

Zygo-Albuside A: New Saponin from *Zygophyllum album* L. with Significant Antioxidant, Anti-Inflammatory and Antiapoptotic Effects against Methotrexate-Induced Testicular Damage

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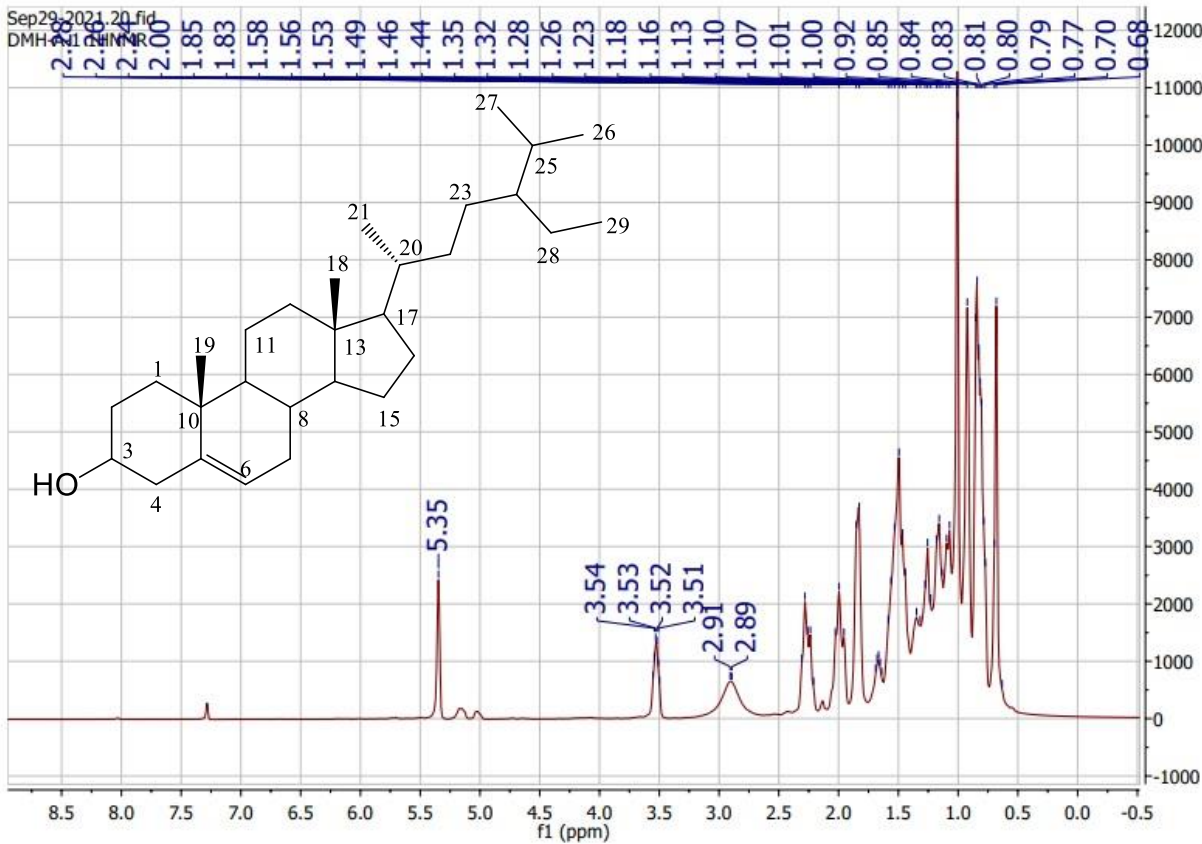


Figure S1: ^1H -NMR spectrum of Compound 1 (500 MHz, CDCl_3).

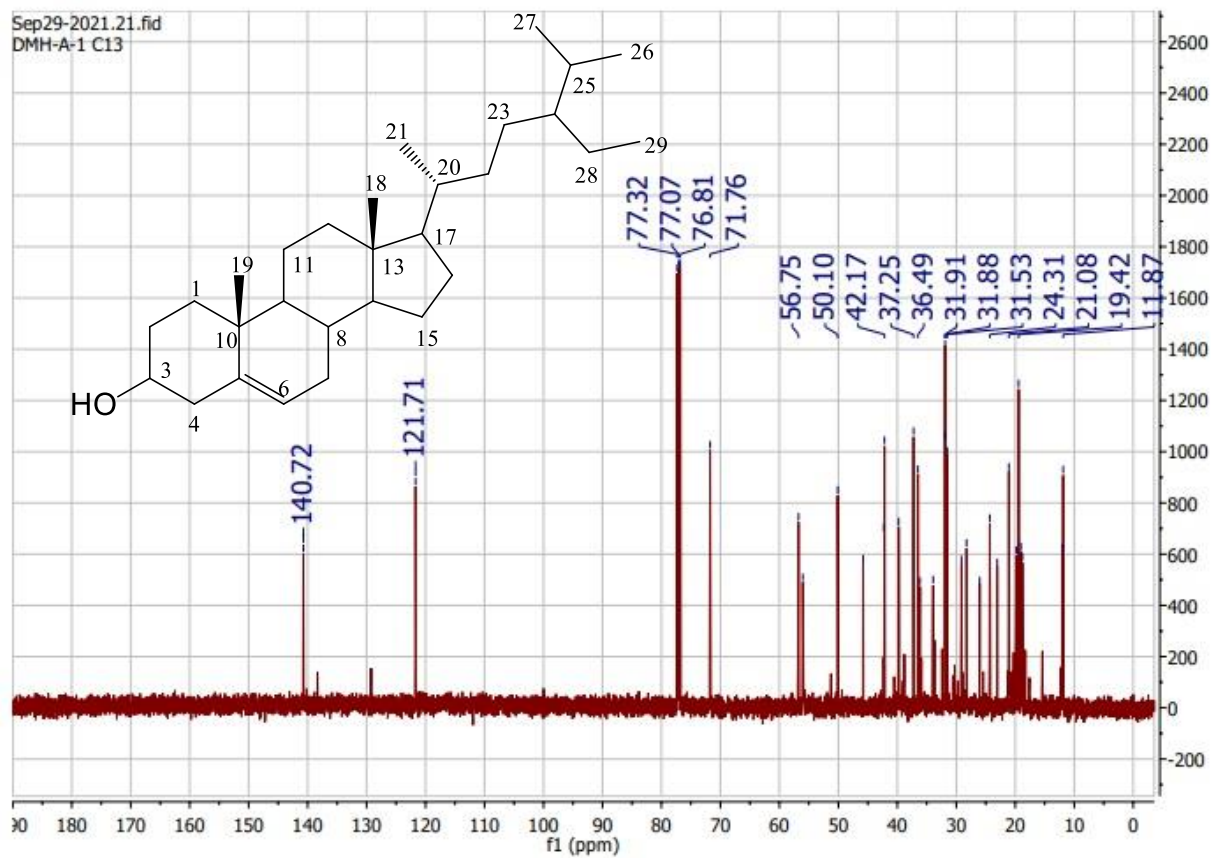


Figure S2: ^{13}C -NMR spectrum of Compound 1 (125 MHz, CDCl_3).

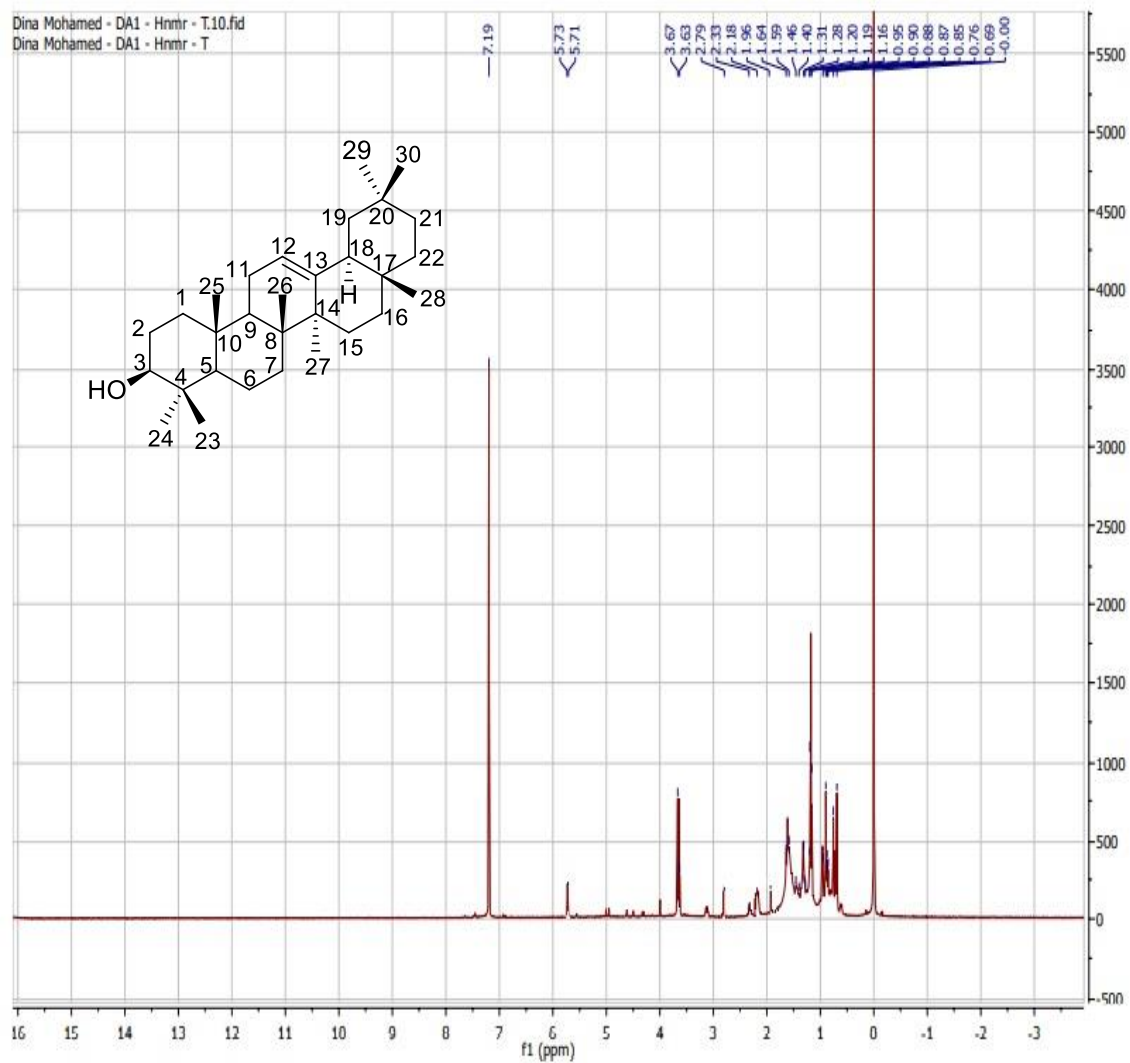


Figure S3: $^1\text{H-NMR}$ spectrum of Compound 2 (500 MHz, CDCl_3).

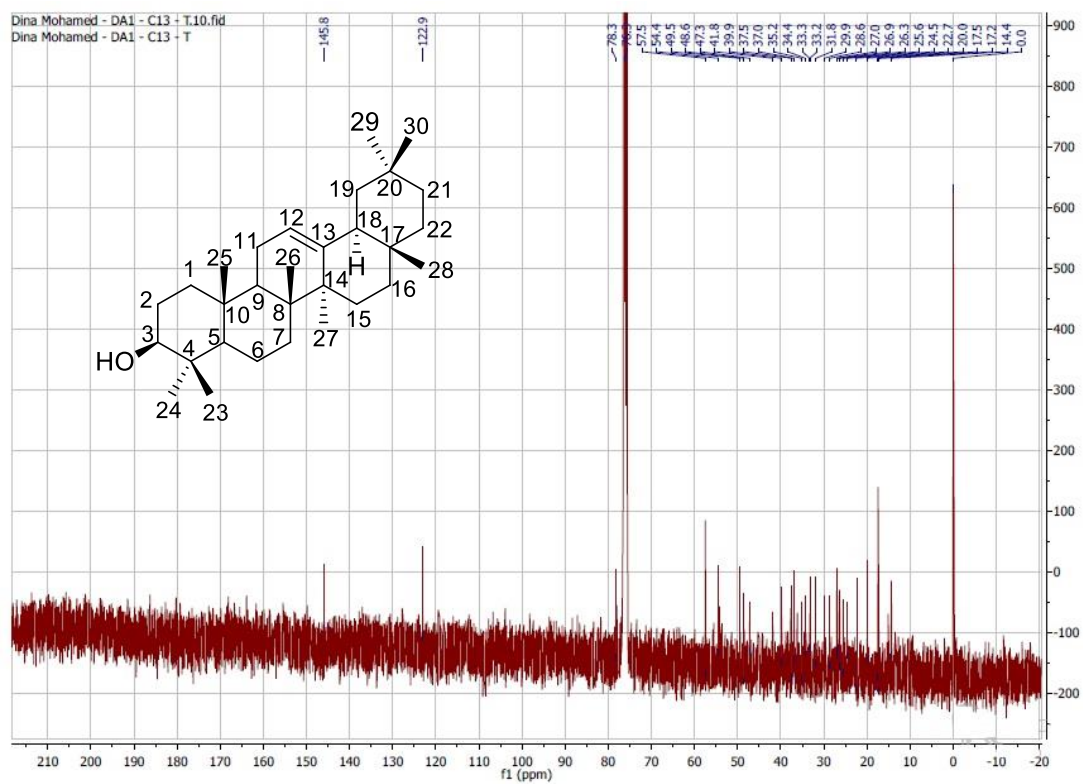


Figure 4: ¹³C-NMR spectrum of Compound 2 (125 MHz, CDCl₃).

