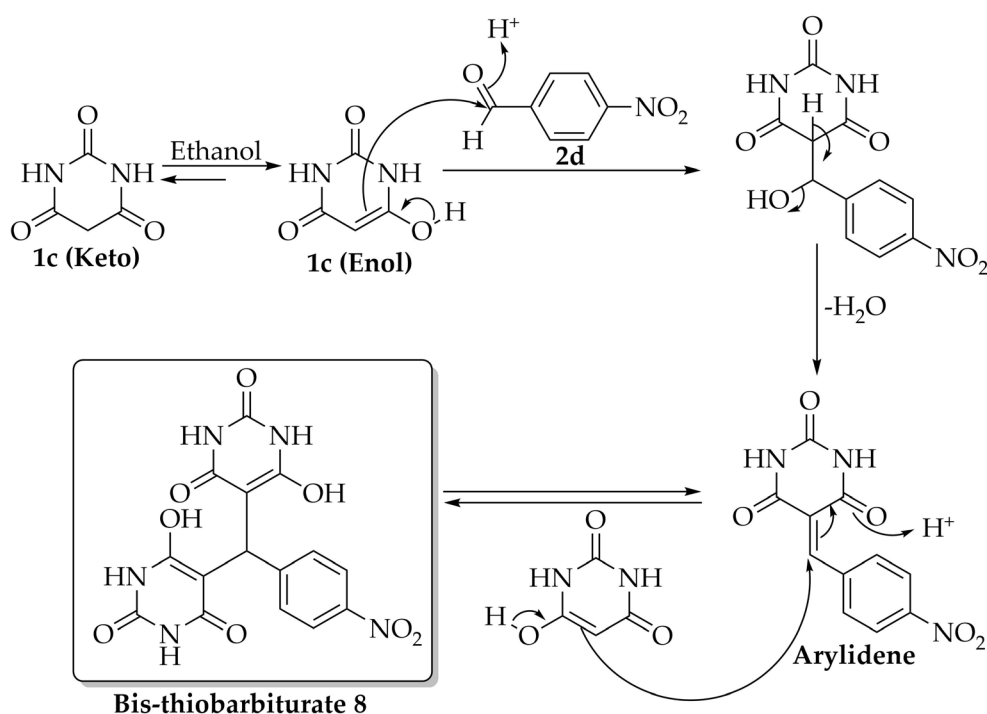


Supplementary Information

Bis-thiobarbiturates as Promising Xanthine Oxidase Inhibitors: Synthesis and Biological Evaluation

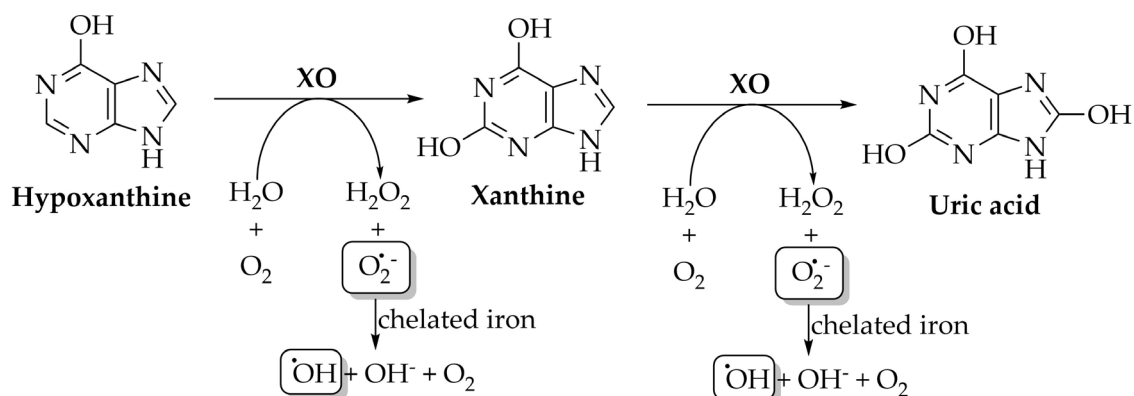


Scheme S1 – Chemical mechanism for the formation of bis-thiobarbiturate 8.

Table S1 – Full *in vitro* data for XO inhibitory activity, DPPH radical scavenging strength and cytotoxicity effects on NHDF, Caco-2 and MCF-7 cells by bis-thiobarbiturates **3-7** and **9-19** and references Febuxostat, Allo, Trolox and 5-FU.^a

Compound	XO inhibition (%)		DPPH (%) scavenging at 30 μ M)	Cytotoxicity (% cell viability at 30 μ M)		
	30 μ M	5 μ M		NHDF	Caco-2	MCF-7
3	80.15 \pm 0.68	10.42 \pm 1.01	34.58 \pm 0.96	88.65 \pm 1.40	91.23 \pm 9.87	89.96 \pm 5.33
4	36.47 \pm 2.95	-	42.64 \pm 0.81	95.57 \pm 7.26	98.58 \pm 5.70	90.64 \pm 3.92
5	86.12 \pm 2.41	52.92 \pm 4.02	34.76 \pm 1.42	86.48 \pm 5.92	62.52 \pm 11.94	90.68 \pm 4.06
6	92.25 \pm 0.09	56.82 \pm 2.16	34.99 \pm 1.32	99.78 \pm 5.43	100.13 \pm 4.45	91.68 \pm 1.48
7	20.86 \pm 2.47	-	32.41 \pm 0.26	96.60 \pm 4.32	97.09 \pm 3.63	98.66 \pm 4.83
9	45.61 \pm 1.69	-	39.63 \pm 2.26	81.35 \pm 8.87	67.49 \pm 9.4	90.18 \pm 5.70
10	23.53 \pm 0.17	-	38.12 \pm 1.56	57.11 \pm 8.12	62.6 \pm 7.74	100.16 \pm 13.01
11	95.72 \pm 1.49	80.92 \pm 0.59	57.25 \pm 0.98	94.27 \pm 7.84	72.31 \pm 9.76	92.15 \pm 3.68
12	72.25 \pm 5.65	-	42.92 \pm 0.61	85.64 \pm 7.17	90.88 \pm 3.84	91.70 \pm 4.91
13	89.73 \pm 1.41	37.42 \pm 2.55	29.90 \pm 0.50	75.80 \pm 2.41	95.43 \pm 4.17	98.22 \pm 3.25
14	90.70 \pm 0.71	56.29 \pm 1.21	28.04 \pm 0.25	80.13 \pm 7.69	97.2 \pm 2.97	88.06 \pm 3.25
15	14.92 \pm 1.68	-	32.33 \pm 0.48	91.18 \pm 7.77	96.91 \pm 4.73	91.25 \pm 4.26
16	61.44 \pm 1.22	-	31.35 \pm 0.91	60.68 \pm 1.72	95.36 \pm 3.30	91.11 \pm 2.00
17	90.75 \pm 2.87	55.2 \pm 1.63	40.90 \pm 0.65	68.89 \pm 9.33	88.72 \pm 7.85	86.62 \pm 5.87
18	98.85 \pm 0.82	63.43 \pm 2.1	31.04 \pm 0.66	86.55 \pm 2.38	94.68 \pm 7.19	88.29 \pm 3.29
19	91.68 \pm 1.87	67.3 \pm 1.27	3.83 \pm 2.44	81.01 \pm 3.57	80.71 \pm 12.98	93.67 \pm 5.68
Febuxostat	95.03 \pm 0.91	93.88 \pm 2.84	-	-	-	-
Allo	88.51 \pm 3.64	26.93 \pm 0.82	0.48 \pm 0.21	-	-	-
Trolox	-	-	54.86 \pm 0.98	-	-	-
5-FU	-	-	-	20.93 \pm 0.83	37.06 \pm 1.51	45.88 \pm 4.72

^a Results are expressed as average values \pm standard deviation (SD) of at least two independent determinations.



Scheme S2 – Diagram of XO oxidative hydroxylation of hypoxanthine over xanthine and uric acid, with reactive oxygen species (superoxide anion radical and hydroxyl radical) generation (adapted from Šmelcerović *et al.* [1¹]).

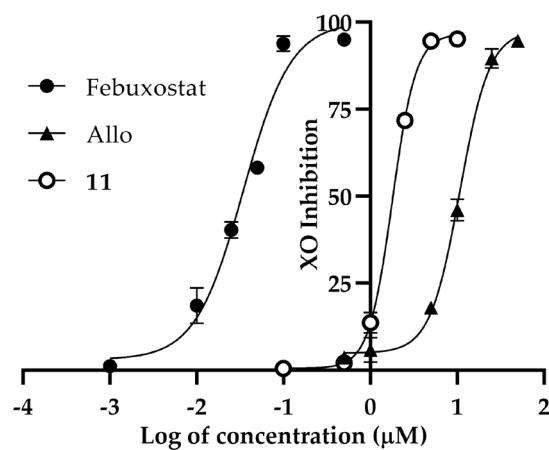


Figure S1 – IC₅₀ curves for XO inhibition by Febuxostat, Allo and bis-thiobarbiturate 11.

