

Article

Evaluation of *Zamia floridana* A. DC. Leaves and Its Isolated Secondary Metabolites as Natural Anti-toxoplasma and Anti-cancer Agents Using In Vitro and In Silico Studies

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Supplementary Materials

Table S1. Toxoplasmodicidal effect of *Z. floridana* methanol extract and its different fractions.

Drug	EC ₅₀ ± SEM (µg/mL)
Cotrimoxazole	4.18±0.3
<i>Z. floridana</i> MeOH extract	8.19±0.4
Pet. ether fraction	31.95±1.3
Chloroform fraction	16.71±0.8
Ethyl acetate fraction	9.74±0.5
<i>n</i> -butanol fraction	7.16±0.4

Table S2. The percent inhibition of the cancer cells' viability under the effect of the different tested concentrations of *Z. floridana* leaves MeOH extracts.

Conc. ($\mu\text{g/mL}$)	HEPG-2	MCF-7	HCT-116	PC3	HELA	WISH
Doxorubicin						
100	6.3	6.2	7.1	8.8	7.3	8.4
50	11.2	10.9	13.9	16.3	12.1	17.1
25	14.1	14.3	18.7	21.7	18.9	24.3
12.5	28.3	26.9	31.4	38.9	30.8	33.5
6.25	45.8	41.5	47.9	59.2	51.7	56.6
3.125	57.6	58.4	60.5	73.6	62.4	69.2
1.56	71.2	69.1	73.8	95.3	74.0	88.6
<i>Z. floridana</i> MeOH extract						
100	26.8	20.1	27.3	35.7	33.1	31.2
50	40.2	31.7	38.1	50.5	42.7	47.3
25	52.1	45.2	47.2	62.6	54.2	54.1
12.5	68.7	52.8	65.0	71.3	66.9	76.2
6.25	84.9	76.3	78.4	92.8	87.0	88.4
3.125	99.4	95.2	91.8	100	100	100
1.56	100	100	100	100	100	100

Table S3. The percent inhibition of the cancer cells' viability under the effect of the different tested concentrations of *Z. floridana* leaves' different fractions.

Conc. (µg/ml)	MCF-7	HCT-116	WISH
PE fraction			
100	34.8	31.6	49.9
50	47.3	41.9	75.2
25	61.2	54.4	81.1
12.5	73.6	65.1	89.9
6.25	88.1	79.6	99.4
3.125	100	98.2	100
1.56	100	100	100
CHCl₃ fraction			
100	28.7	27.8	48.9
50	41.0	38.2	66.2
25	57.2	49.3	75.7
12.5	61.4	63.7	91.4
6.25	80.5	78.6	98.1
3.125	98.9	95.1	100
1.56	100	100	100
EtOAc fraction			
100	23.8	7.9	34.6
50	34.9	16.3	52.5
25	48.0	27.0	60.0
12.5	54.7	38.2	84.5
6.25	75.2	54.1	97.1
3.125	93.1	78.4	100
1.56	100	95.9	100
<i>n</i>-BuOH fraction			
100	5	21.3	37.4
50	26.3	28.2	56.7
25	35.6	37.8	64.9
12.5	40.9	51.4	89.9
6.25	62.4	75.1	97.2
3.125	81.2	93.9	100
1.56	96.1	100	100

Table S4. Cytotoxic effect of *Z. floridana* methanol extract against different cell lines.

Drug	<i>In vitro</i> Cytotoxicity IC ₅₀ ± SEM (µg/mL)					
	HEPG-2	MCF-7	HCT-116	PC3	HELA	WISH
Doxorubicin	4.50±0.2	4.17±0.2	5.23±0.3	8.87±0.6	5.57±0.4	7.79±0.5
Total MeOH extract of <i>Z. floridana</i>	31.87±2.6	20.57±1.7	27.33±2.3	48.48±3.5	36.36±2.9	40.29±3.2

Table S5. Cytotoxic effect of *Z. floridana* different fractions against MCF-7, HCT-116 and WISH cell lines.

Drug	<i>In vitro</i> Cytotoxicity IC ₅₀ ± SEM (µg/mL)		
	MCF-7	HCT-116	WISH
Doxorubicin	4.17±0.2	5.23±0.3	7.79±0.5
Pet. ether fraction	45.06±3.1	33.78±2.5	95.61±4.6
Chloroform fraction	32.39±2.3	28.16±2.1	84.15±4.4
Ethyl acetate fraction	22.89±1.8	9.04±0.8	54.26±3.1
<i>n</i> -Butanol fraction	12.33±1.1	17.88±1.4	59.44±3.2

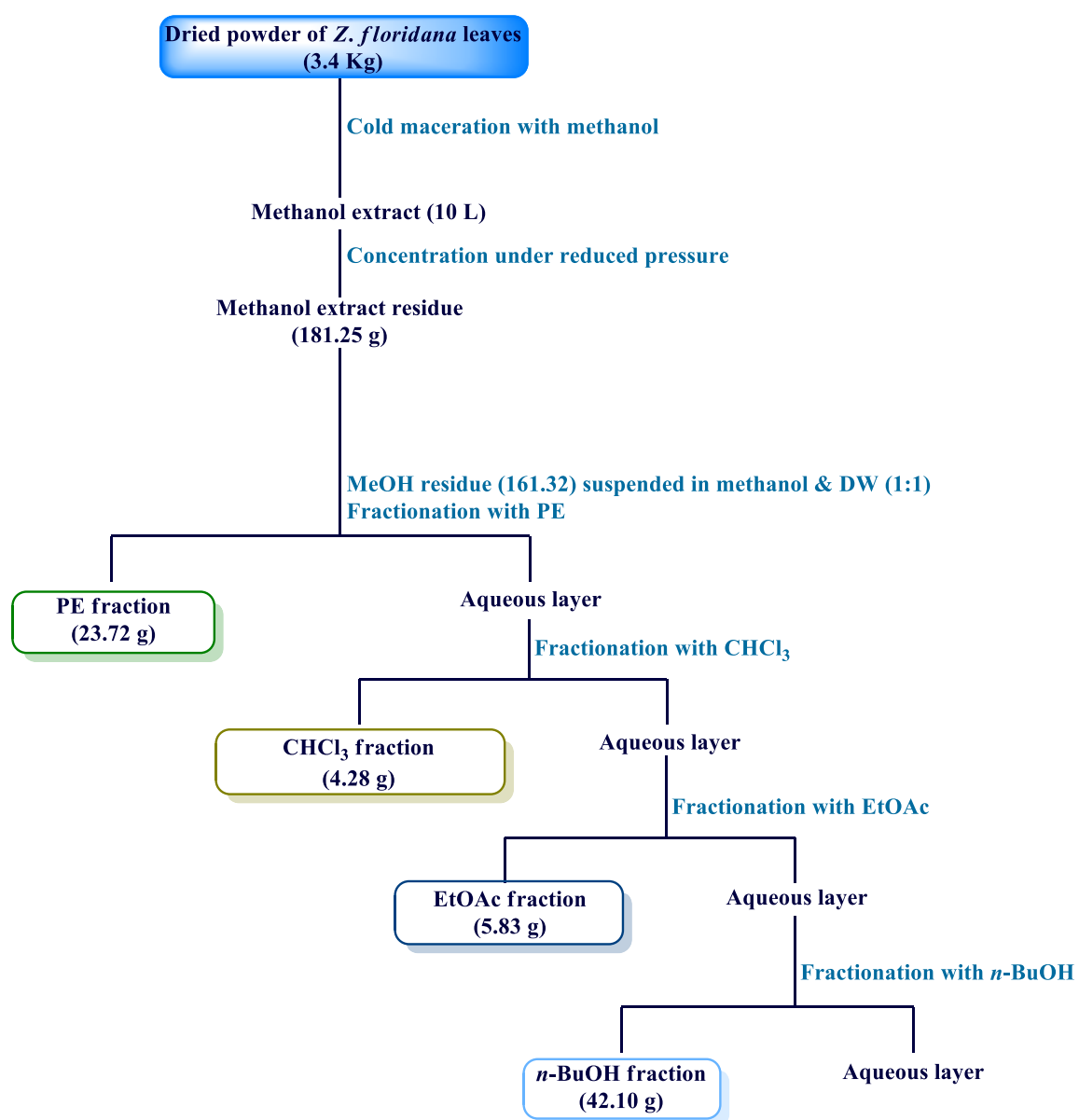


Figure S1. Extraction and fractionation steps of *Z. floridana* leaves

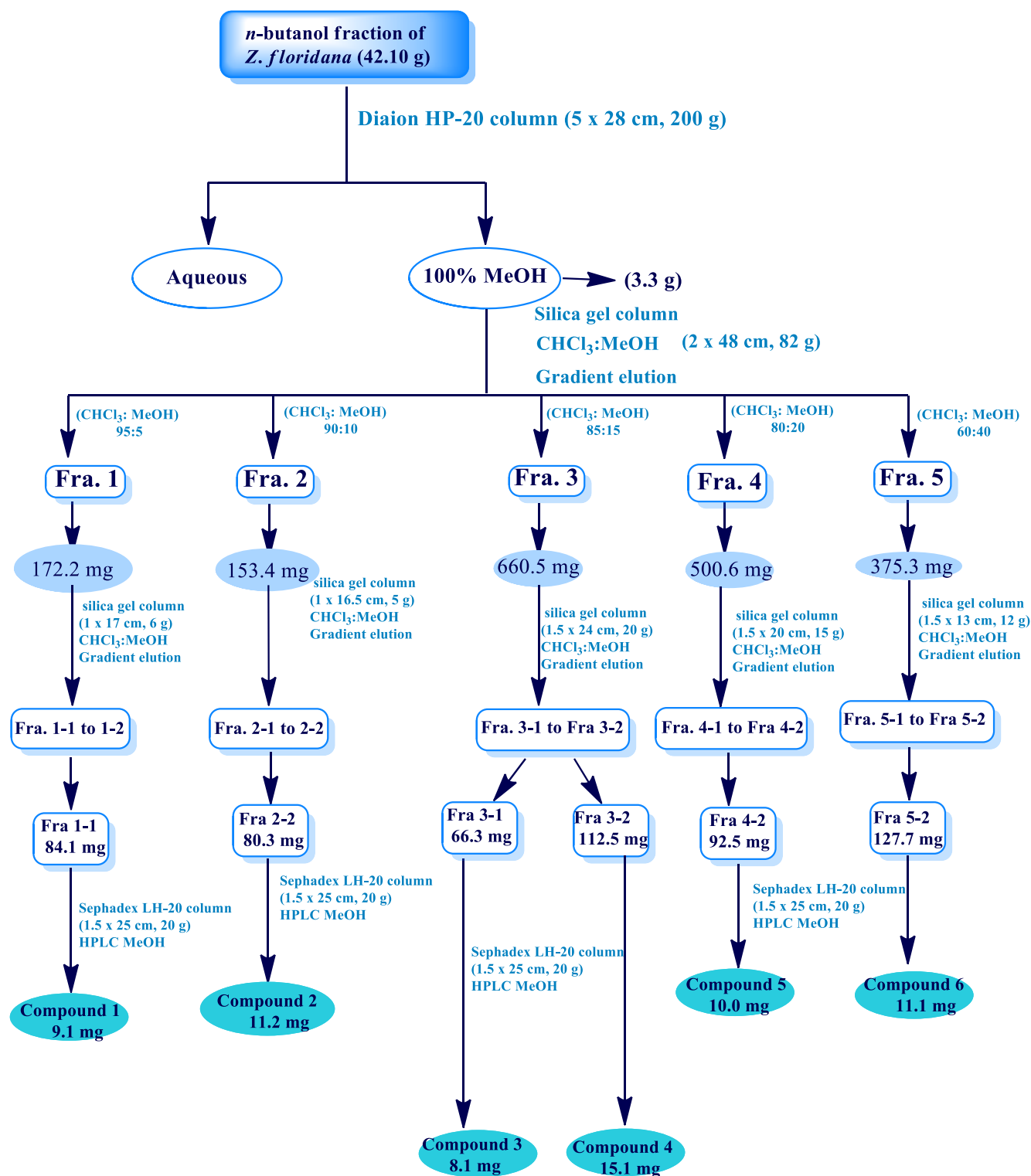


Figure S2. Isolation steps of six pure compounds from *Z. floridana* n-BuOH fraction