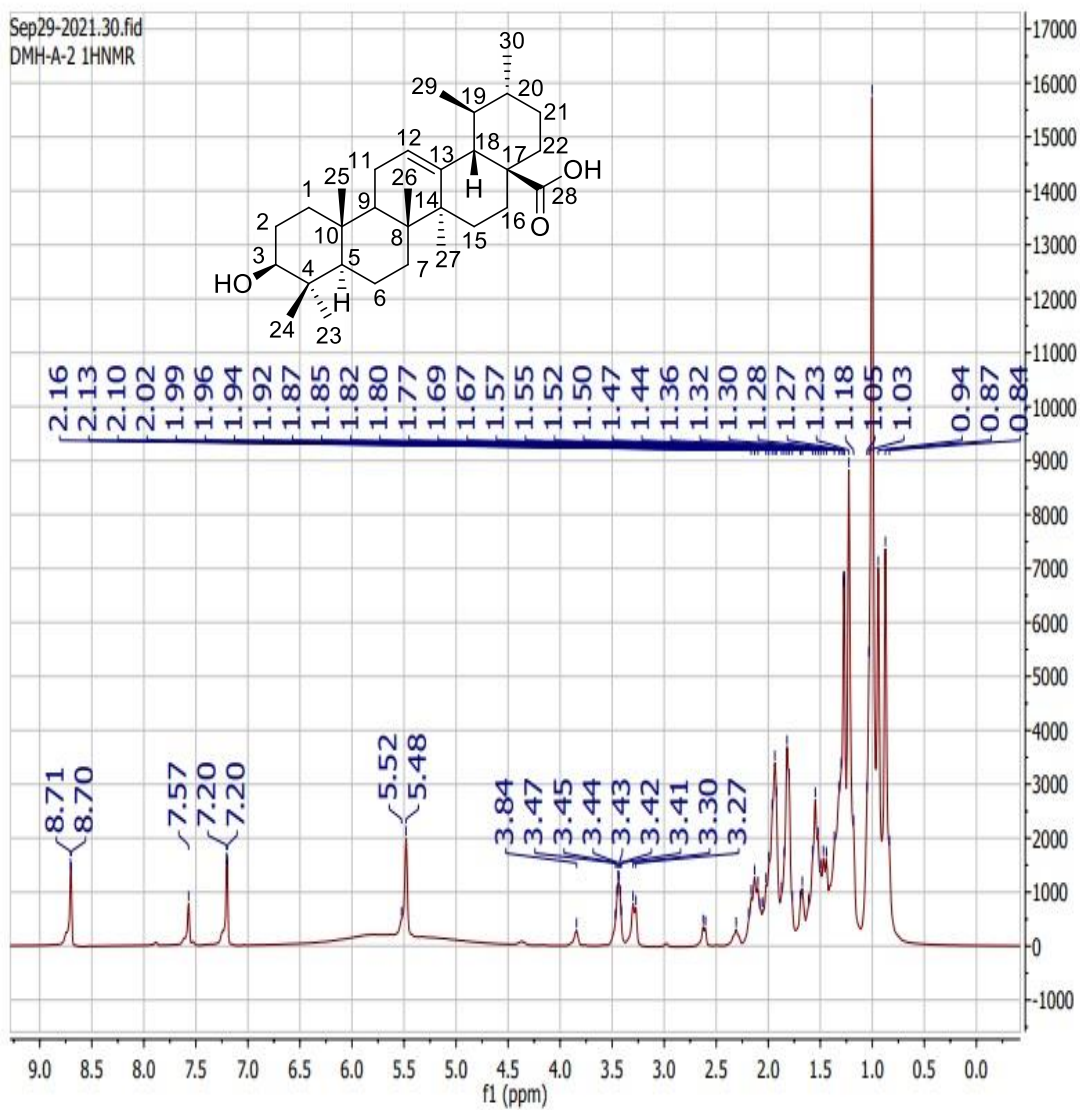


Figure S5: ¹H-NMR spectrum of Compound 3 (500 MHz, C₅D₅N).

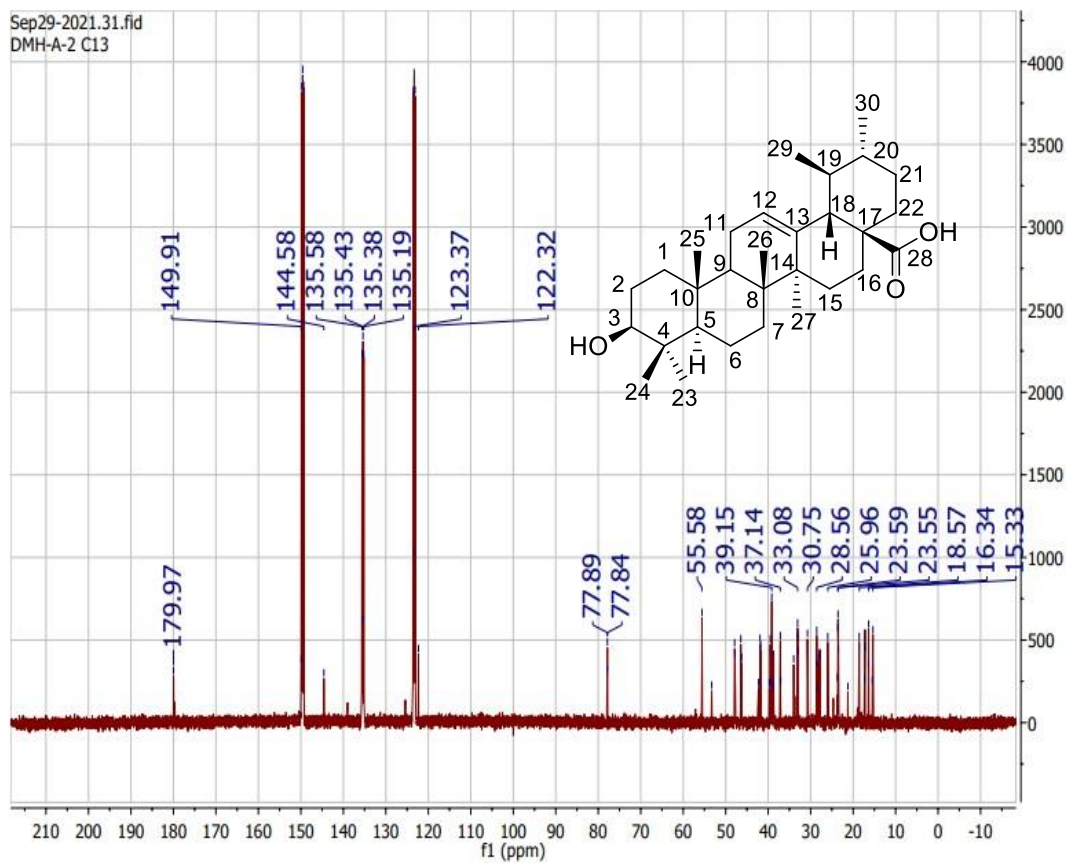


Figure S6: ^{13}C -NMR spectrum of Compound 3 (125 MHz, $\text{C}_5\text{D}_5\text{N}$).

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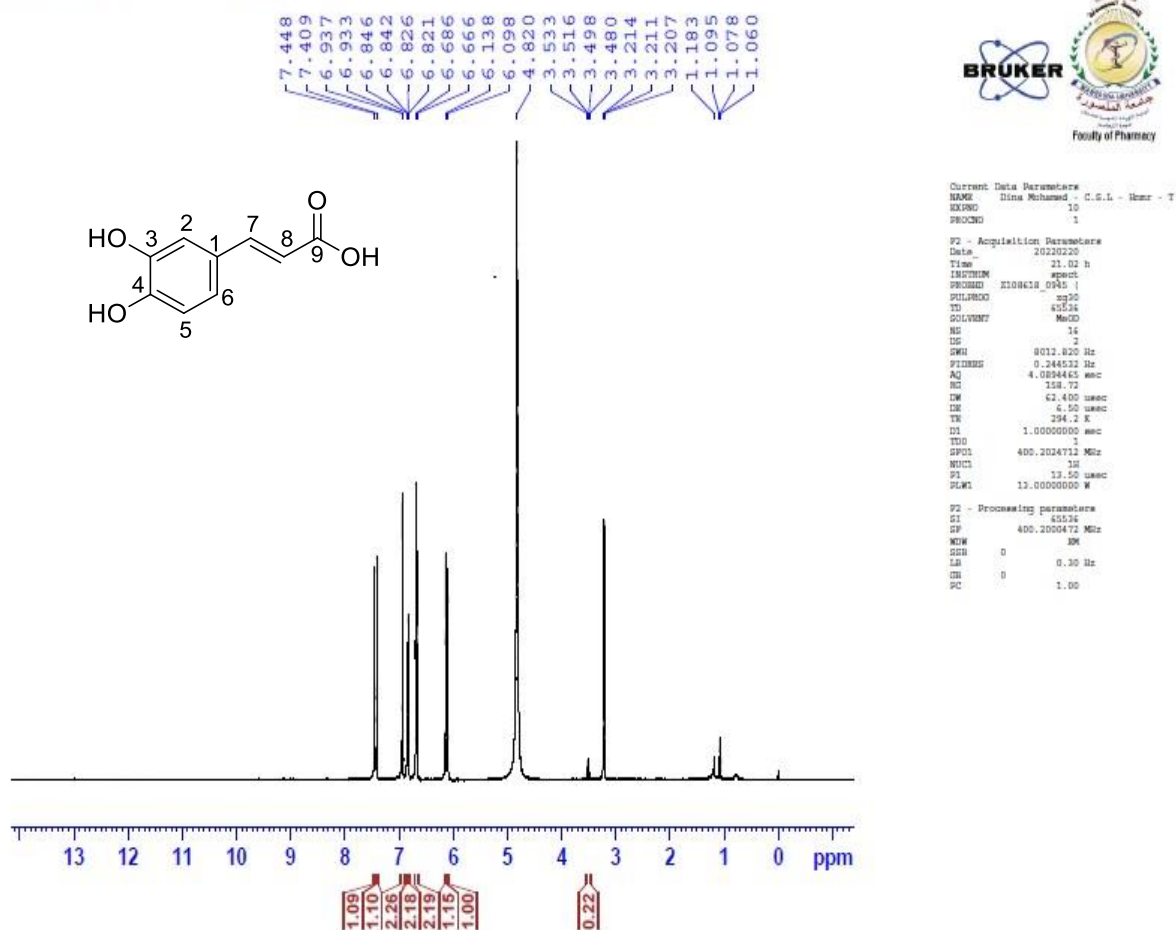


Figure S7: ¹H-NMR spectrum of Compound 4 (500 MHz, MeOH).

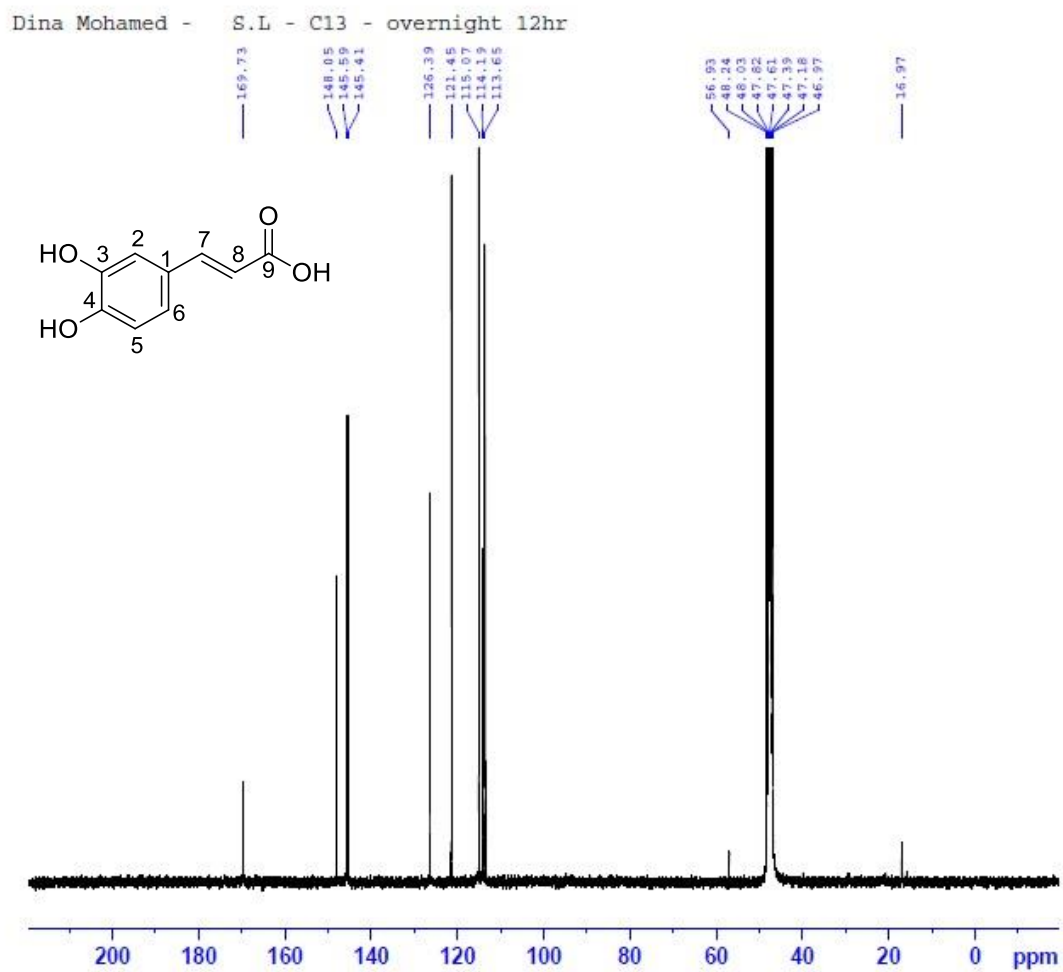


Figure S8: ^{13}C -NMR spectrum of Compound 4 (125 MHz, MeOH).

