Supporting information

Identification of Spiro-Fused Pyrrolo[3,4-*a*]pyrrolizines and Tryptanthrines as Potential Antitumor Agents: Synthesis and *in vitro* Evaluation

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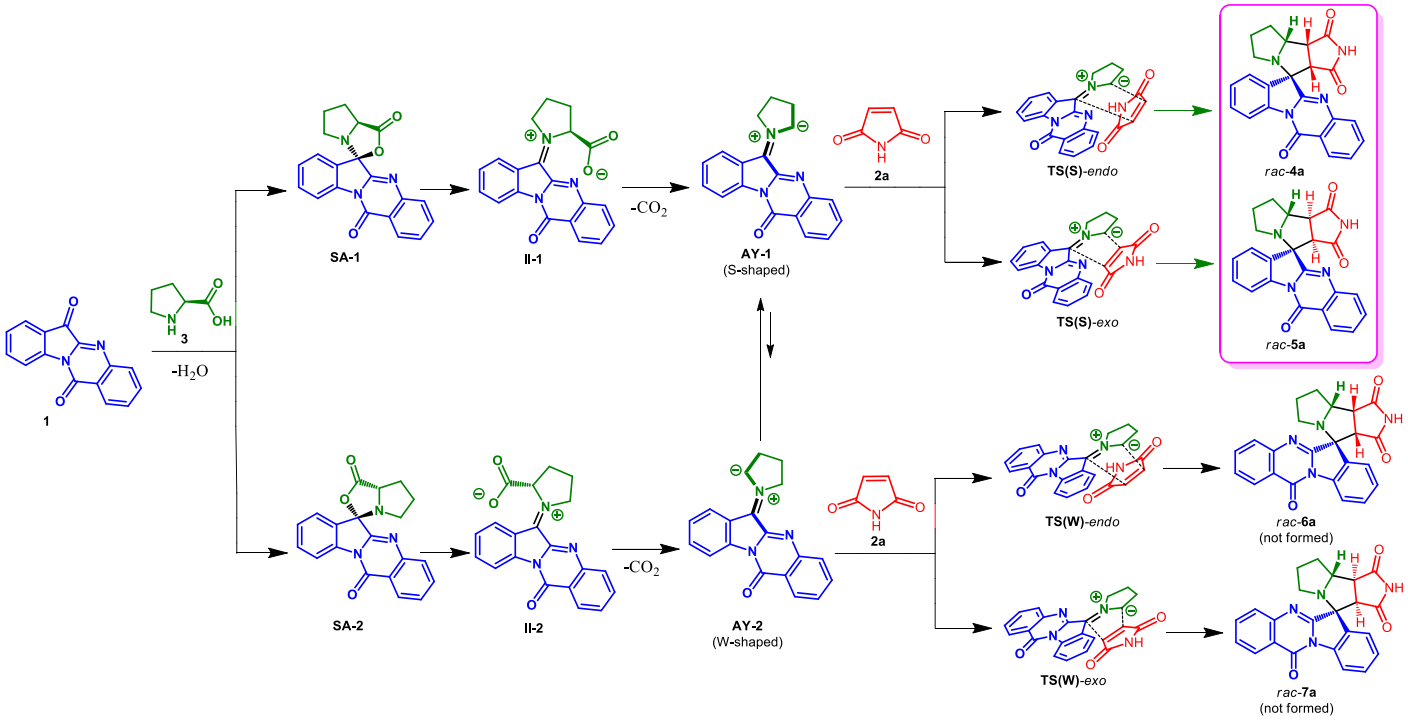
1. Plausible reaction mechanism for the formation of compounds 4 and 5



Scheme S1. Plausible reaction mechanism for the formation of compounds **4** and **5**

Comment: In the first stage, spiro-pyrrolooxazoles **SA-1** and **SA-2** are formed from tryptanthrine (**1**) and L-proline (**3**). Further, **SA-1** and **SA-2** through the stage of iminium intermediates **II-1** and **II-2** are converted into *S*- and *W*-shaped azomethine ylides **AY-1** and **AY-2**, respectively. At the last stage, 1,3-dipolar cycloaddition occurs between azomethine ylides and maleimide (**2a**). As a result of this reaction, it is possible to identify only compounds **4a** and **5a** - the products of the interaction of S-ylide **AY-1** with maleimide (**2a**).

2. Copies of ^{1}H and ^{13}C NMR spectra of compounds 4 and 5

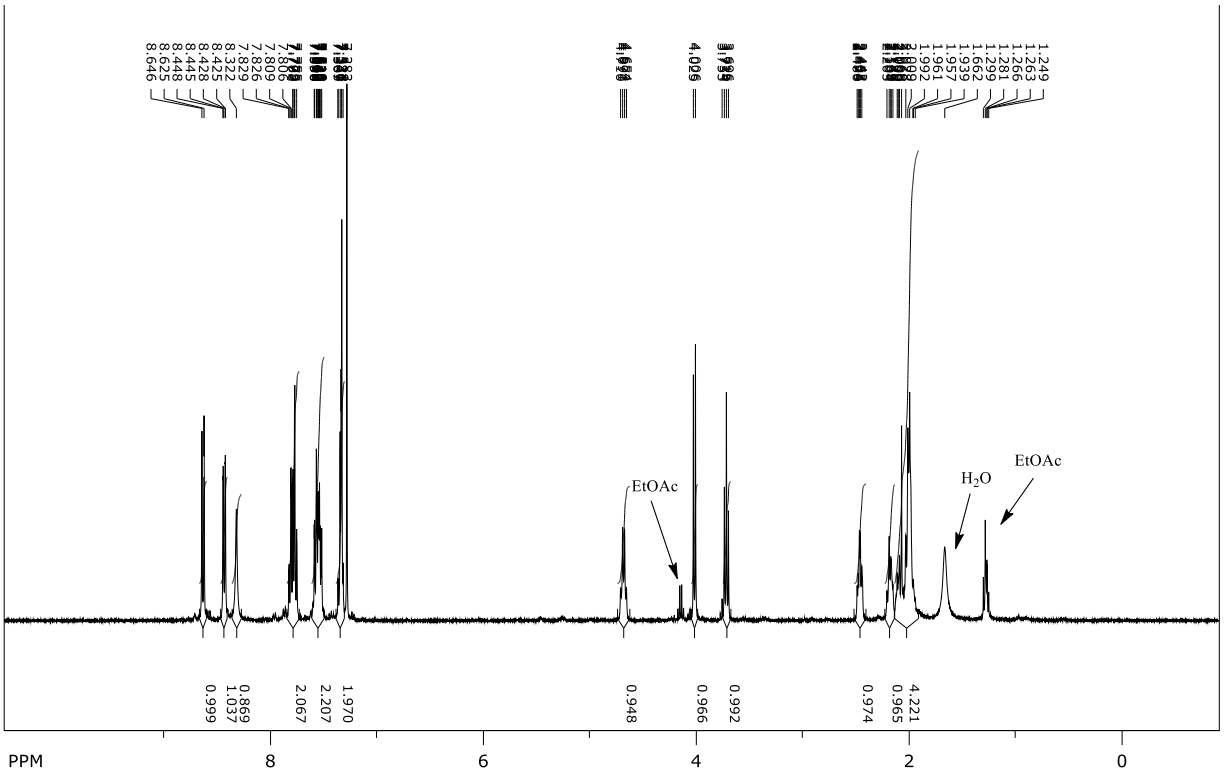


Figure S1. ^{1}H NMR spectrum of compound **4a** ($CDCl_3$, 400 MHz)