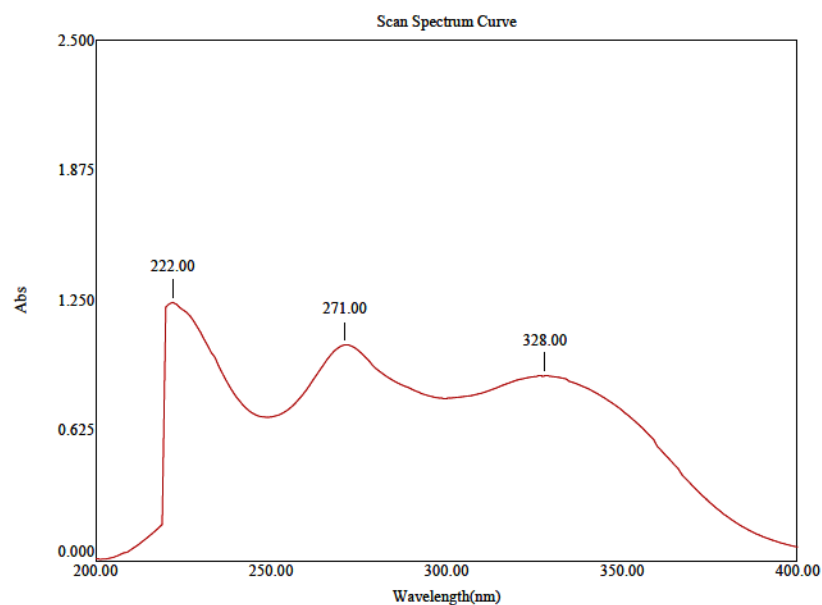


Figure S3. UV spectrum of compound (1) in MeOH

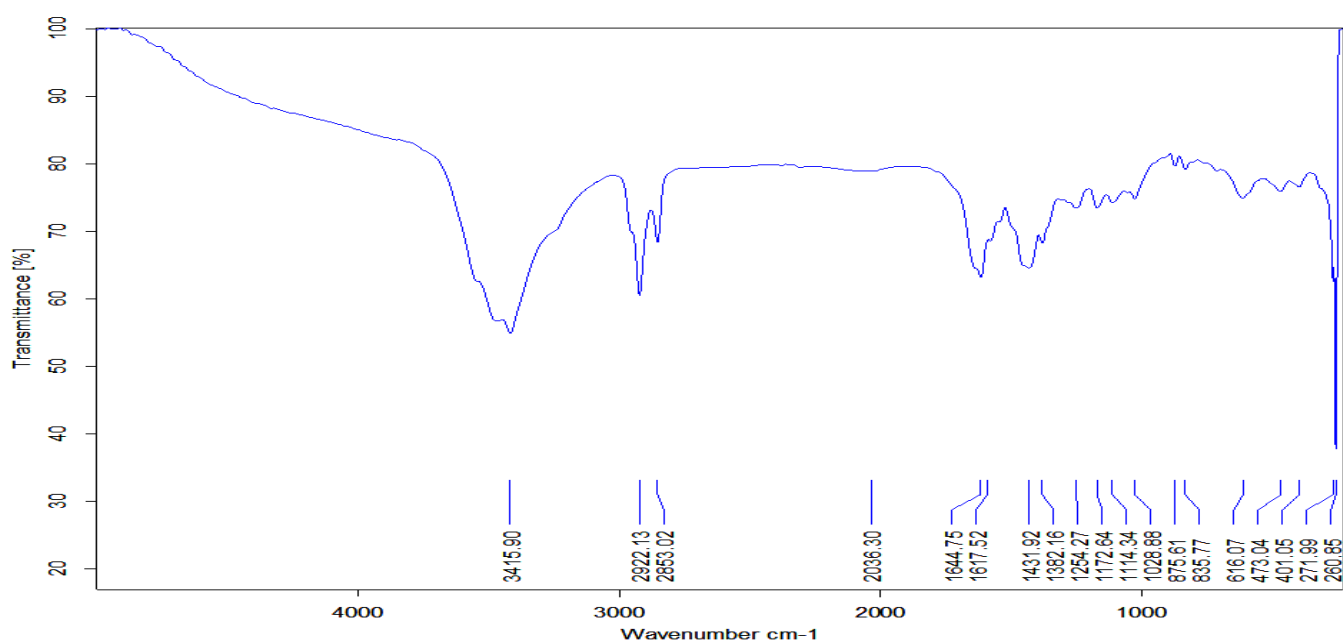


Figure S4. IR spectrum of compound (1) in KBr disc

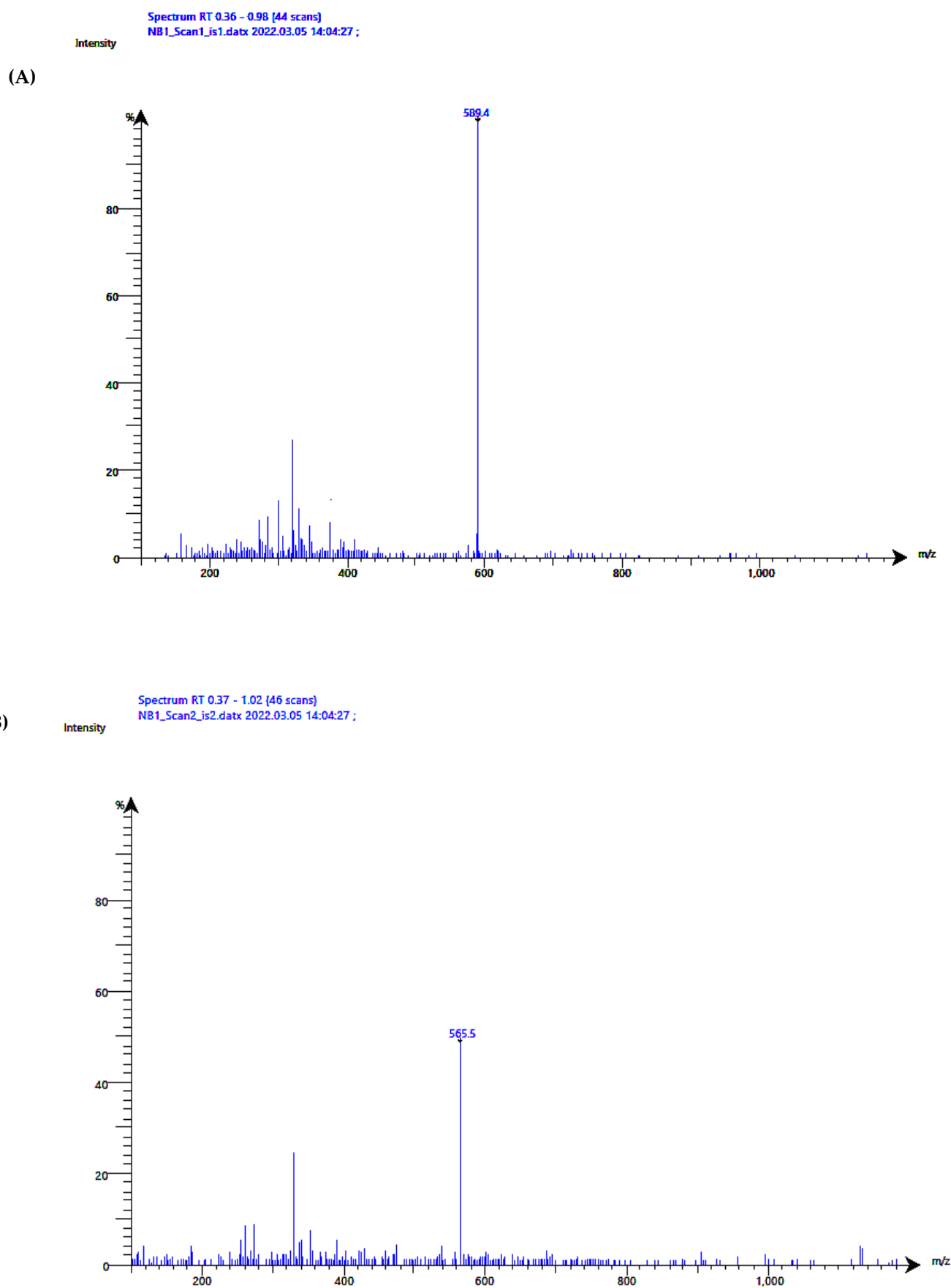


Figure S6. ESIMS "positive (A) and negative modes (B)" of compound (1)

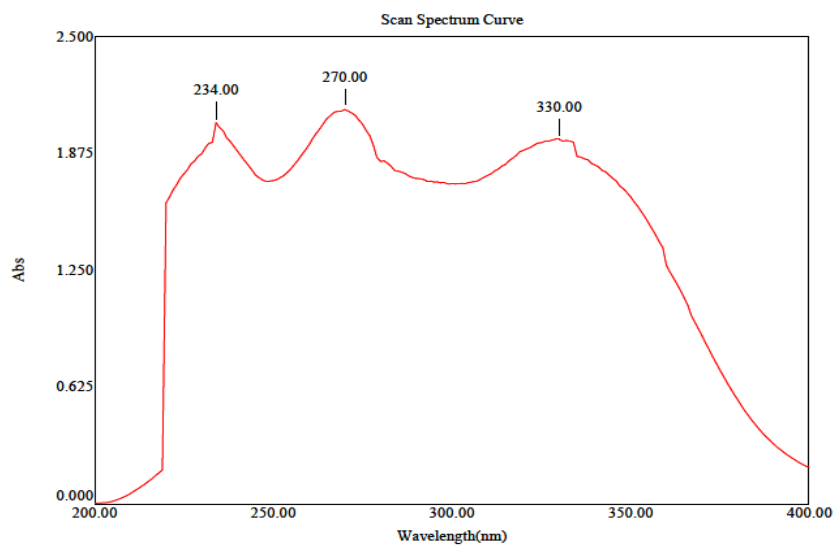


Figure S7. UV spectrum of compound (2) in MeOH

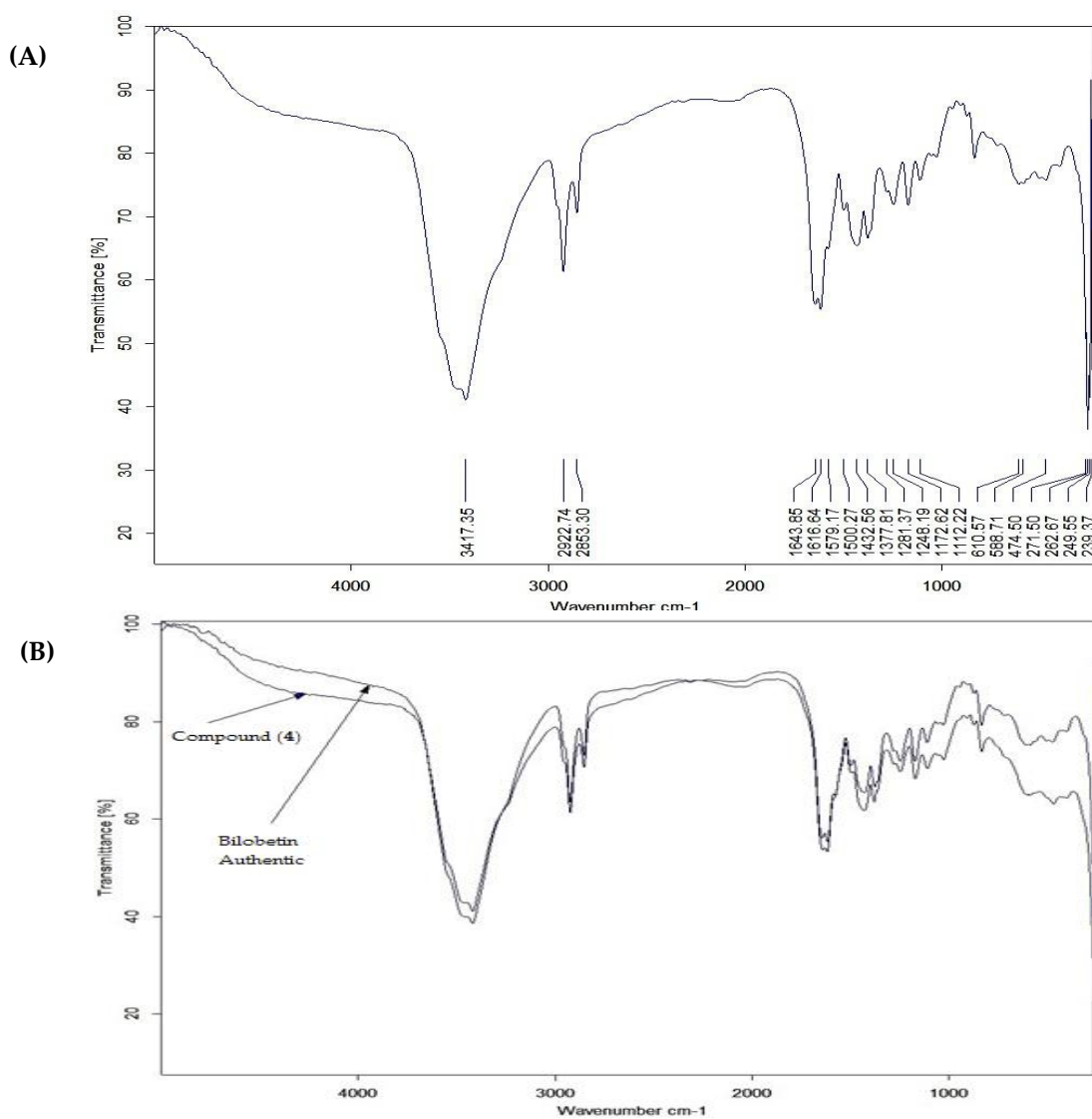
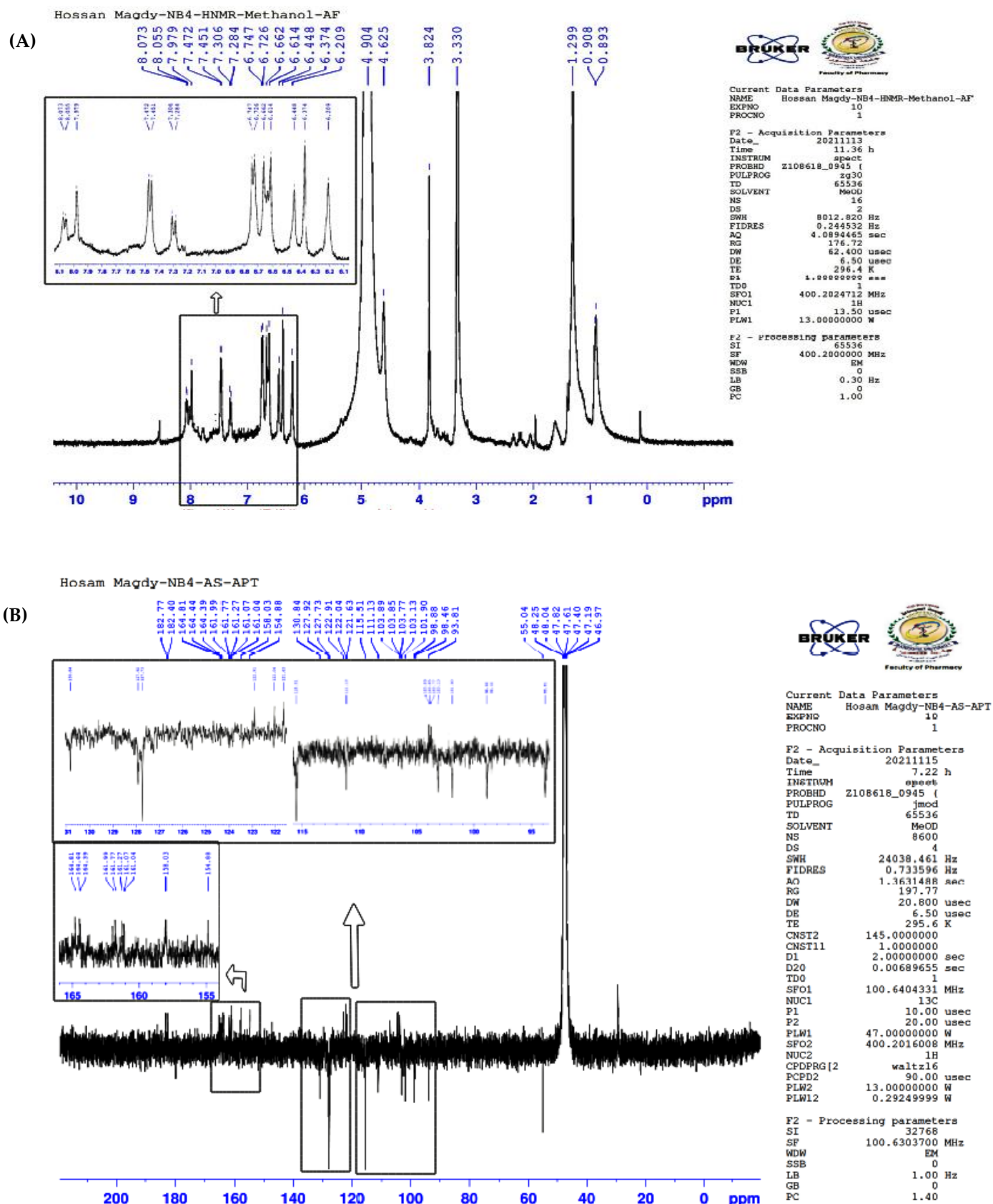