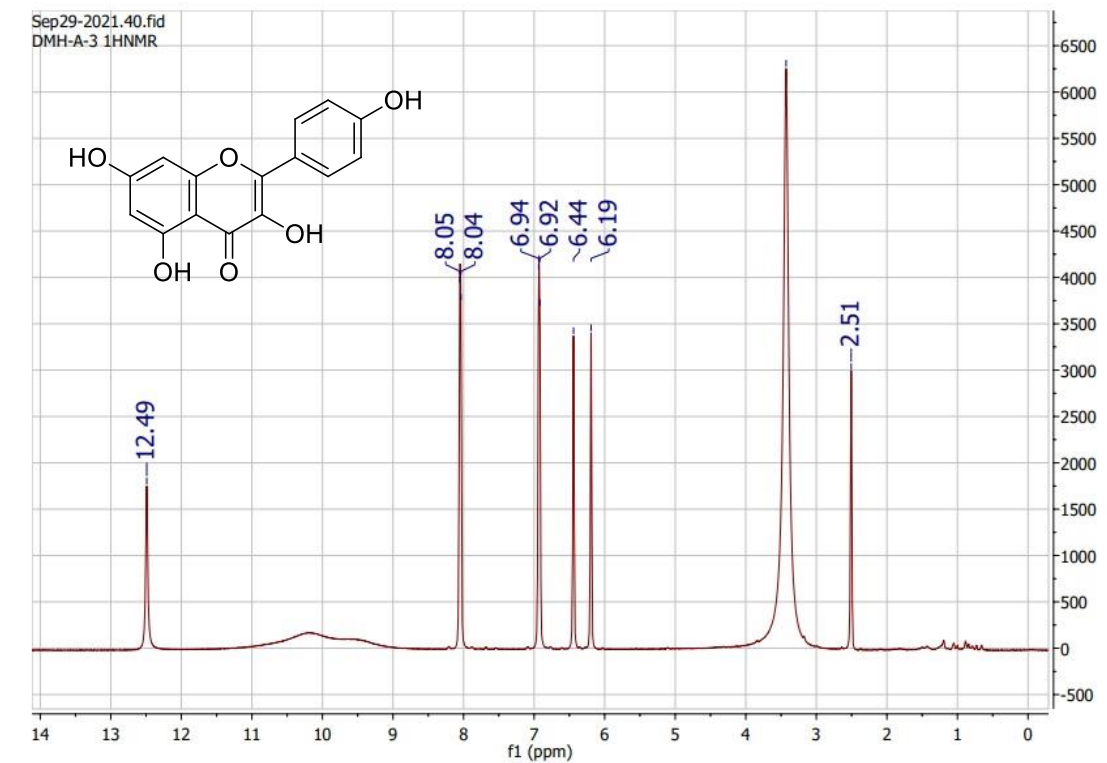


Figure S9: ^1H -NMR spectrum of Compound 5 (500 MHz, DMSO- d_6).

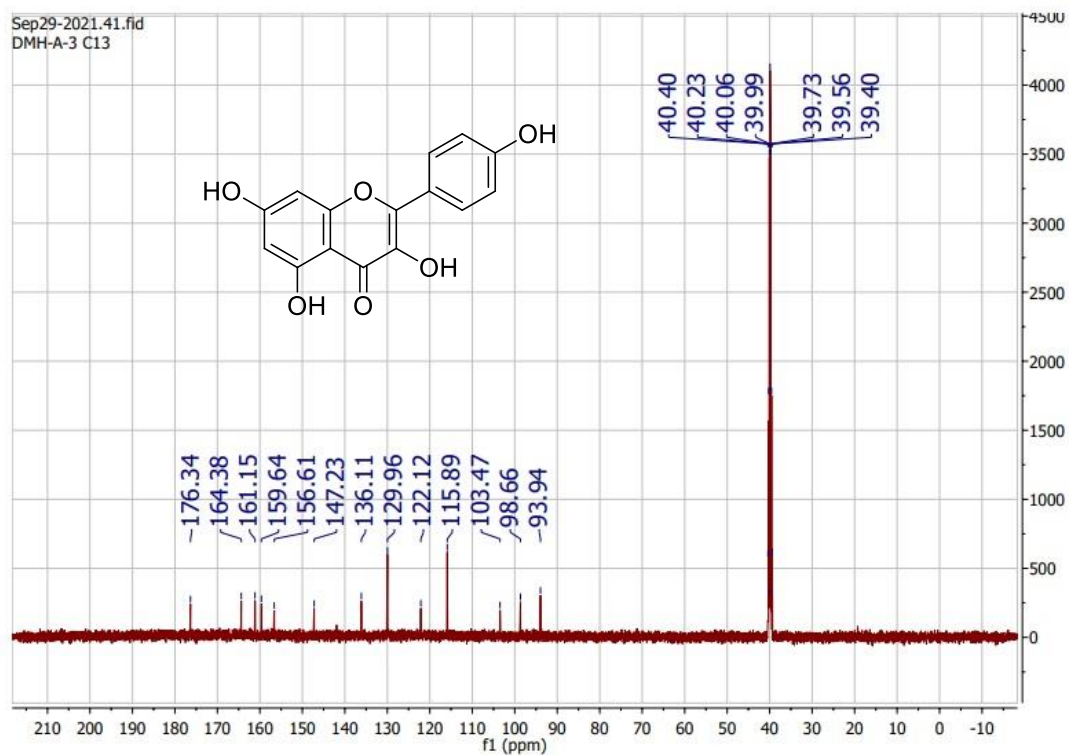


Figure S10: ^{13}C -NMR spectrum of Compound 5 (125 MHz, DMSO- d_6).

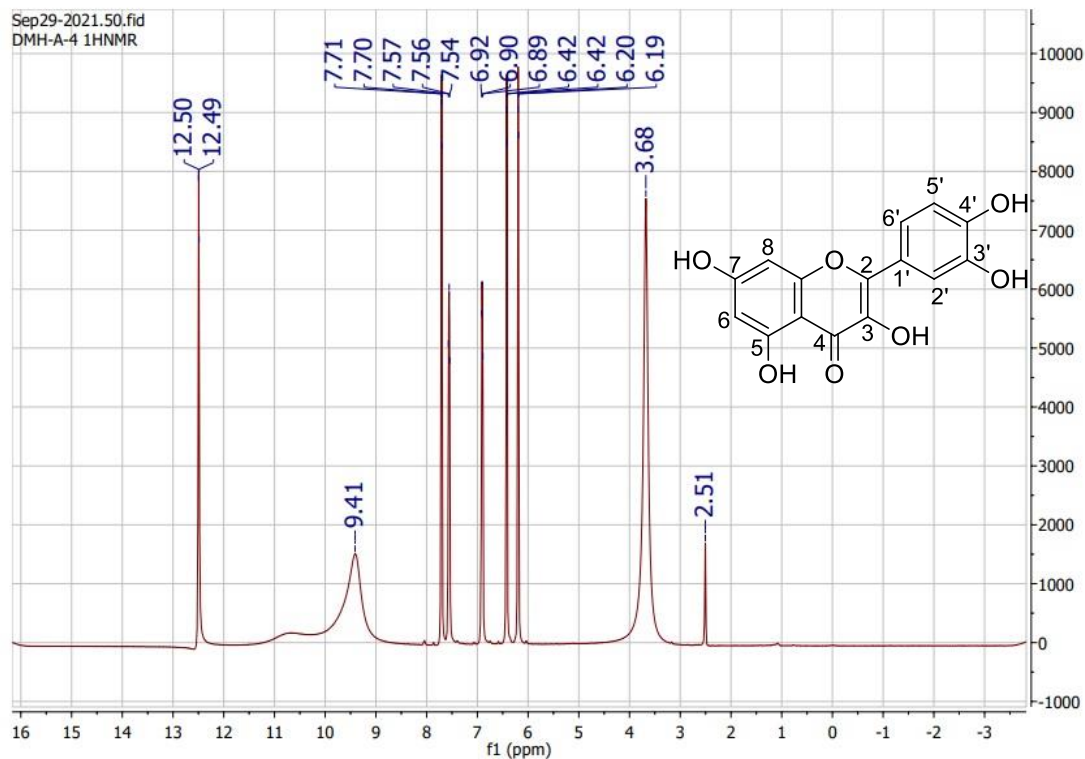


Figure S11: ^1H -NMR spectrum of Compound 6 (500 MHz, DMSO- d_6).

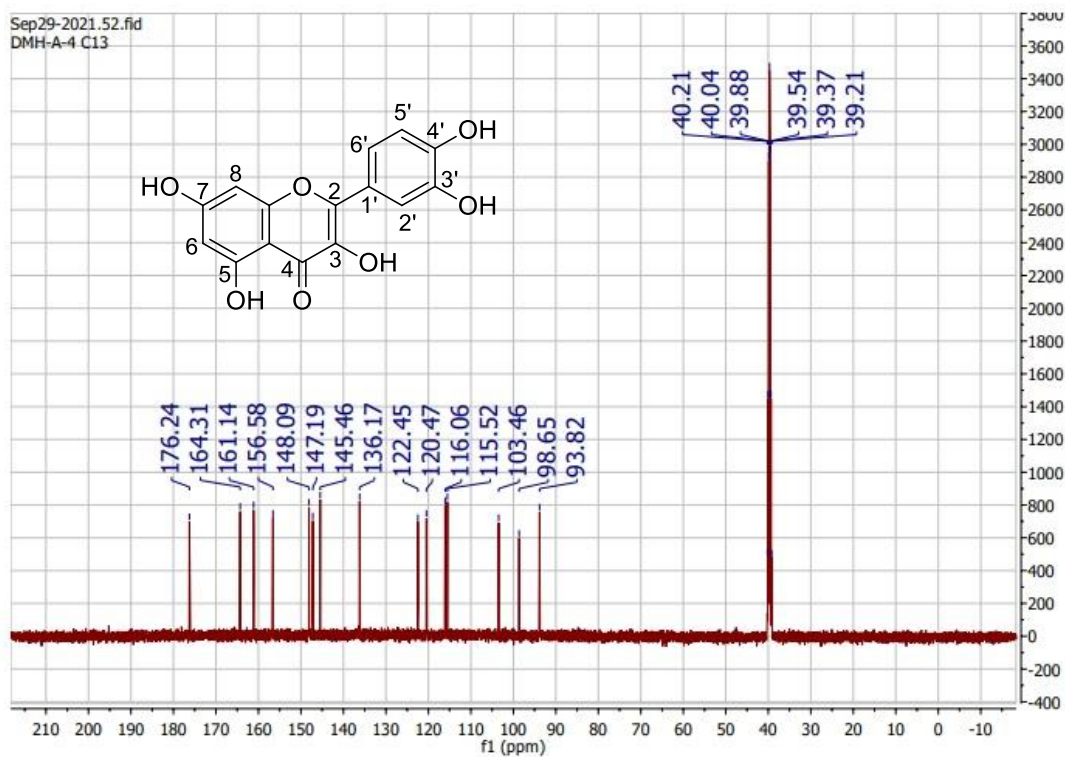


Figure S12: ^{13}C -NMR spectrum of Compound 6 (125 MHz, DMSO- d_6).