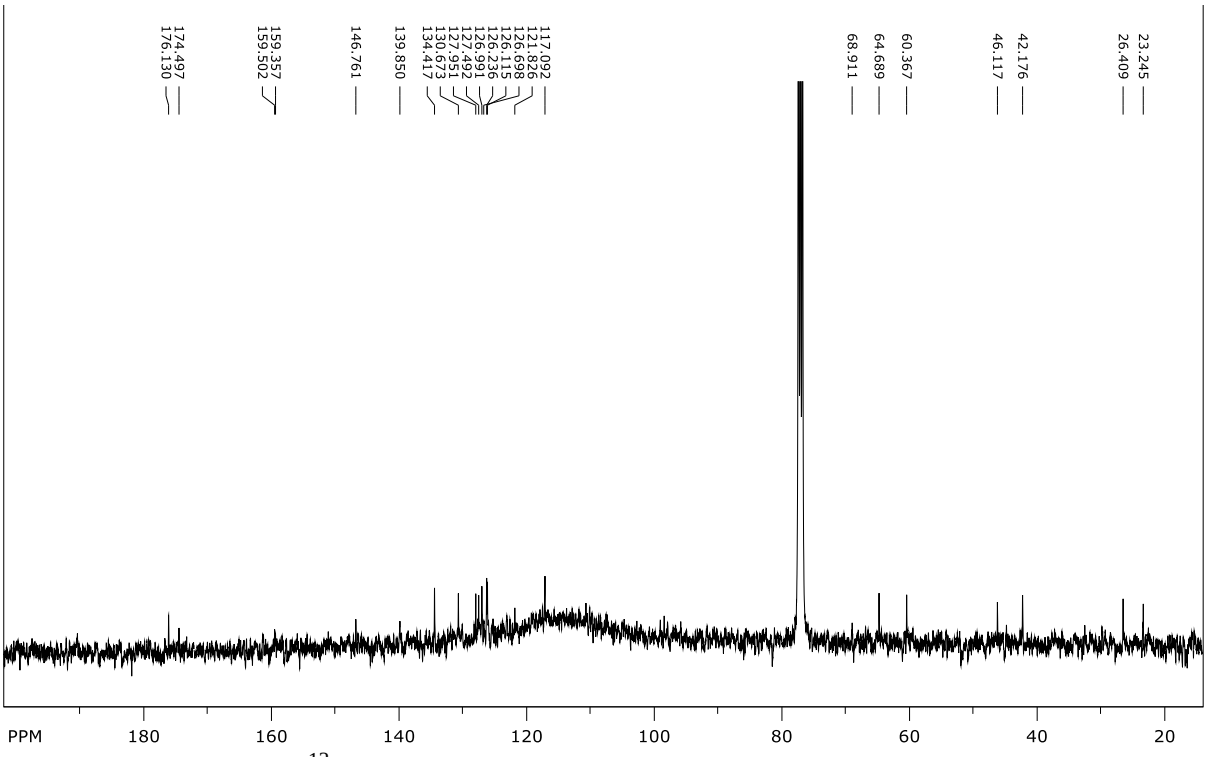


Figure S2. ^{13}C NMR spectrum of compound **4a** ($CDCl_3$, 101 MHz)

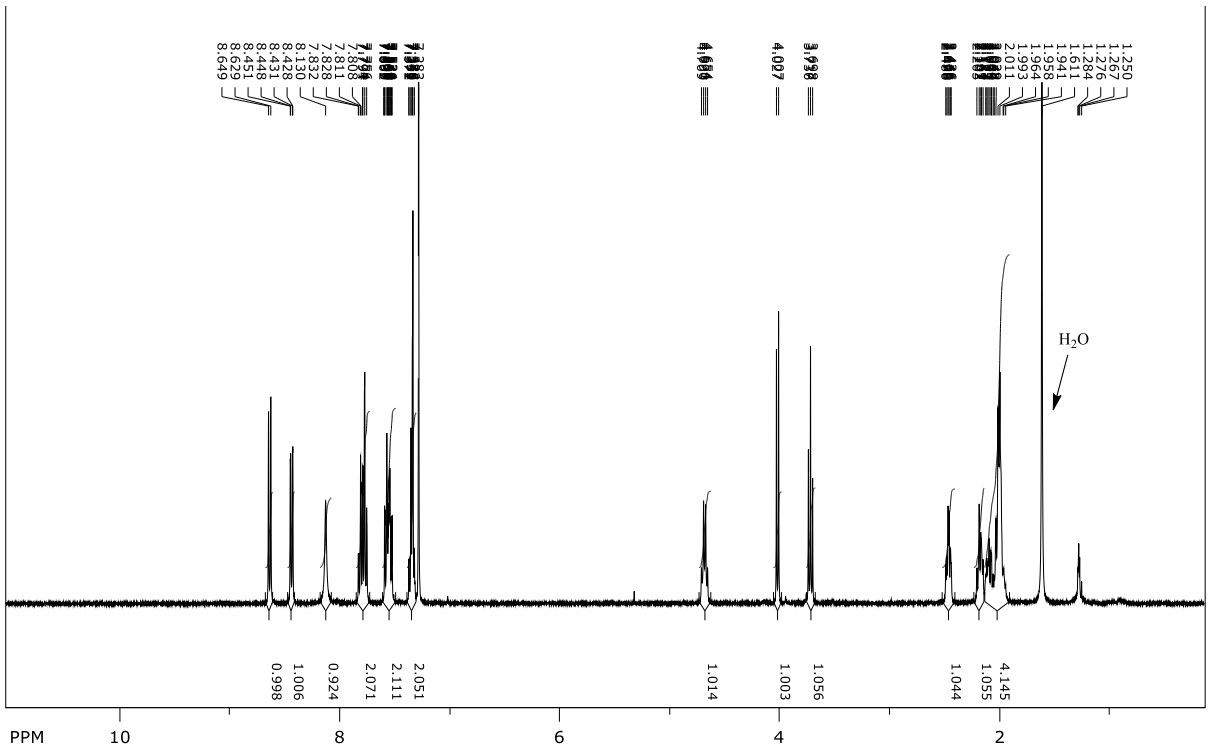


Figure S3. ^{1}H NMR spectrum of compound **5a** ($CDCl_3$, 400 MHz)

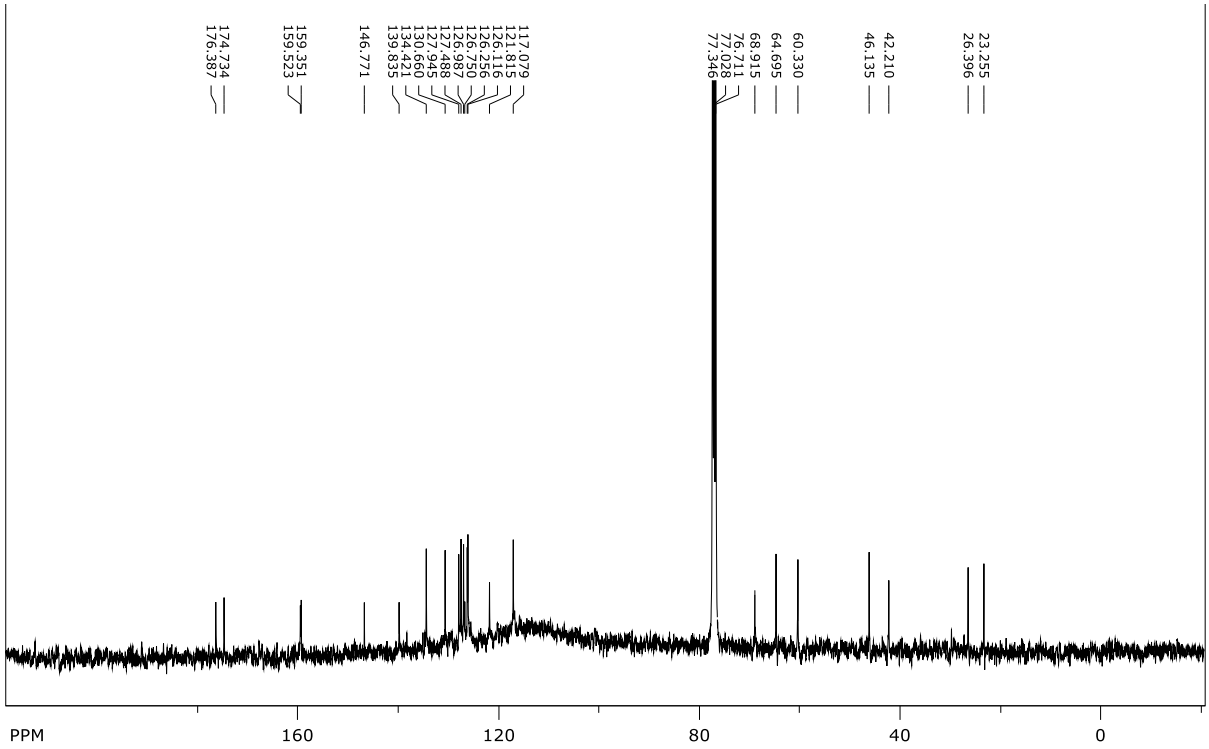


Figure S4. ^{13}C NMR spectrum of compound **5a** ($CDCl_3$, 101 MHz)