Figure S8. (A) IR spectrum of compound (2) in KBr disc
(B) IR fingerprint spectrum of compound (2) and bilobetin authentic sample in KBr

Figure S9. (A) ^1H and (B) APT NMR spectrum of compound (2) (CD_3OD)

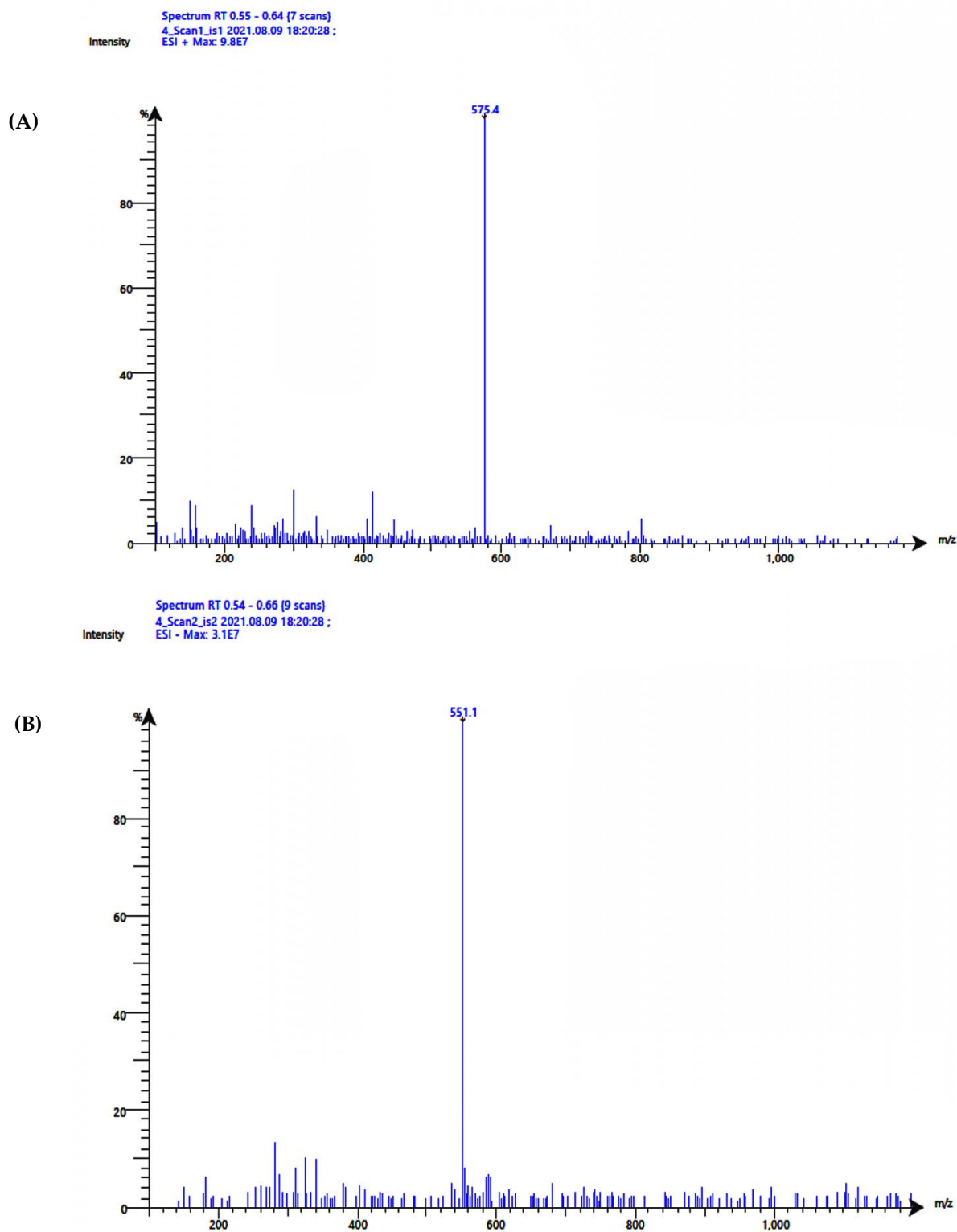


Figure S10. ESIMS "positive (A) and negative modes (B)" of compound (2)

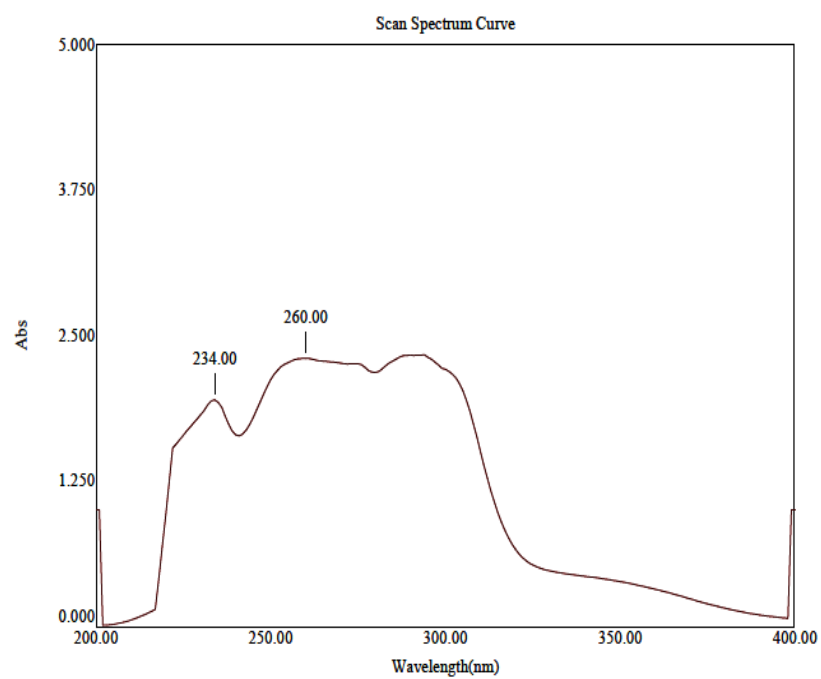
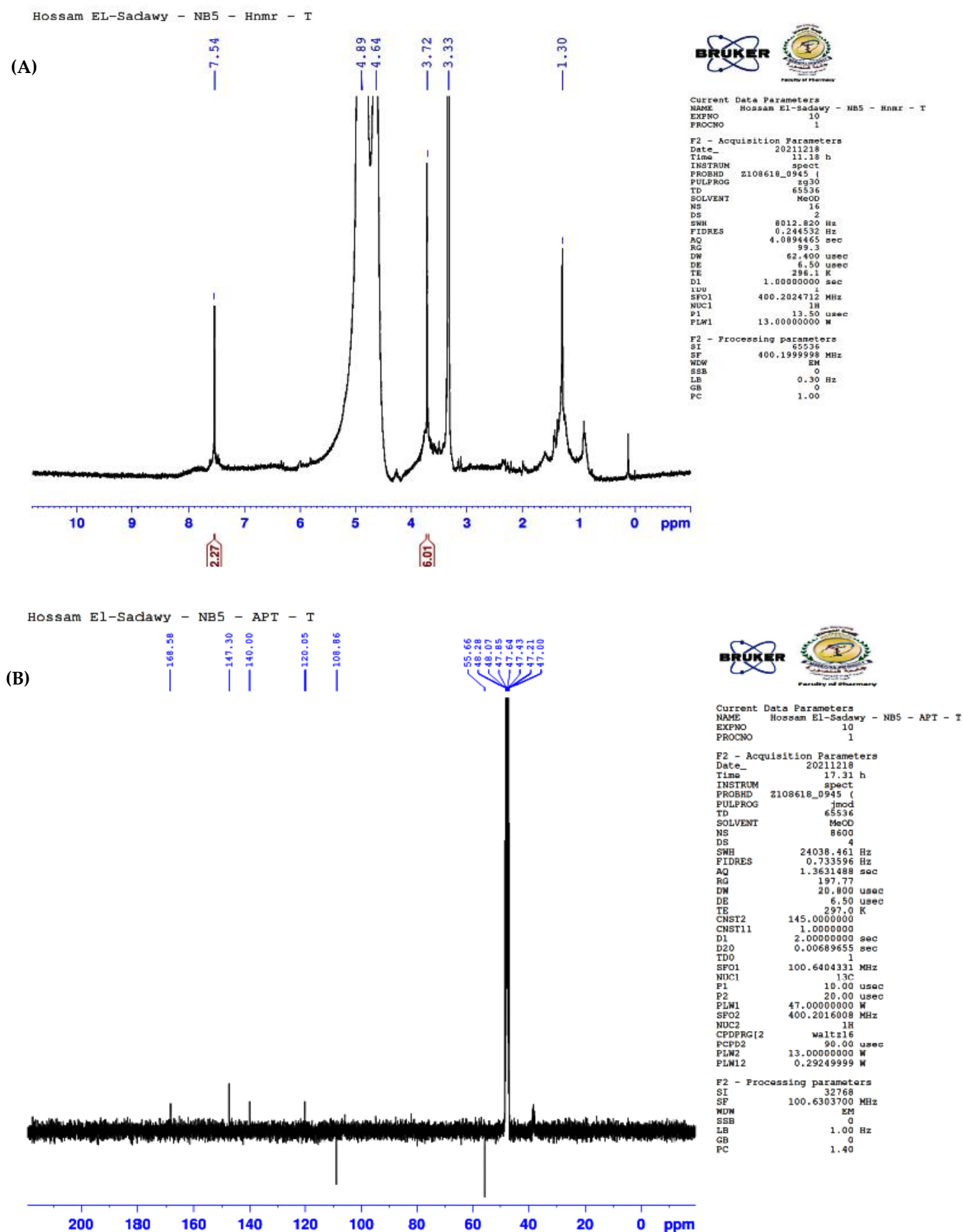


Figure S11. UV spectrum of compound (3) in MeOH

Figure S12. (A) ^1H and (B) APT NMR spectrum of compound (3) (CD_3OD)