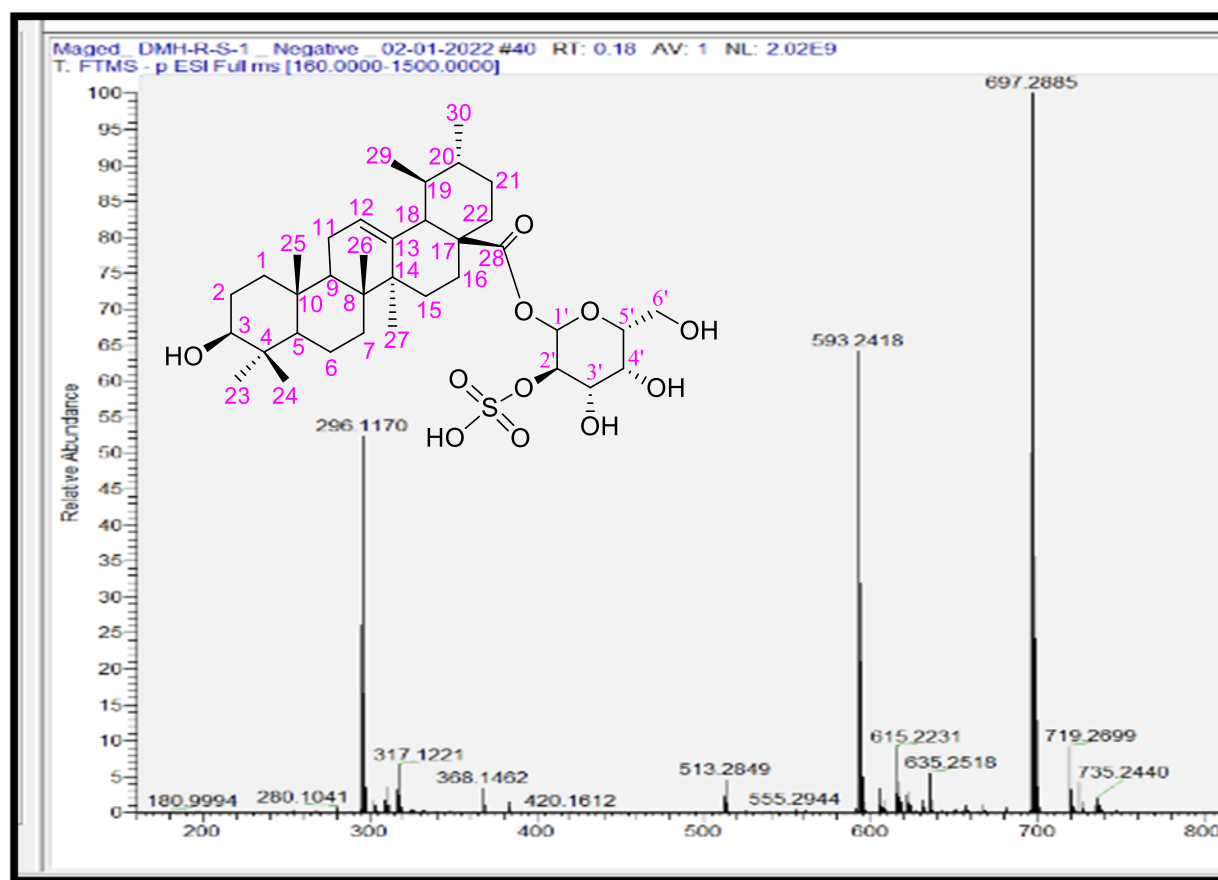


Figure S13: HRMS of Compound 7

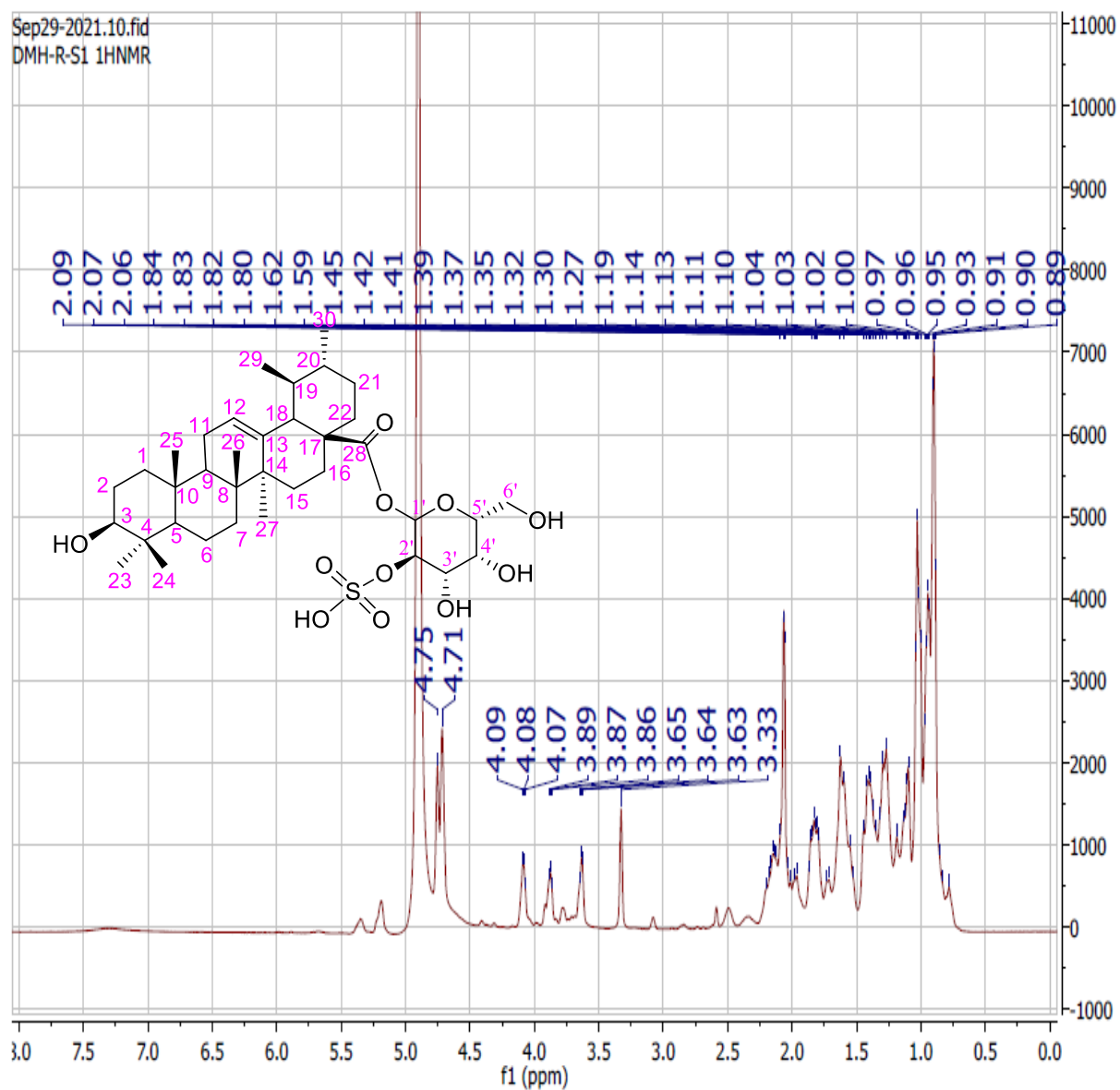


Figure S14: ^1H -NMR spectrum of Compound 7 (500 MHz, MeOH).

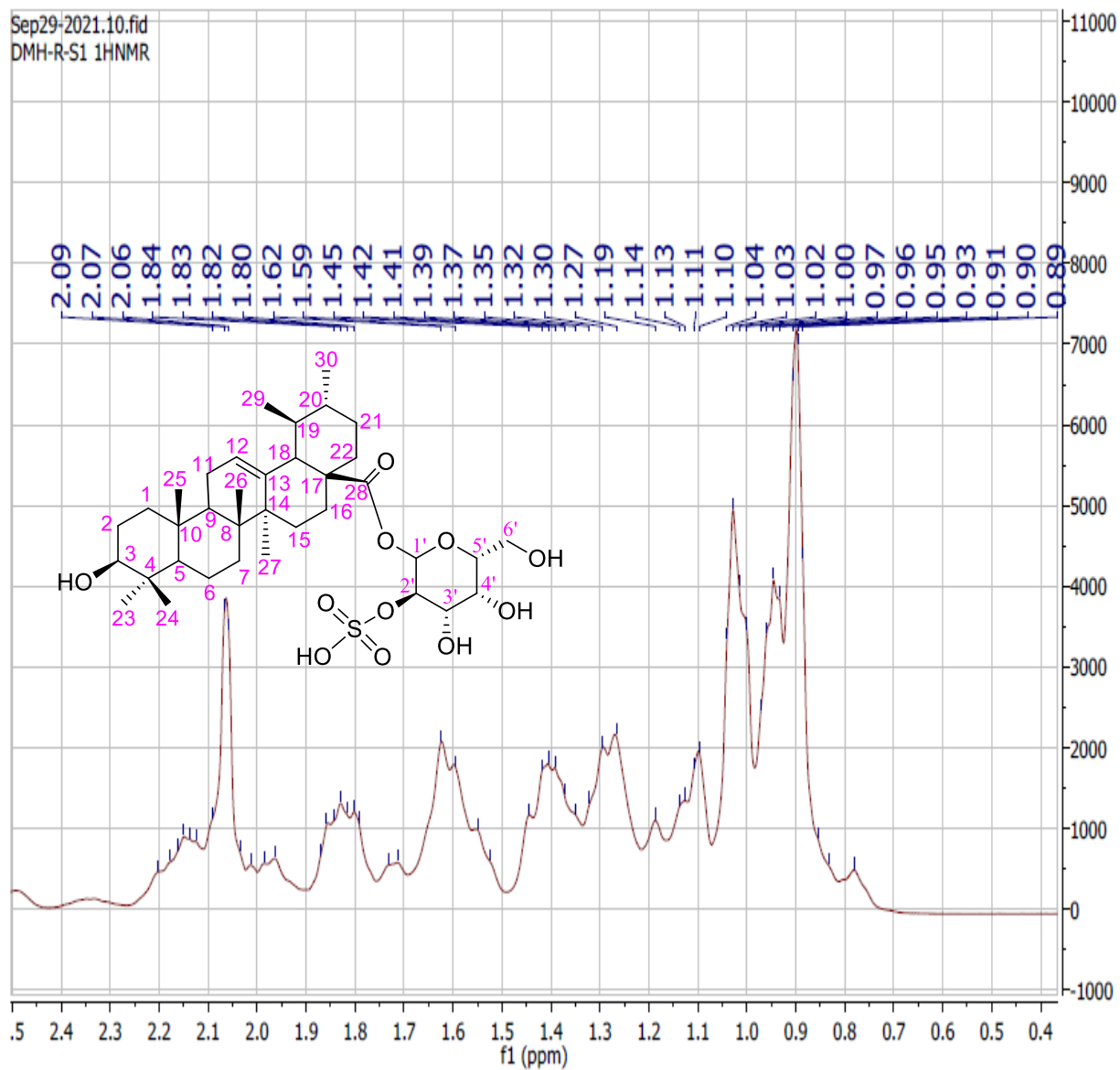


Figure S15: Partial expansion of ^1H -NMR spectrum of Compound 7 (500 MHz, MeOH).

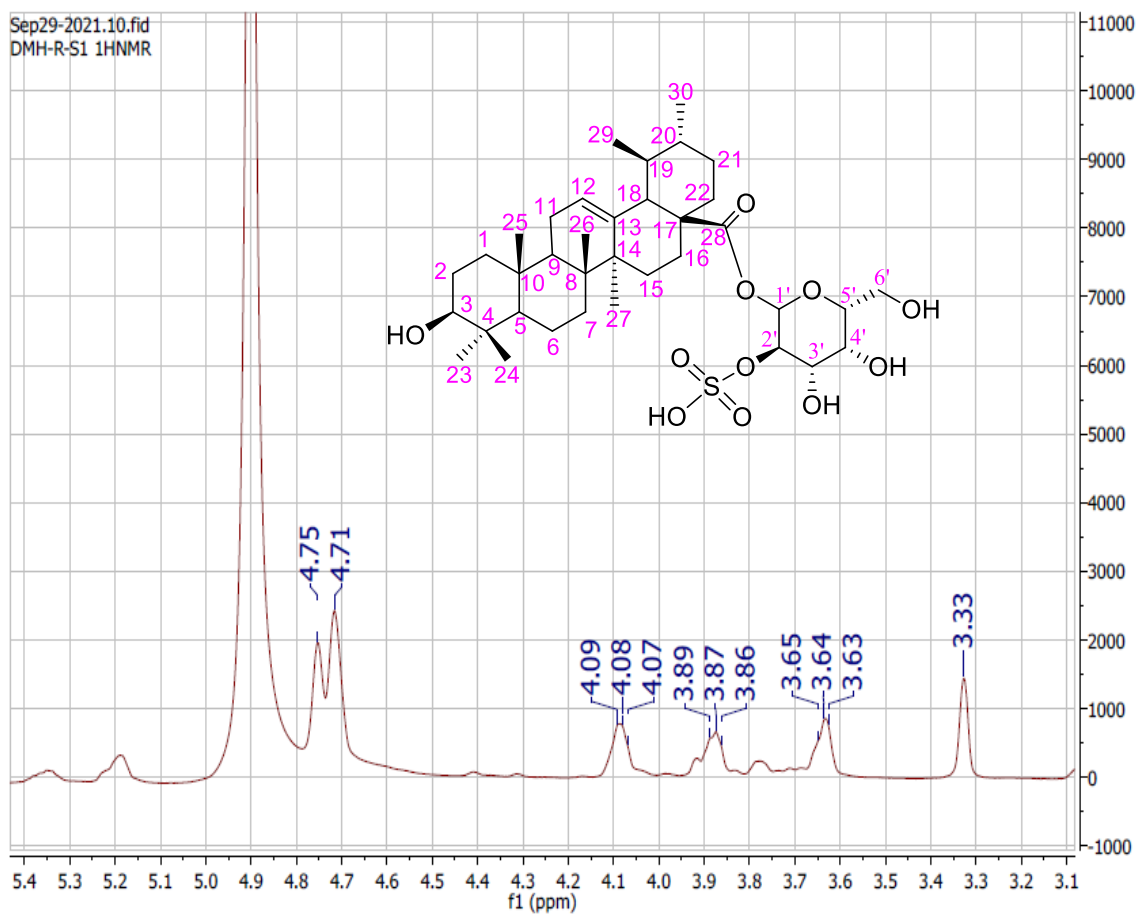


Figure S16: Partial expansion of ^1H -NMR spectrum of Compound 7 (500 MHz, MeOH).