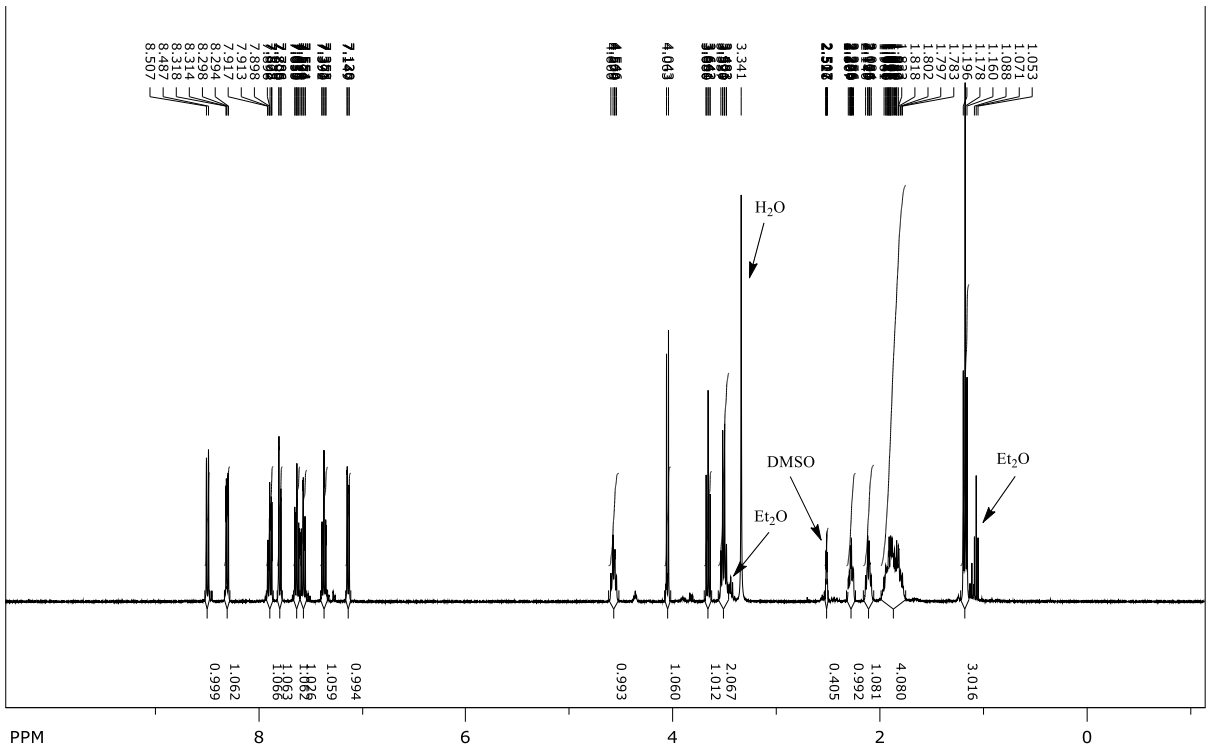


Figure S5. ^{1}H NMR spectrum of compound **4b** (DMSO-*d6*, 400 MHz)

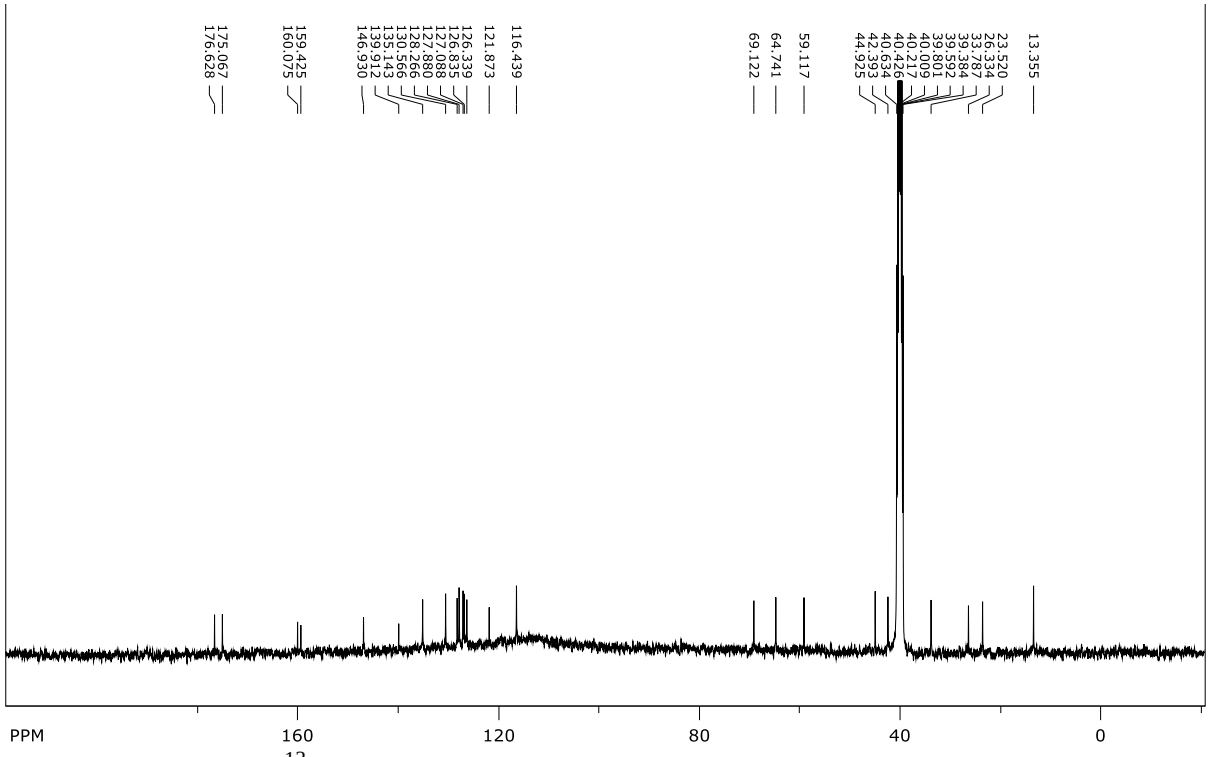


Figure S6. ^{13}C NMR spectrum of compound **4b** (DMSO-*d6*, 101 MHz)