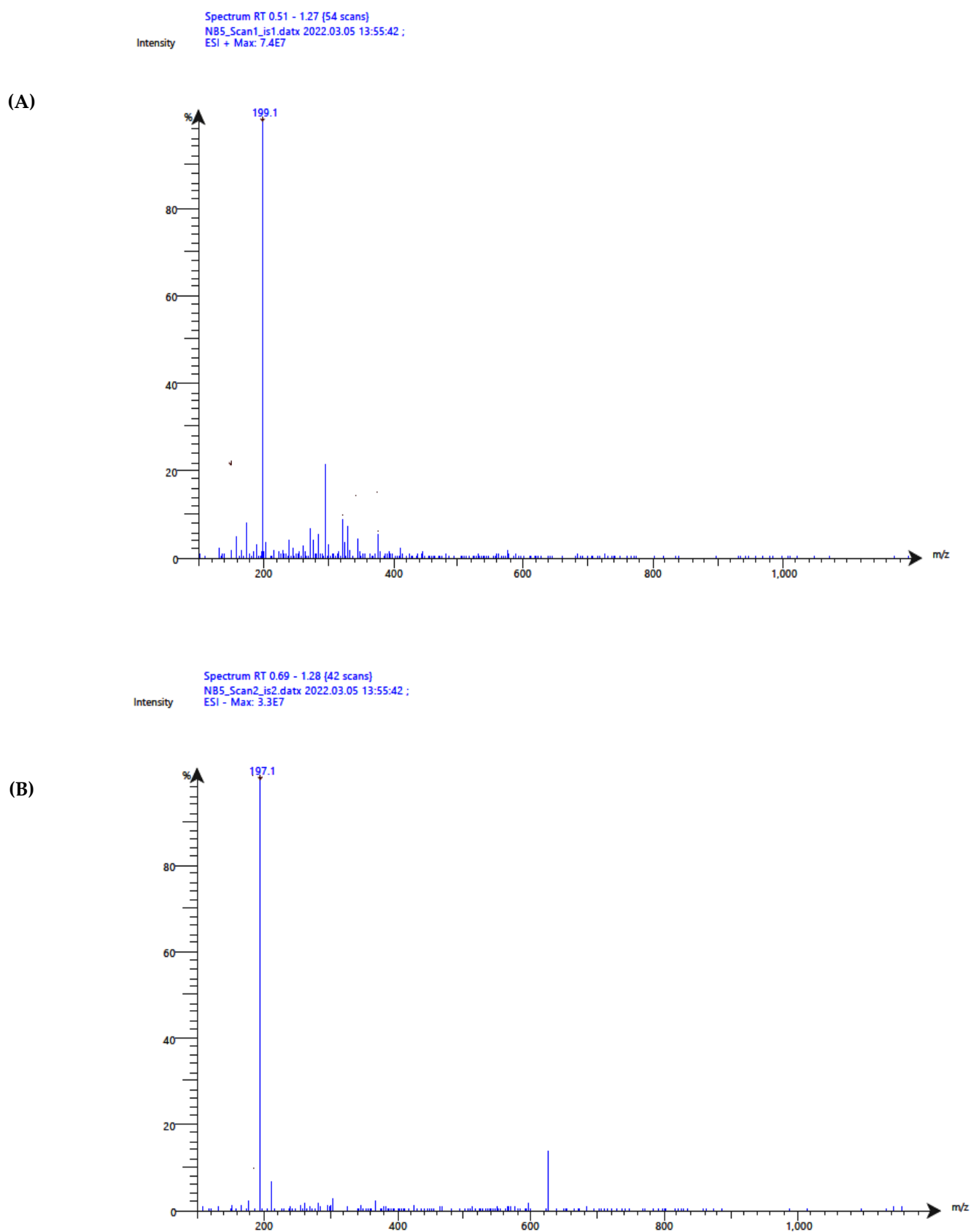


Figure S13. ESIMS "positive (A) and negative modes (B)" of compound (3)

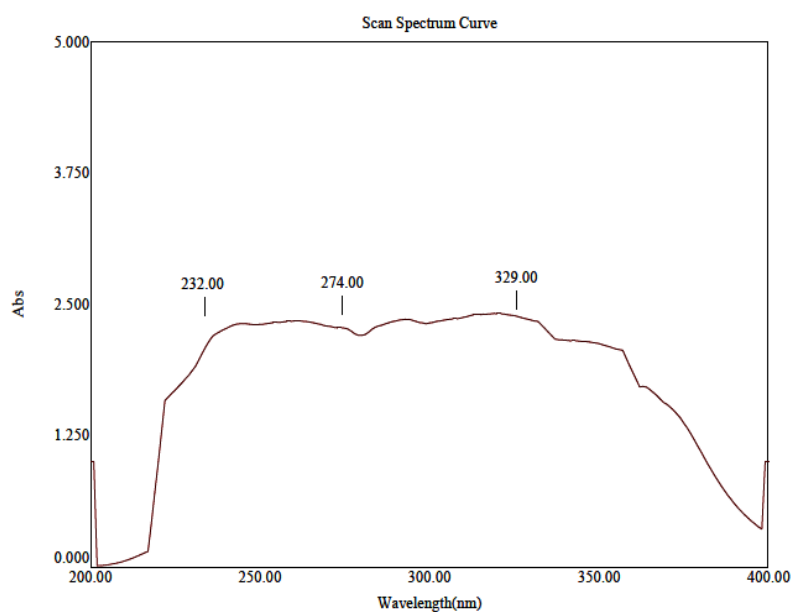
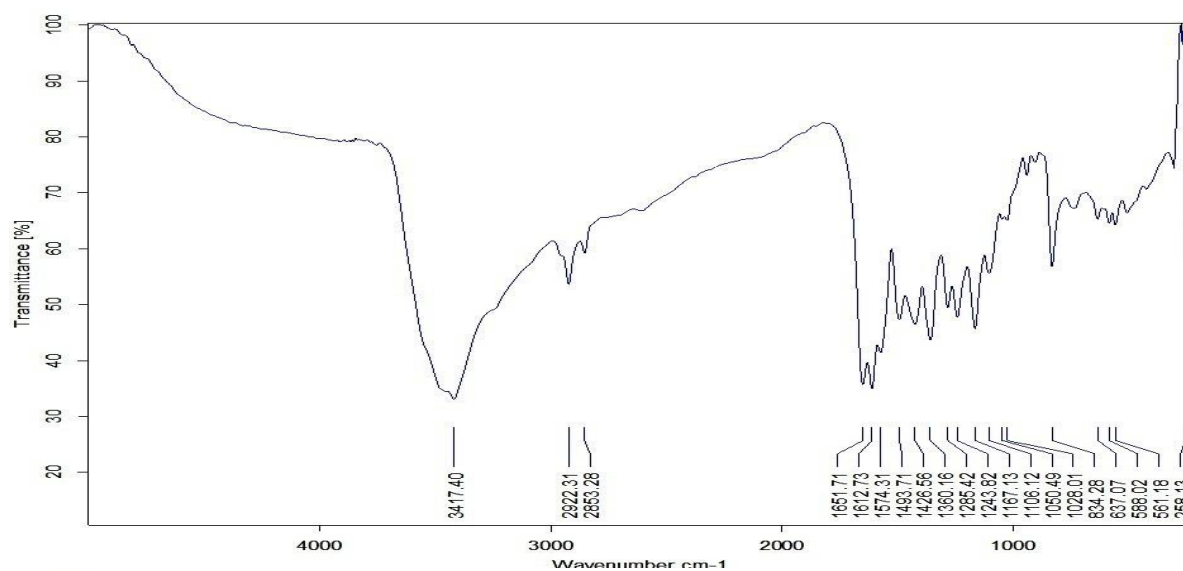


Figure S14. UV spectrum of compound (4) in MeOH

(A)



(B)

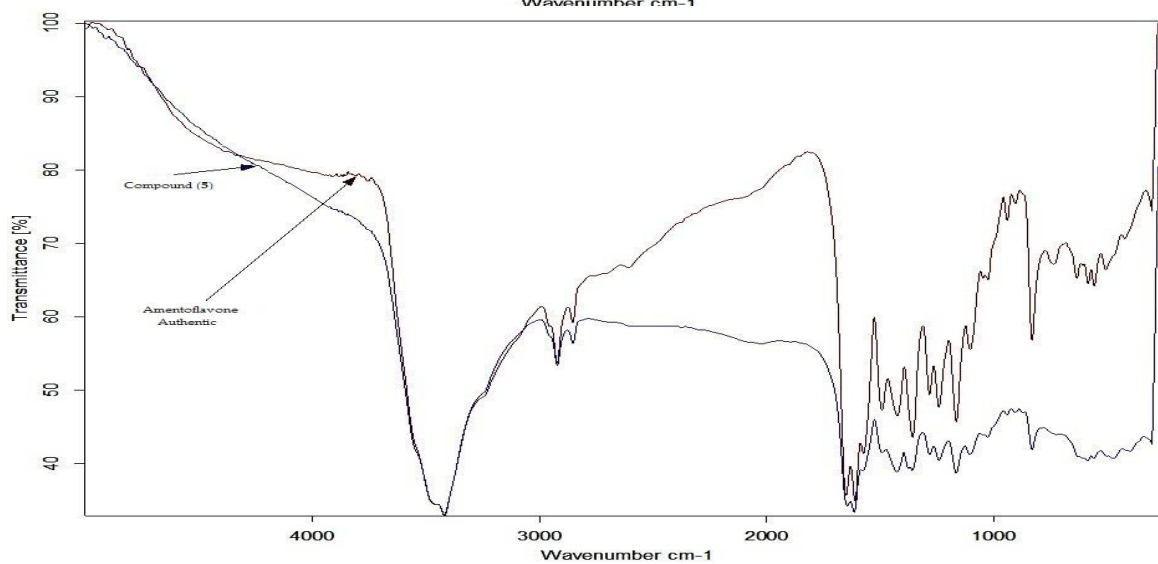
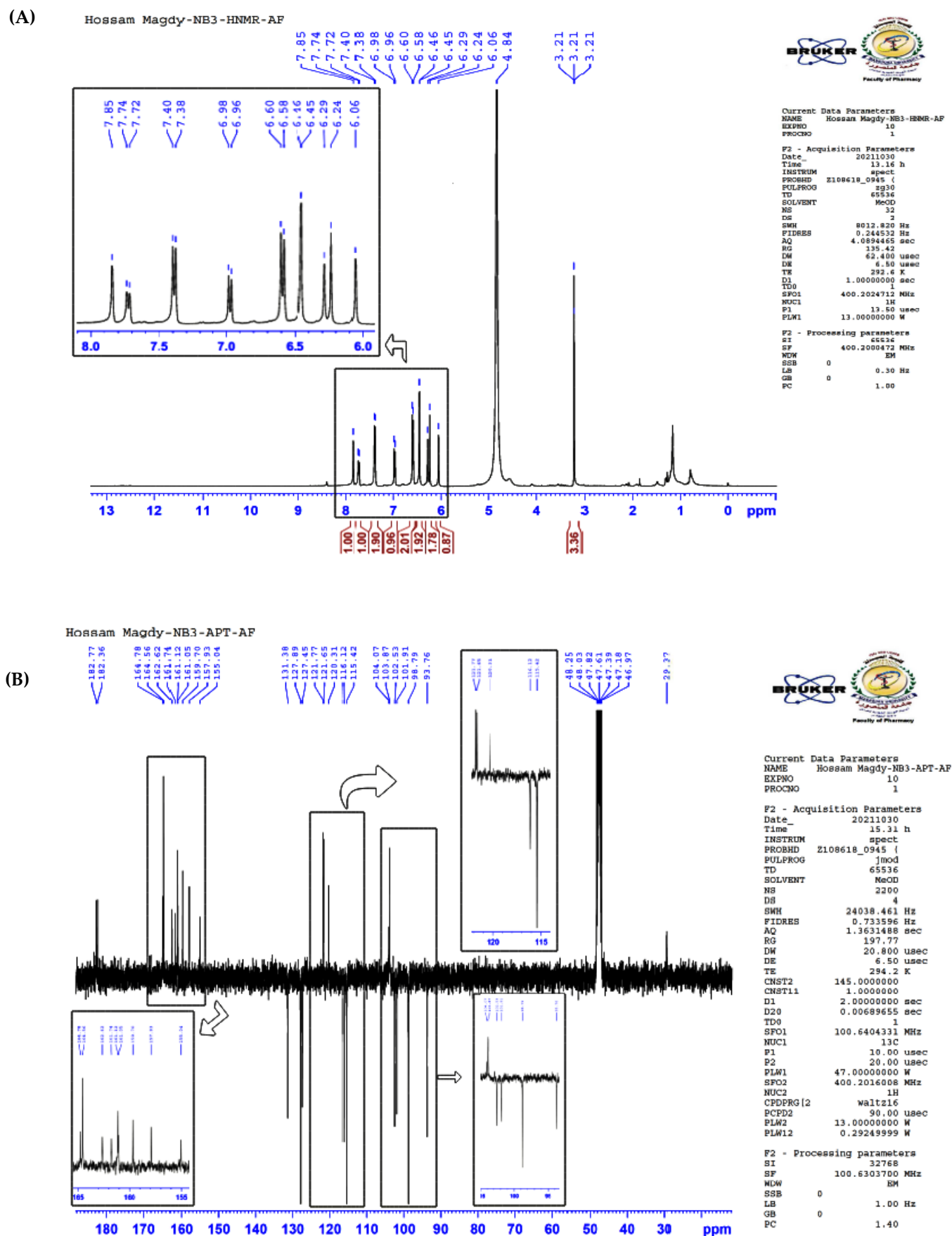
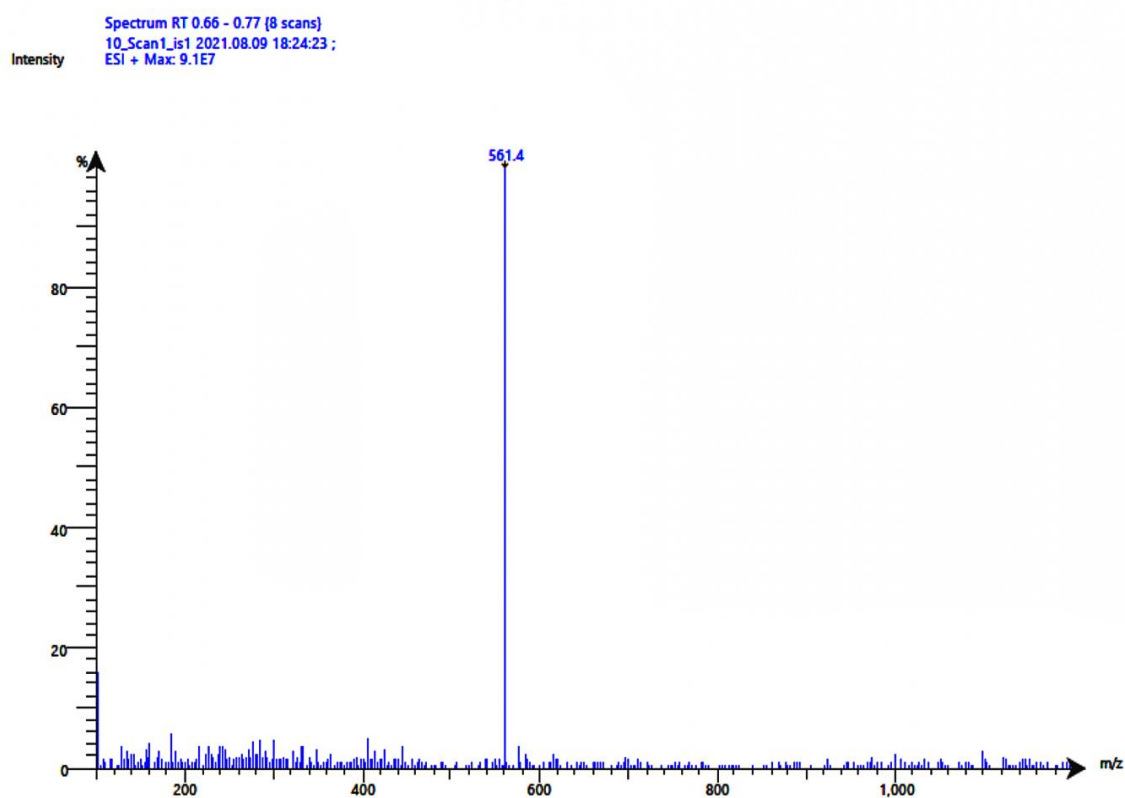