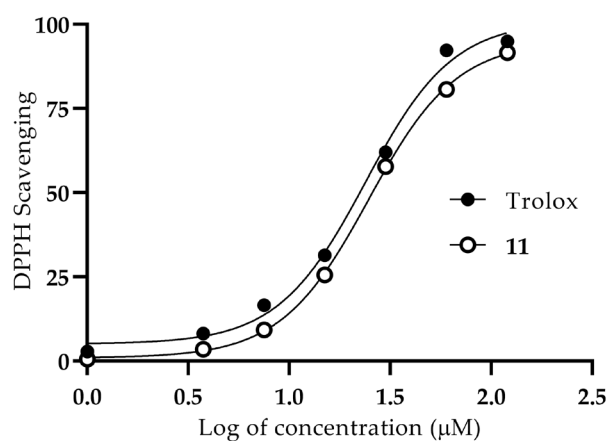


Figure S2 – IC₅₀ curves for DPPH scavenging activity of Trolox and bis-thiobarbiturate 11.

¹ Šmelcerović, A.; Tomović, K.; Šmelcerović, Ž.; Petronijević, Ž.; Kocić, G.; Tomašić, T.; Jakopin, Ž.; Anderluh, M. Xanthine oxidase inhibitors beyond allopurinol and febuxostat; an overview and selection of potential leads based on in silico calculated physico-chemical properties, predicted pharmacokinetics and toxicity. *Eur J Med Chem* **2017**, 135, 491-516.

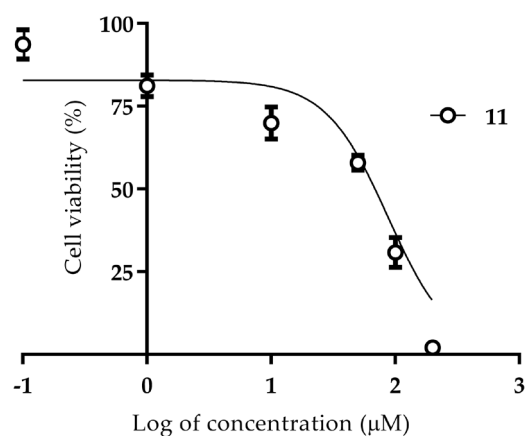


Figure S3 – IC₅₀ curve for cytotoxicity of bis-thiobarbiturate **11** on NHDF cell line.

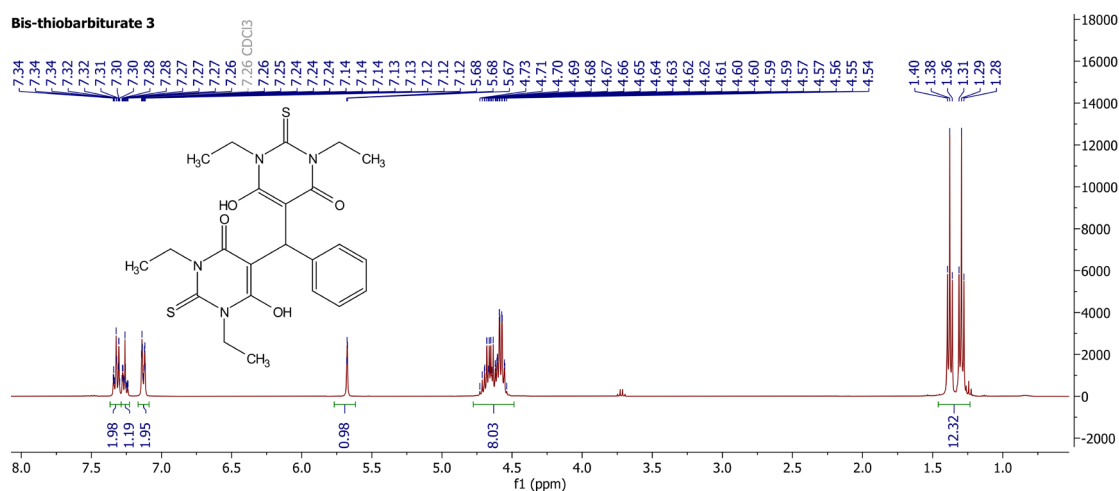


Figure S4 – ¹H NMR of bis-thiobarbiturate **3** in CDCl₃.

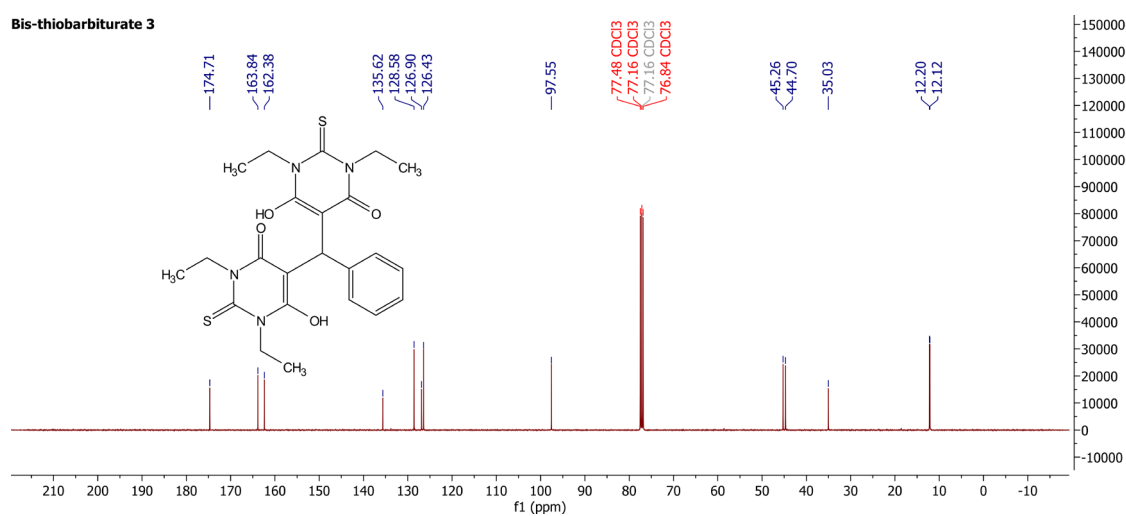


Figure S5 – ¹³C NMR of bis-thiobarbiturate **3** in CDCl₃.

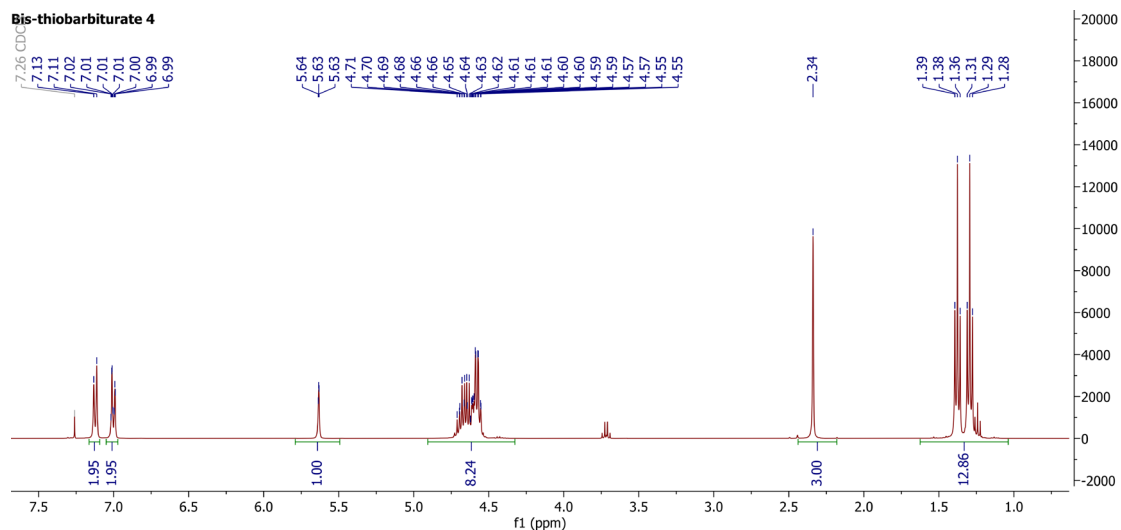


Figure S6 – ^1H NMR of bis-thiobarbiturate 4 in CDCl_3 .

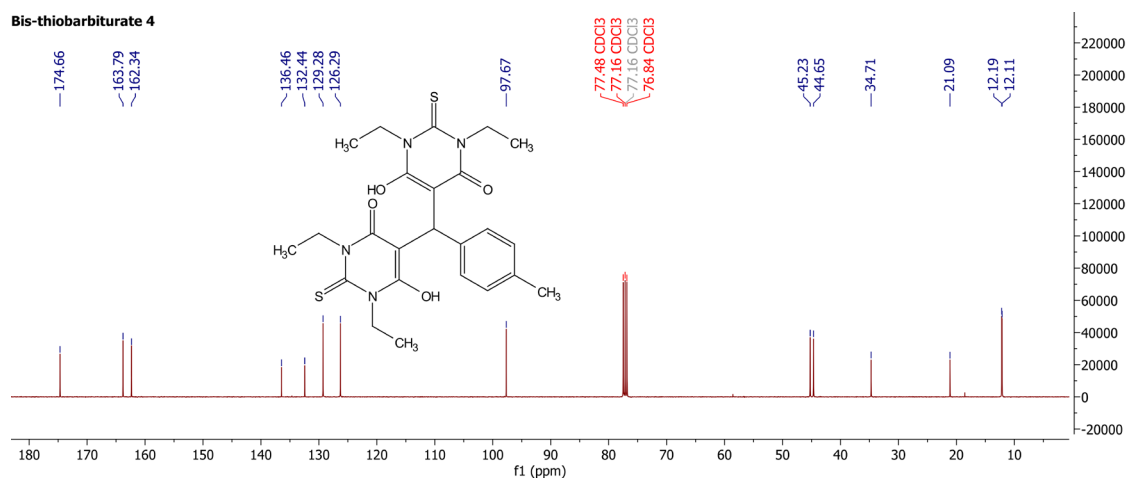


Figure S7 – ^{13}C NMR of bis-thiobarbiturate 4 in CDCl_3 .

