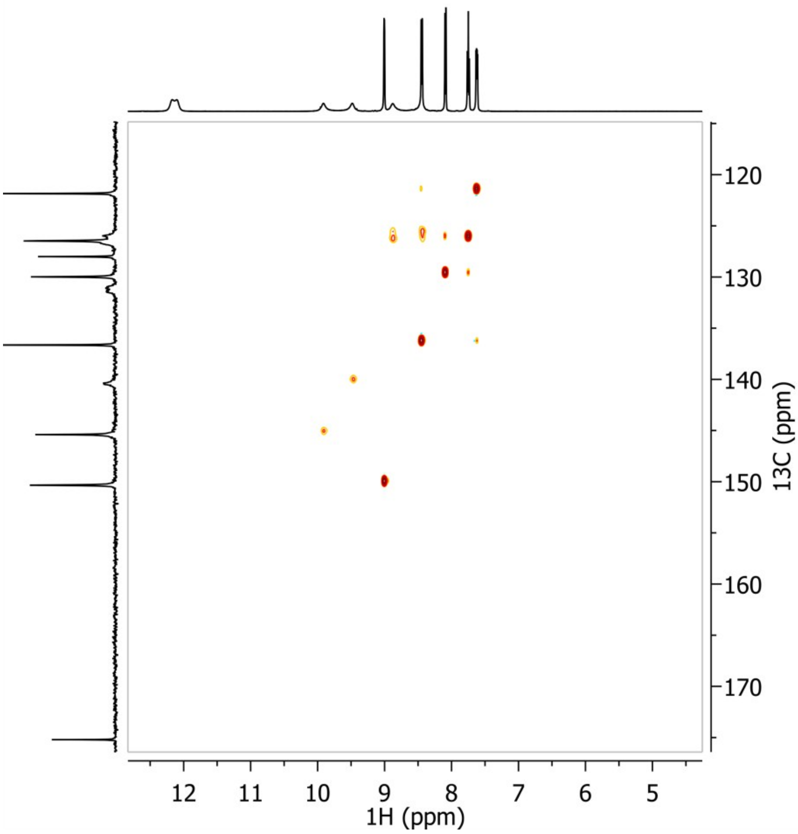


Figure S 54. ^{1}H–^{13}C HSQC spectrum of **S2**.

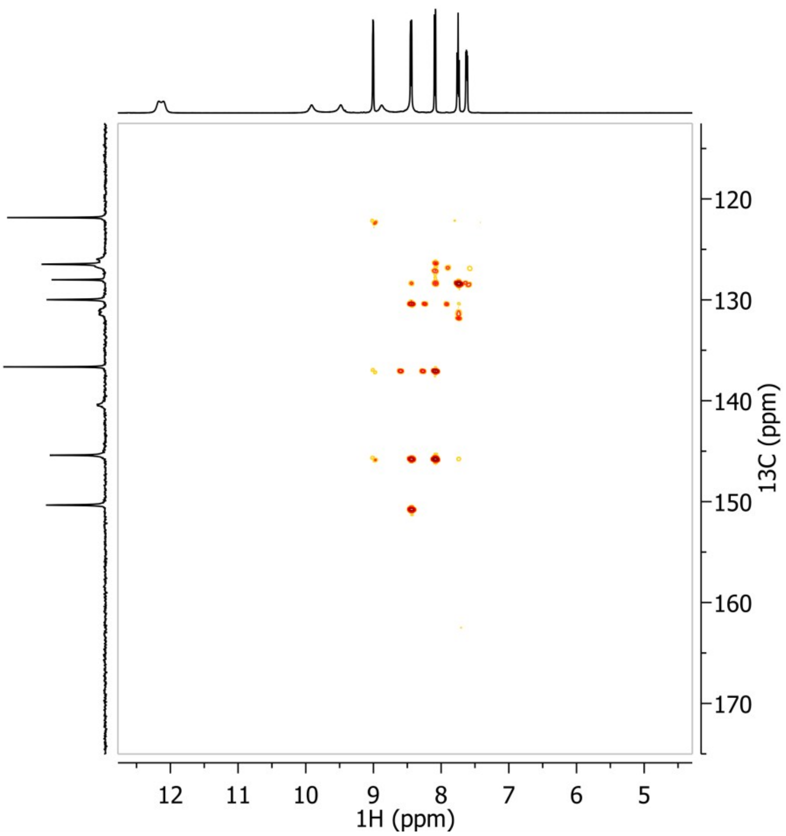


Figure S 55. ^{1}H–^{13}C HMBC spectrum of **S2**.

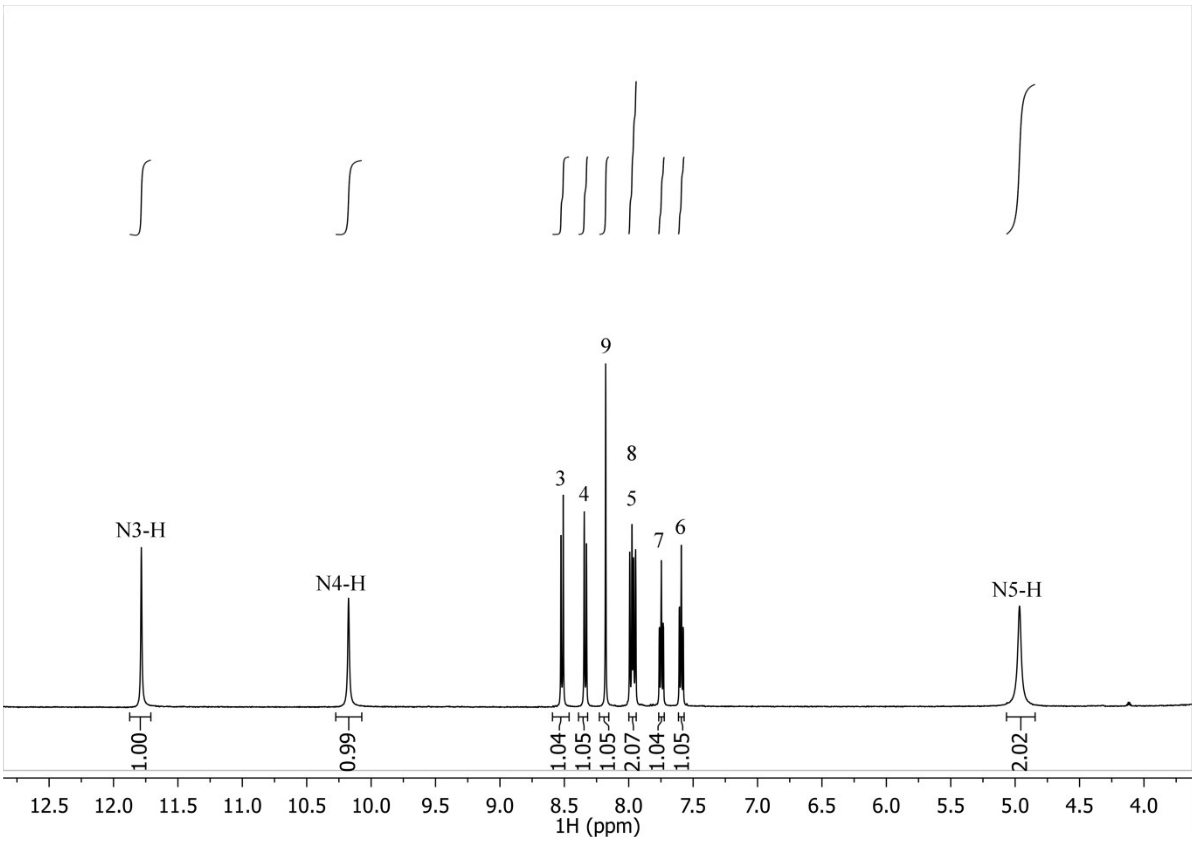


Figure S 56. ^{1}H NMR spectrum of **S3** in DMSO-d_6.