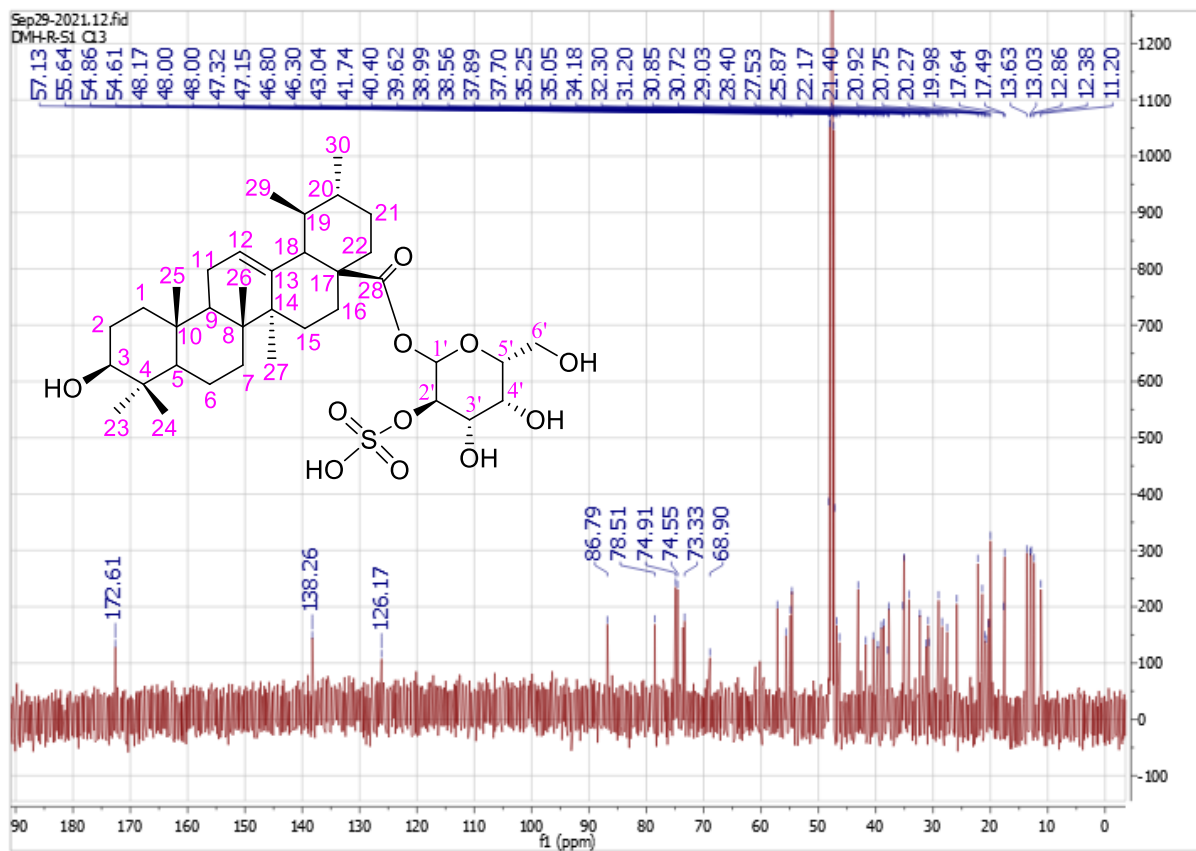


Figure S17: ^{13}C -NMR spectrum of Compound 7 (125 MHz, MeOH).

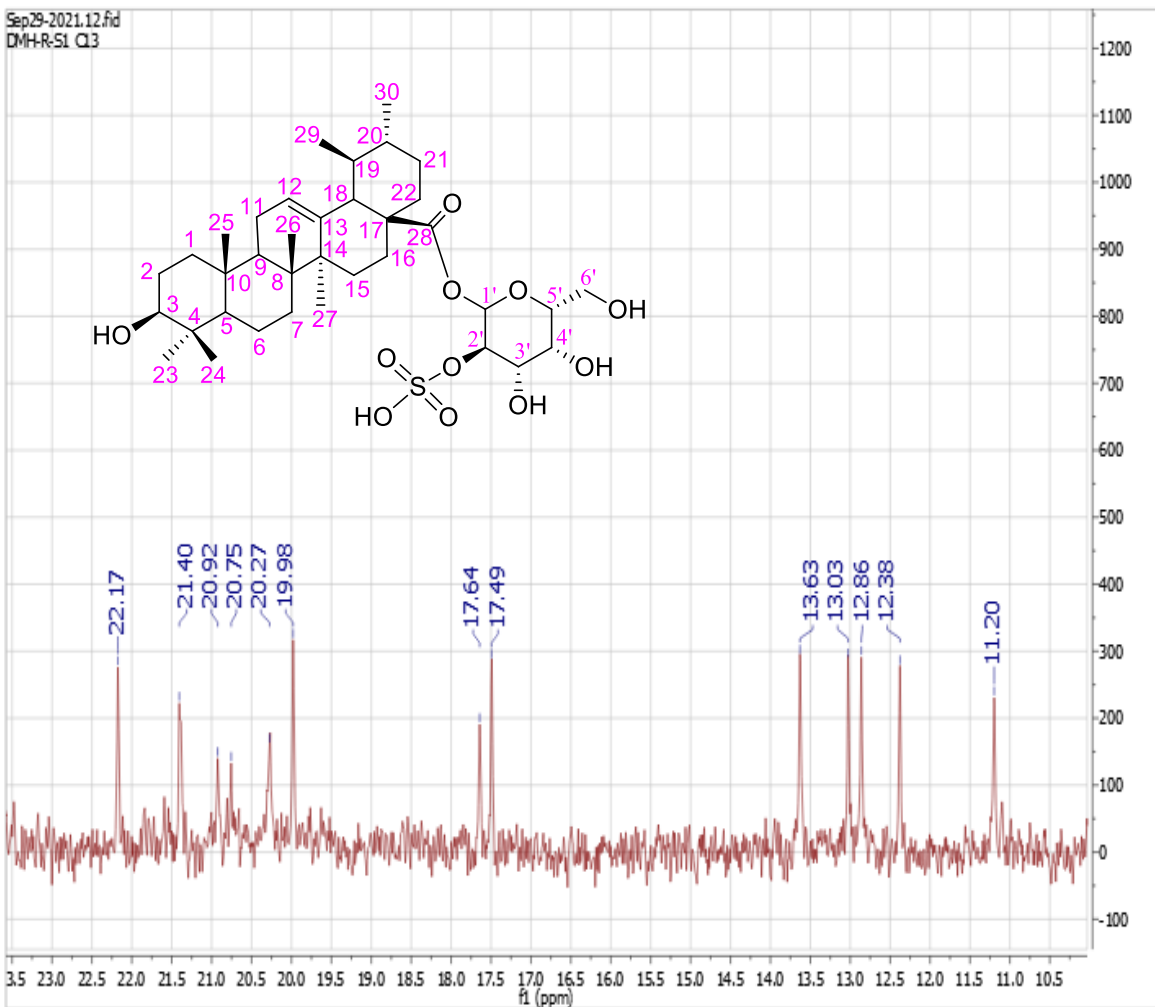


Figure S18: Partial expansion of ¹³C-NMR spectrum of Compound 7 (125 MHz, MeOH).

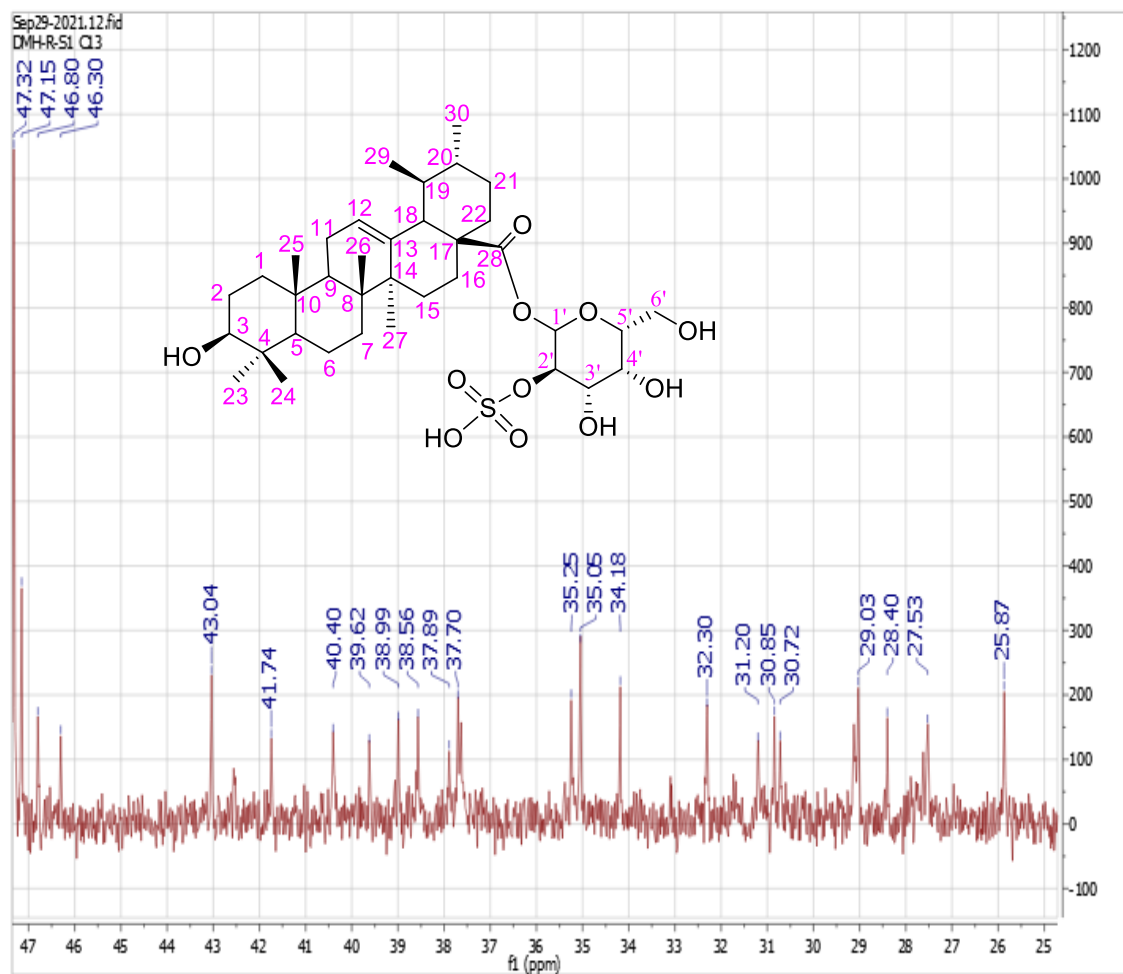


Figure S19: Partial expansion of ^{13}C -NMR spectrum of Compound 7 (125 MHz, MeOH).

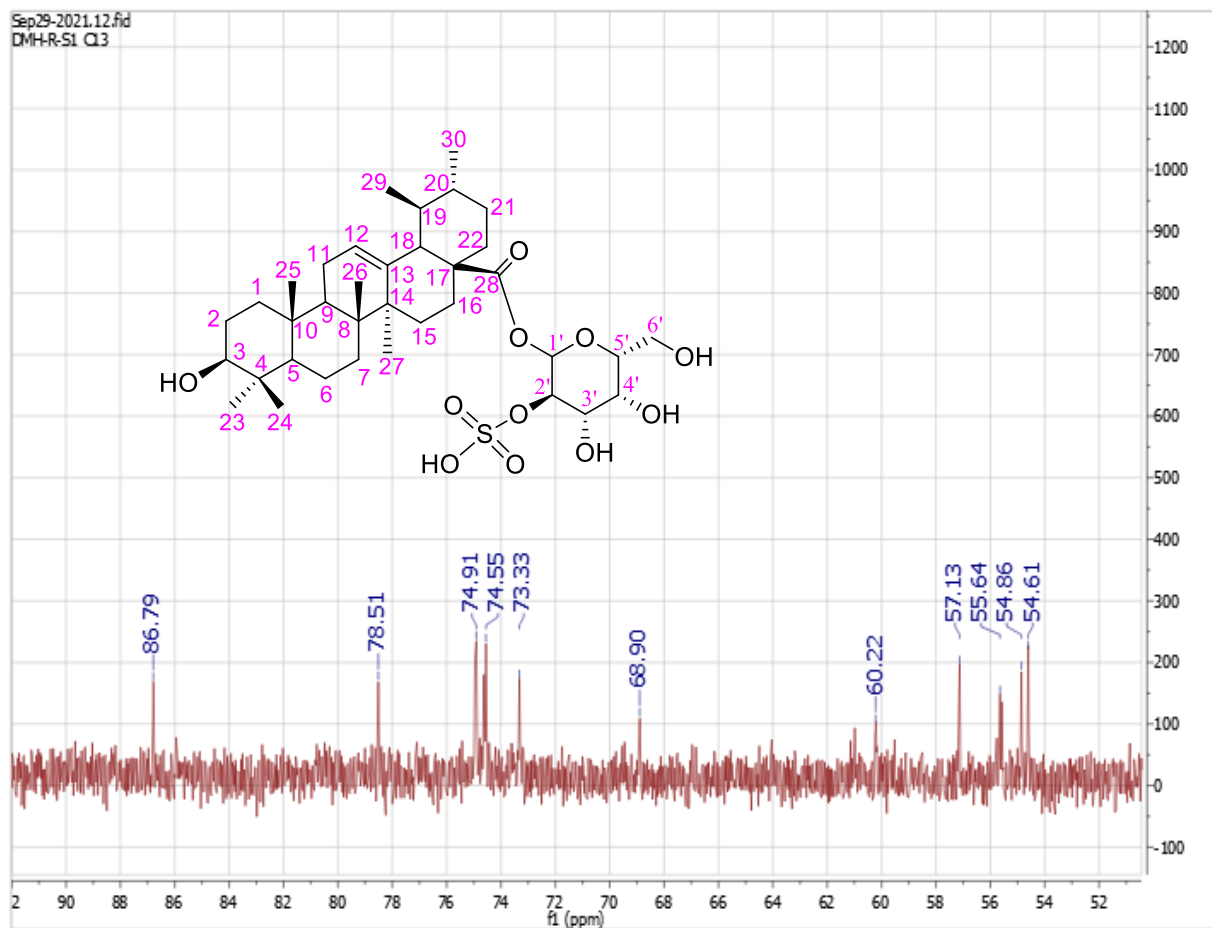


Figure S20: Partial expansion of ¹³C-NMR spectrum of Compound 7 (125 MHz, MeOH).