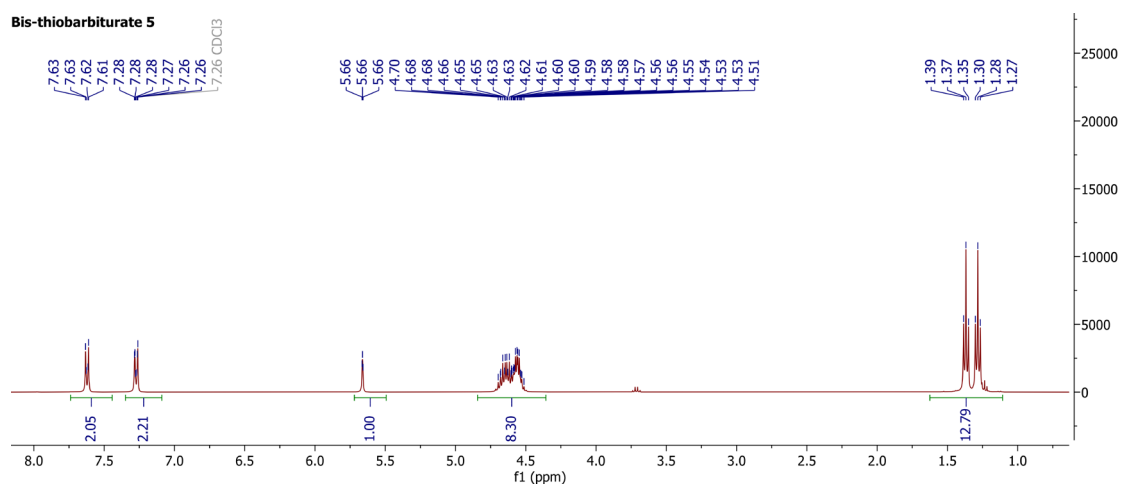


Figure S8 – ^1H NMR of bis-thiobarbiturate 5 in CDCl_3 .

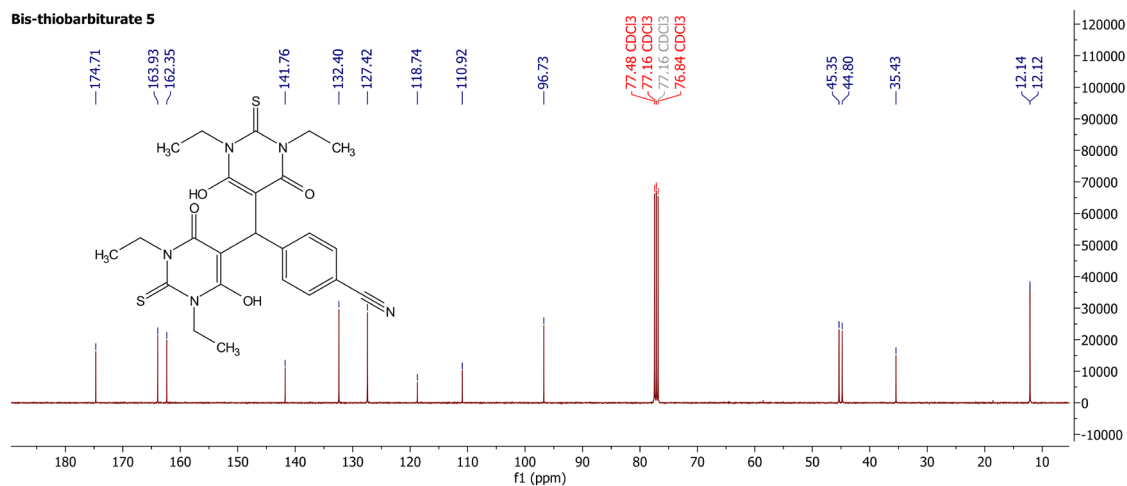


Figure S9 – ^{13}C NMR of bis-thiobarbiturate 5 in CDCl_3 .

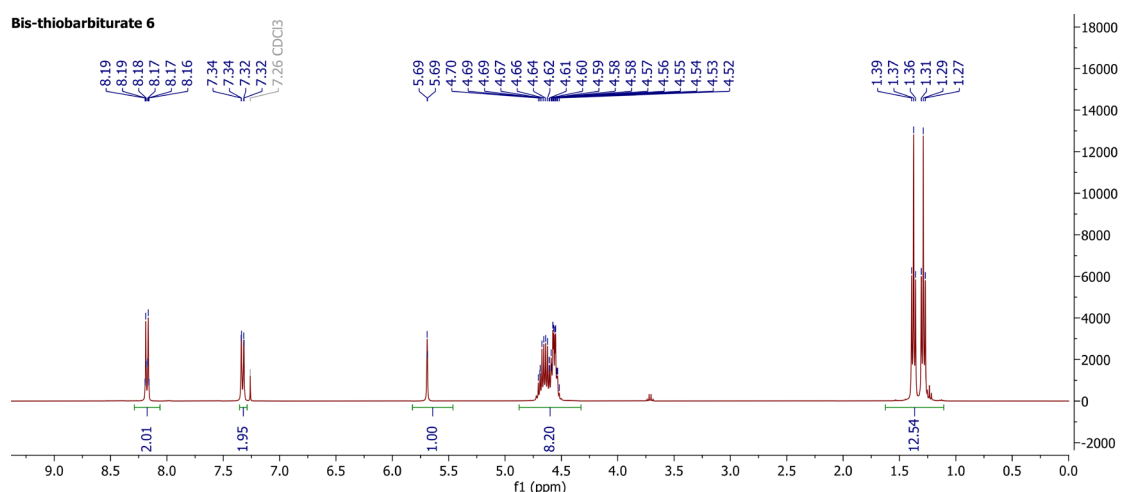


Figure S10 – ^1H NMR of bis-thiobarbiturate 6 in CDCl_3 .

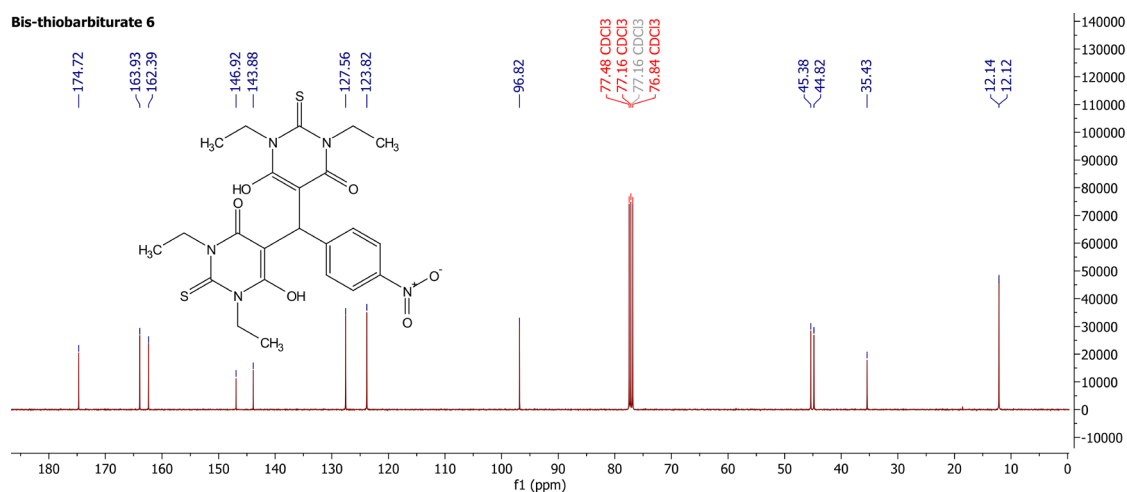


Figure S11 – ^{13}C NMR of bis-thiobarbiturate 6 in CDCl_3 .