Figure S15. (A) IR spectrum of compound (4) in KBr disc

(B) IR fingerprint spectrum of compound (4) and Amentoflavone authentic sample in KBr disc

Figure S16. (A) ^1H and (B) APT NMR spectrum of compound (4) (CD_3OD)

(A)



(B)

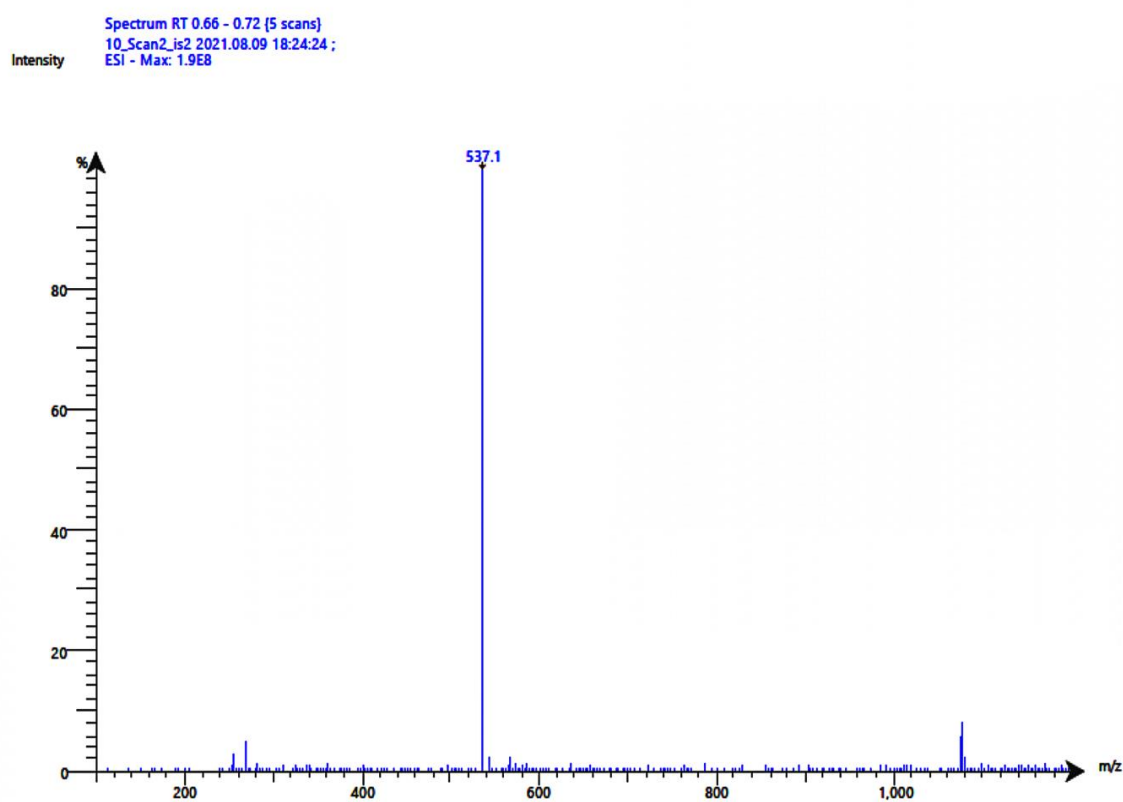


Figure S17. ESIMS "positive (A) and negative modes (B)" of compound (4)

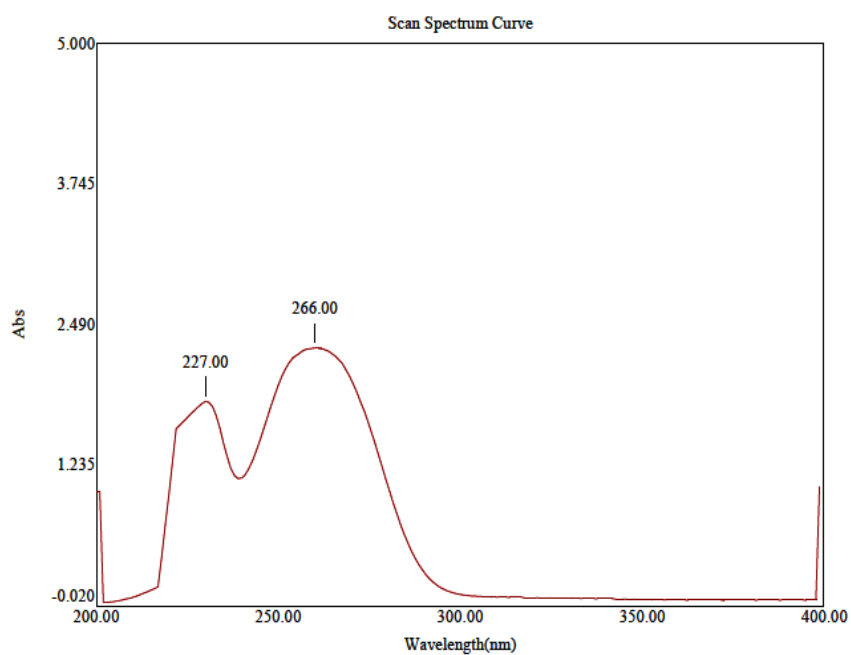


Figure S18. UV spectrum of compound (5) in MeOH

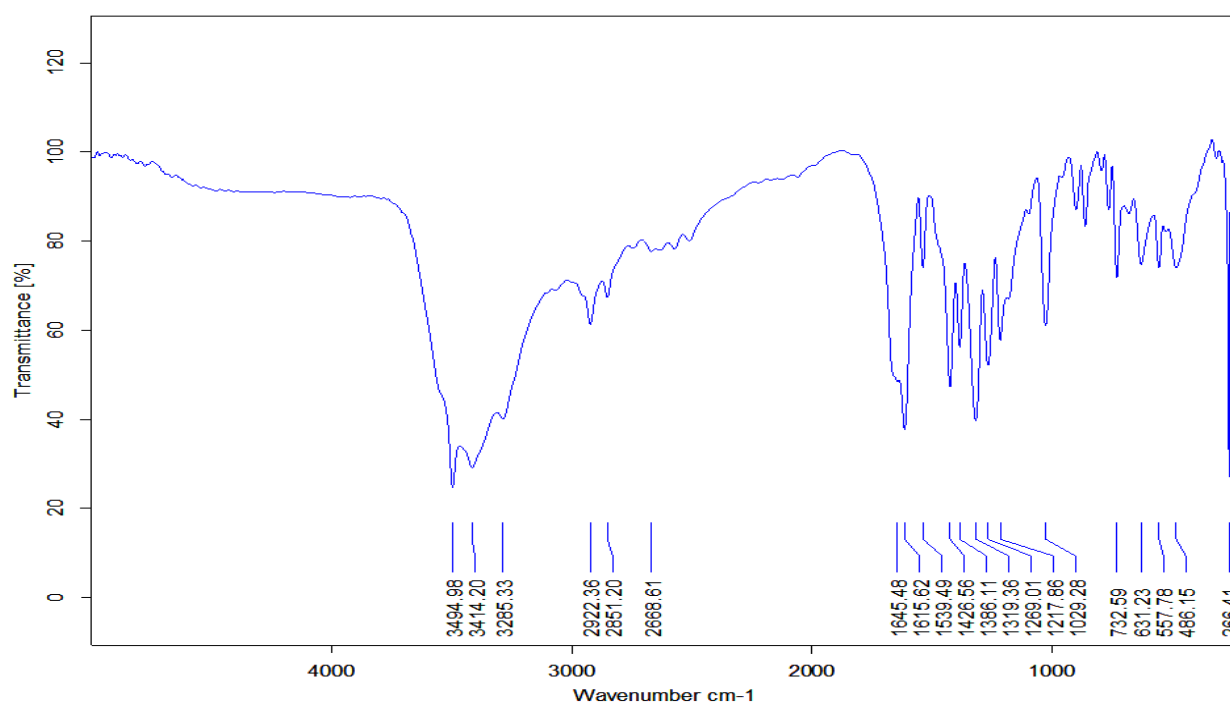
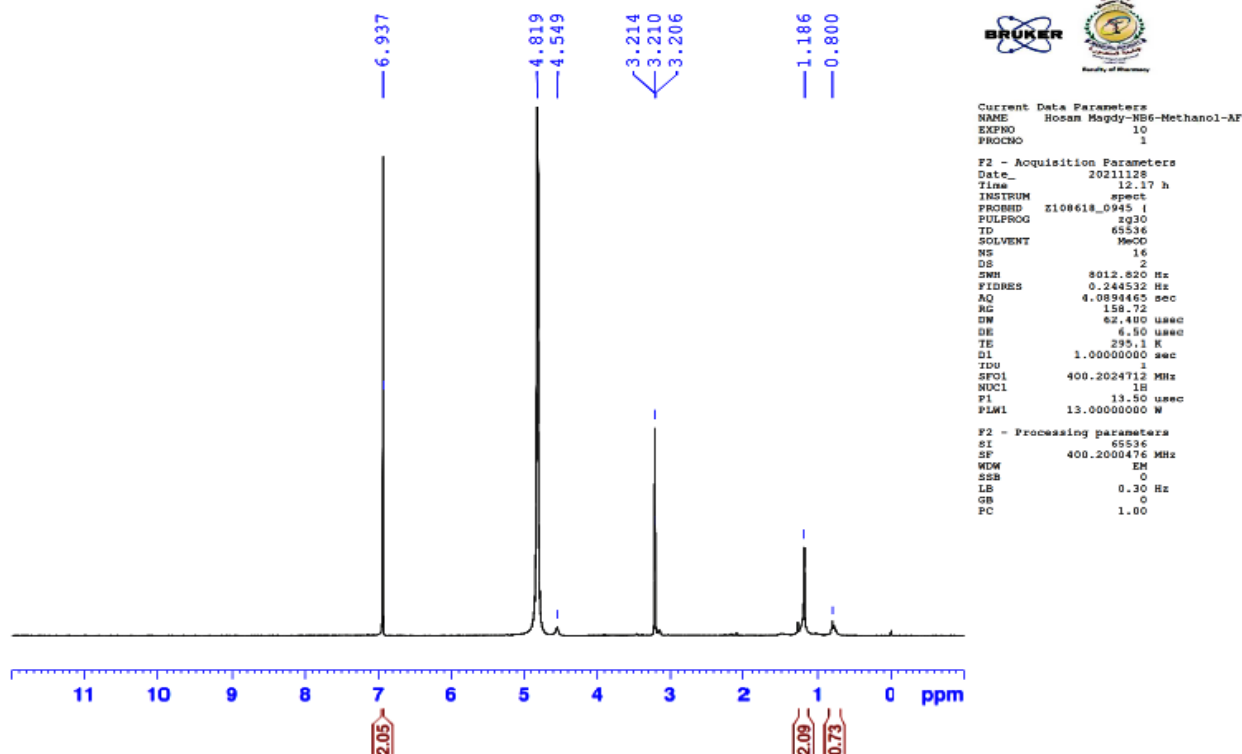


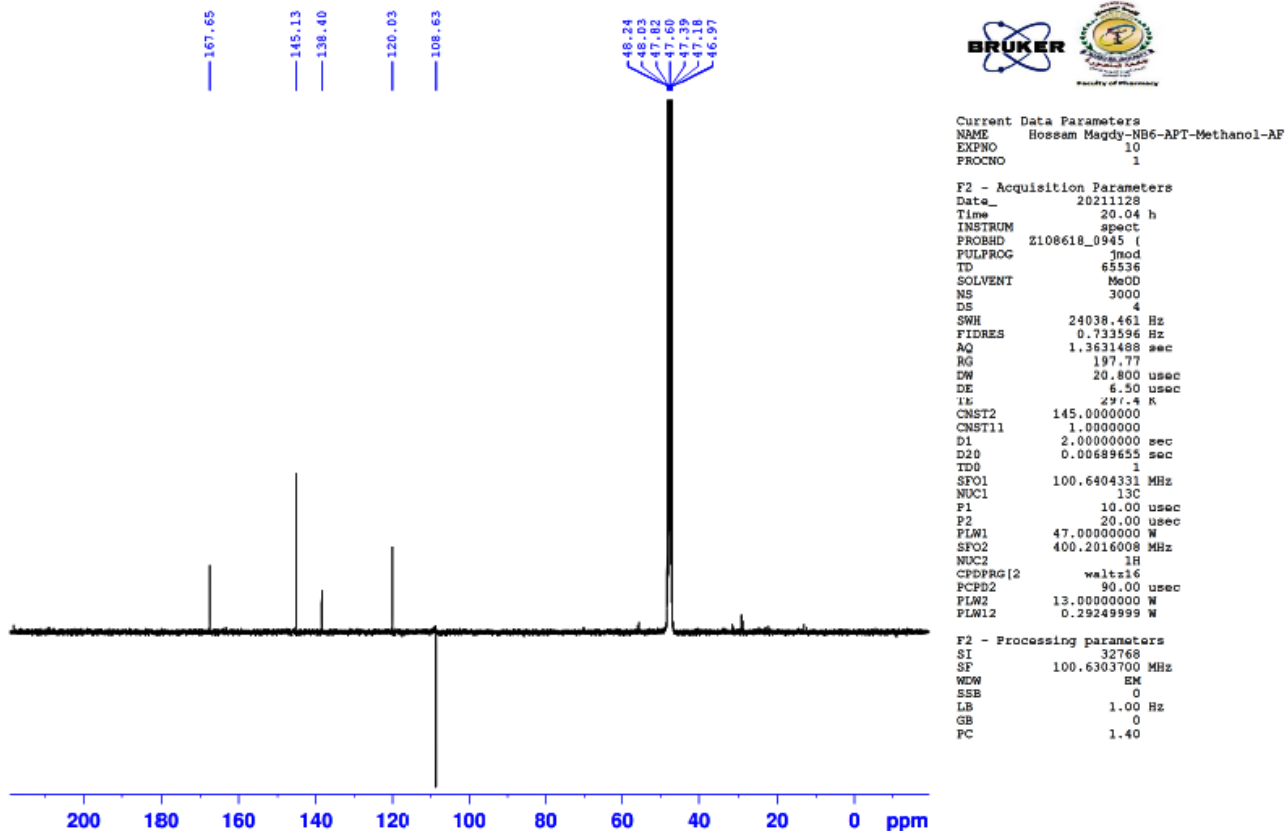
Figure S19. IR spectrum of compound (5) in KBr disc