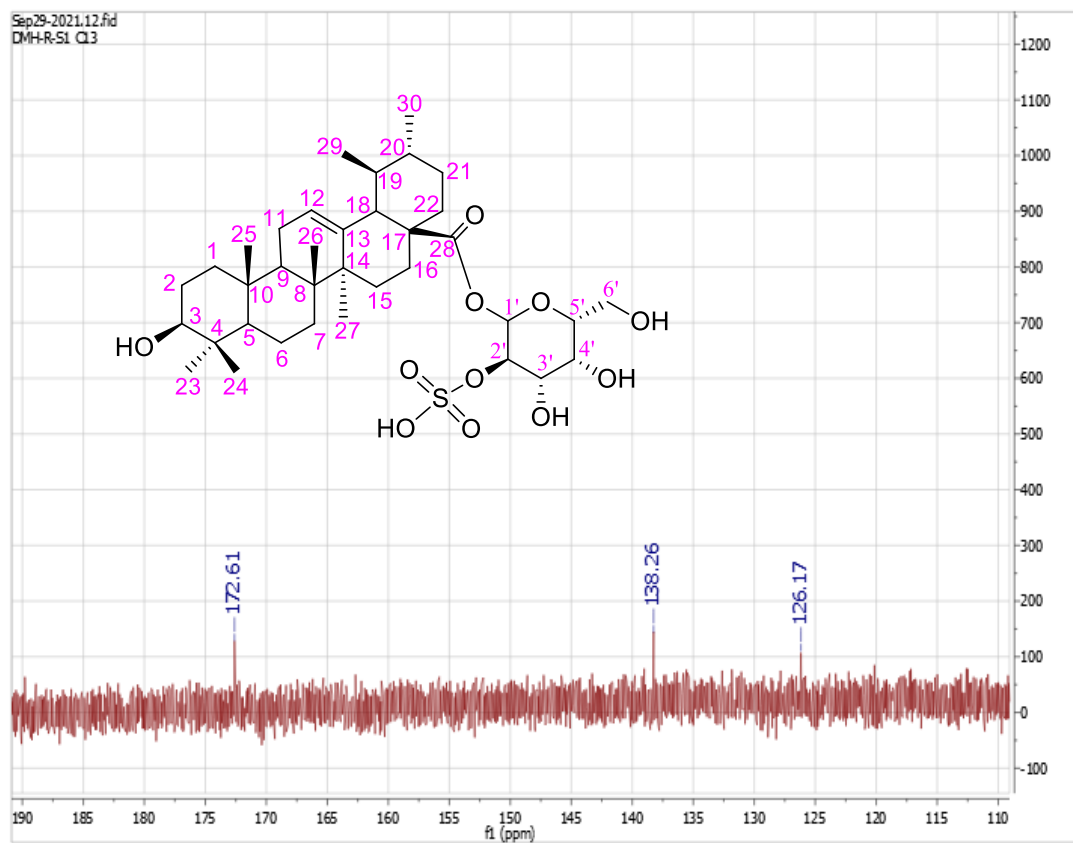


Figure S21: Partial expansion of ¹³C-NMR spectrum of Compound 7 (125 MHz, MeOH).

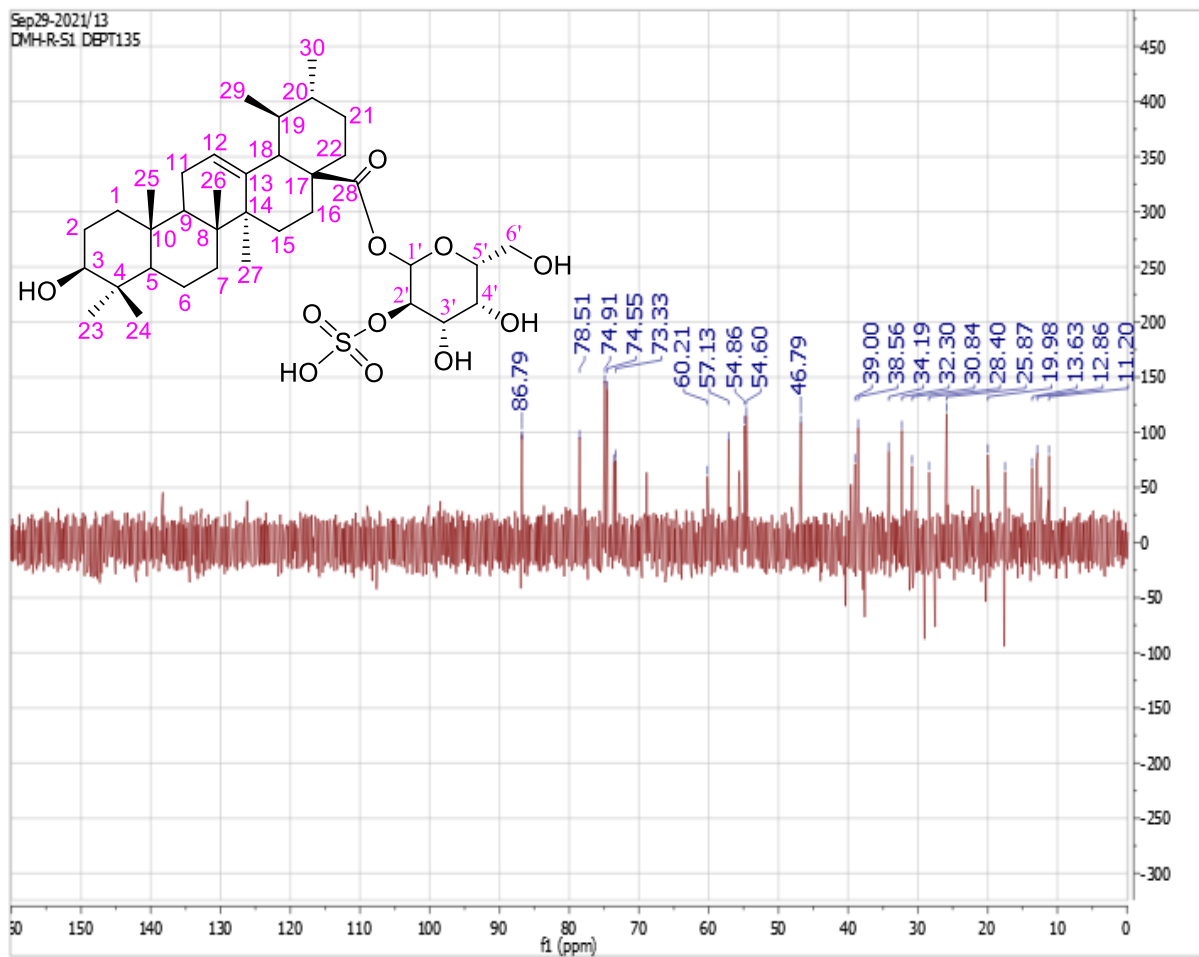


Figure S22: DEPT 135 of Compound 7 (125 MHz, MeOH).

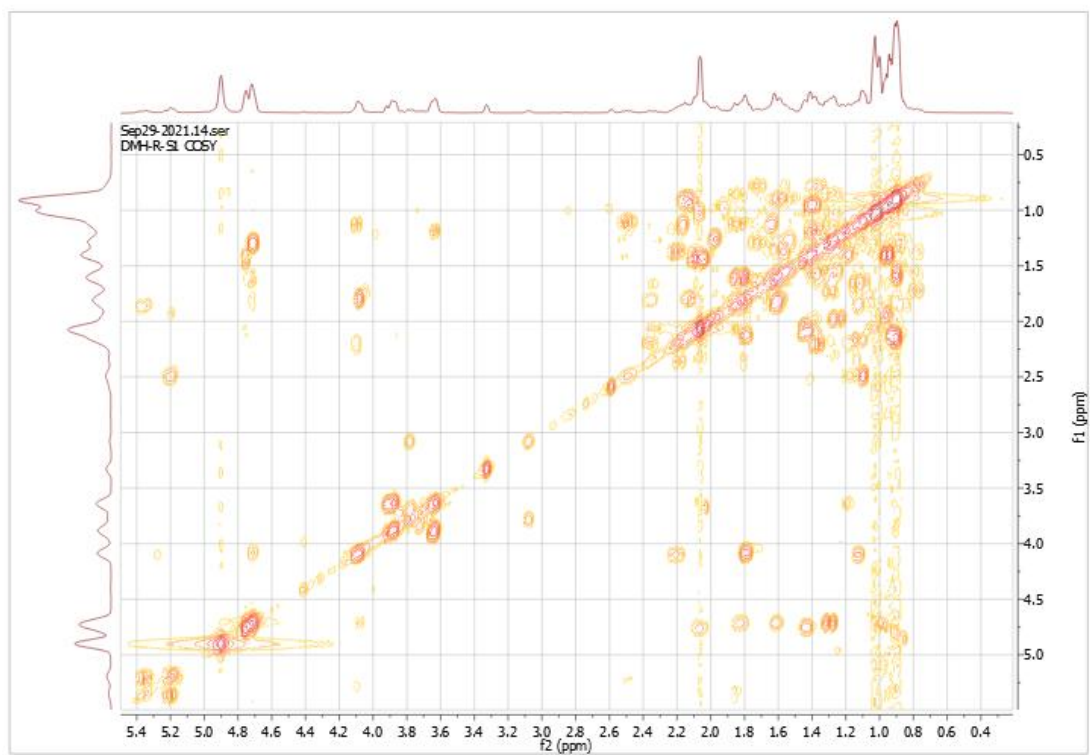


Figure S23: COSY of Compound 7

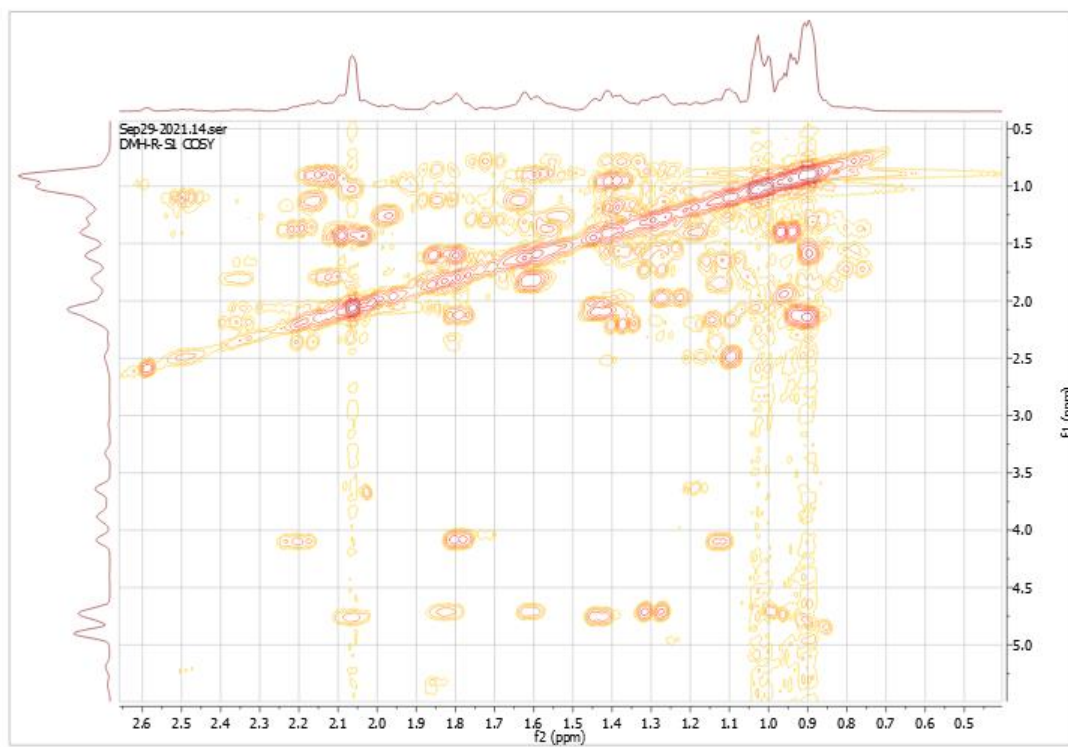


Figure S24: Partial expansion of COSY of Compound 7.