Bis-thiobarbiturate 7

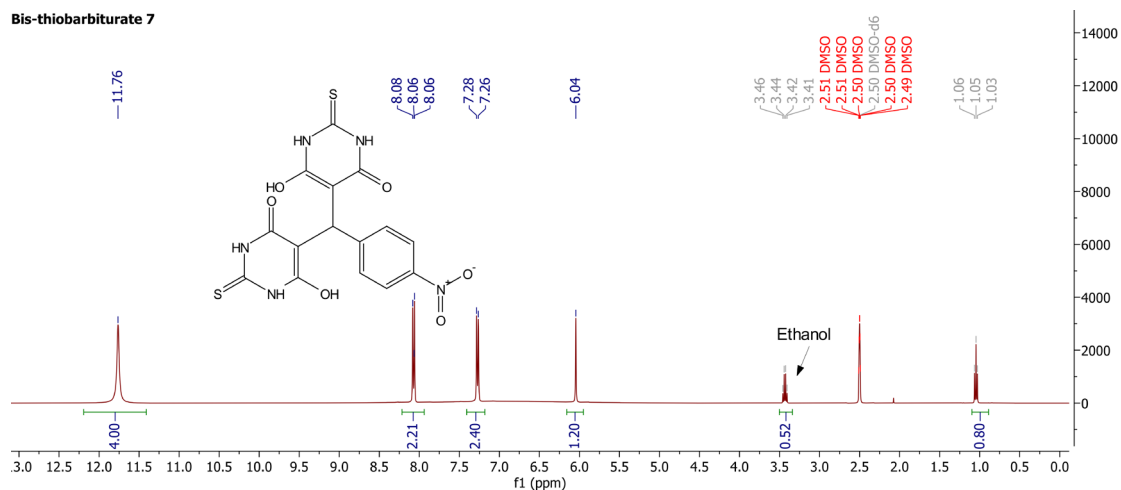


Figure S12 – ^1H NMR of bis-thiobarbiturate 7 in $\text{DMSO-}d_6$.

Bis-thiobarbiturate 7

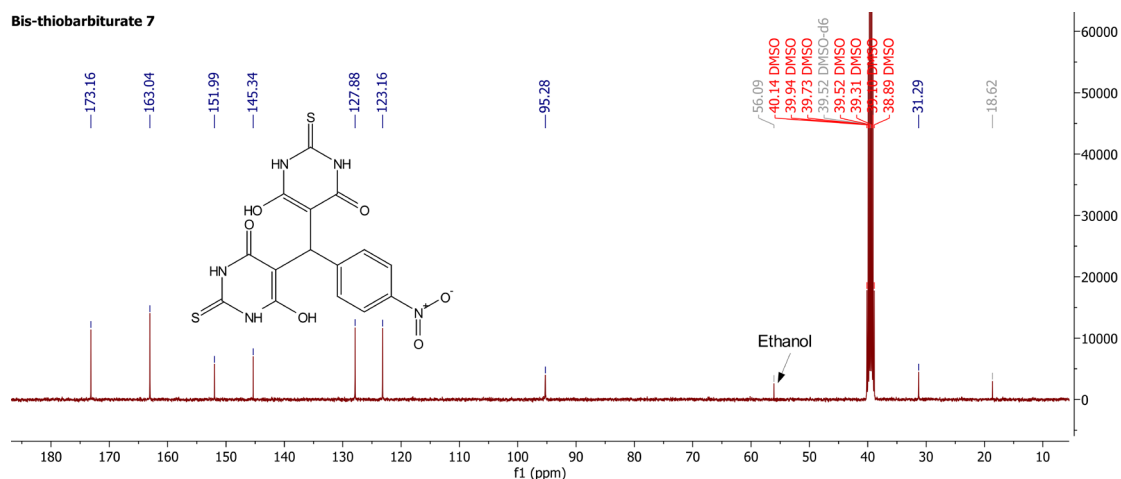


Figure S13 – ^{13}C NMR of bis-thiobarbiturate 7 in $\text{DMSO-}d_6$.

Bis-barbiturate 8

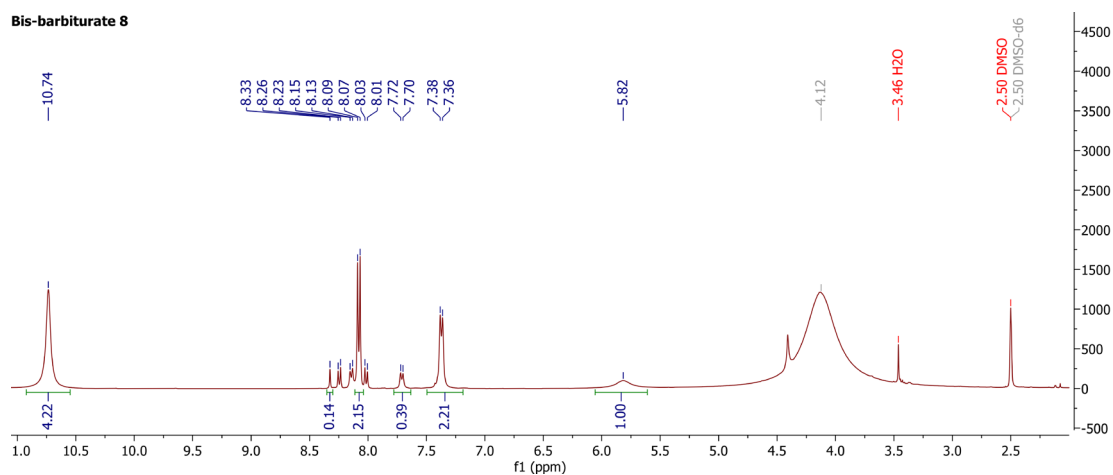


Figure S14 – ^1H NMR of bis-barbiturate 8 in $\text{DMSO-}d_6$.

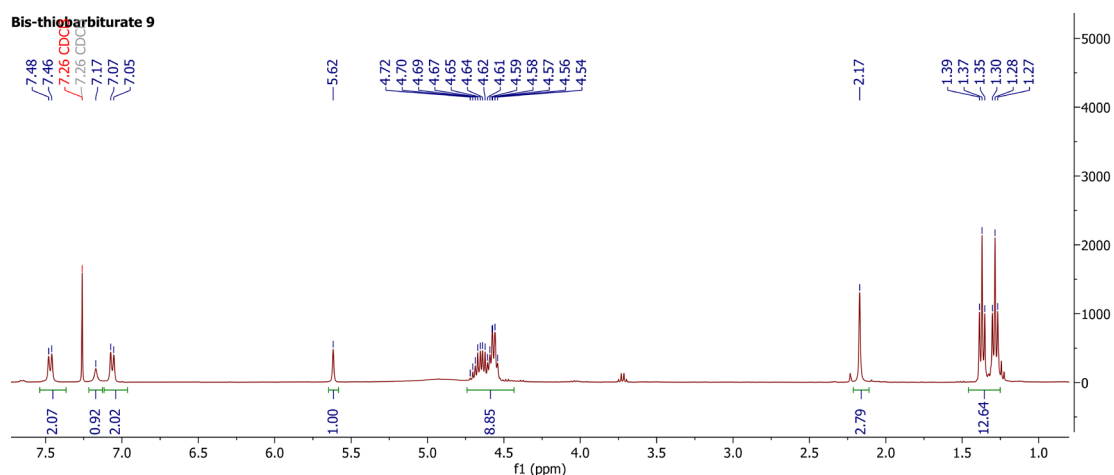


Figure S15 – ¹H NMR of bis-thiobarbiturate **9** in CDCl₃.

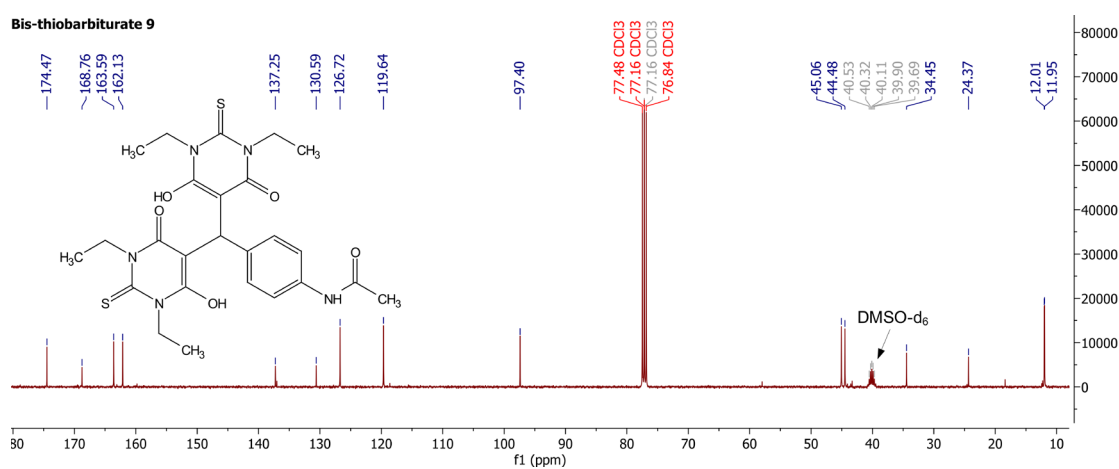


Figure S16 – ¹³C NMR of bis-thiobarbiturate **9** in CDCl₃.