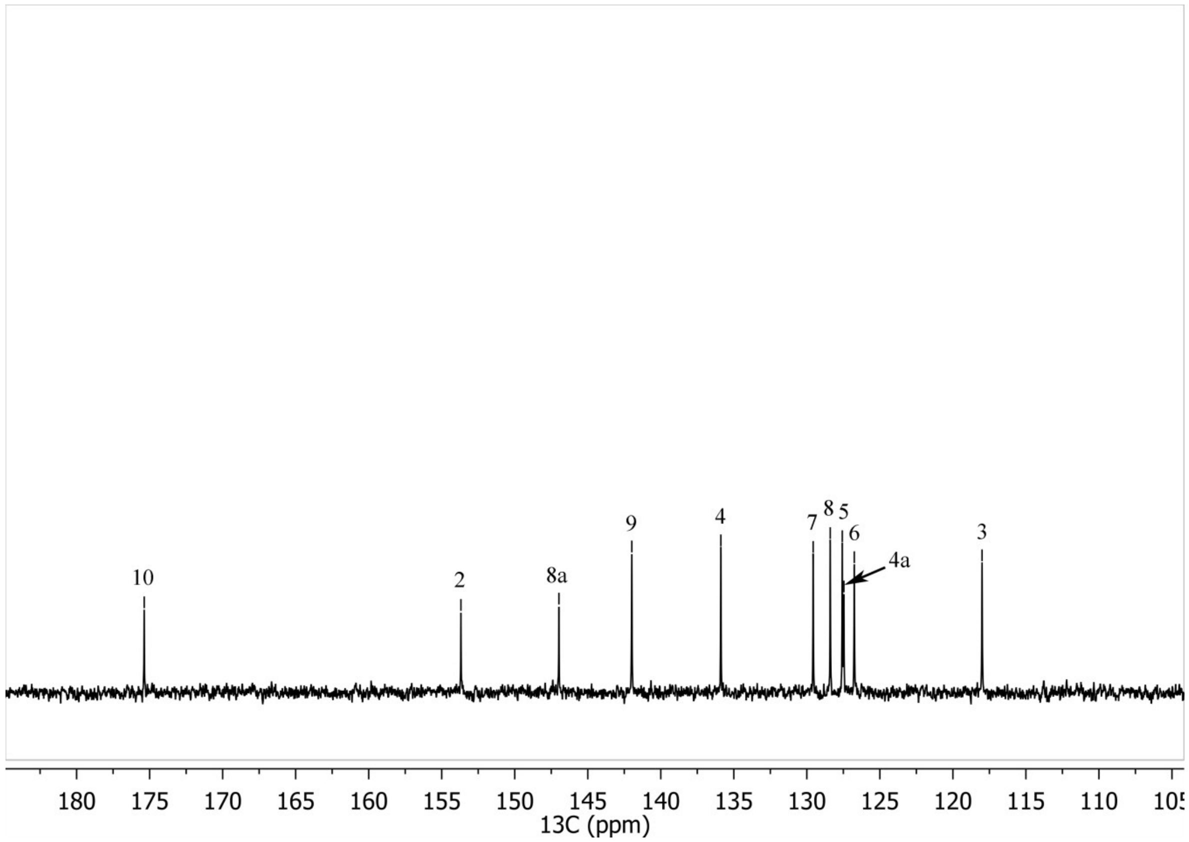


Figure S 57. ^{13}C NMR spectrum of **S3** in DMSO-d_6.

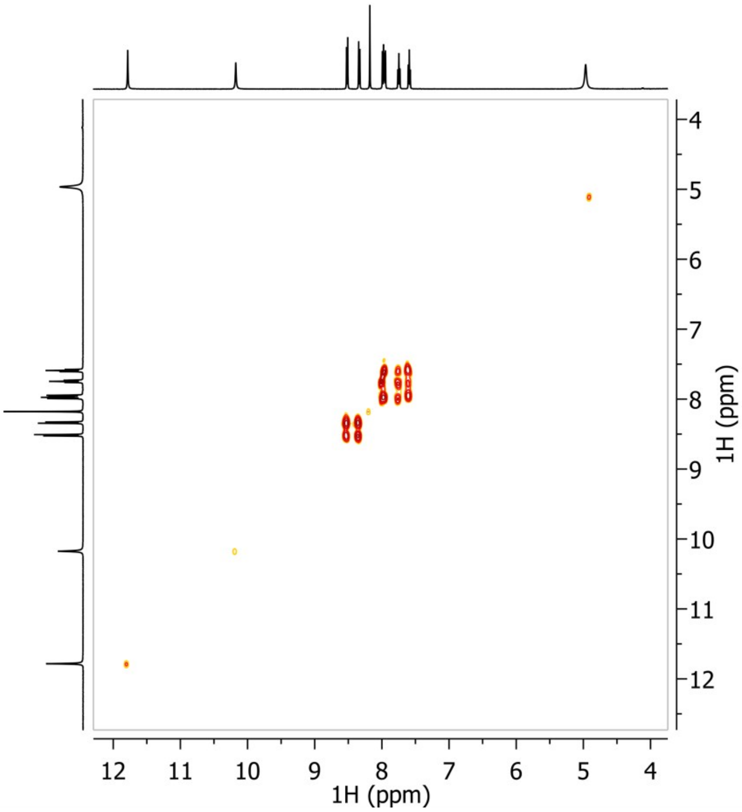


Figure S 58. COSY spectrum of **S3**.

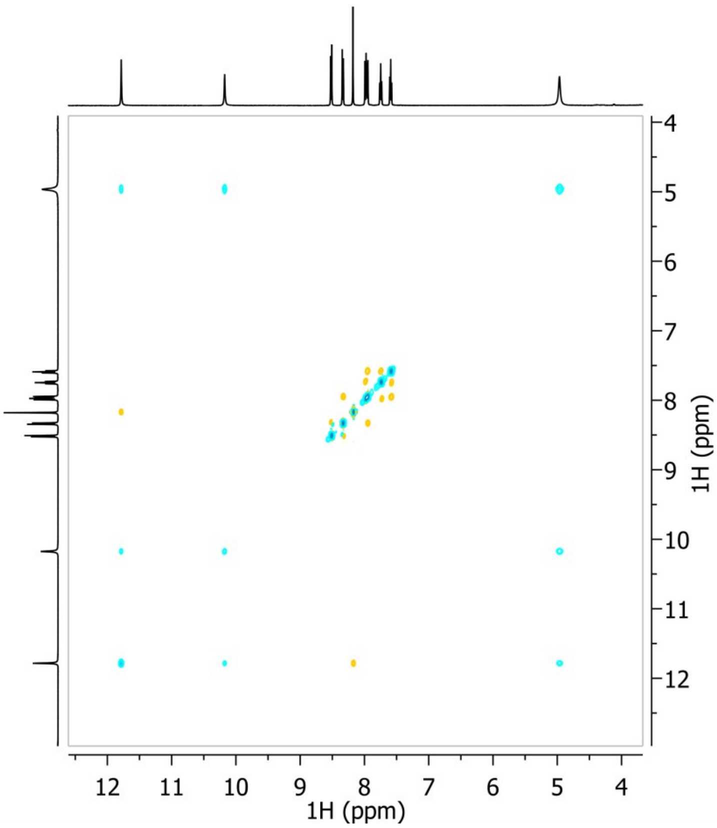


Figure S 59. NOESY spectrum of **S3**.