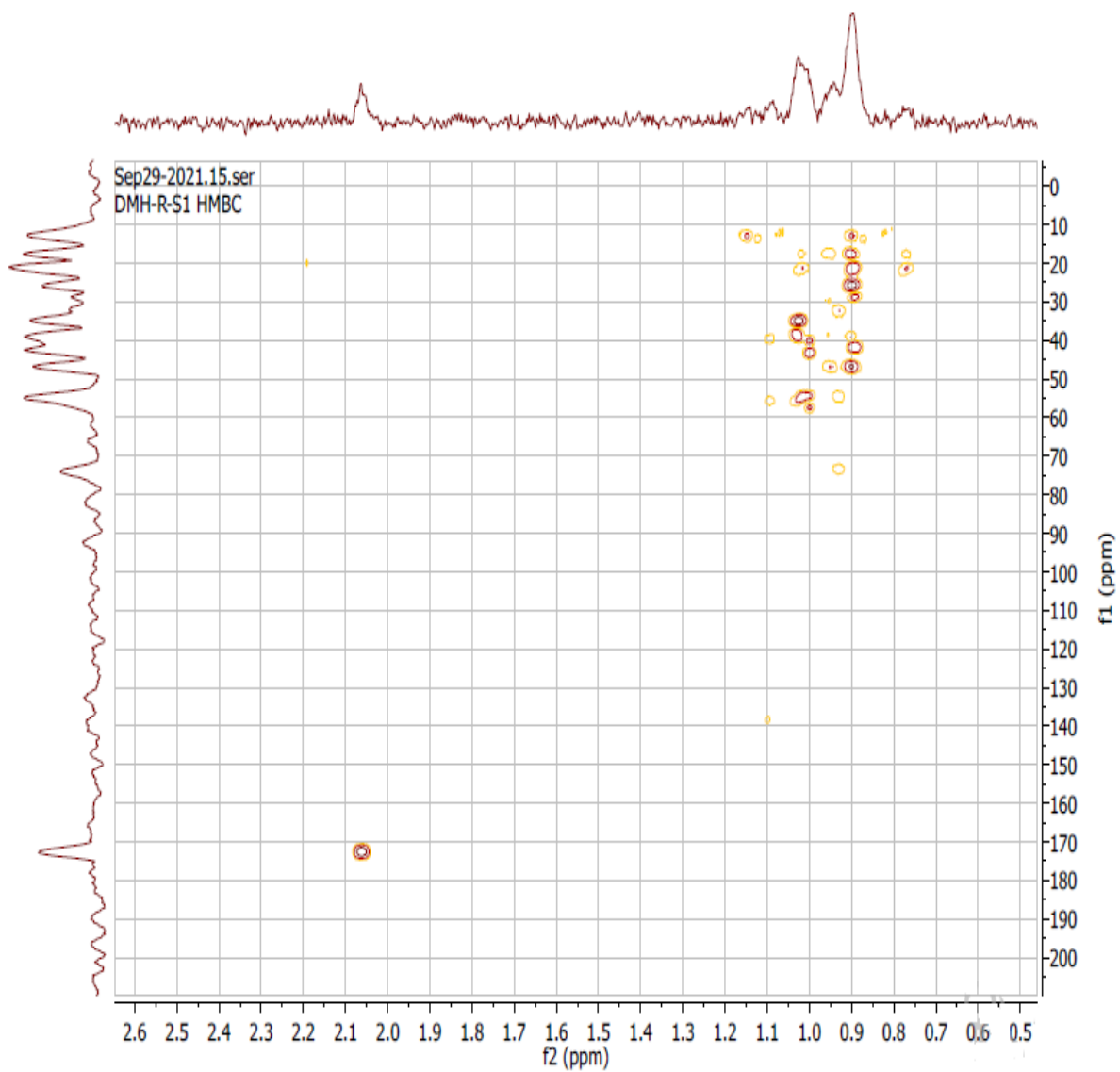


Figure S25: HMBC of compound 7

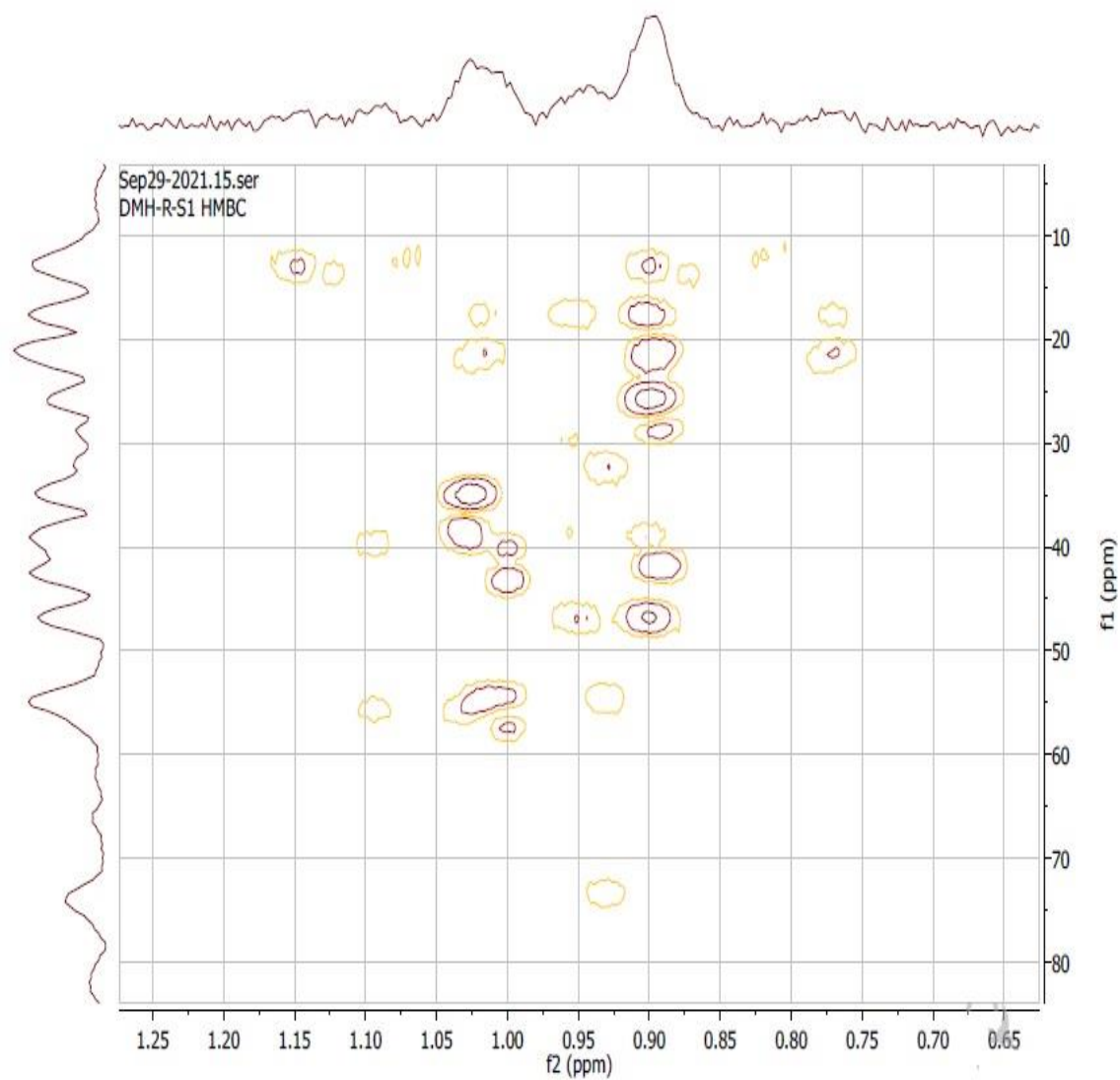


Figure S26: Expansion of HMBC of compound 7.