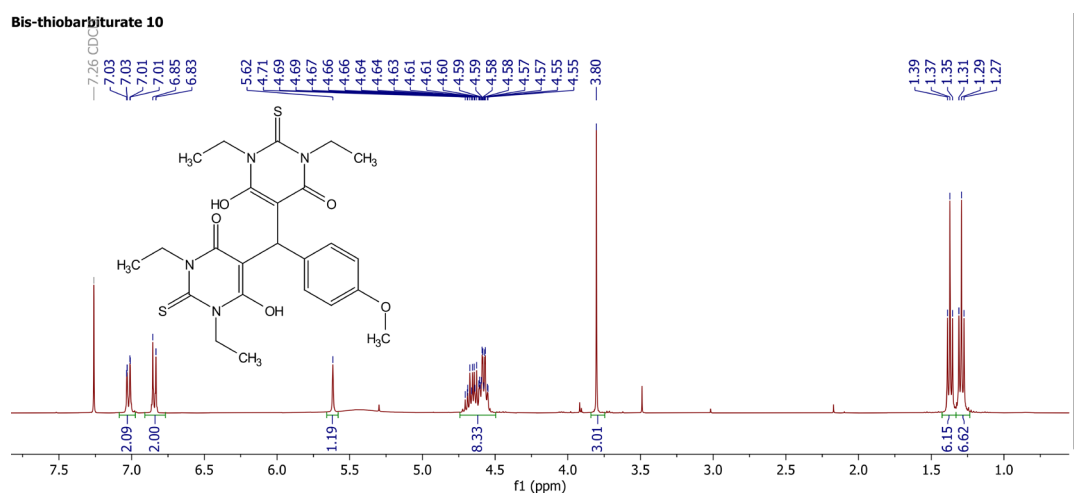


Figure S17 – ¹H NMR of bis-thiobarbiturate **10** in CDCl₃.

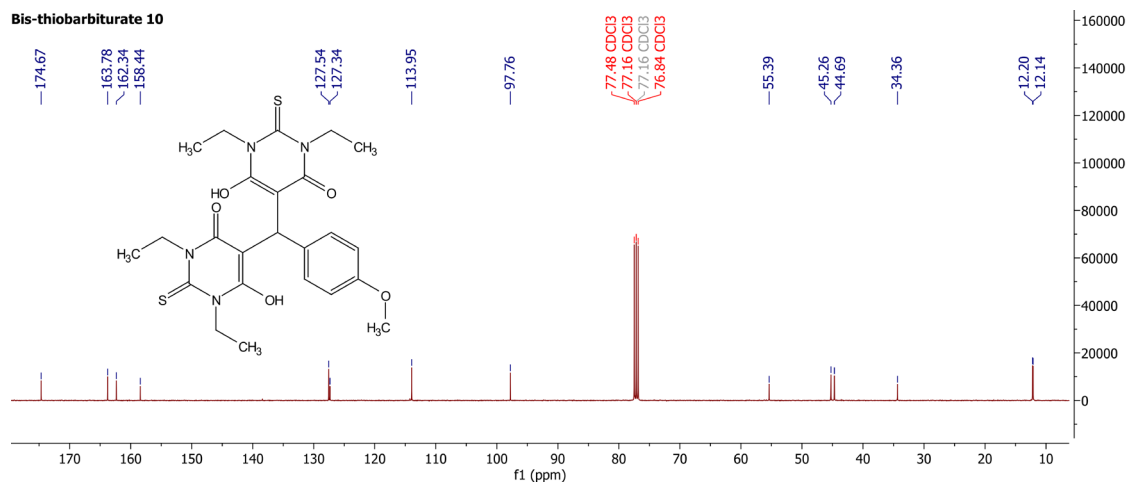


Figure S18 – ¹³C NMR of bis-thiobarbiturate 10 in CDCl₃.

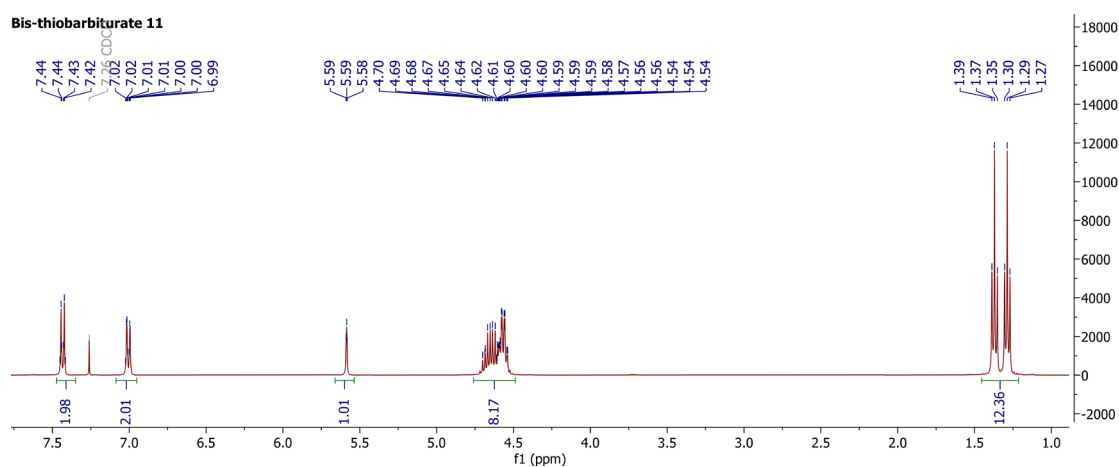


Figure S19 – ¹H NMR of bis-thiobarbiturate 11 in CDCl₃.

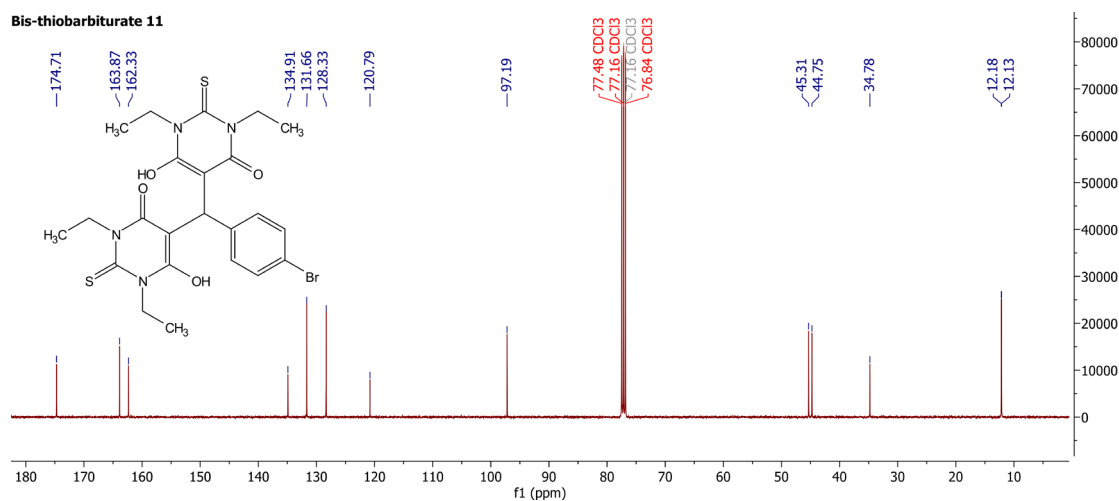


Figure S20 – ¹³C NMR of bis-thiobarbiturate 11 in CDCl₃.

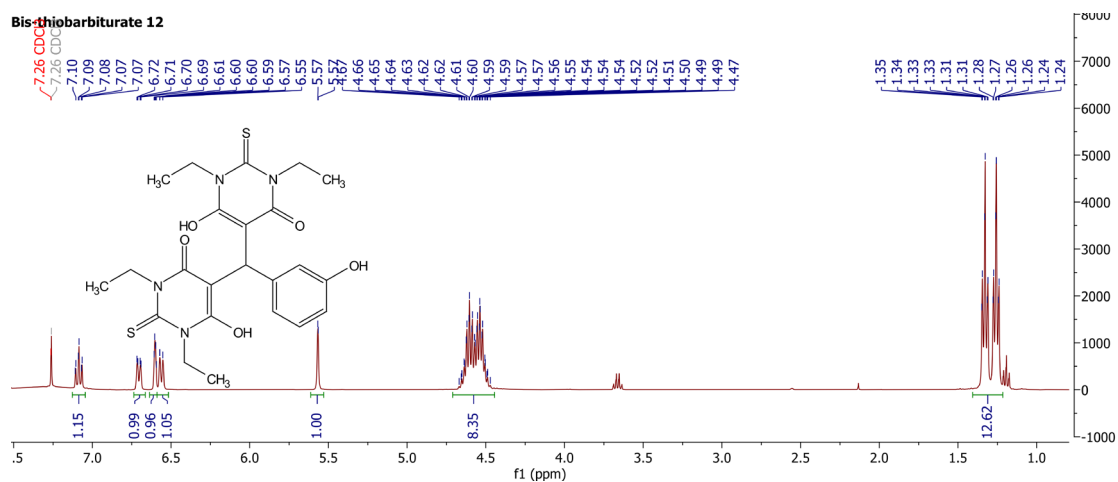


Figure S21 – ^1H NMR of bis-thiobarbiturate **12** in CDCl_3 .