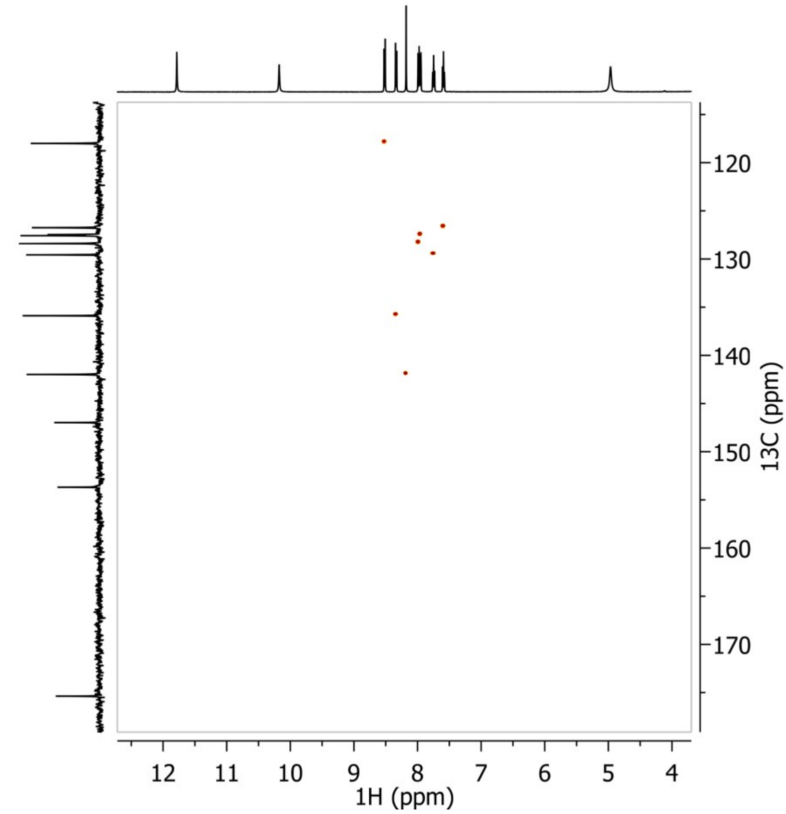


Figure S 60. ^{1}H–^{13}C HSQC spectrum of **S3**.

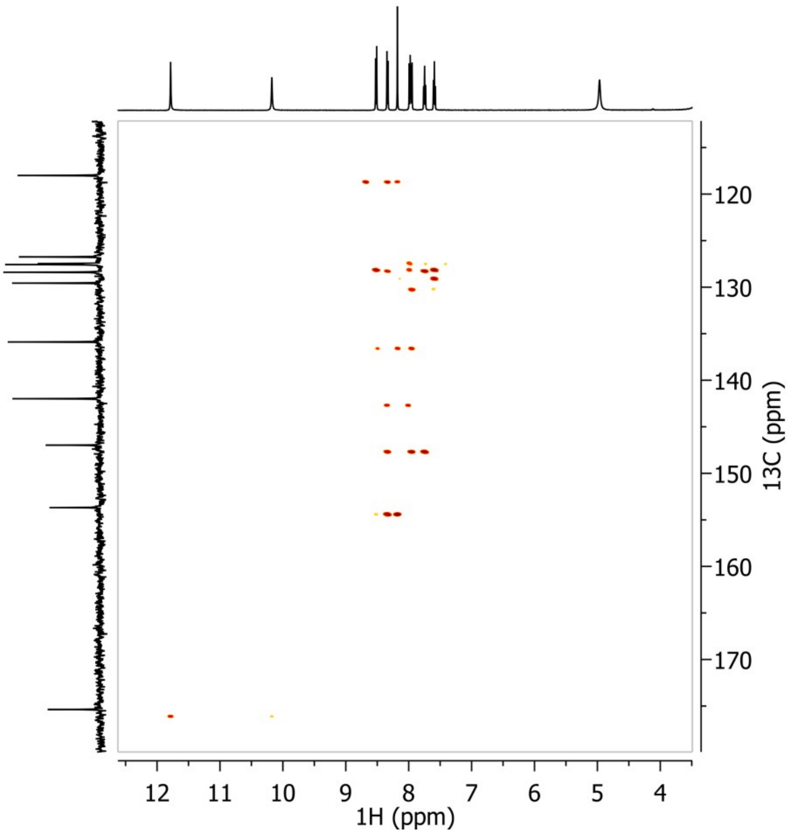


Figure S 61. ^{1}H–^{13}C HMBC spectrum of **S3**.

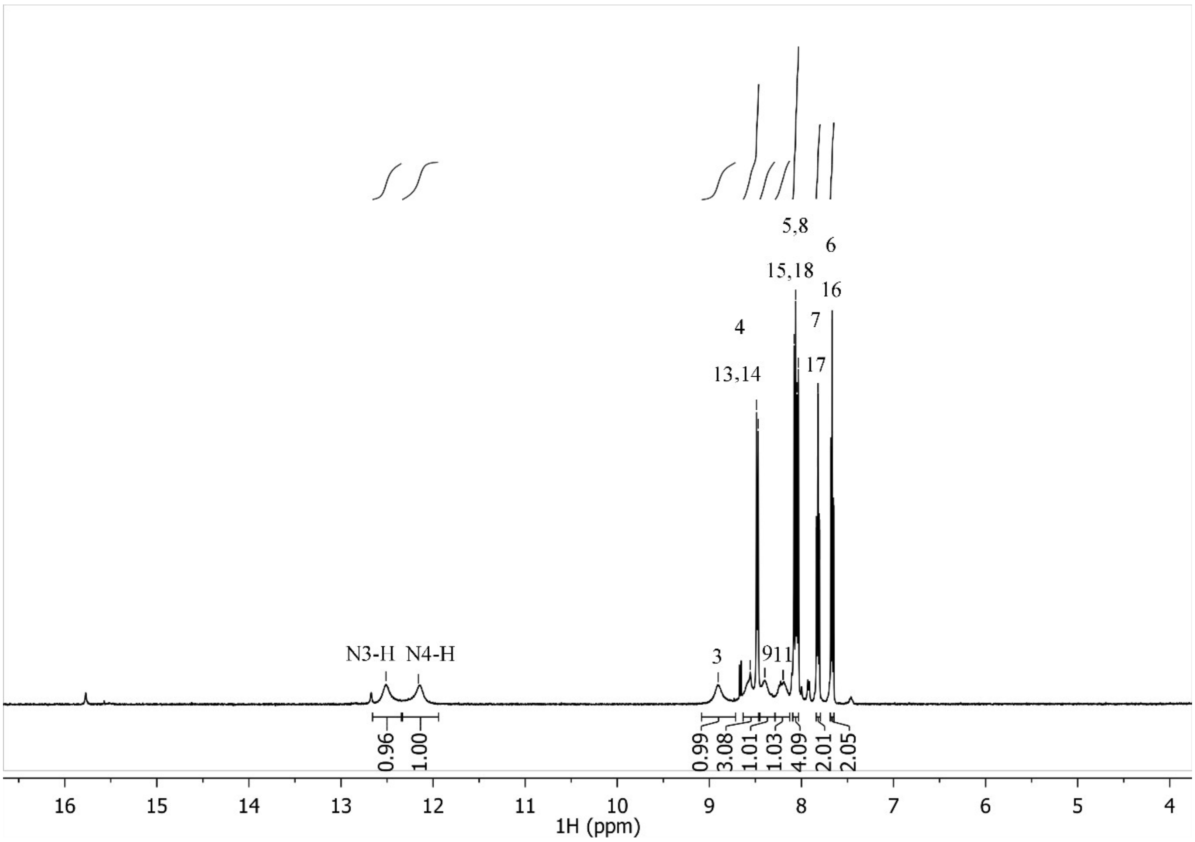


Figure S 62. ^{1}H NMR spectrum of **S4** in DMSO-d_6.