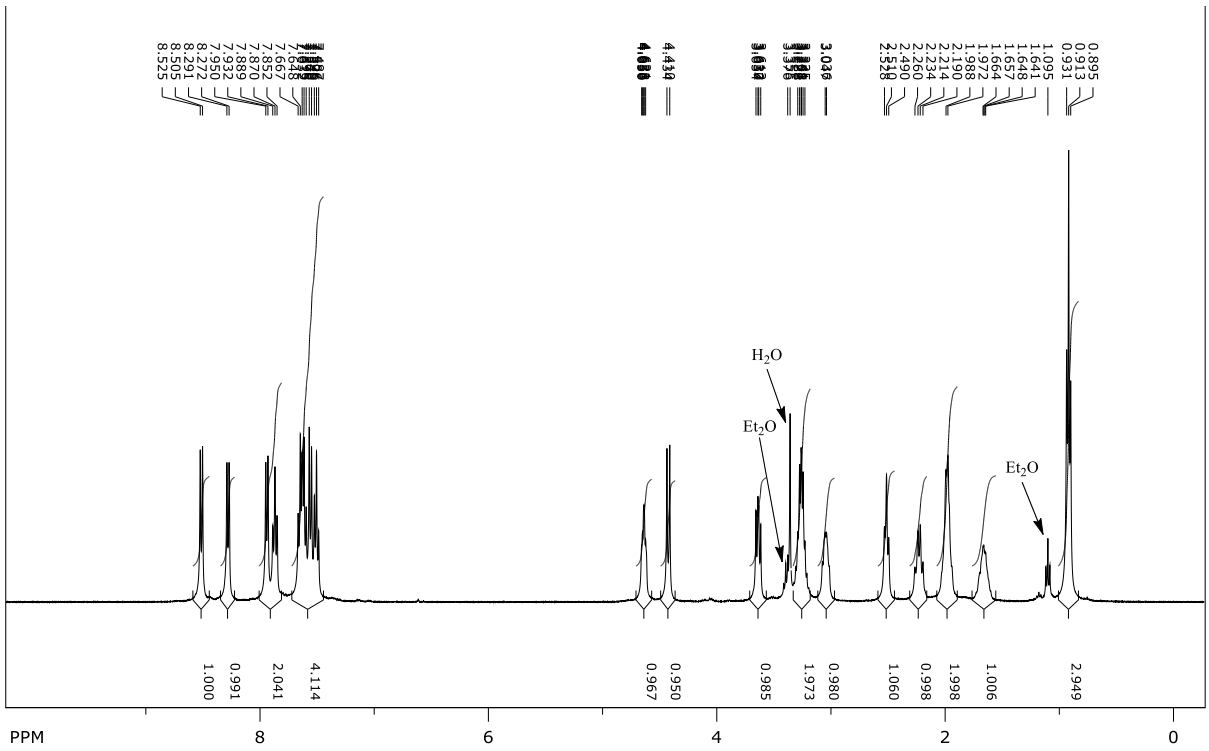


Figure S7. ^{1}H NMR spectrum of compound **5b** (DMSO-*d6*, 400 MHz)

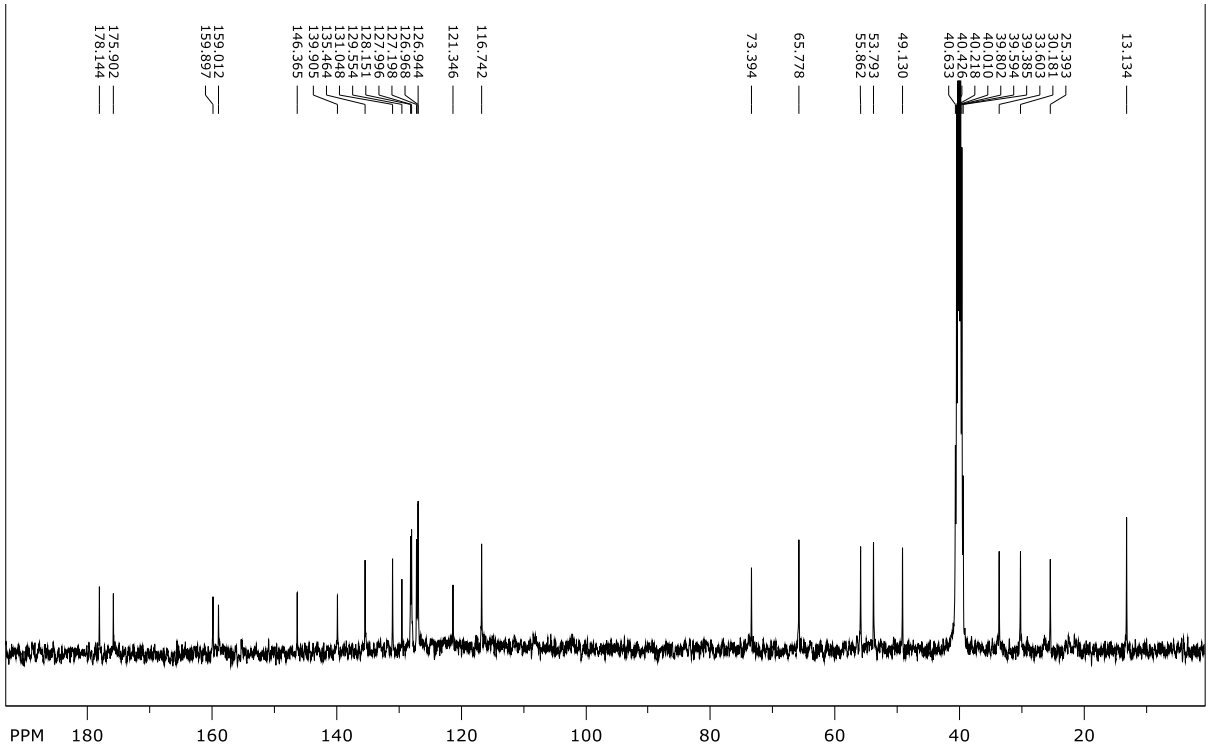


Figure S8. ^{13}C NMR spectrum of compound **5b** (DMSO-*d6*, 101 MHz)

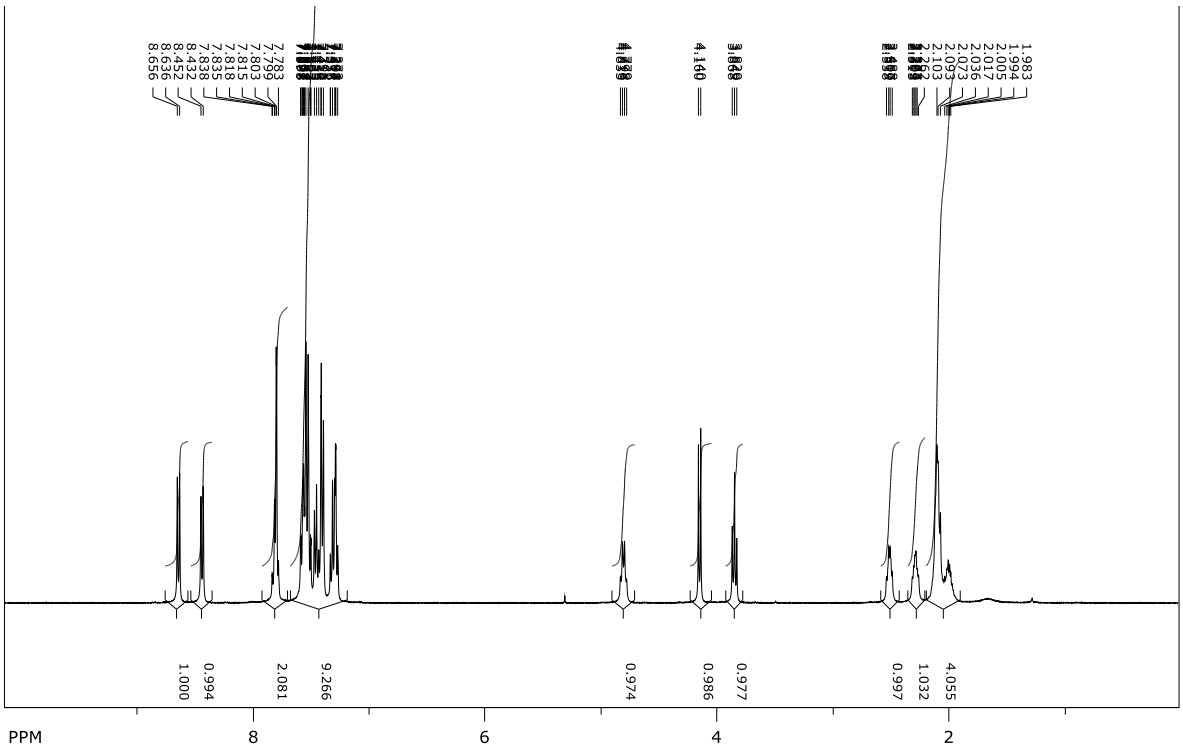


Figure S9. ^{1}H NMR spectrum of compound **4c** ($CDCl_3$, 400 MHz)