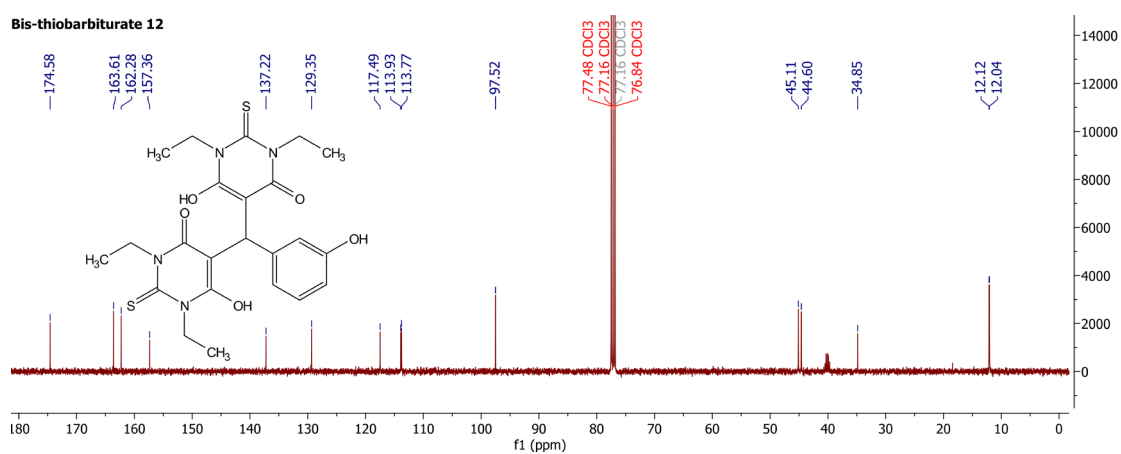


Figure S22 – ^{13}C NMR of bis-thiobarbiturate **12** in CDCl_3 .

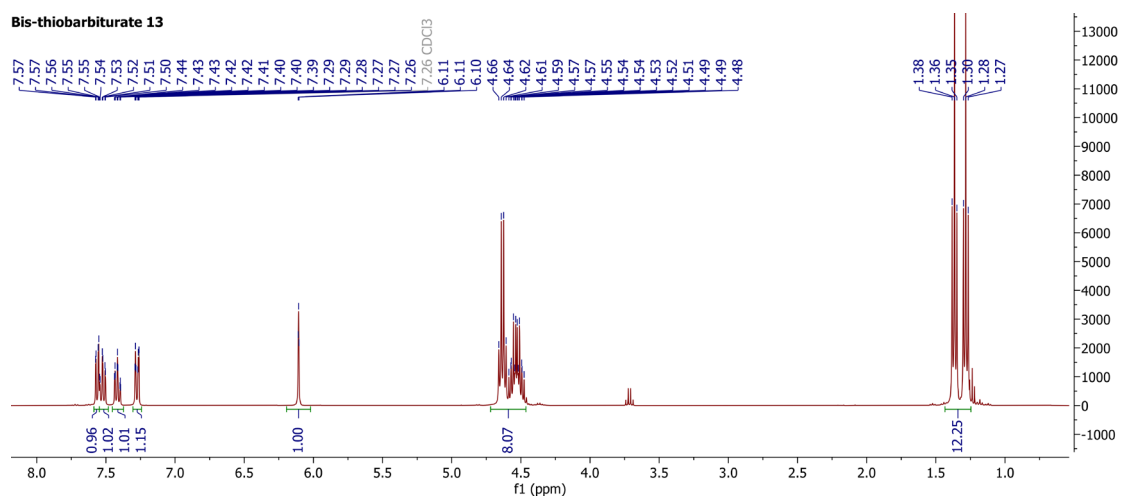


Figure S23 – ^1H NMR of bis-thiobarbiturate **13** in CDCl_3 .

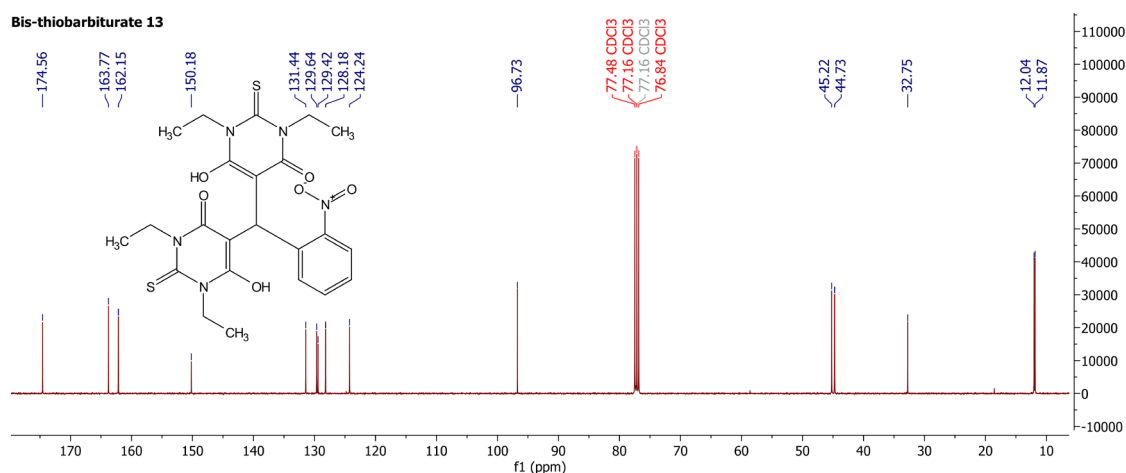


Figure S24 – ¹³C NMR of bis-thiobarbiturate 13 in CDCl₃.

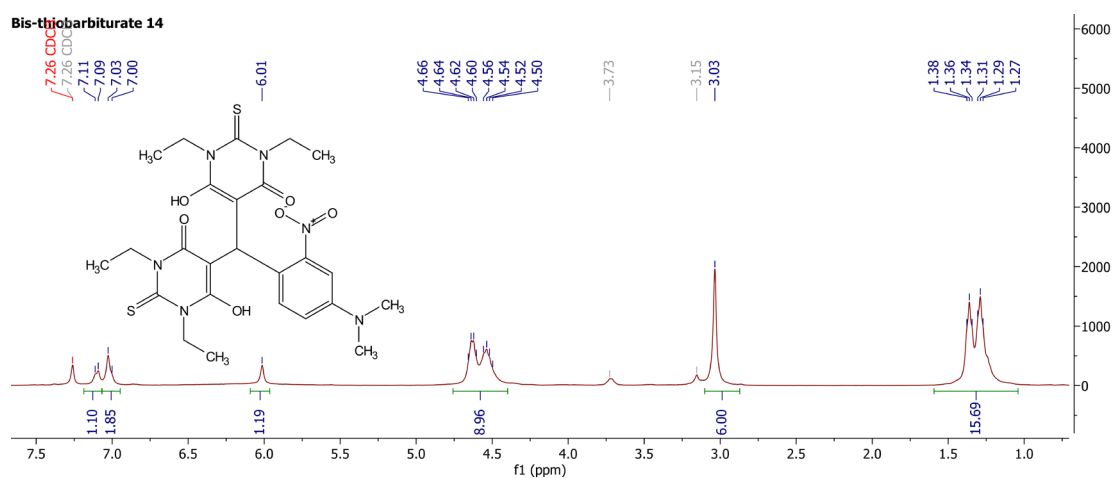


Figure S25 – ¹H NMR of bis-thiobarbiturate 14 in CDCl₃.

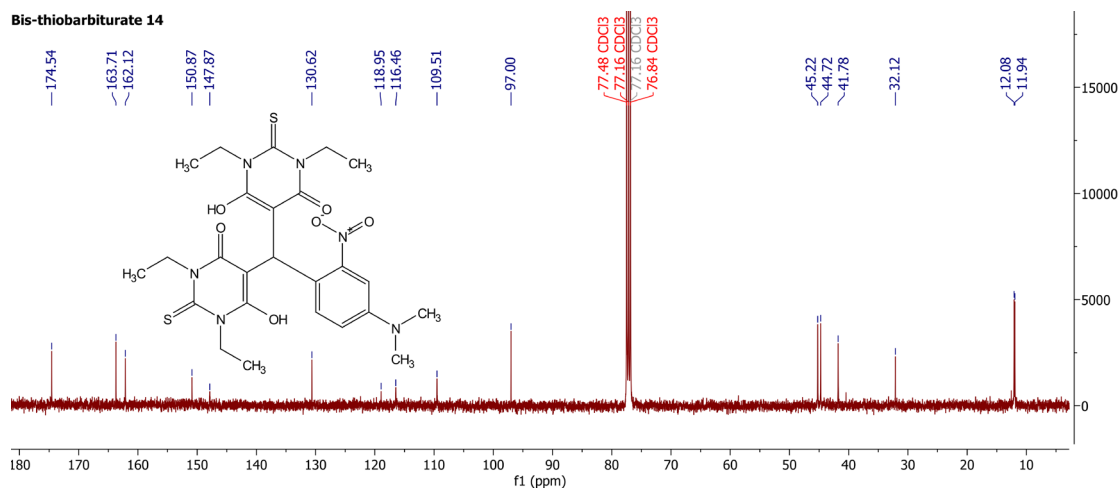


Figure S26 – ¹³C NMR of bis-thiobarbiturate 14 in CDCl₃.