(A) Hosam Magdy-NB6-Methanol-AF



(B)

Hossam Magdy-NB6-APT-Methanol-AF

Figure S20. (A) ^1H and (B) APT NMR spectrum of compound (5) (CD_3OD)

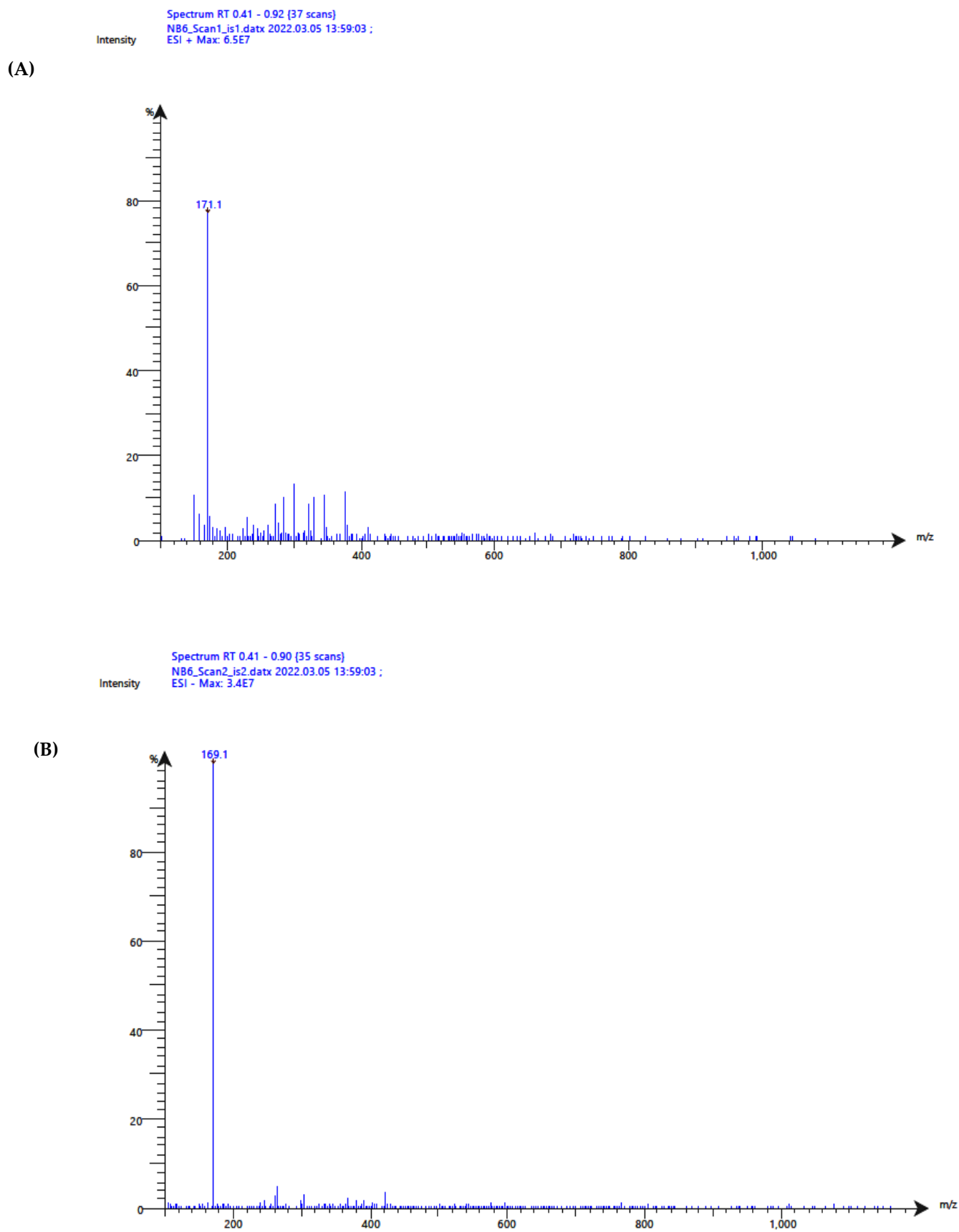


Figure S21. ESIMS "positive (A) and negative modes (B)" of compound (5)

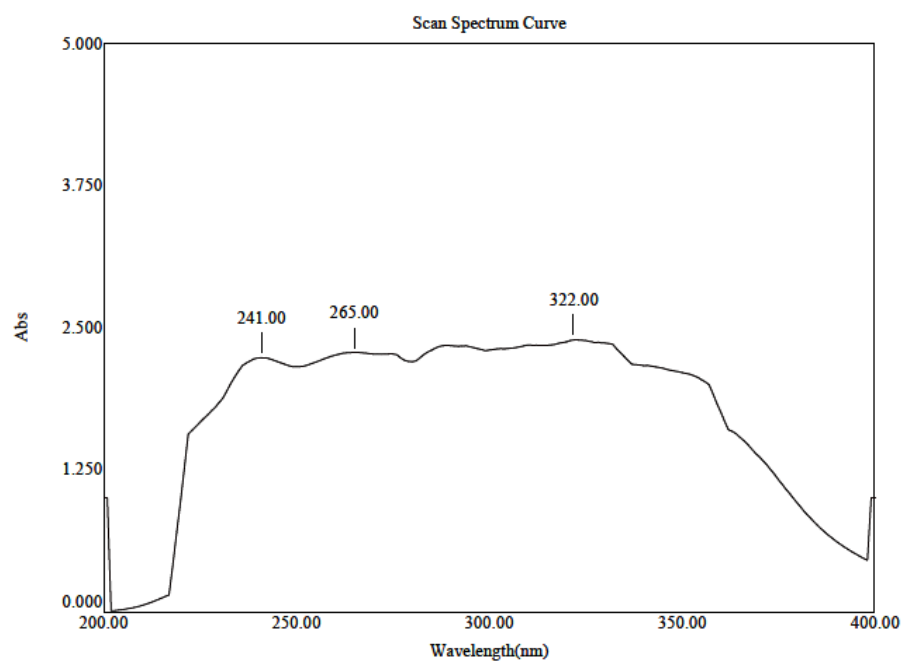


Figure S22. UV spectrum of compound (6) in MeOH

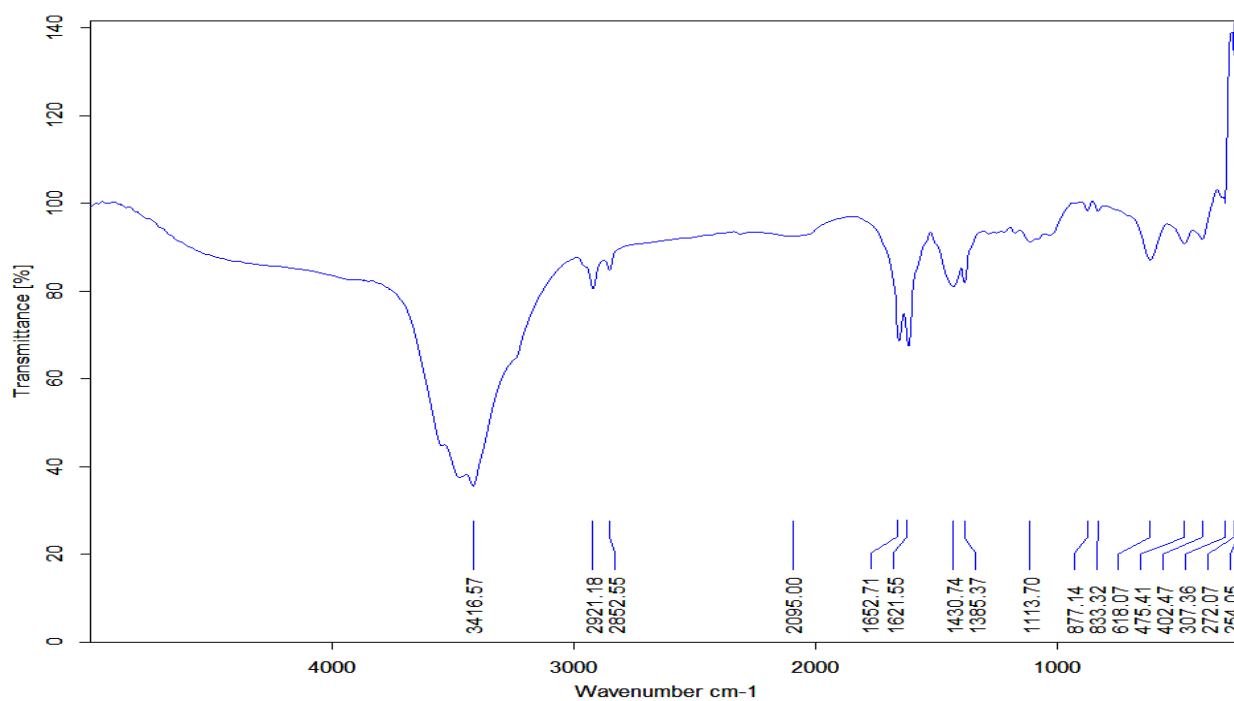
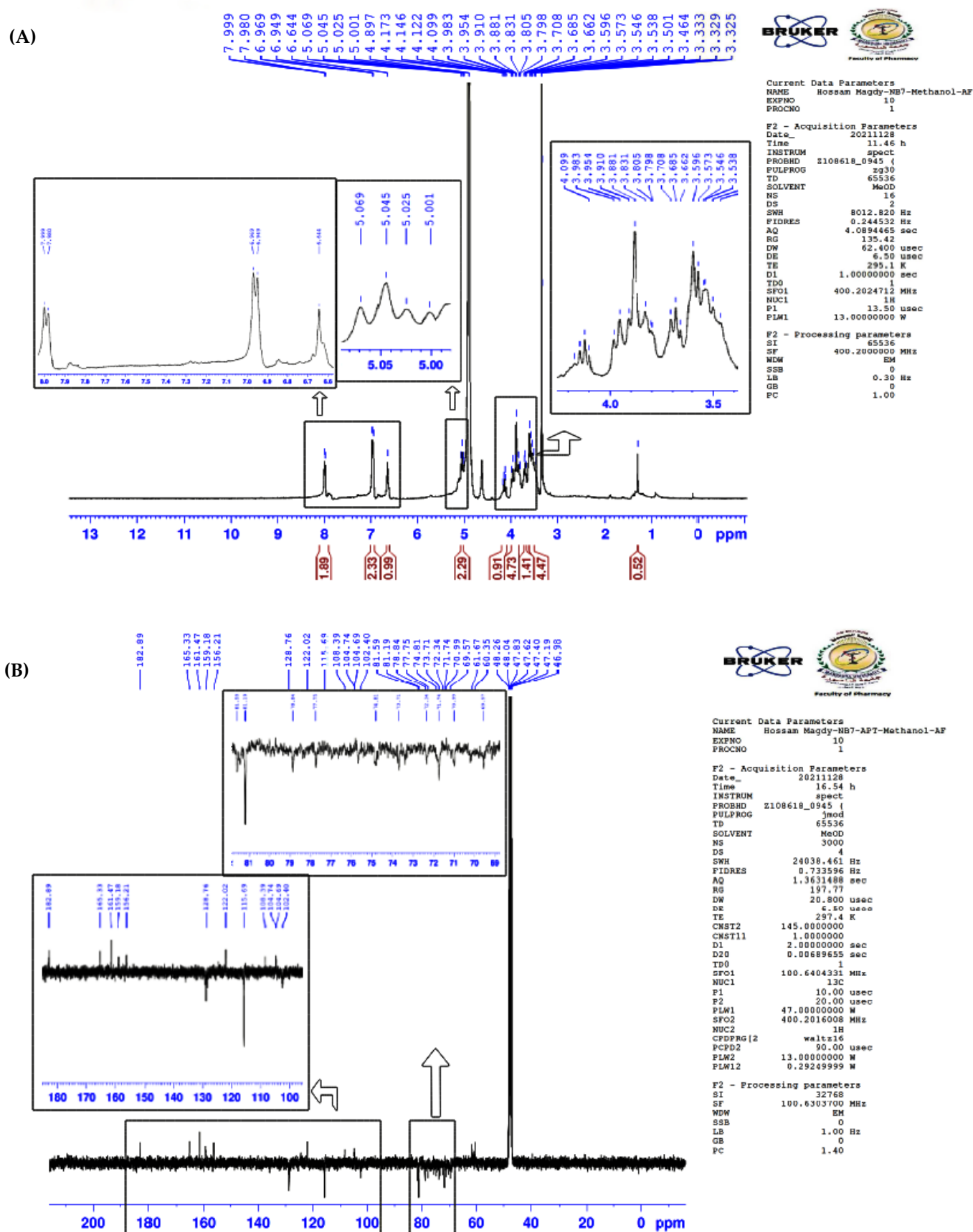