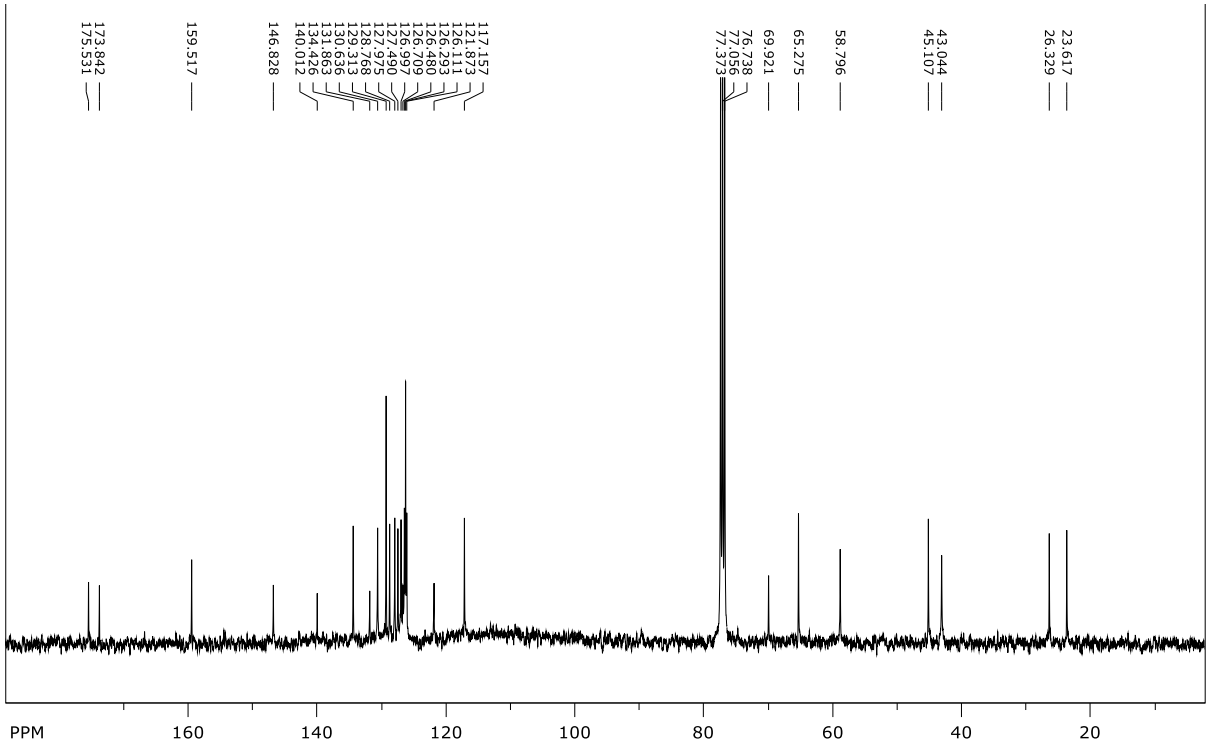


Figure S10. ^{13}C NMR spectrum of compound **4c** ($CDCl_3$, 101 MHz)

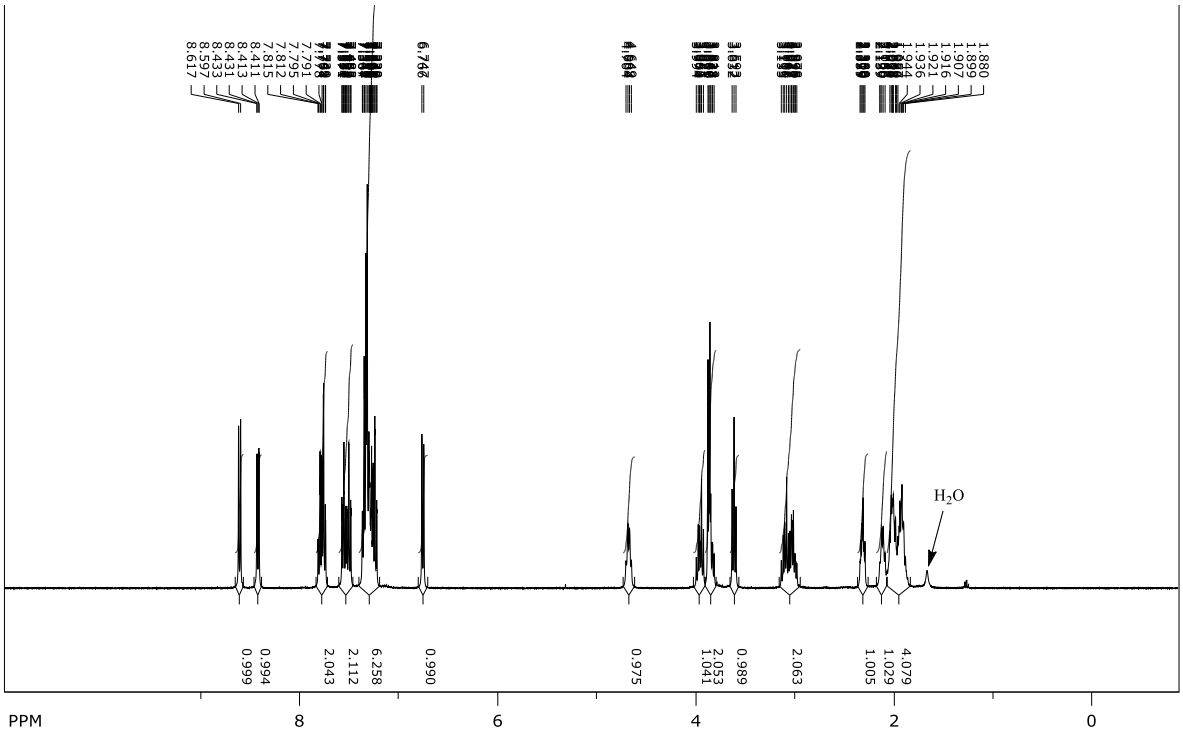


Figure S11. ^{1}H NMR spectrum of compound **4d** ($CDCl_3$, 400 MHz)

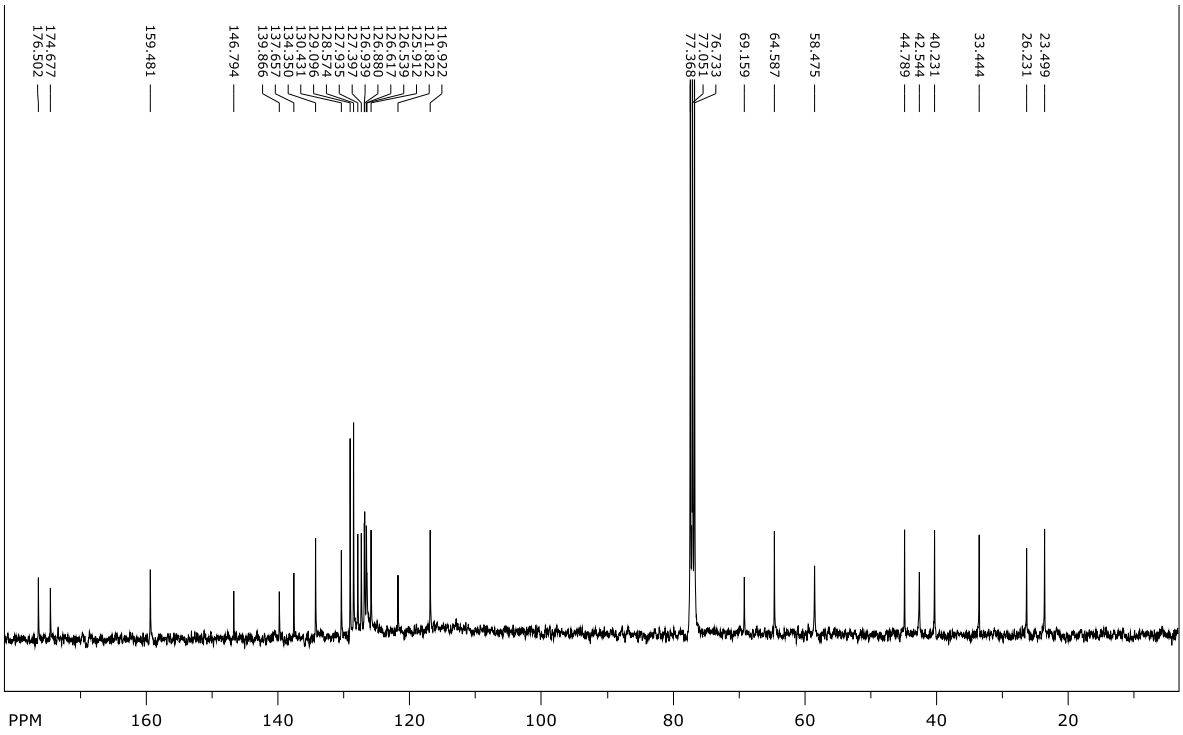


Figure S12. ^{13}C NMR spectrum of compound **4d** ($CDCl_3$, 101 MHz)