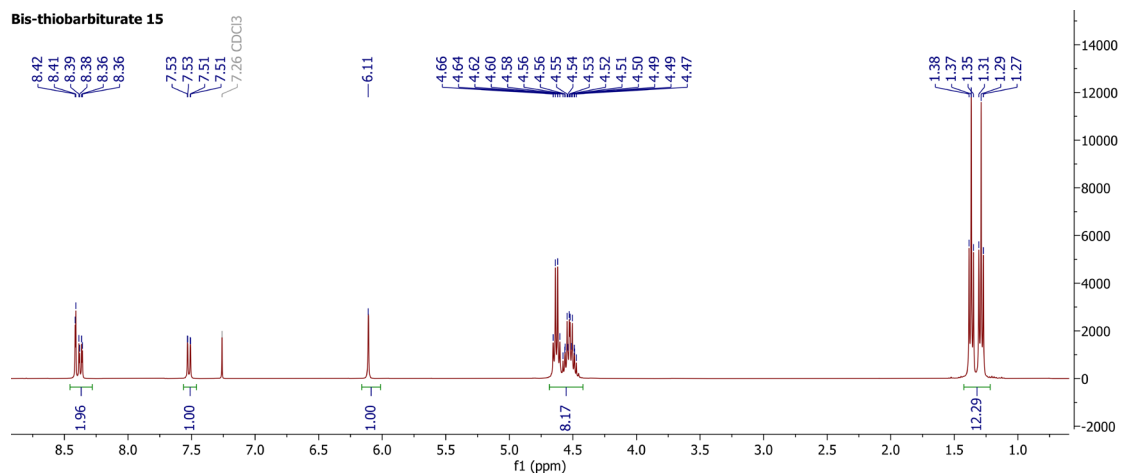


Figure S27 – ¹H NMR of bis-thiobarbiturate **15** in CDCl₃.

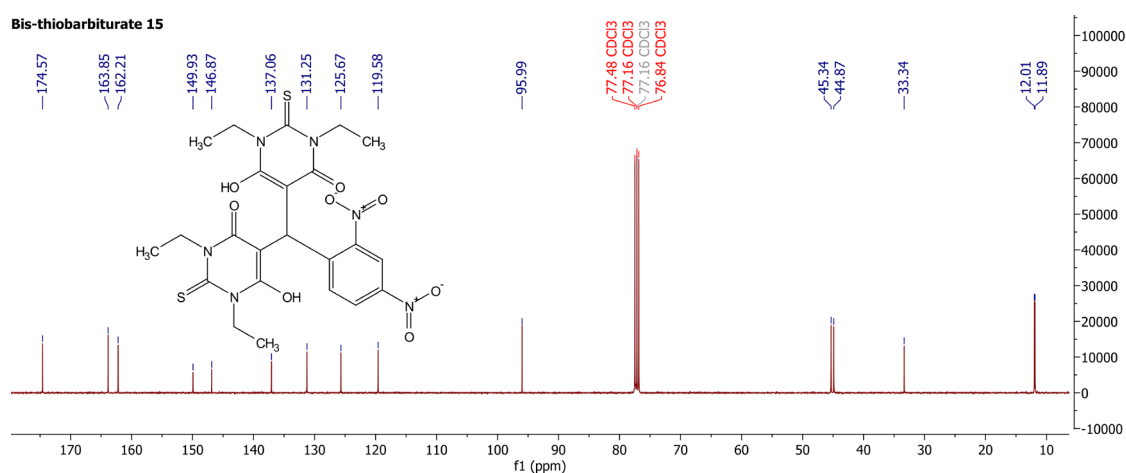


Figure S28 – ¹³C NMR of bis-thiobarbiturate **15** in CDCl₃.

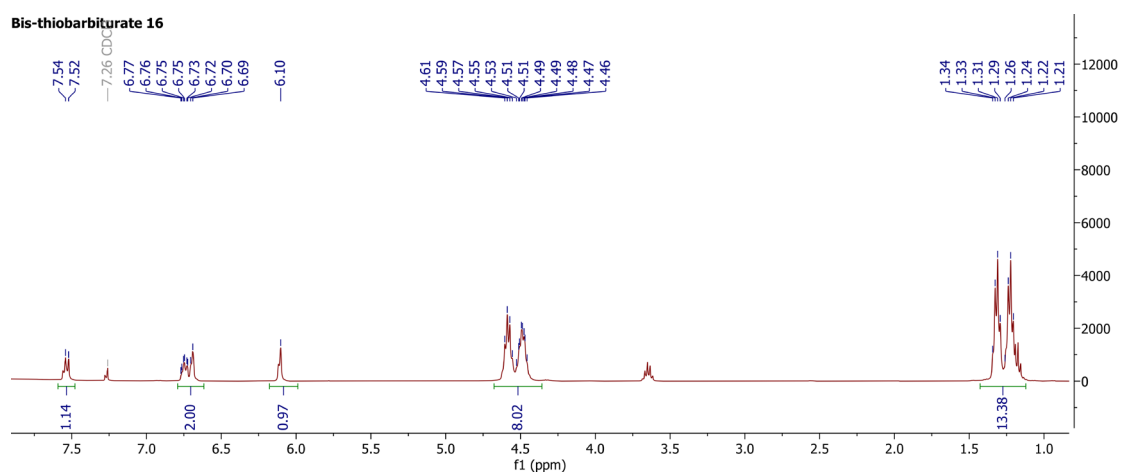


Figure S29 – ¹H NMR of bis-thiobarbiturate **16** in CDCl₃.

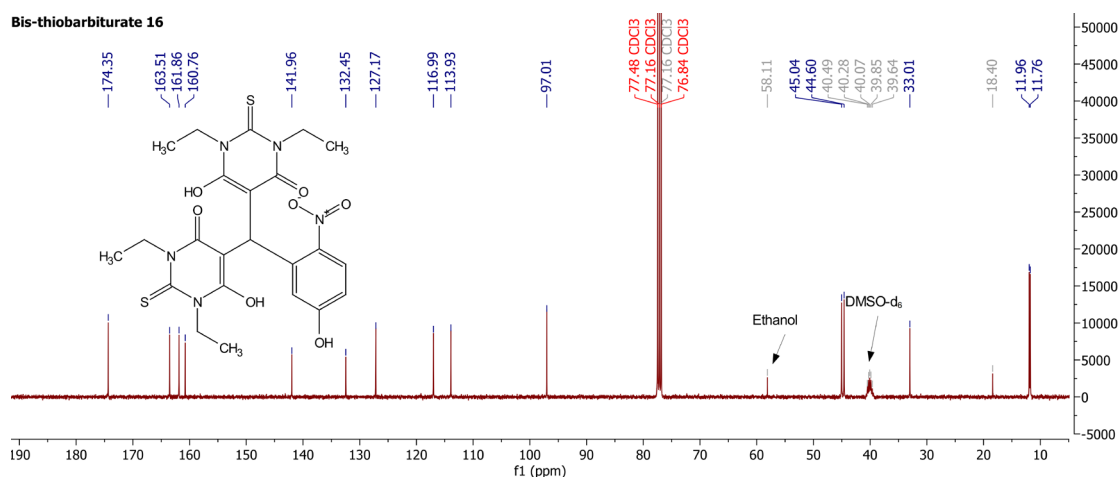


Figure S30 – ¹³C NMR of bis-thiobarbiturate 16 in CDCl₃.

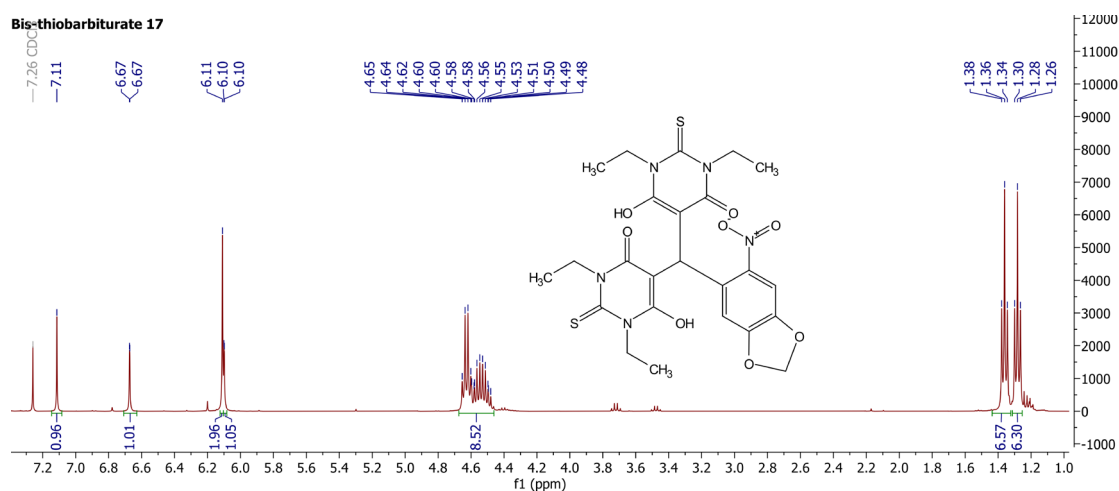


Figure S31 – ¹H NMR of bis-thiobarbiturate 17 in CDCl₃.