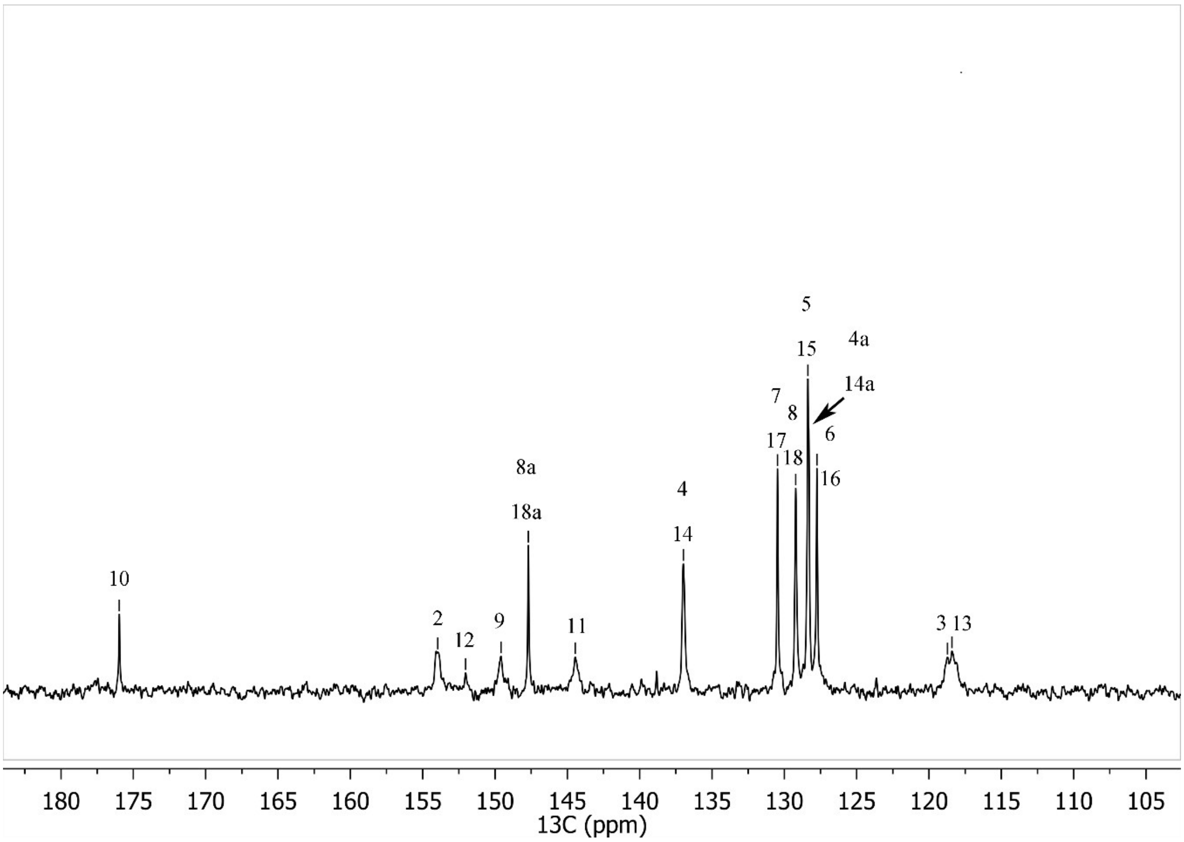


Figure S 63. ^{13}C NMR spectrum of **S4** in DMSO-d_6.

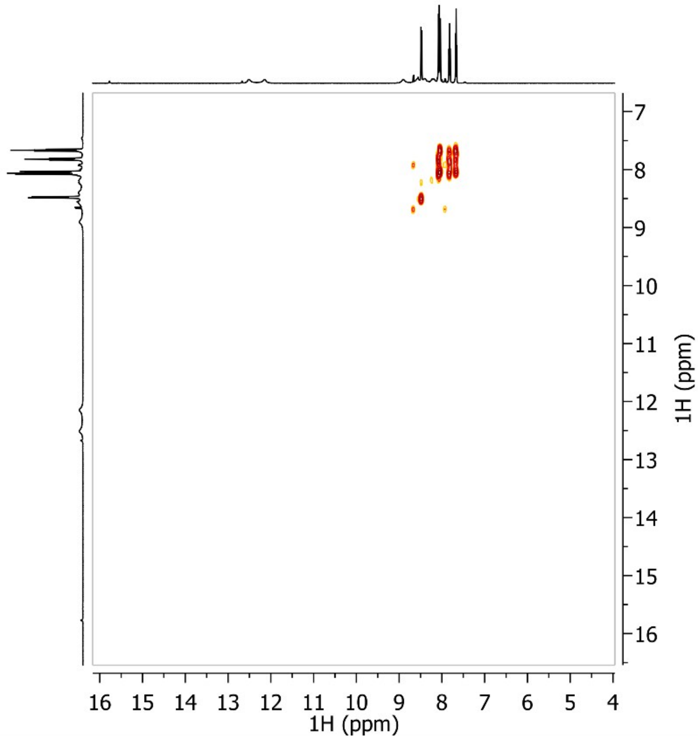


Figure S 64. COSY spectrum of **S4**.

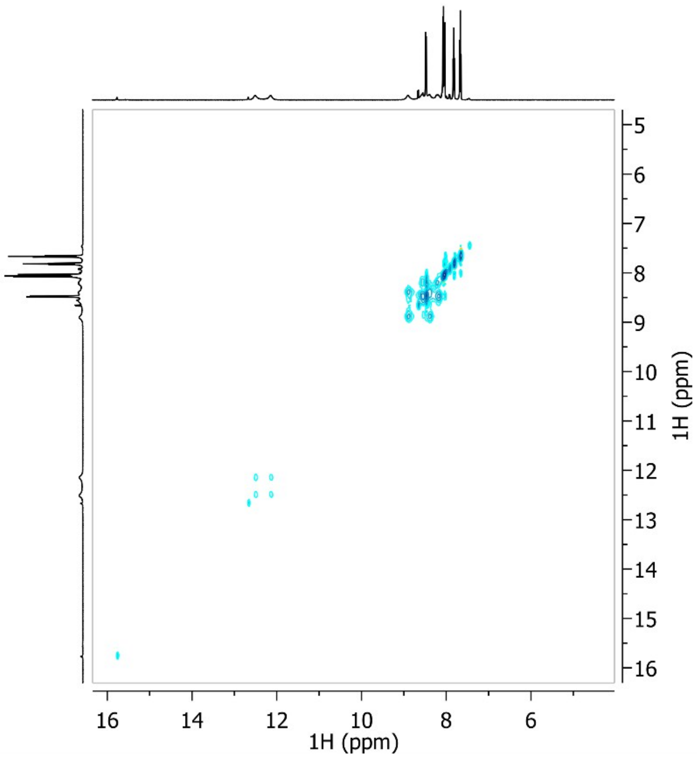


Figure S 65. NOESY spectrum of **S4**.

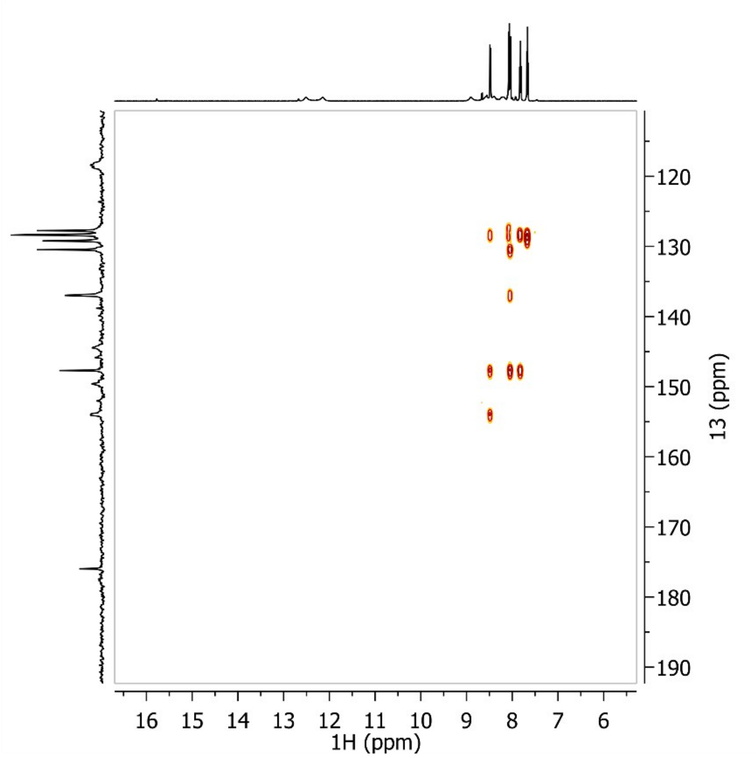


Figure S 66. ^{1}H–^{13}C HSQC spectrum of **S4**.

