Copper-assisted anti-cancer activity of hydroxycinnamic acid terpyridine conjugates on triple-negative breast cancer

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Electronic Supplementary Information

Table of Contents

S12. *ct*-DNA binding studies (UV-Visible absorbance studies)

S13. *ct*-DNA binding studies (*ct*-DNA melting studies)

S14. *ct*-DNA binding studies (Circular Dichroism studies)

S15. Live-dead imaging of 4T1 cells treated with **4** and **5**

S16. Clonogenic assay in 4T1 cells treated with **4** and **5**

S17. HPLC traces of **4**

S18. HPLC traces of **5**

S19. HPLC traces of **Cu-1**

S20. Stability of **4** & **5** in TE Buffer

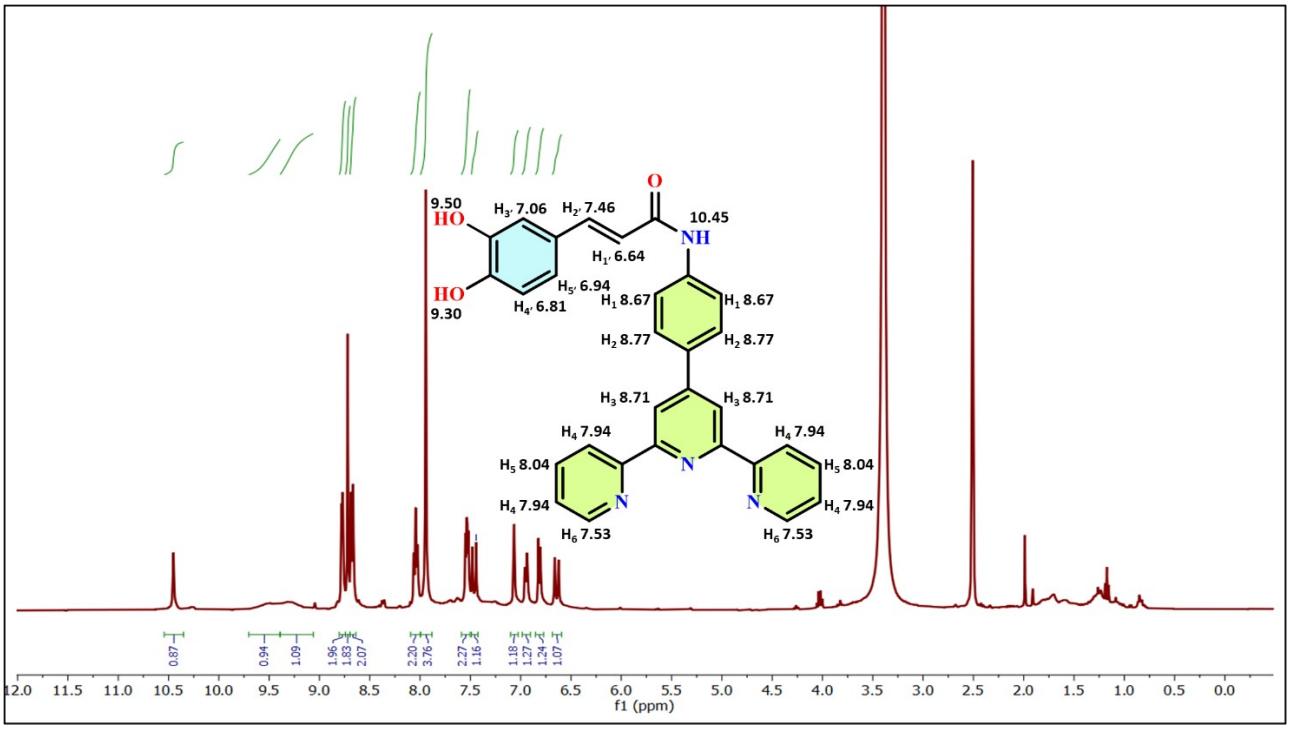


Figure S1. ^{1}H NMR (400 MHz) spectrum of **2** in DMSO – d_6.