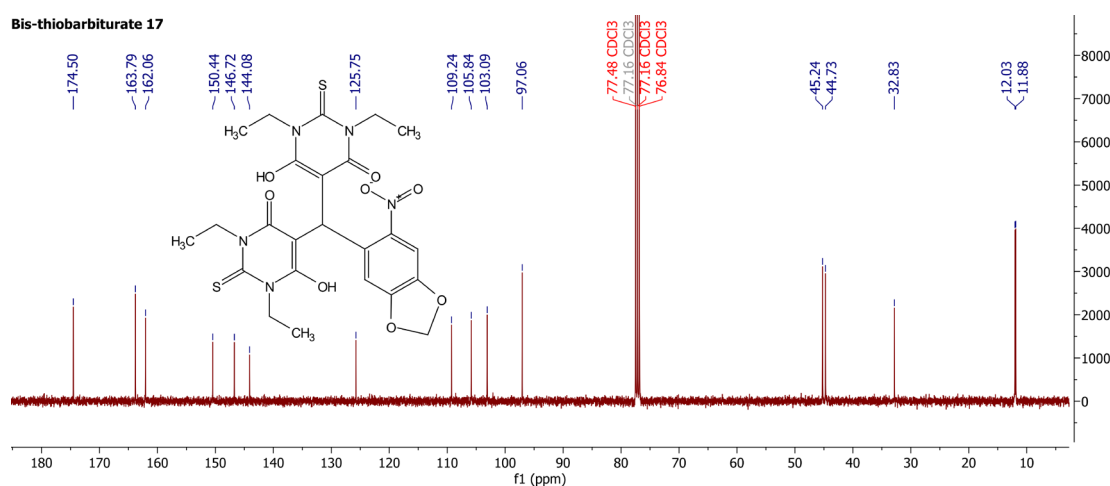


Figure S32 – ¹³C NMR of bis-thiobarbiturate 17 in CDCl₃.

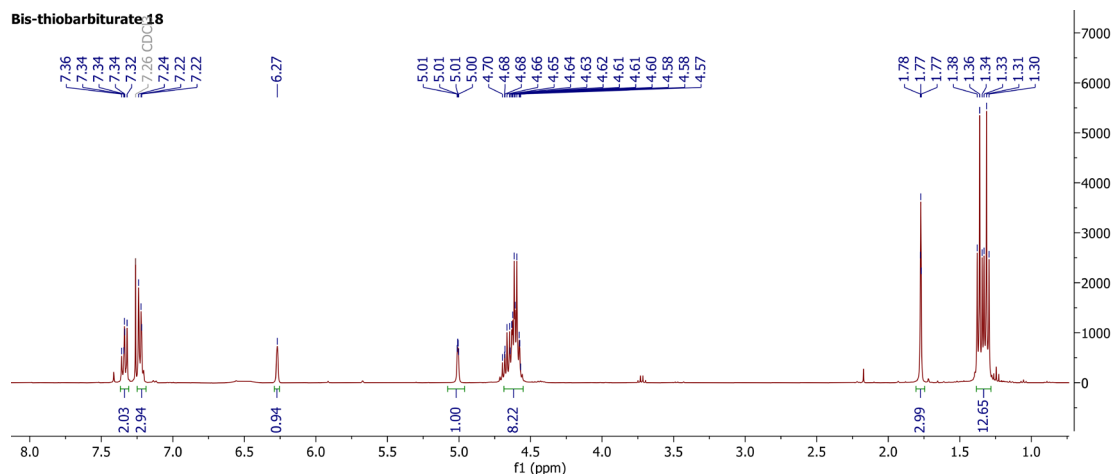


Figure S33 – ^1H NMR of bis-thiobarbiturate **18** in CDCl_3 .

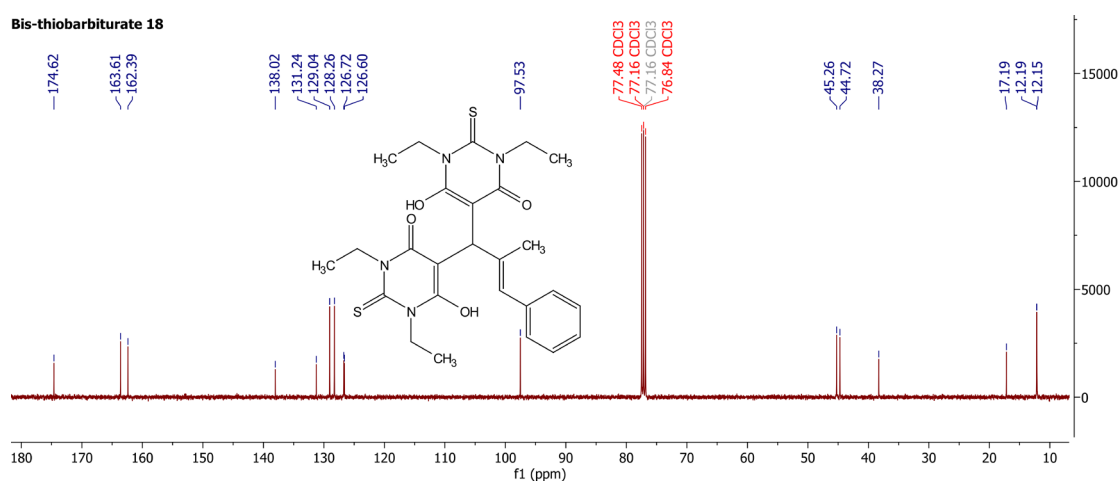


Figure S34 – ^{13}C NMR of bis-thiobarbiturate **18** in CDCl_3 .

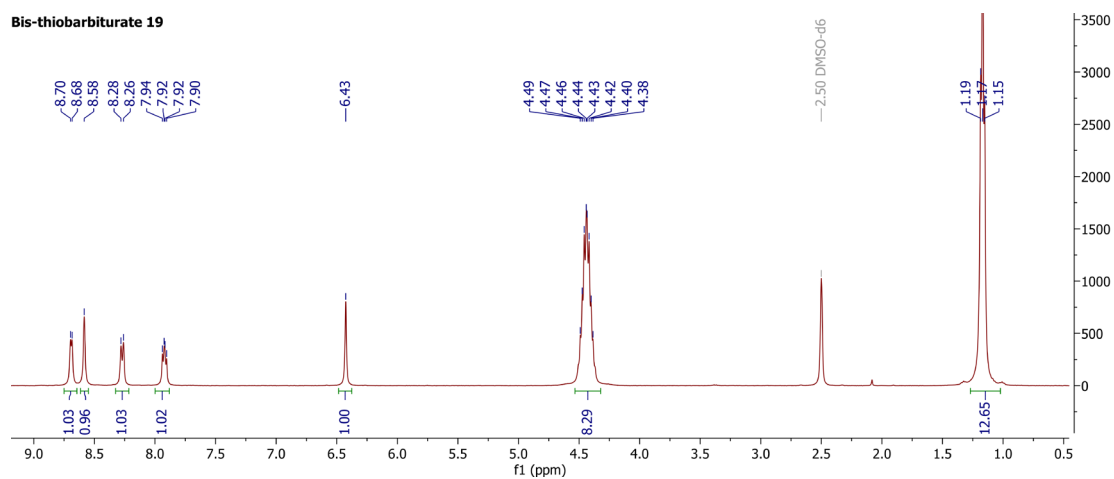


Figure S35 – ^1H NMR of bis-thiobarbiturate **19** in $\text{DMSO}-d_6$.

Bis-thiobarbiturate **19**

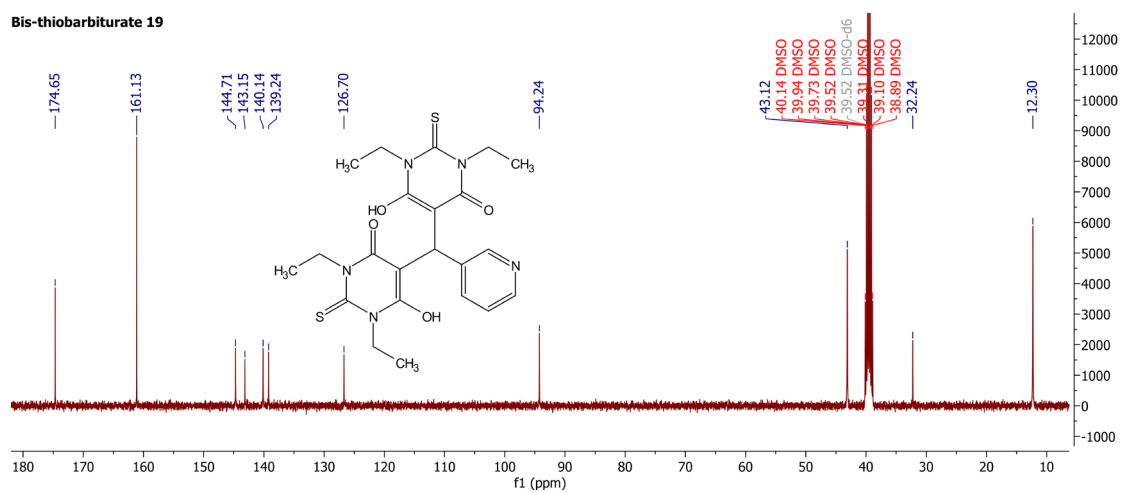


Figure S36 – ¹³C NMR of bis-thiobarbiturate **19** in DMSO-*d*₆.