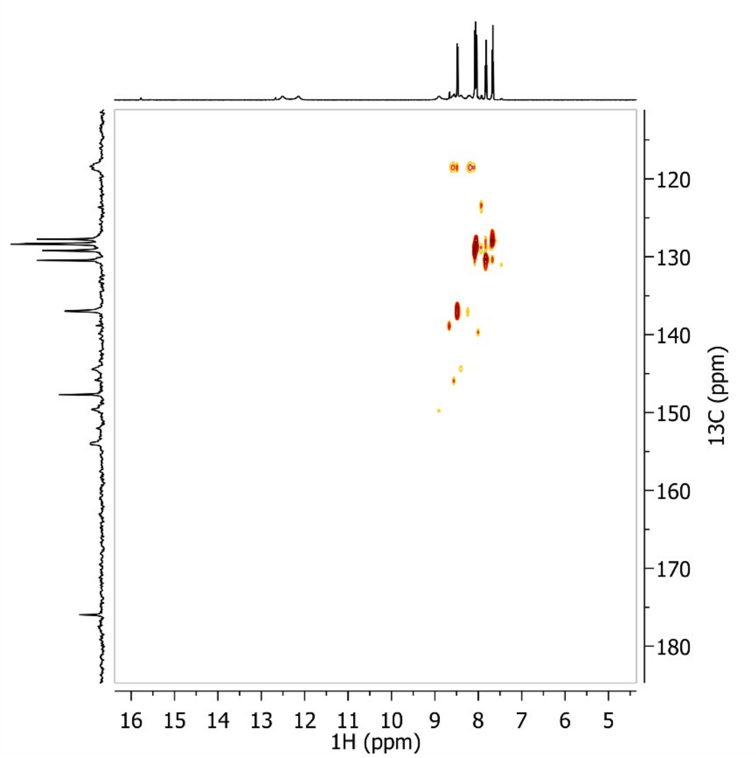


Figure S 67. ^{1}H–^{13}C HMBC spectrum of **S4**.

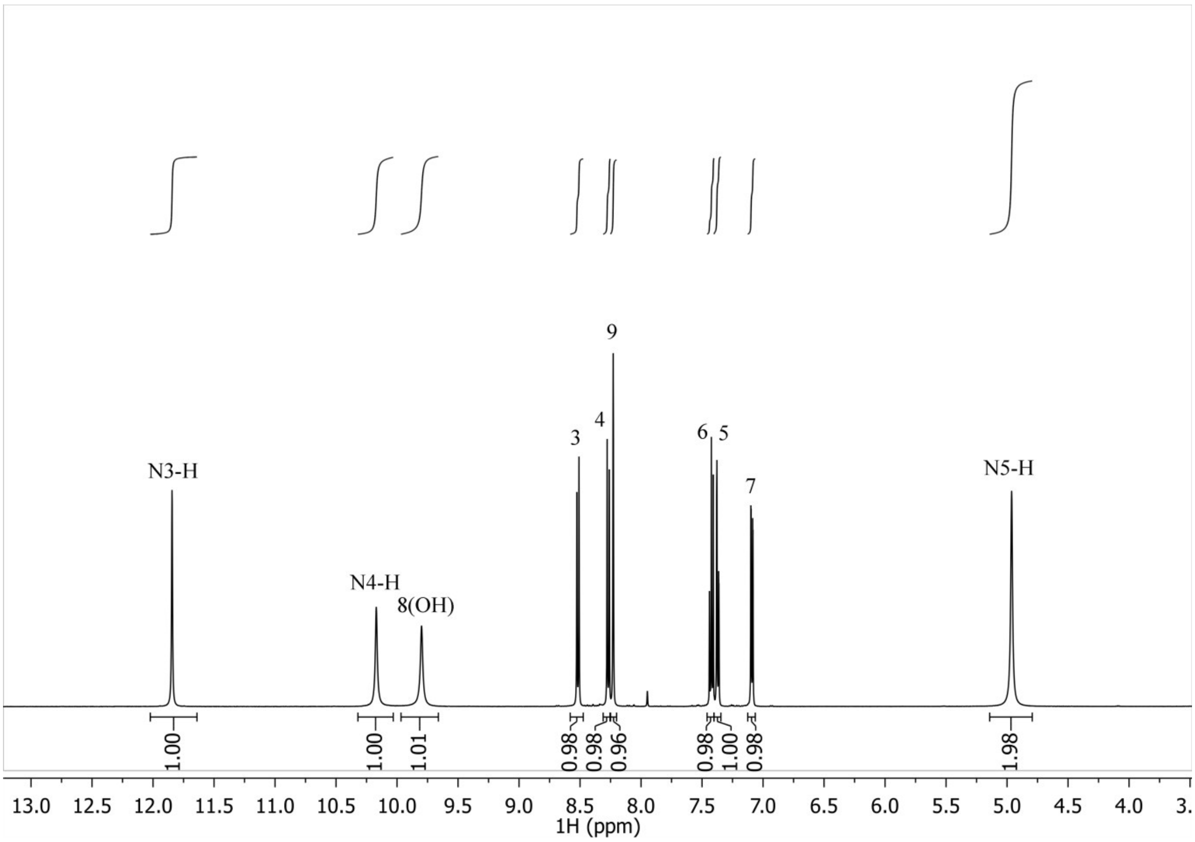


Figure S 68. ^{1}H NMR spectrum of **S5** in DMSO-d_6.

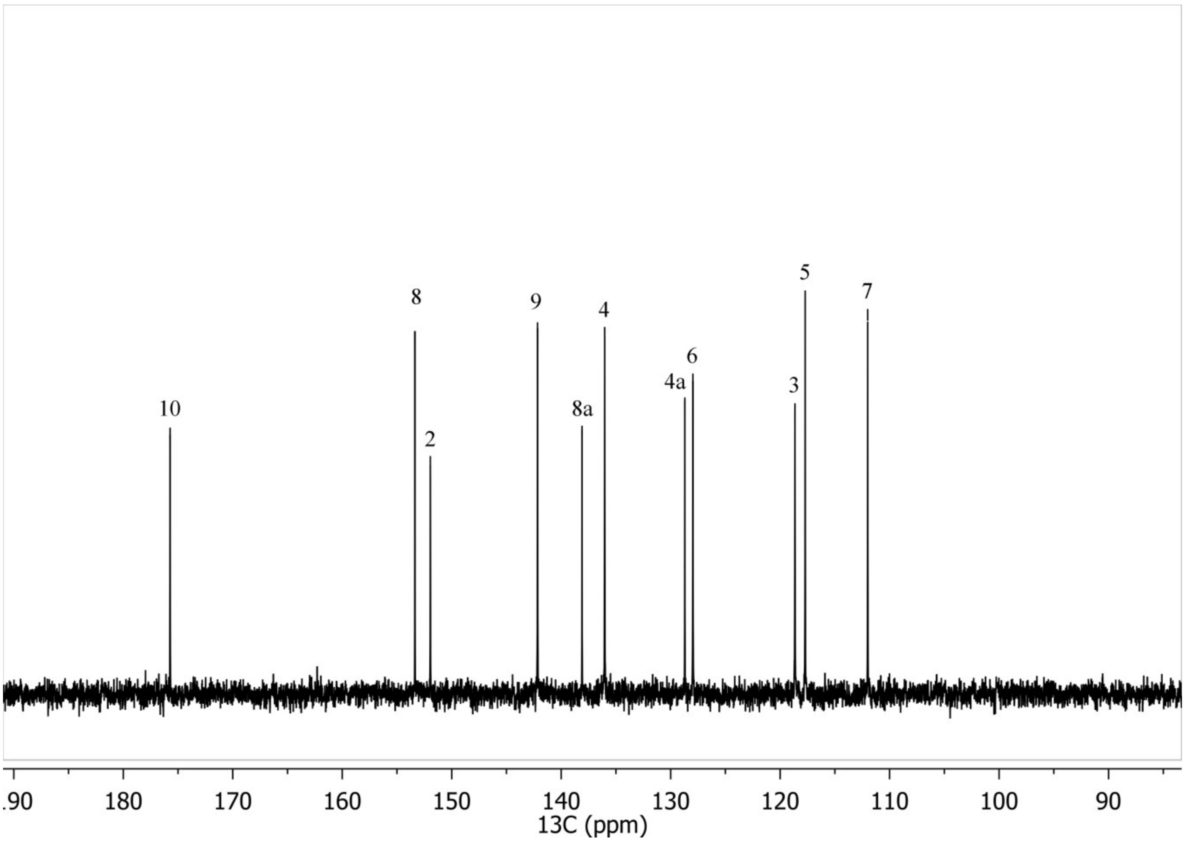


Figure S 69. ^{13}C NMR spectrum of **S5** in DMSO-d_6.