Figure S23. IR spectrum of compound (6) in KBr disc

Figure S24. (A) ^1H and (B) APT NMR spectrum of compound (6) (CD_3OD)

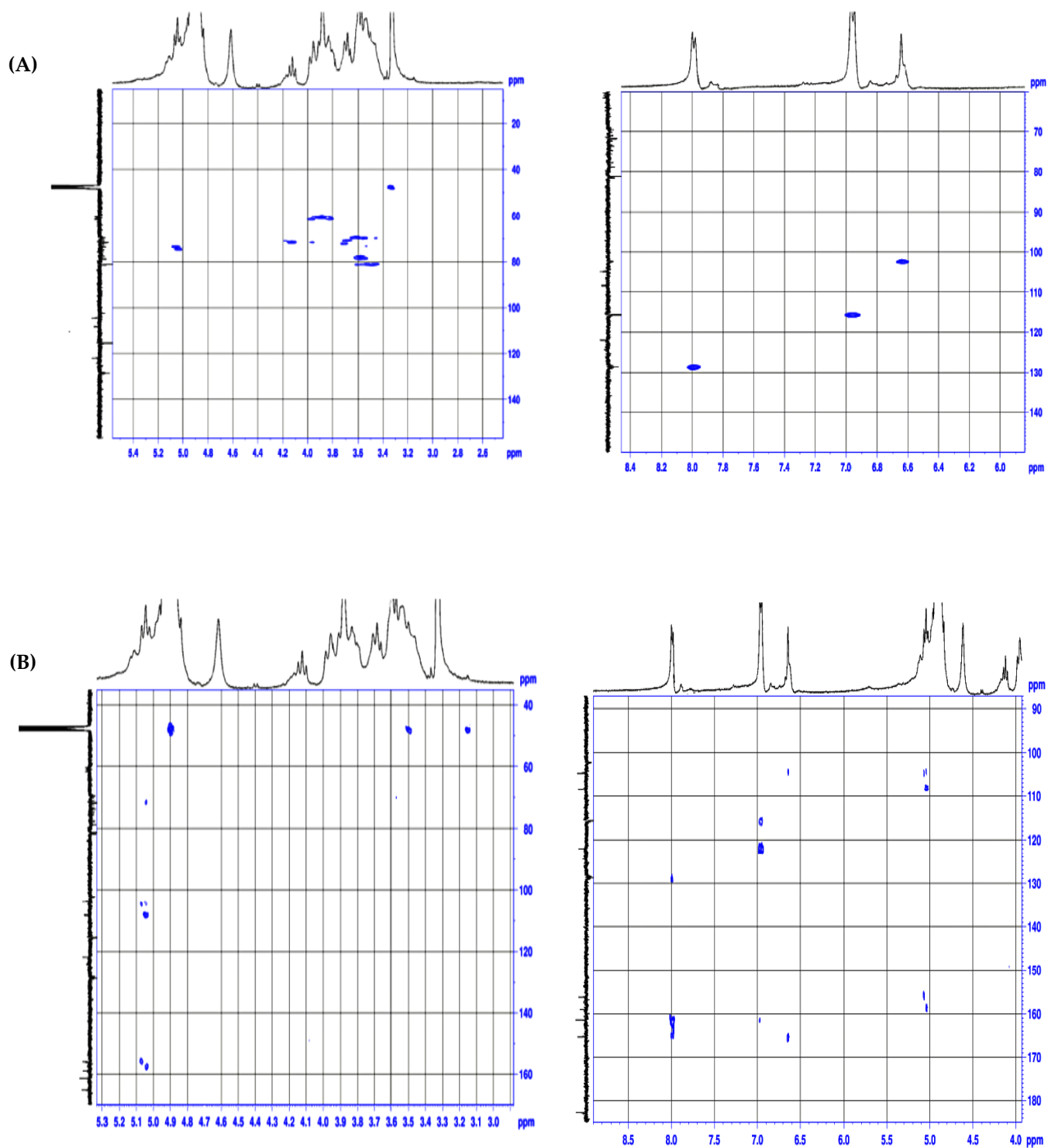


Figure S25. (A) HSQC and (B) HMBC spectrums of compound (6) (CD₃OD)

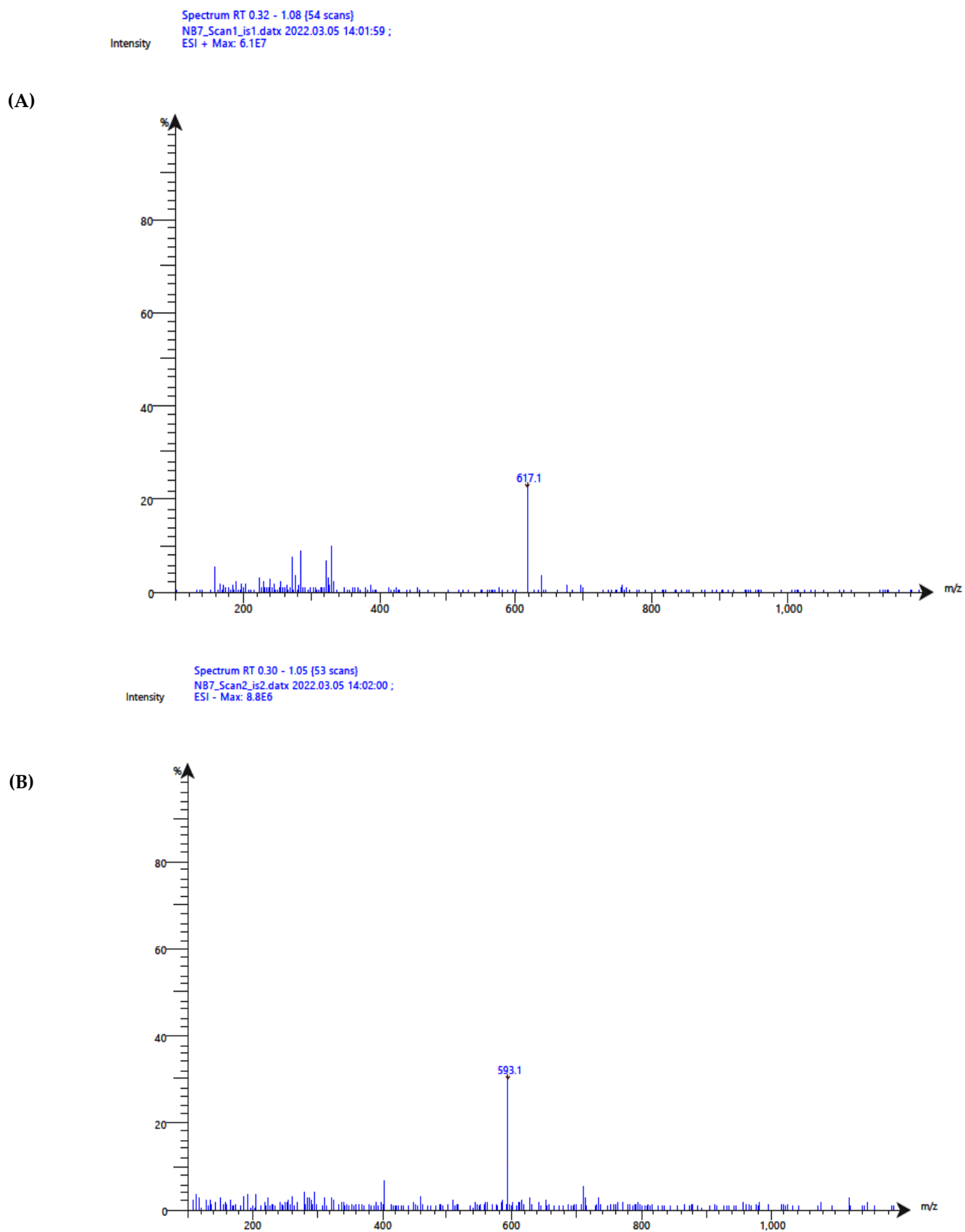
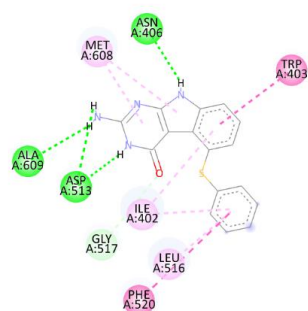


Figure S26. ESIMS "positive (A) and negative modes (B)" of compound (6)

(A)



(B)

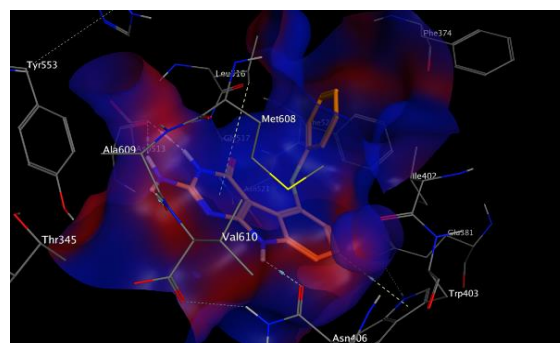
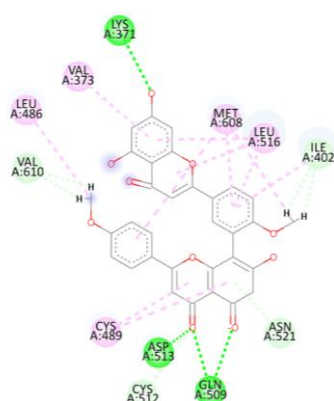


Figure S27. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **1UE** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink and violet lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

(A)



(B)

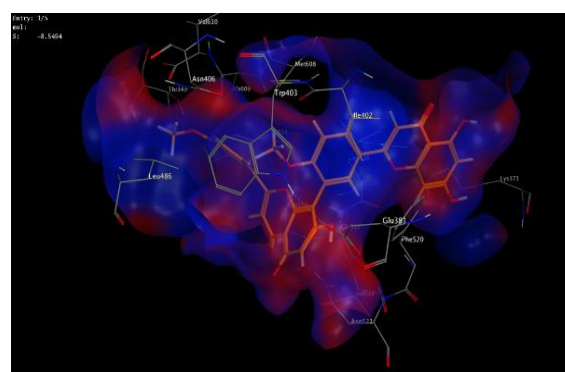
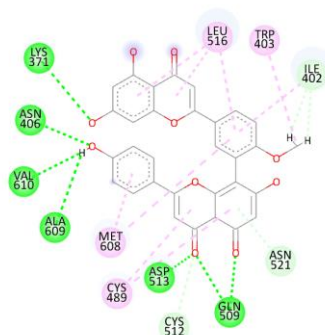


Figure S28. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **Compound (1)** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

(A)



(B)

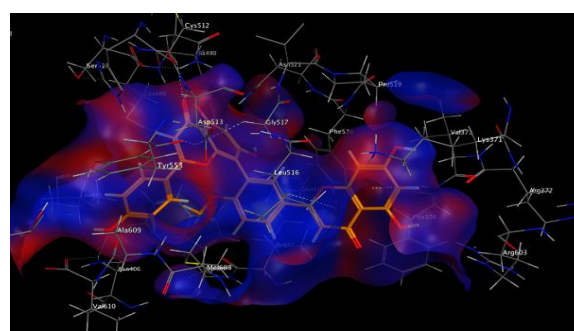


Figure S29. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **Compound (2)** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

(A)

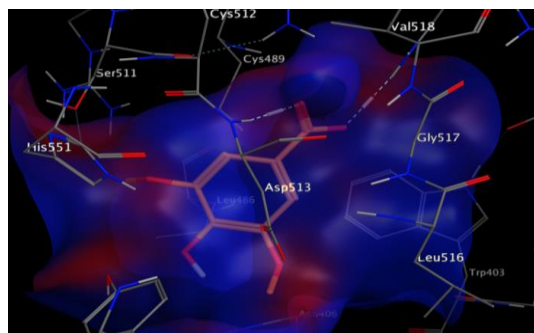
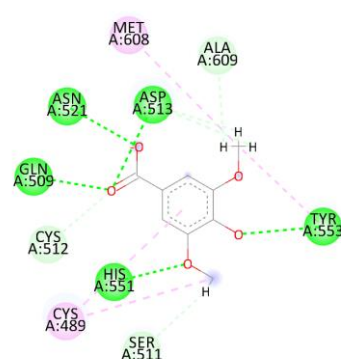
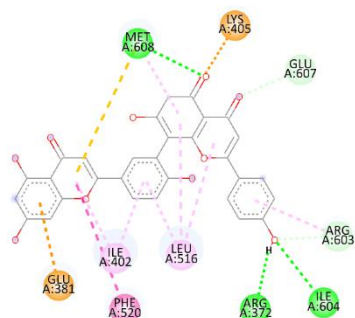


Figure S30. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **Compound (3)** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

(A)



(B)

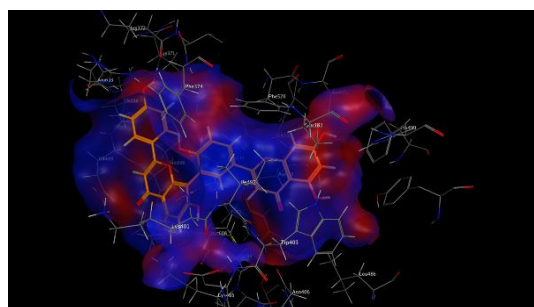
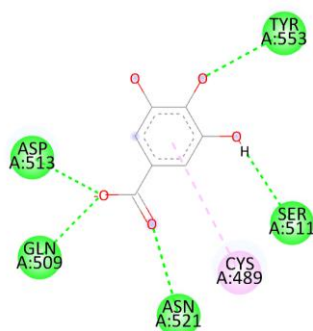