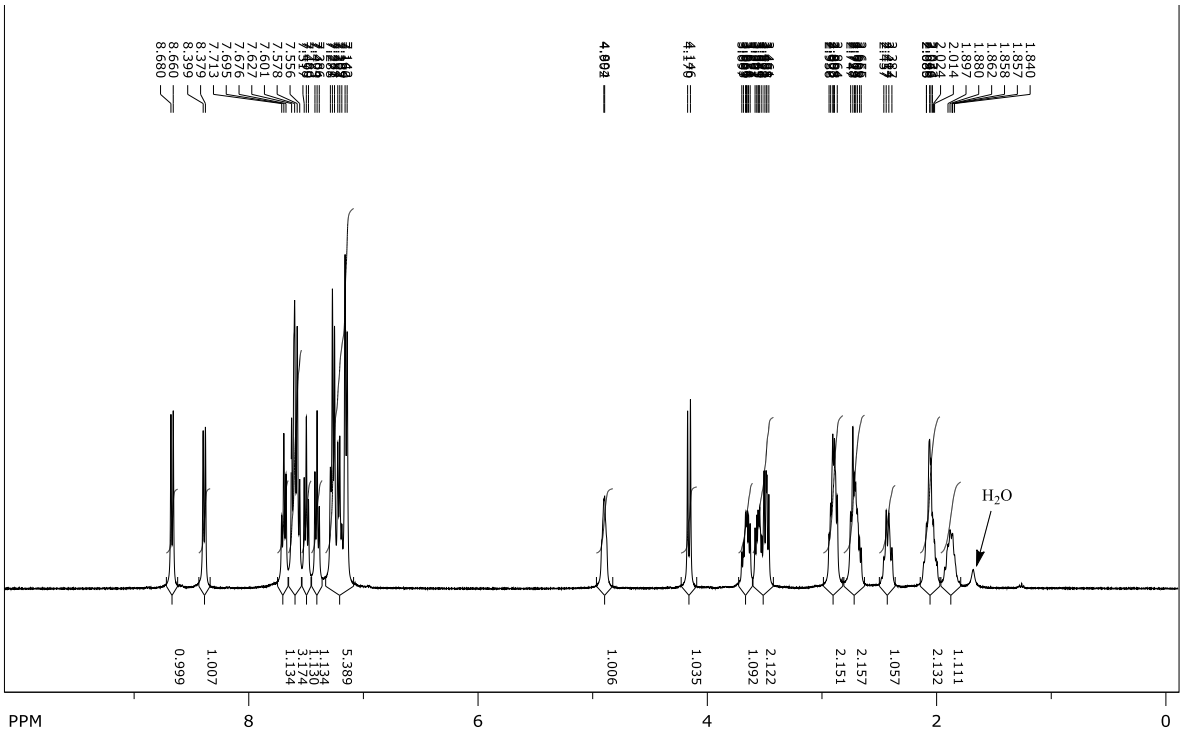


Figure S13. ^{1}H NMR spectrum of compound **5d** ($CDCl_3$, 400 MHz)

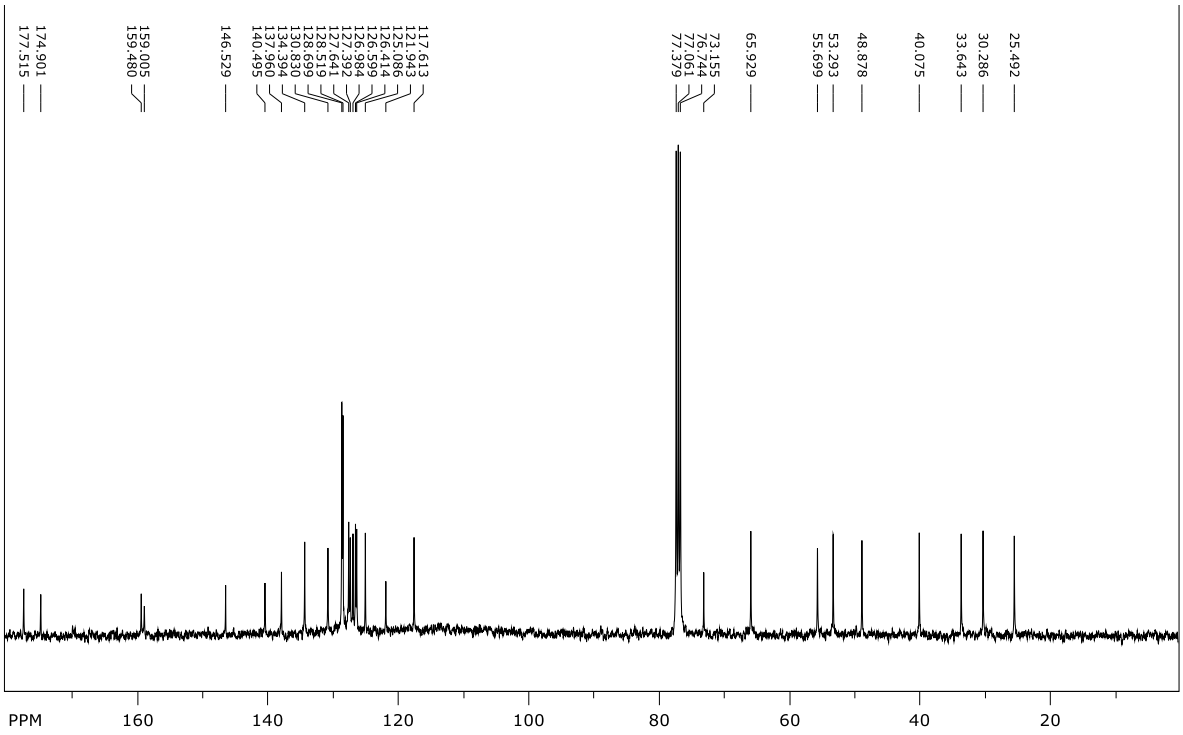


Figure S14. ^{13}C NMR spectrum of compound **5d** ($CDCl_3$, 101 MHz)

3. 2D NMR spectra of compound 4b and 5b

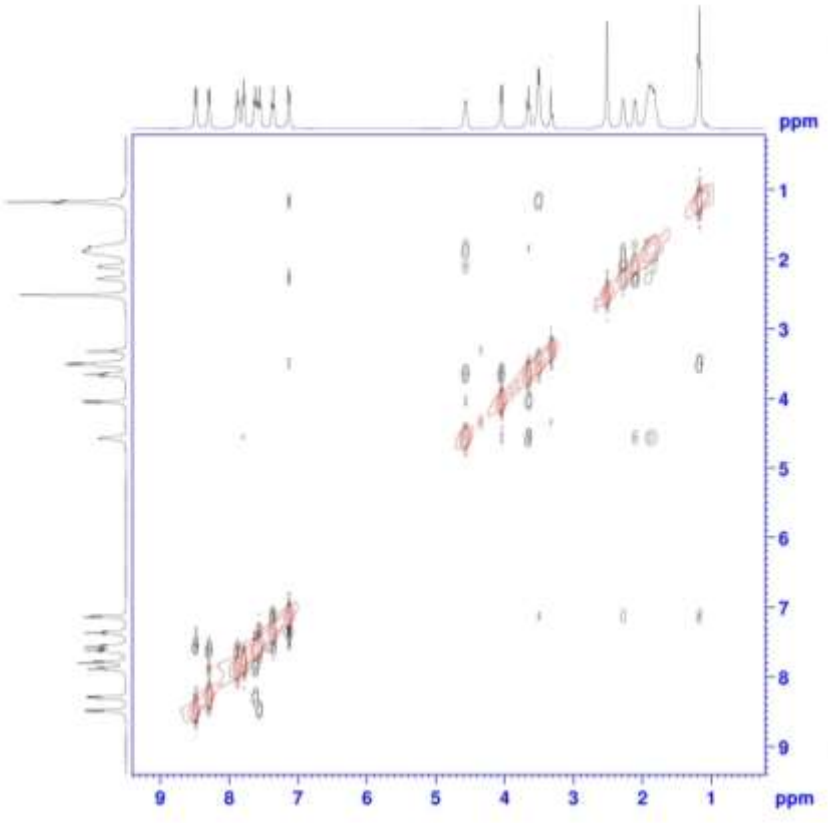


Figure S15. NOESY spectrum of compound **4b** (DMSO-*d6*, 400 MHz)