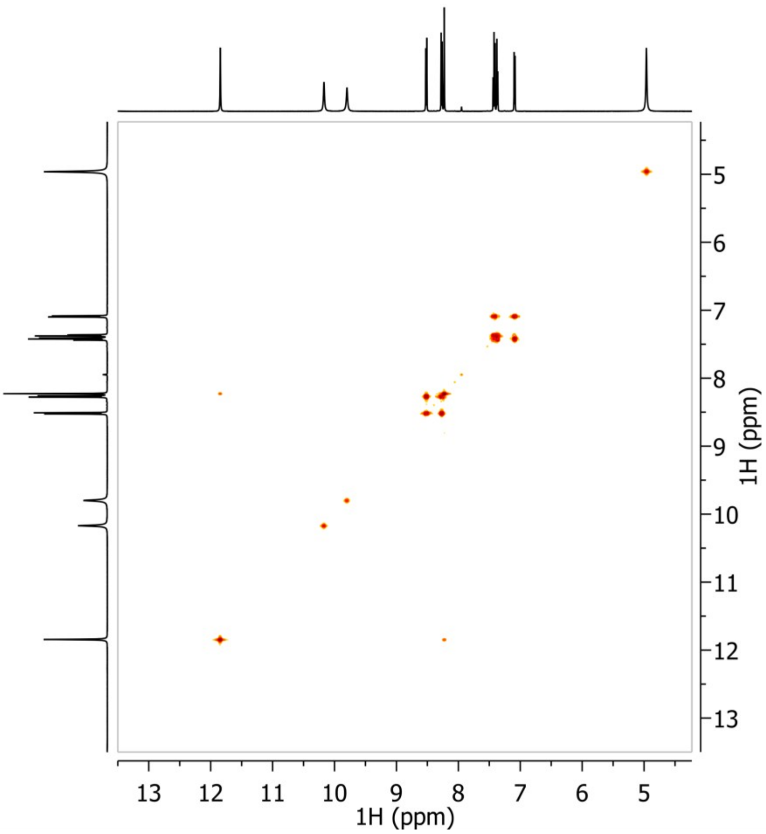


Figure S 70. COSY spectrum of **S5**.

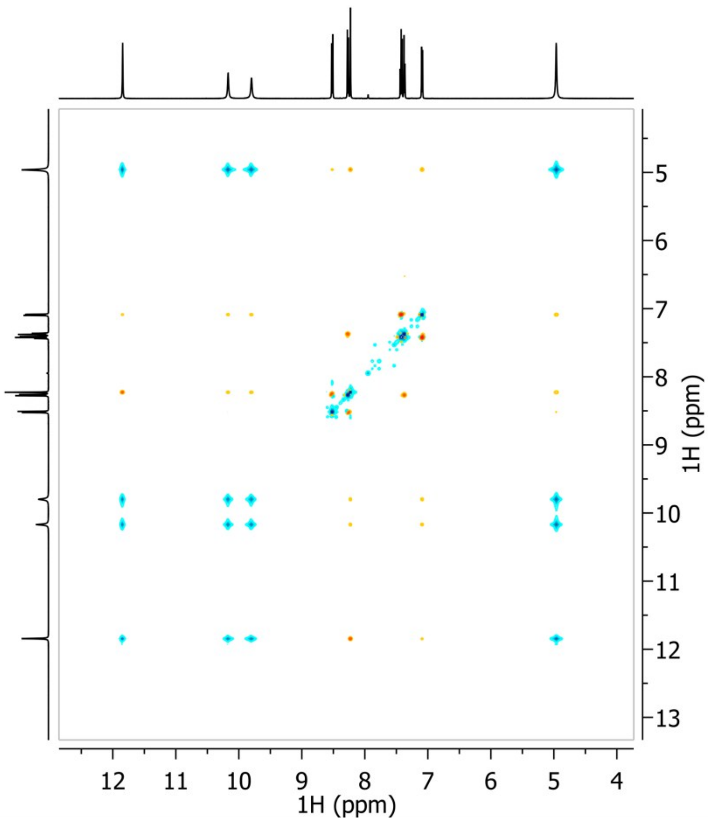


Figure S 71. NOESY spectrum of **S5**.

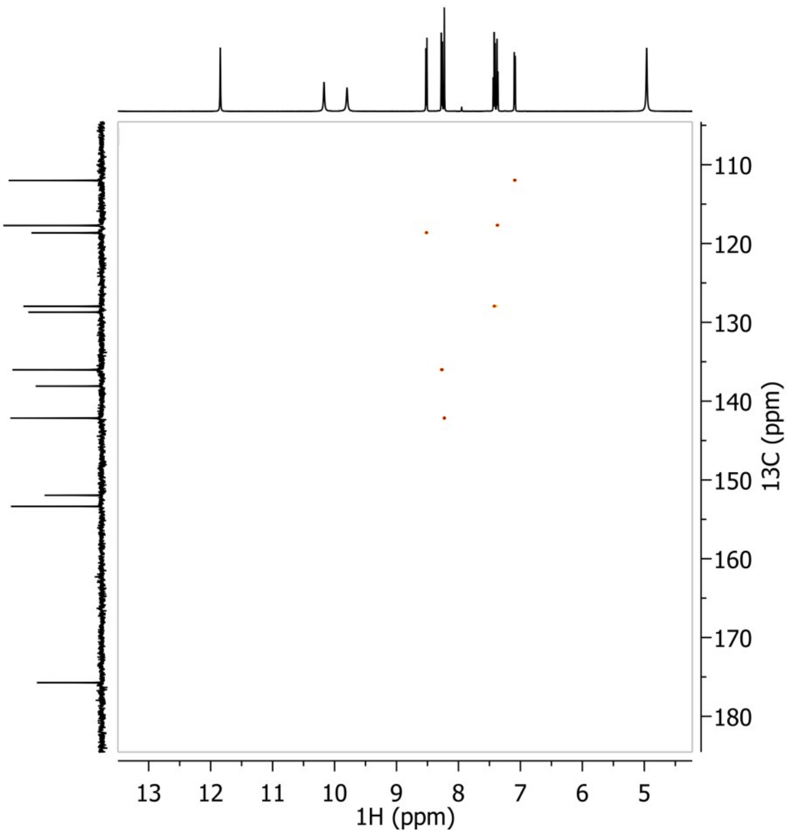


Figure S 72. ^{1}H–^{13}C HSQC spectrum of **S5**.

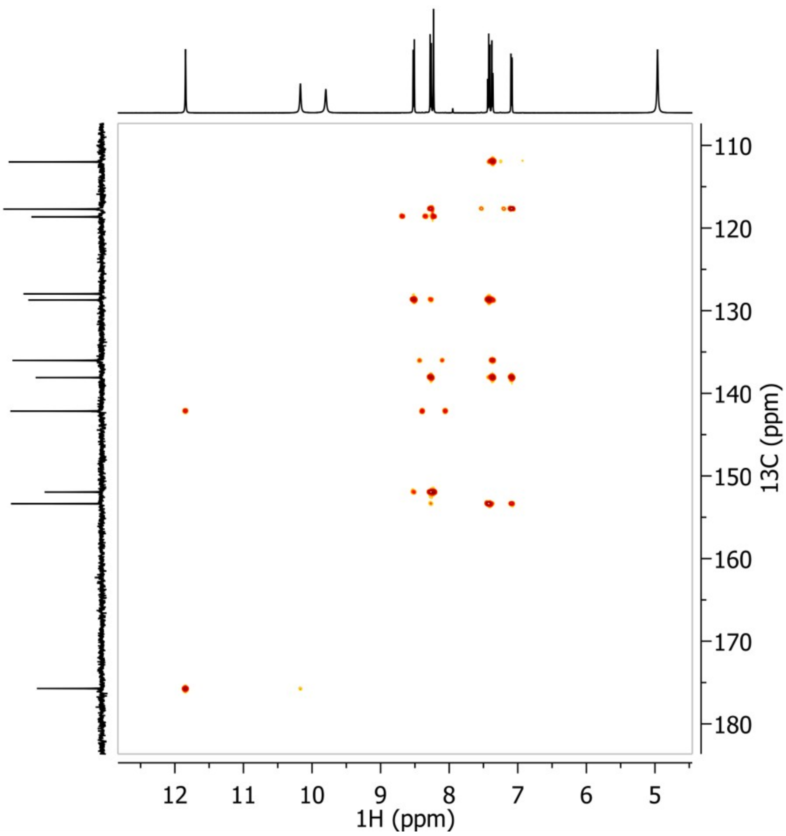


Figure S 73. ^{1}H–^{13}C HMBC spectrum of **S5**.