Figure S31. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **Compound (4)** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink, violet and orange lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

(A)



(B)

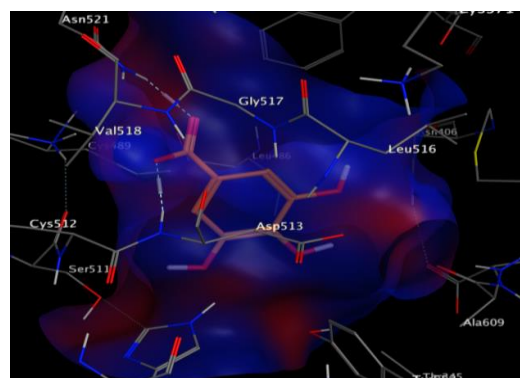


Figure S32. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **Compound (5)** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

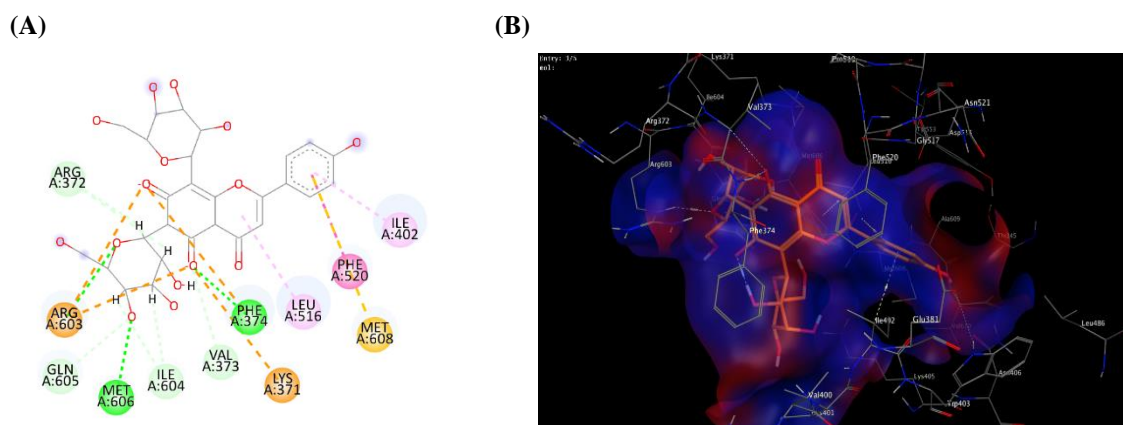


Figure S33. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **Compound (6)** into active site of TS-DHFR (PDB code: 4KY4). (A) Dotted pink, violet and orange lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

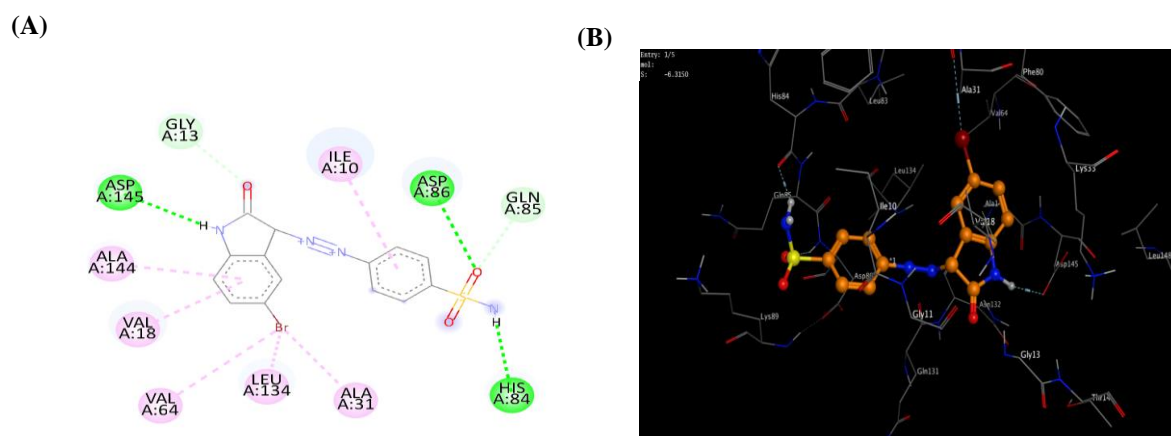


Figure S34. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **(106)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

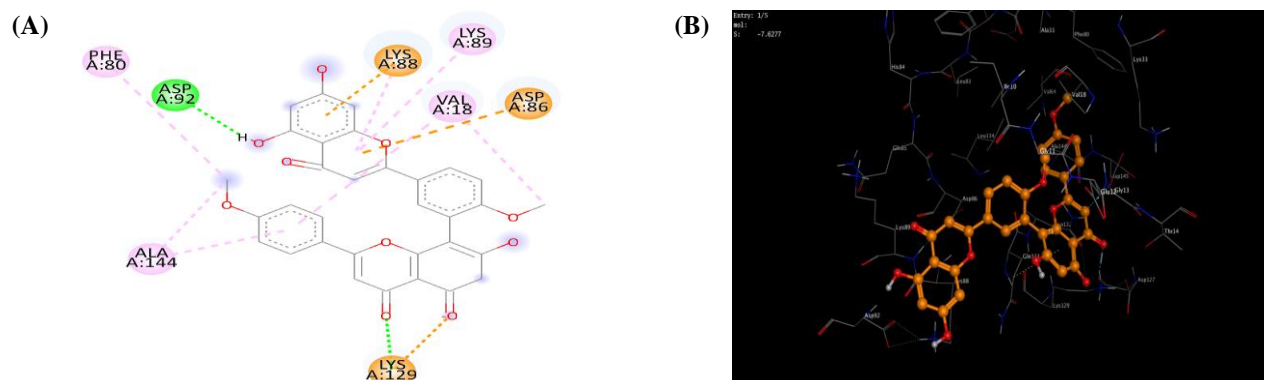


Figure S35. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **compound (1)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink and orange lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

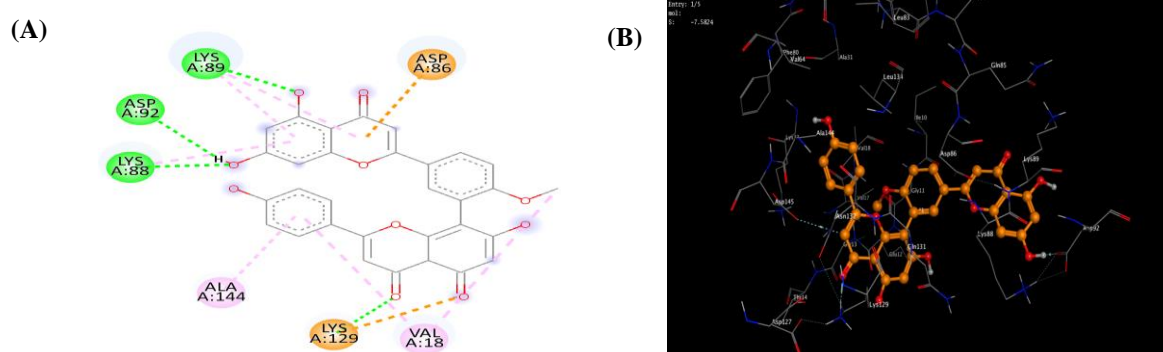


Figure S36. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **compound (2)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink, violet and orange lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

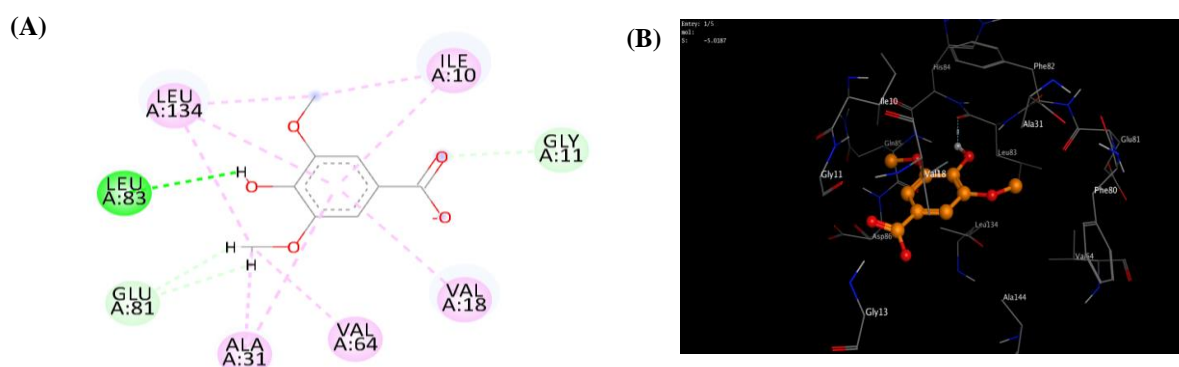


Figure S37. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **compound (3)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

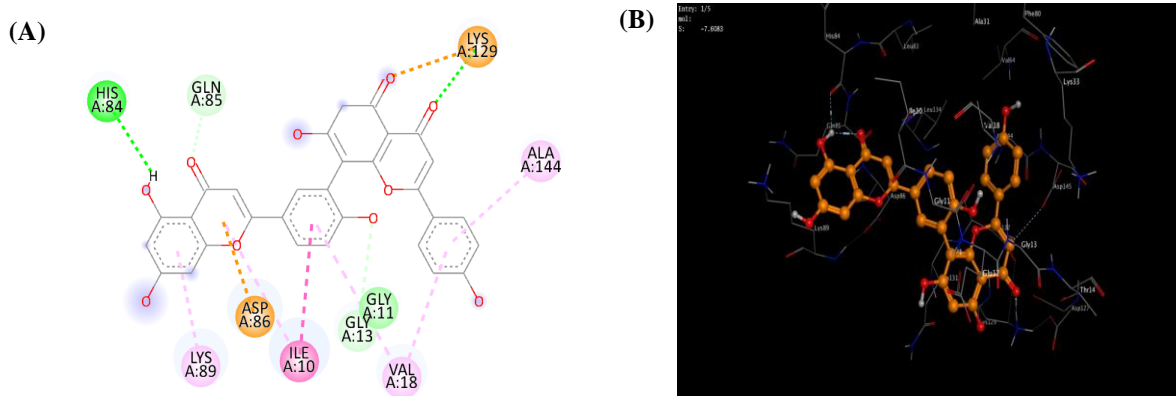


Figure S38. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **compound (4)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink, violet and orange lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

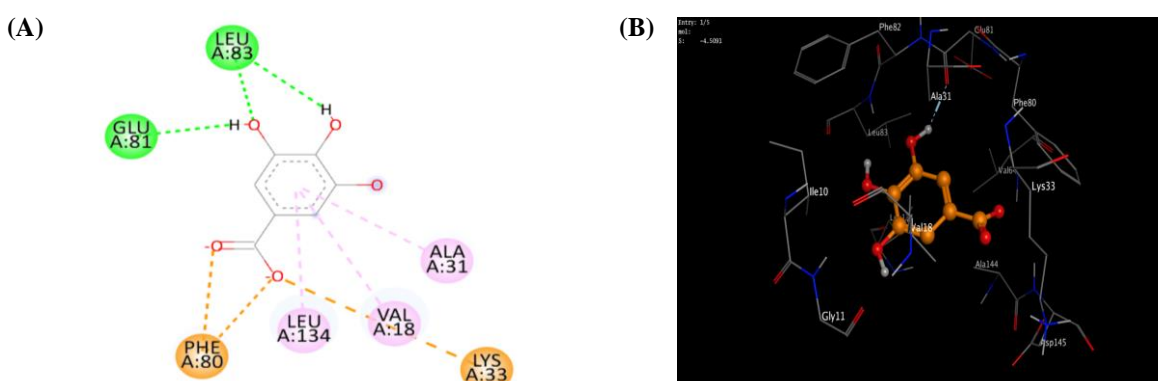


Figure S39. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **compound (5)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink and orange lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.

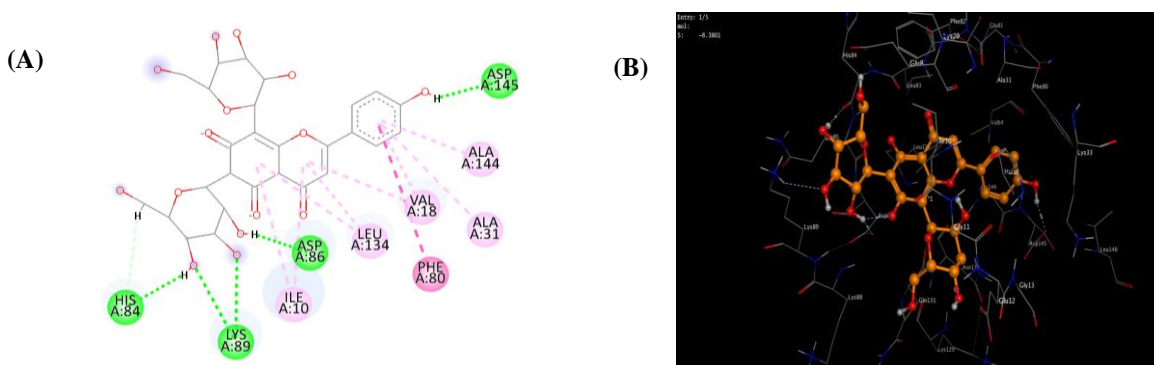


Figure S40. 2D (A) and 3D (B) patterns demonstrating the binding interaction of **compound (6)** into active site of CDK-2 (PDB ID: 1FVT). (A) Dotted pink and violet lines indicate hydrophobic interaction, and dotted green lines indicate hydrogen bonds. (B) Blue color surface refers a hydrophobic area and red color surface refers hydrophilic area.