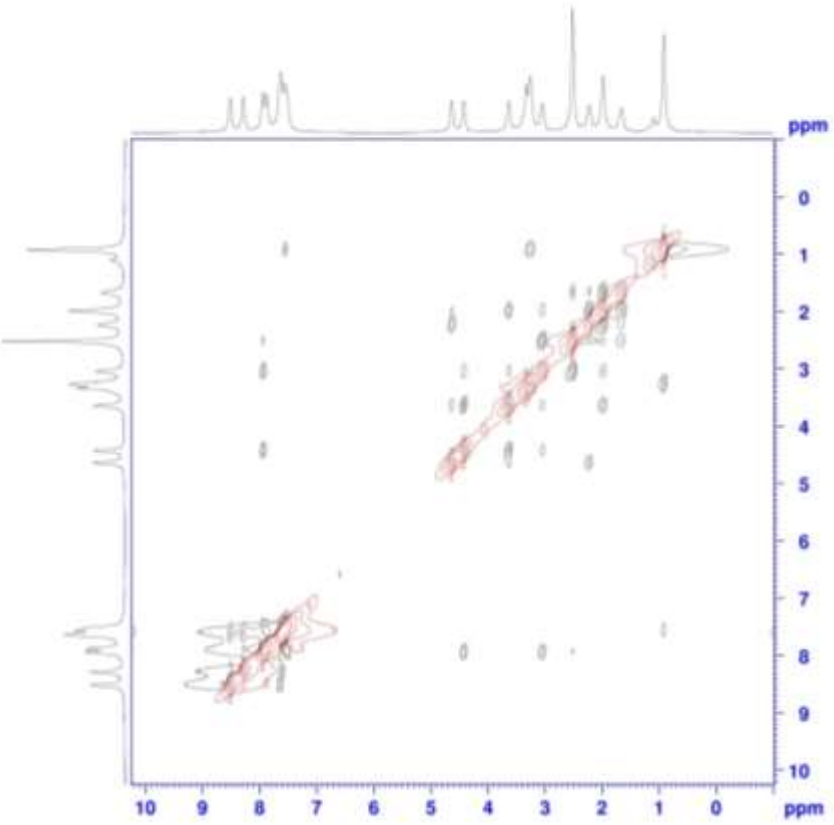


Figure S16. NOESY spectrum of compound **5b** (DMSO-*d6*, 400 MHz)

4. Bioassay details

Table S1. K562 cells viability after the treatment for 72 h

Compound	Cell viability							
	1 μg/ml	2 μg/ml	5 μg/ml	10 μg/ml	20 μg/ml	30 μg/ml	50 μg/ml	100 μg/ml
4a	100.4±4.7	45.8±3.9	43.7±3.1	36.7±4.6	12.1±2.2	n.d.[1]	10.2±1.4	12.0±3.4
5a	101.9±1.6	101.0±8.0	79.6±2.2	62.8±3.7	41.4±1.7	11.3±1.8	n.d.	n.d.
4b	110.3±1.5	96.5±2.6	71.3±9.2	38±2.1	18.1±5.8	8.3±1.2	n.d.	n.d.
5b	102.1±4.0	94.2±5.5	90.3±4.1	76.3±7.2	66.7±4.6	n.d.	48.8±4.2	27.8±4.2
4c	90.4±0.9	82±1.5	65.1±3.4	57.3±2.6	50.8±5.5	n.d.	n.d.	n.d.
5c	99.6±1.8	93.9±4.1	81.9±3.8	66.2±4.1	53.7±3.4	n.d.	n.d.	n.d.
4d	91.7±4.8	79.9±4.2	59.6±1.3	56.0±2.5	50.4±4.3	n.d.	36.8±6.8	n.d.
5d	96.5±5.6	88.2±4.7	69.6±0.6	61.5±0.6	60.1±5.5	n.d.	58±4.0	n.d.

[1] Not determined.

Table S2. HeLa cells viability after the treatment for 72 h

Compound	Cell viability							
	1 μg/ml	2 μg/ml	5 μg/ml	10 μg/ml	20 μg/ml	30 μg/ml	50 μg/ml	100 μg/ml
4a	101.4±2.1	101.6±1.9	95.2±5.4	2.5±0.1	3.2±0.1	n.d.[1]	1.4±0.3	5.6±1.1
5a	93.4±2.4	87.6±2.5	91.5±1.6	73.5±6.6	38.9±5.3	2.7±0.2	n.d.	n.d.
4b	92.2±1.4	92.9±1.3	94.2±3.0	90±2.5	62.8±5.5	n.d.	33.1±2.3	1.1±1
5b	101.1±0.8	102.3±4.1	105.1±3.3	102.5±5.4	89.5±4.9	n.d.	46.9±3.8	31.2±3.8

[1] Not determined.

Table S3. Annexin V-FITC/Propidium iodide (PI) dual staining assay of K562 cells treated with cycloadducts **4a** and **5a** at concentrations 5, 10 and 20 μg/ml using flow cytometry

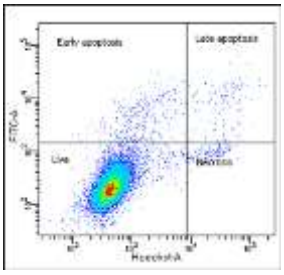
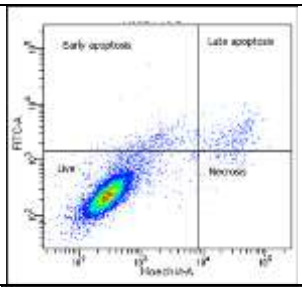
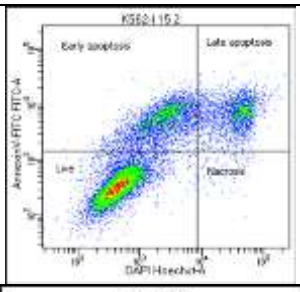
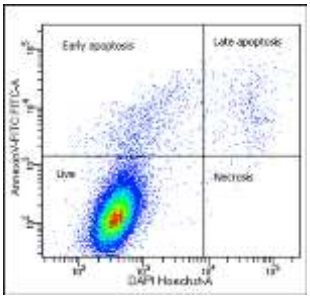
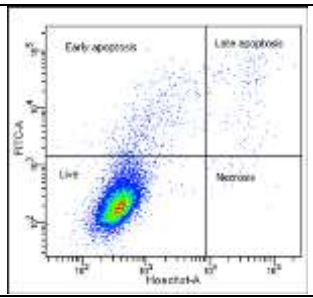
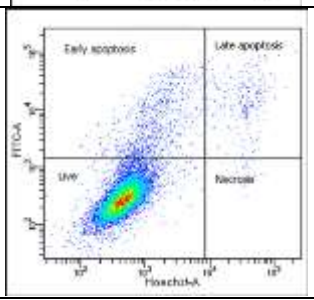
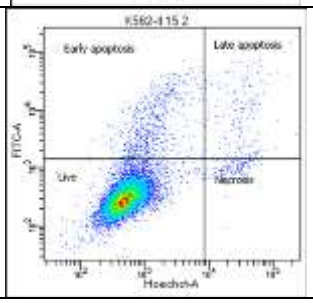
Compound	Concentration, μg/ml			Control
	5	10	20	
4a				
5a				

Table S4. Annexin V-FITC/Propidium iodide (PI) dual staining assay of HeLa cells treated with cycloadducts **4a** and **5a** at concentrations 5, 10 and 20 μg/ml using flow cytometry