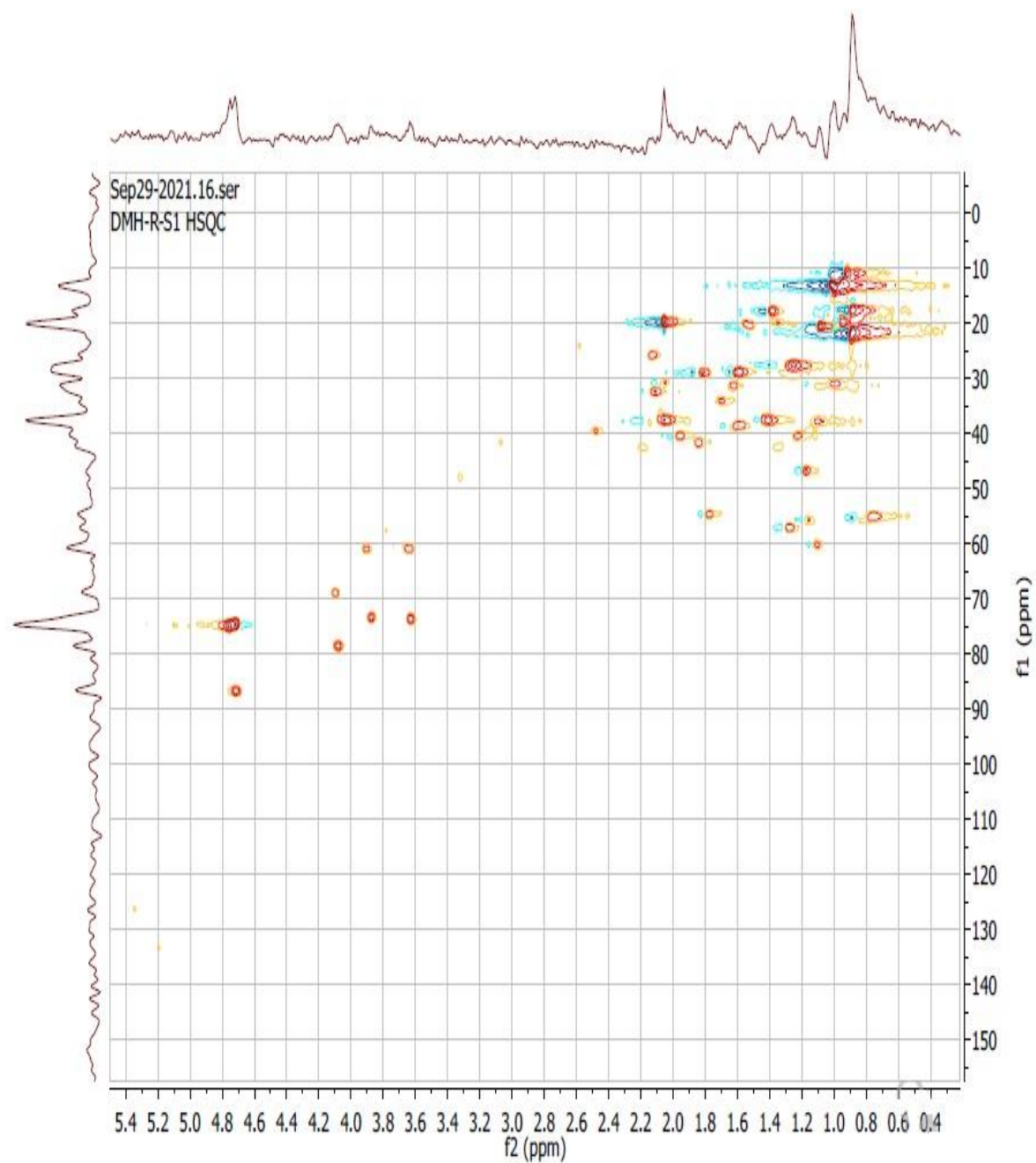


Figure S27: HSQC of compound 7

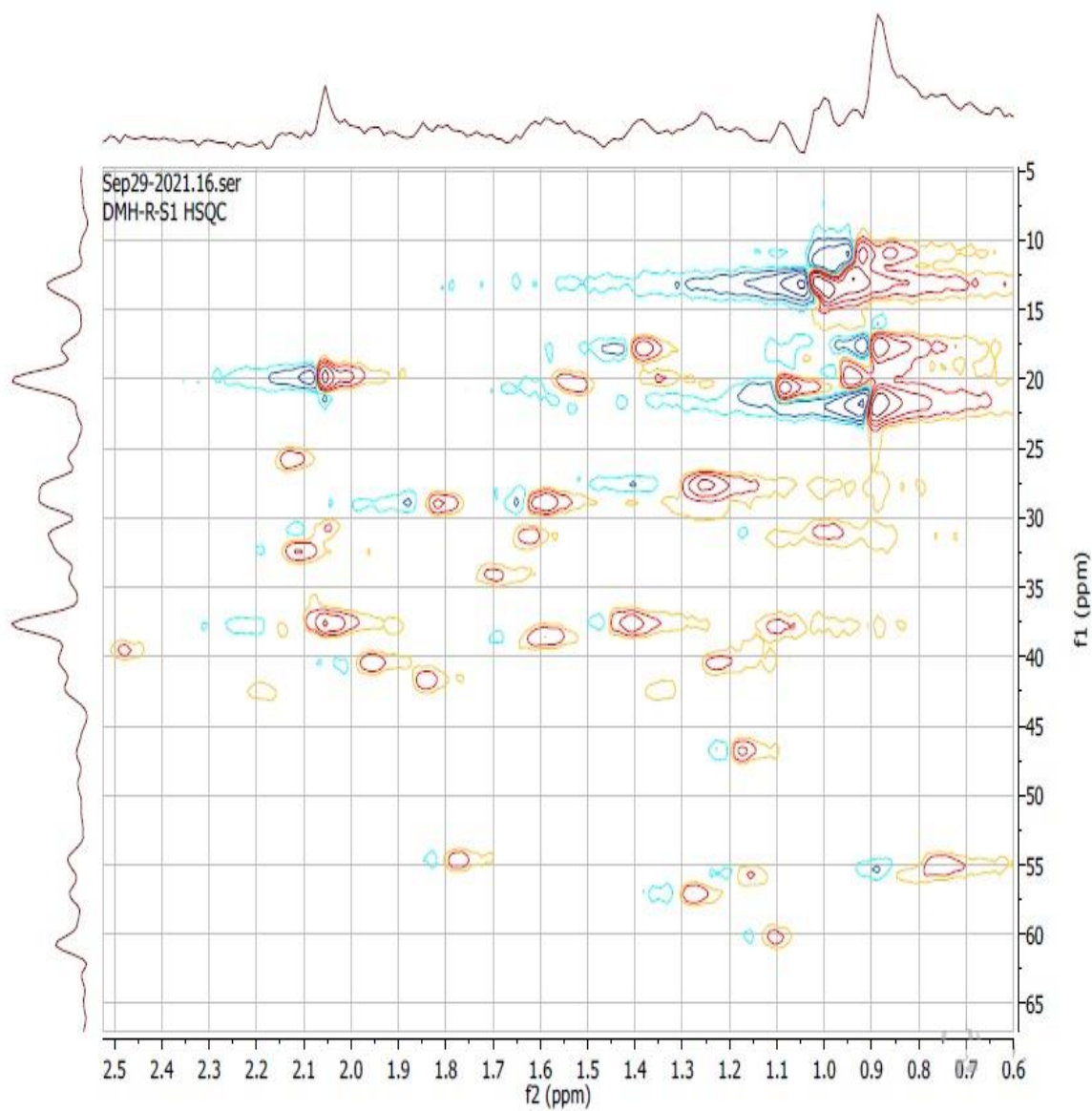


Figure S28: Expansion of HSQC of compound 7.

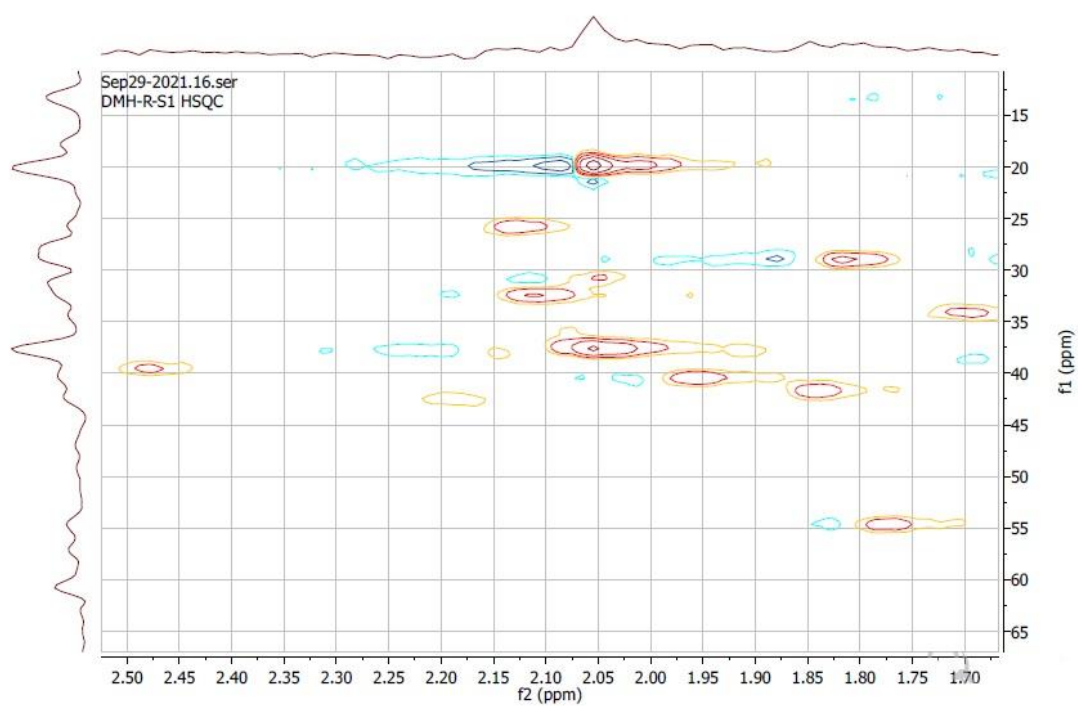


Figure S29: Expansion of HSQC of compound 7.

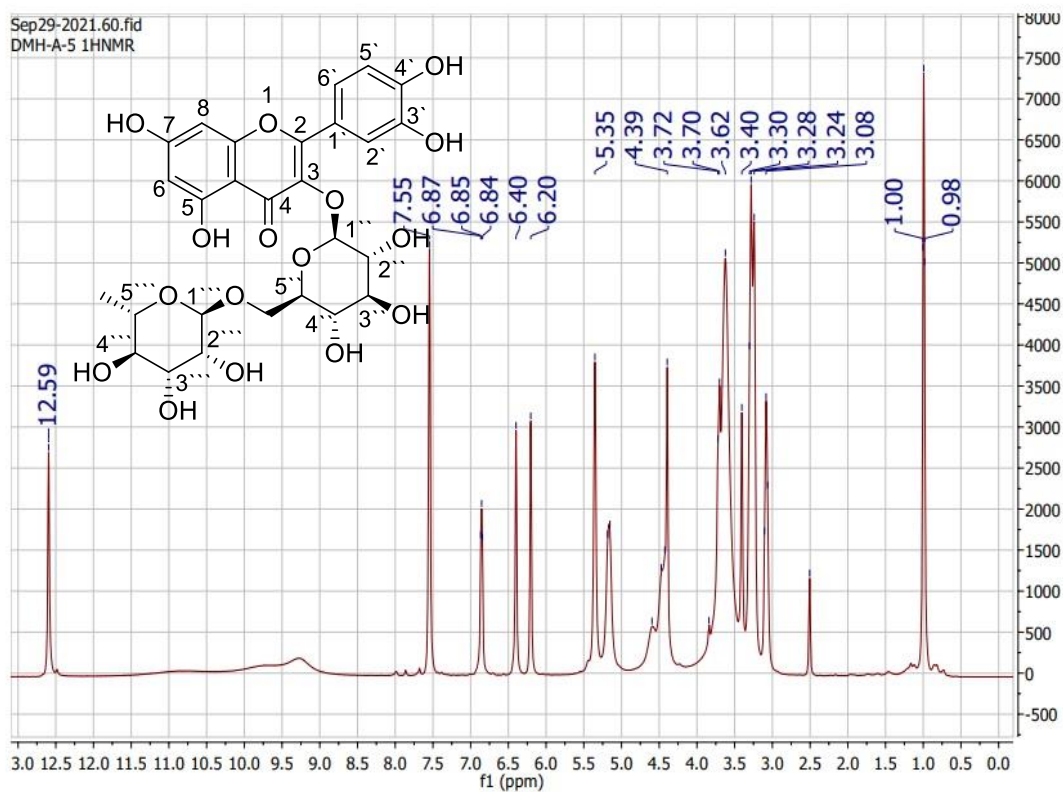


Figure S30: ^1H -NMR spectrum of Compound 8 (500 MHz, DMSO-d_6).

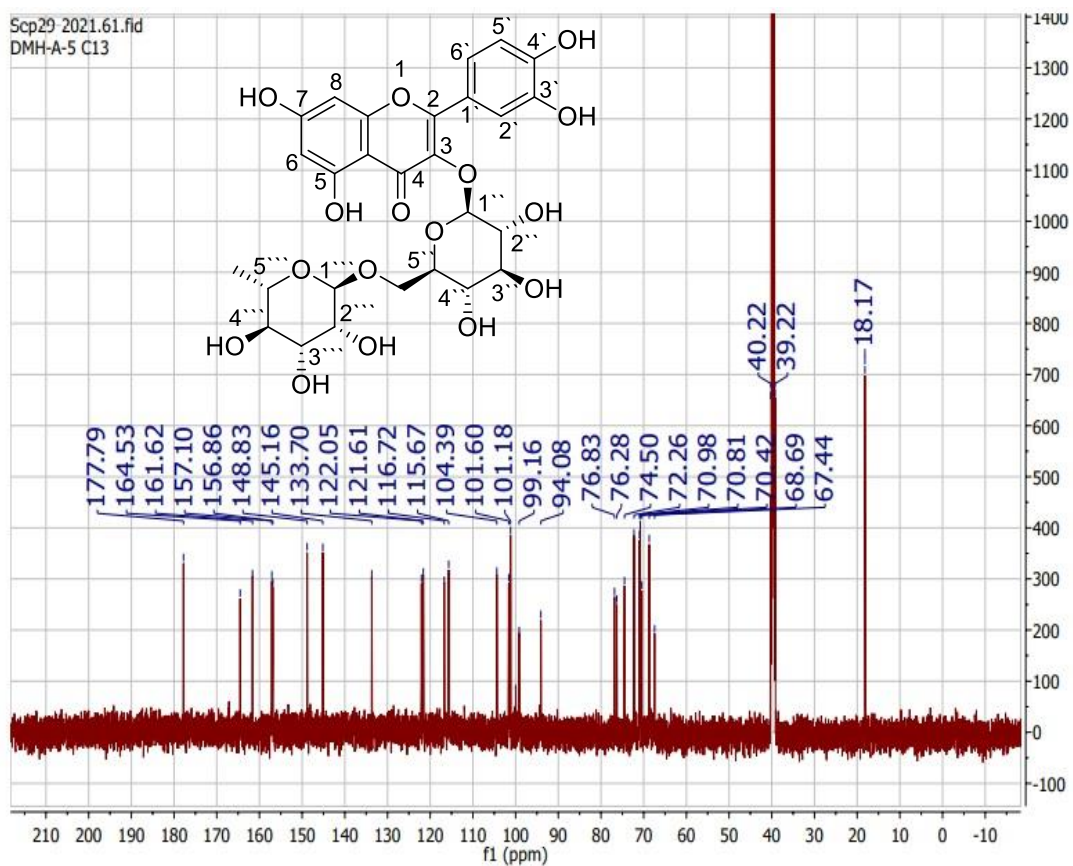


Figure 31: ^{13}C -NMR spectrum of Compound 8 (125 MHz, DMSO- d_6).

Table S1. Liver enzymes and kidney markers in the groups of the preliminary study

	ALT (IU/L)	AST (IU/L)	Urea (mg/dL)	Creatinine (mg/dL)
Normal control	45.60 ± 5.00	50.79 ± 5.90	19.03 ± 3.23	0.92 ± 0.13
<i>Z. album</i> extract (100 mg/kg)	44.22 ± 4.11	50.36 ± 5.21	20.29 ± 3.25	0.90 ± 0.20
Zygo-albuside A (10 mg/kg)	46.32 ± 5.03	51.04 ± 4.32	20.19 ± 3.29	0.91 ± 0.18
Rutin (10 mg/kg)	45.29 ± 5.08	49.89 ± 4.90	20.21 ± 3.50	0.92 ± 0.19

Values are expressed as mean ± SD (n = 5). Data were analyzed using ANOVA followed by Tukey's post hoc test.

Table S2. Primer sequences and annealing temperatures for genes amplification.

	Forward primer	Reverse primer	Annealing temperature
NF- κ b	5'-CAATGGCTACACAGGACCA-3'	5'-CACTGTCACCTGGAACCAGA-3'	52 °C
TNF- α	5'-TCTACTGAACTTCGGGGTGATCG-3'	5'-TGATCTGAGTGTGAGGGTCTGGG-3'	56 °C
p53	5'-ACCGCCGACCTATCCTTACC-3'	5'-TCTTCTGTACGGCGGTCTCTC-3'	56 °C
β -actin	5'-ACGGCCAGGTCATCACTATTG-3'	5'-CAAGAAGGAAGGCTGGAAAAGA-3'	52 °C
miR-29a	5'-GCGCACTGATTCTTTTGGTGTTCAG-3'	5'-GCGAGCACAGAATTAATACGAC-3'	51 °C
RNU6B	5'-CTCGCTTCGGCAGCACATA-3'	5'-CGCTTCACGAATTTGCGTG-3'	53 °C