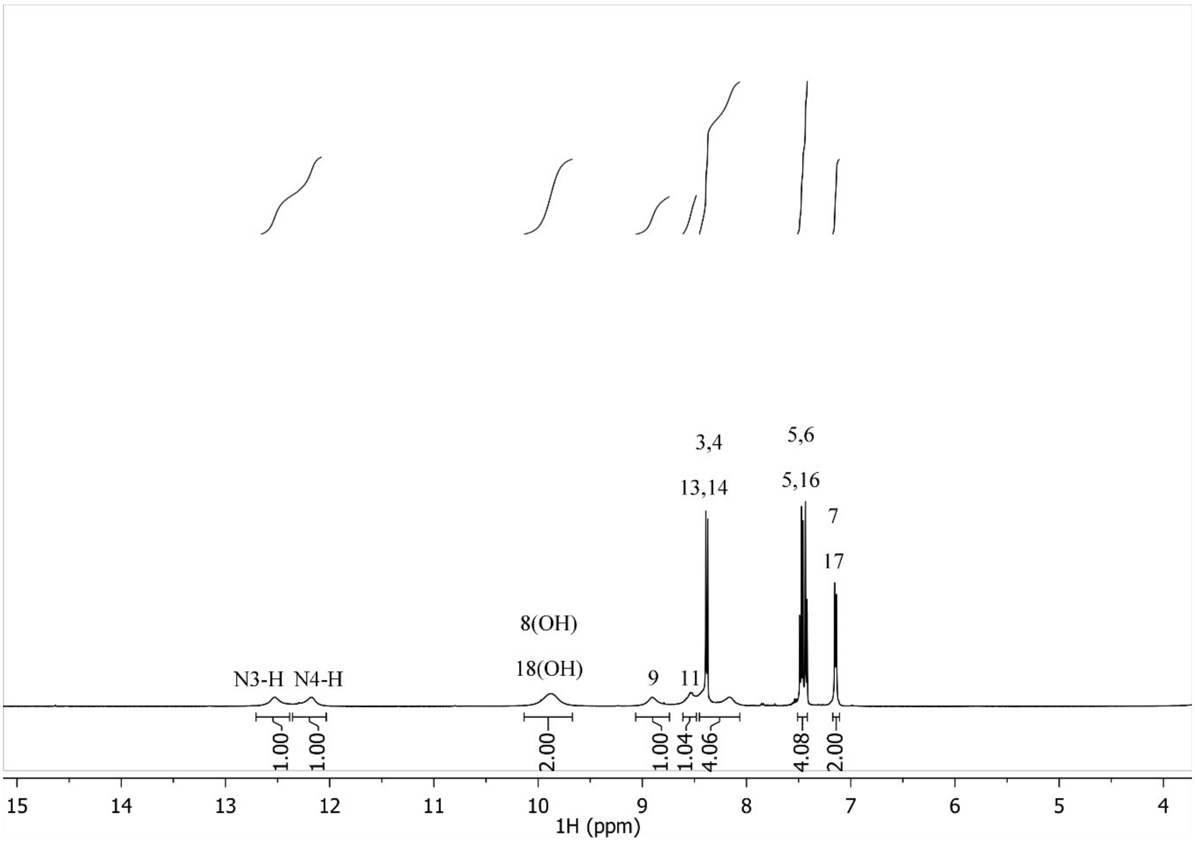


Figure S 74. ^{1}H NMR spectrum of **S6** in DMSO-d_6.

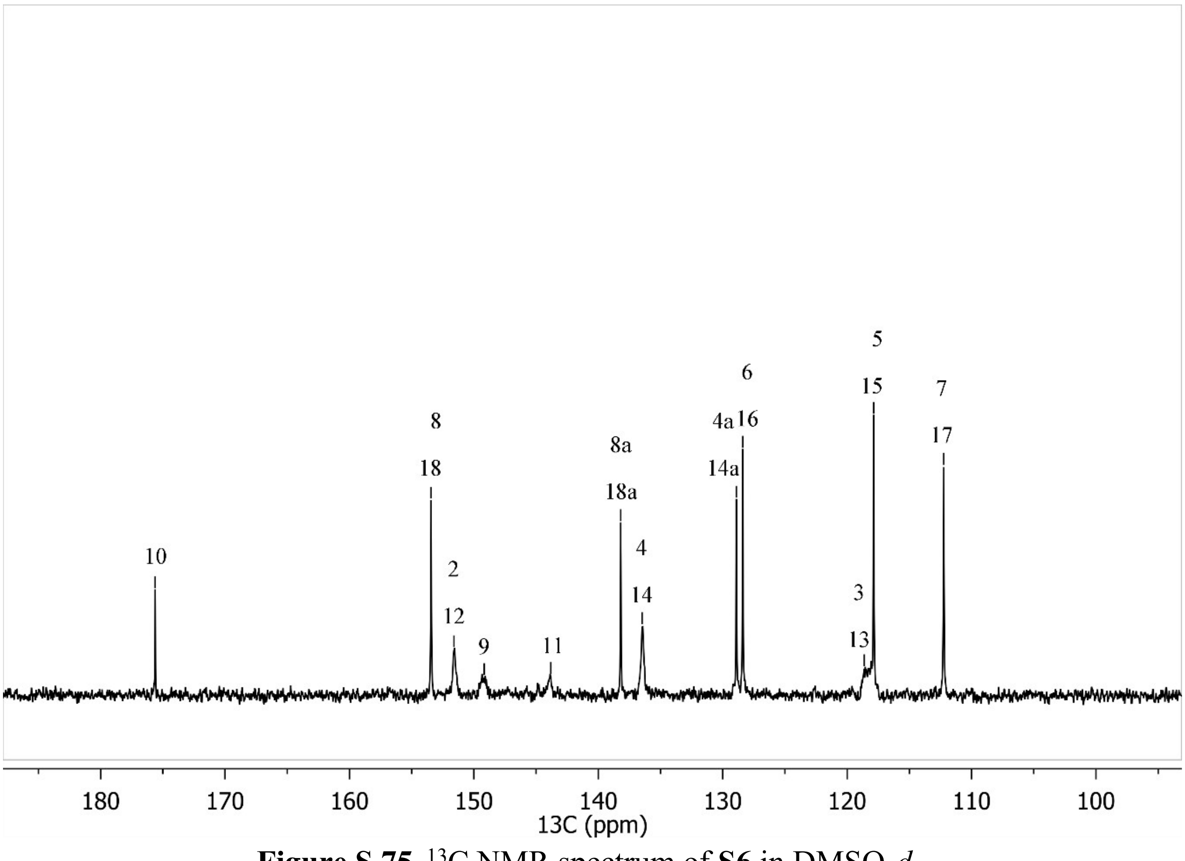


Figure S 75. ^{13}C NMR spectrum of **S6** in DMSO-d_6.