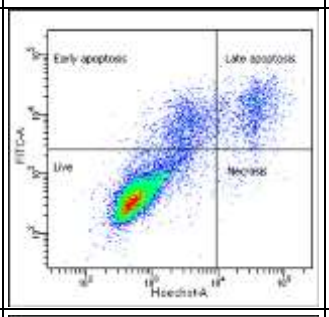
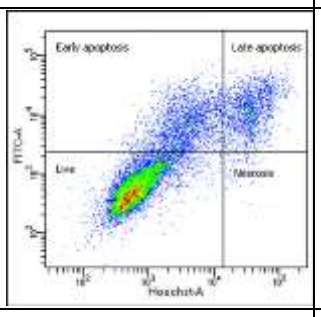
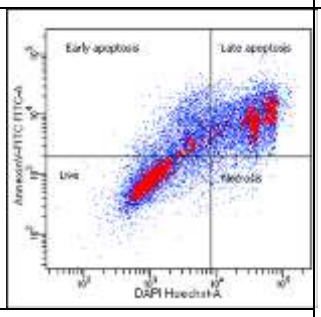
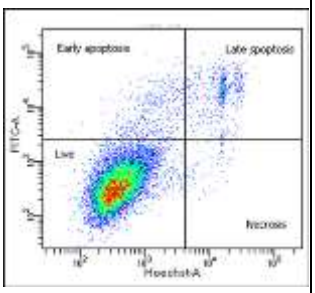
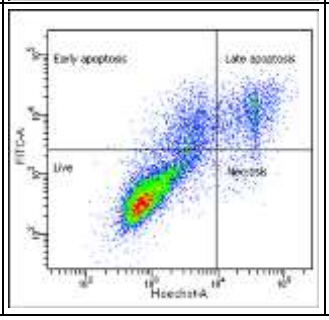
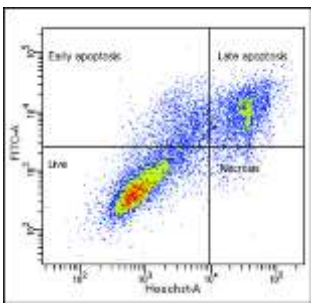
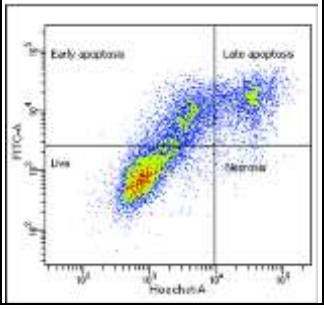
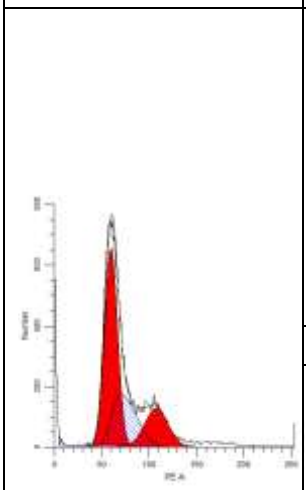
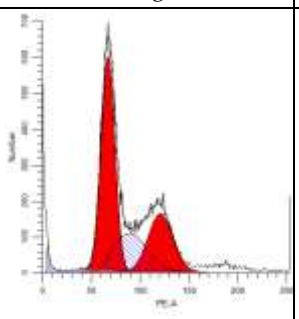
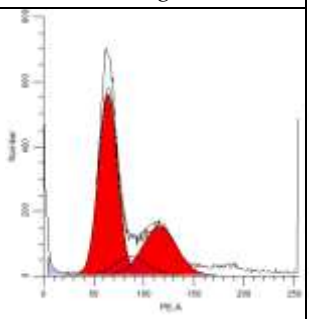
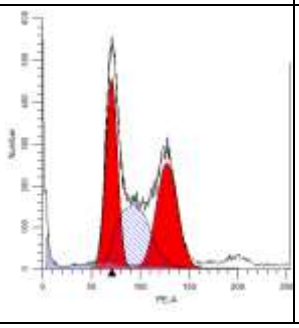
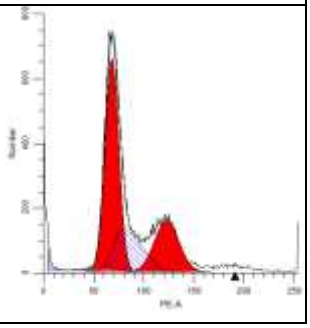
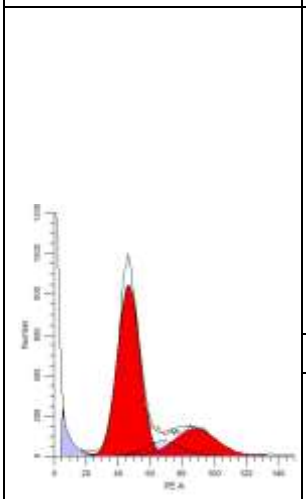
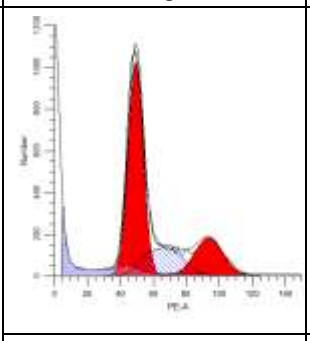
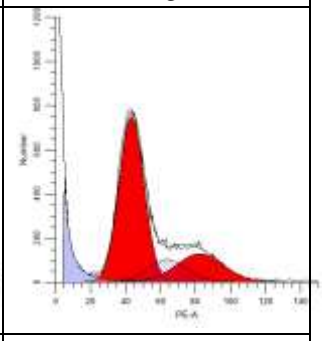
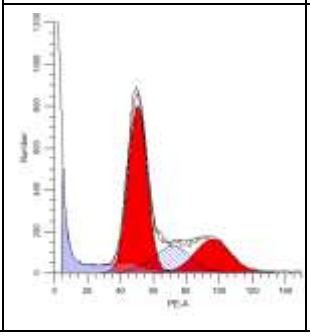
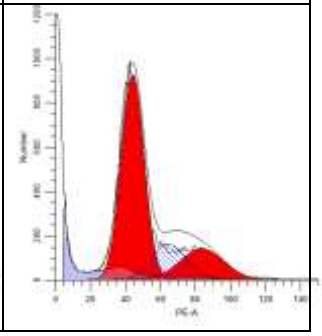
Compound	Concentration, μg/ml			Control
	5	10	20	
4a				
5a				

Table S5. Effect of compounds **4a** and **5a** at concentrations 5 and 10 μg/ml on the distribution of HeLa cells in the cell cycle after 24h treatment.

Control	**4a**, 5 μg/ml	**5a**, 5 μg/ml
		
	4a, 10 μg/ml	**5a**, 10 μg/ml
		

	Control	**4a** (5 μg/ml)	**4a** (10 μg/ml)	**5a** (5 μg/ml)	**5a** (10 μg/ml)
SubG1 (%)	2.41±0.19	4.22±0.25	6.07±0.83	1.08±0.12	4.21±0.51
G0/G1 (%)	53.03±1.67	49.75±1.33	31.82±3.18	59.91±1.45	51.86±2.13
S (%)	26.38±1.05	21.73±1.36	30.83±2.79	10.22±0.62	21.59±1.46
G2/M (%)	18.85±0.93	24.30±1.25	31.73±3.02	28.79±1.48	22.34±1.67

Table S6. Effect of compounds **4a** and **5a** at concentrations 5 and 10 μg/ml on the distribution of K562 cells in the cell cycle after 24h treatment.

Control	**4a**, 5 μg/ml	**5a**, 5 μg/ml
		
	4a, 10 μg/ml	**5a**, 10 μg/ml
		

	Control	**4a** (5 μg/ml)	**4a** (10 μg/ml)	**5a** (5 μg/ml)	**5a** (10 μg/ml)
SubG1 (%)	3.62±0.23	11.32±0.95	14.36±1.22	9.82±0.70	10.31±1.21
G0/G1 (%)	67.51±1.12	53.04±1.01	50.62±1.92	58.91±1.72	57.78±2.04
S (%)	8.93±0.74	17.91±1.07	15.60±0.98	12.31±1.13	15.35±2.99
G2/M (%)	19.94±1.12	17.73±0.95	19.15±2.25	18.96±1.52	16.56±2.16

Table S7. IC_{50} values (μM) of screened compounds for K562 and HeLa cell line.

	4a	**5a**	**4b**	**5b**	**4c**	**5c**	**4d**	**5d**
K562	4.8±0.2	37.4±0.5	18.3±0.4	110.3±0.7	>100	>100	40.8±0.7	>100
HeLa	17.3±0.4	40.7±0.5	69.7±0.7	109.9±1.1	–	–	–	–

Table S8. The values of the signal intensities calculated by processing the images of the obtained Western blots in ImagJ.

	4a	**5a**	Control
GAPDH	14617 14342 14584	13427 13381 13042	12034 11721 12148
p53	12939 12847 13445	3826 3836 4218	3457 3244 3663
MDM2	8475 10399 7096	11781 12466 12518	16474 17393 16723