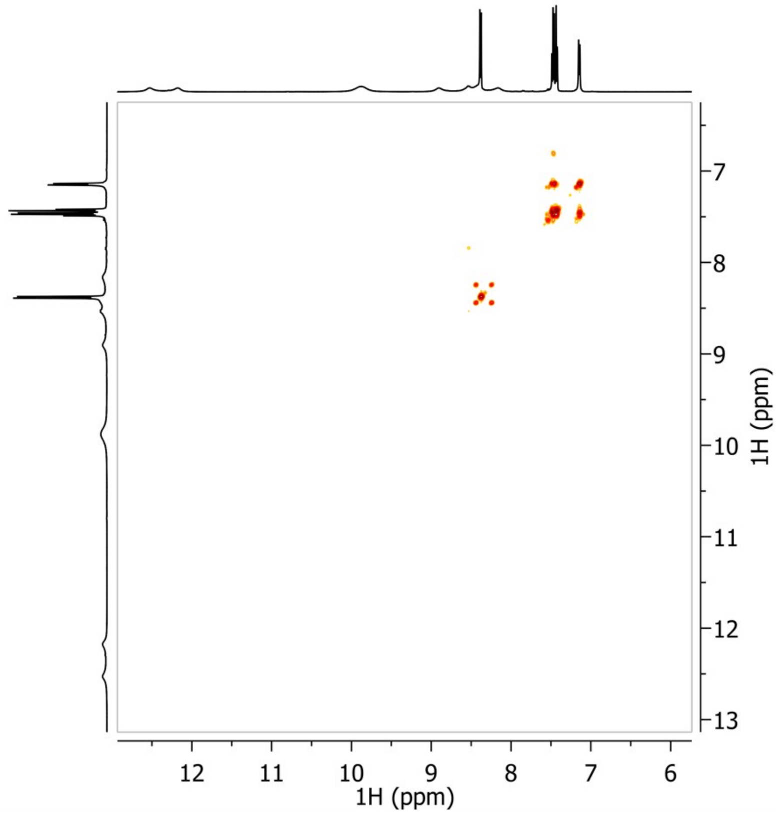


Figure S 76. COSY spectrum of **S6**.

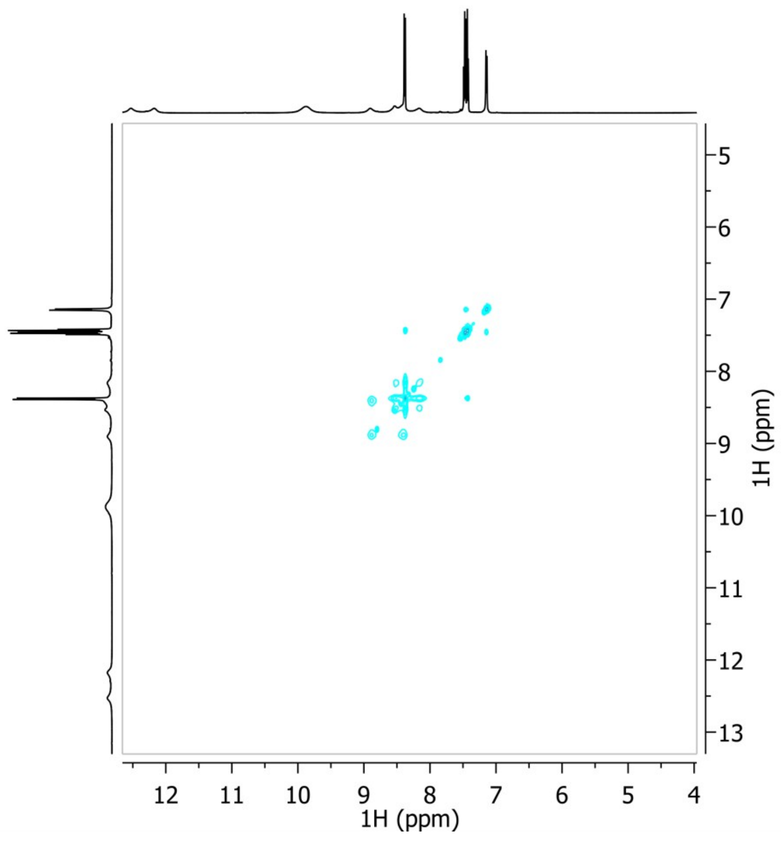


Figure S 77. NOESY spectrum of **S6**.

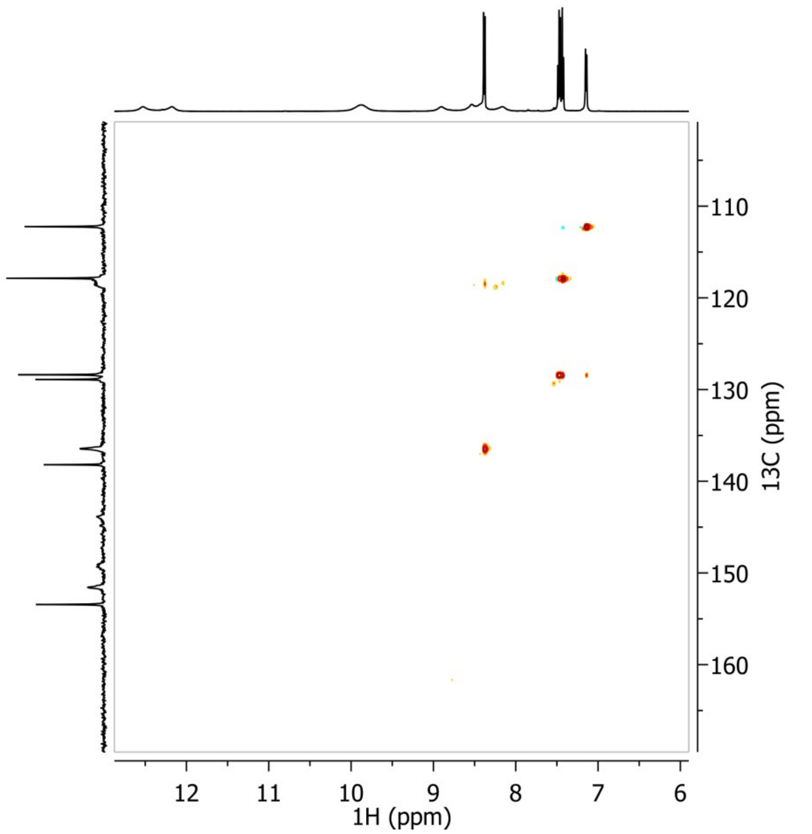


Figure S 78. ^{1}H–^{13}C HSQC spectrum of **S6**.

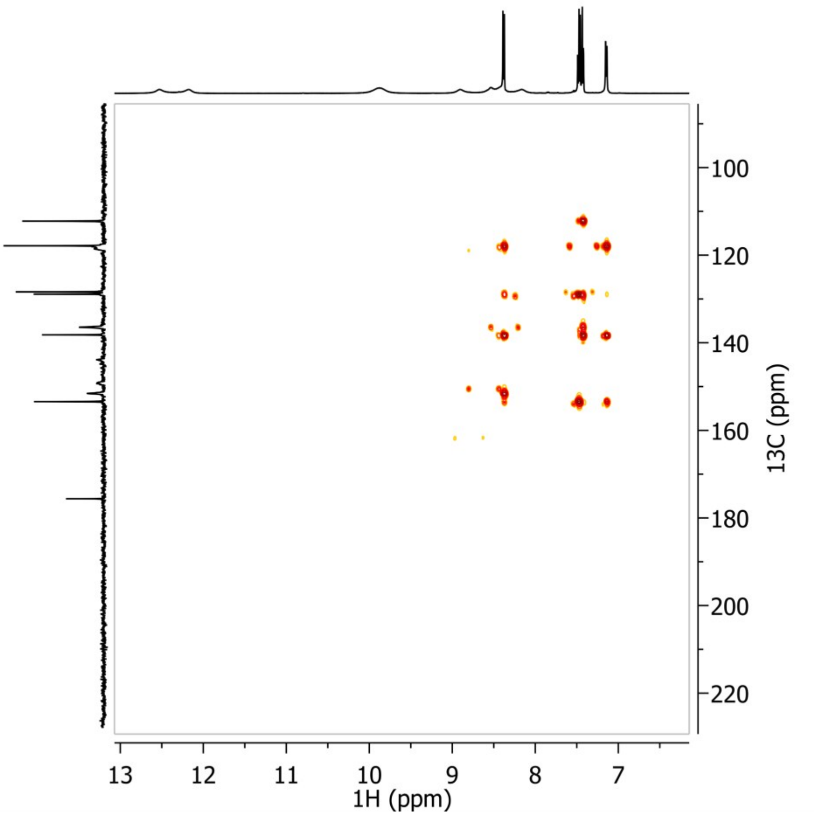


Figure S 79. ^{1}H–^{13}C HMBC spectrum of **S6**.