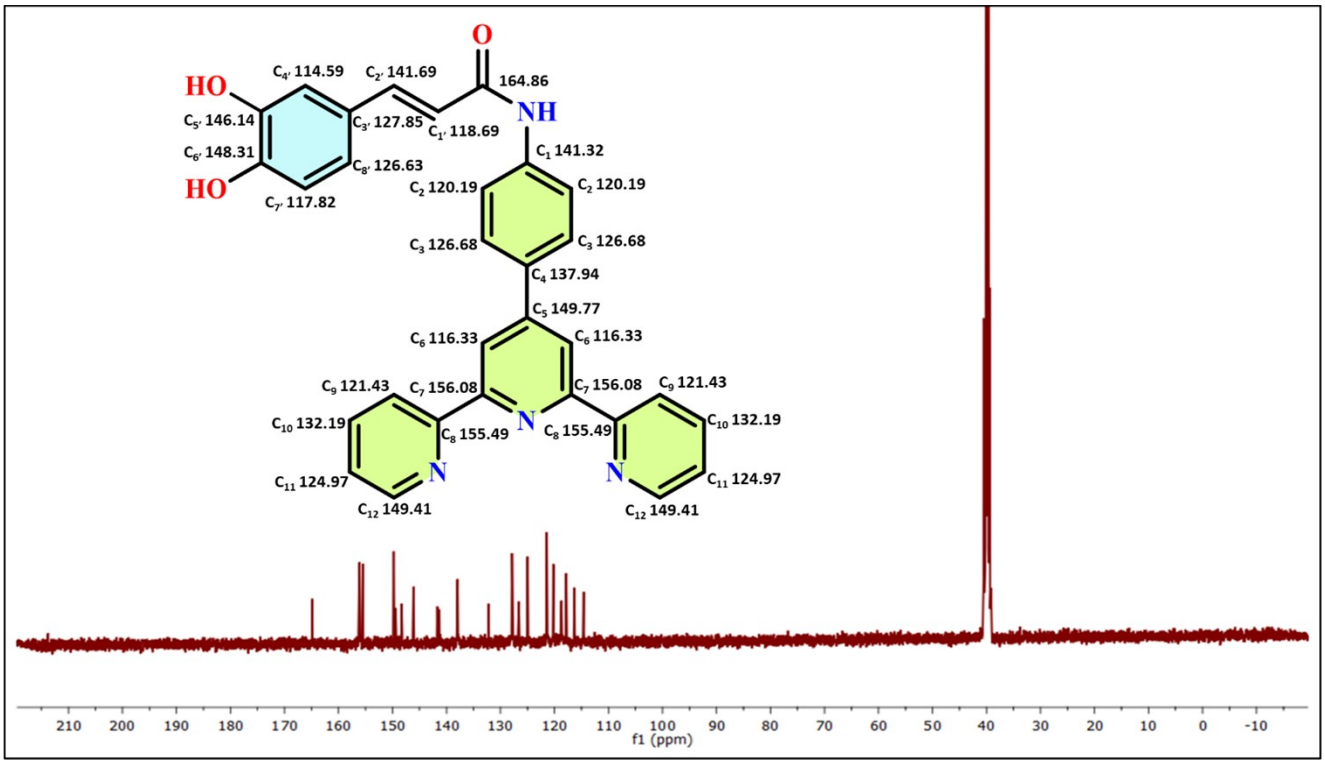


Figure S2. ^{13}C NMR (400 MHz) spectrum of **2** in DMSO – d_6.

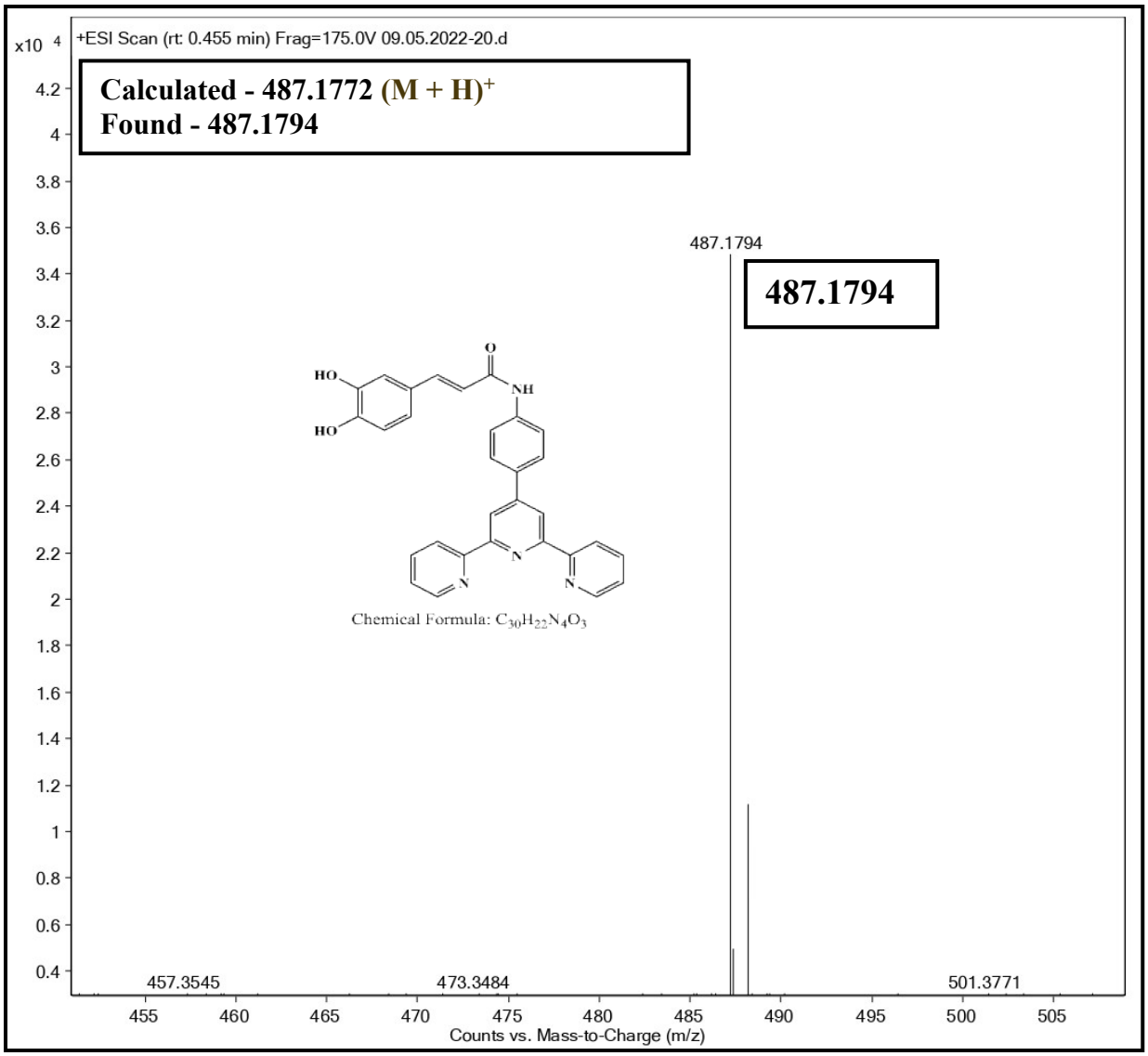


Figure S3. ESI-MS spectrum of **2.**

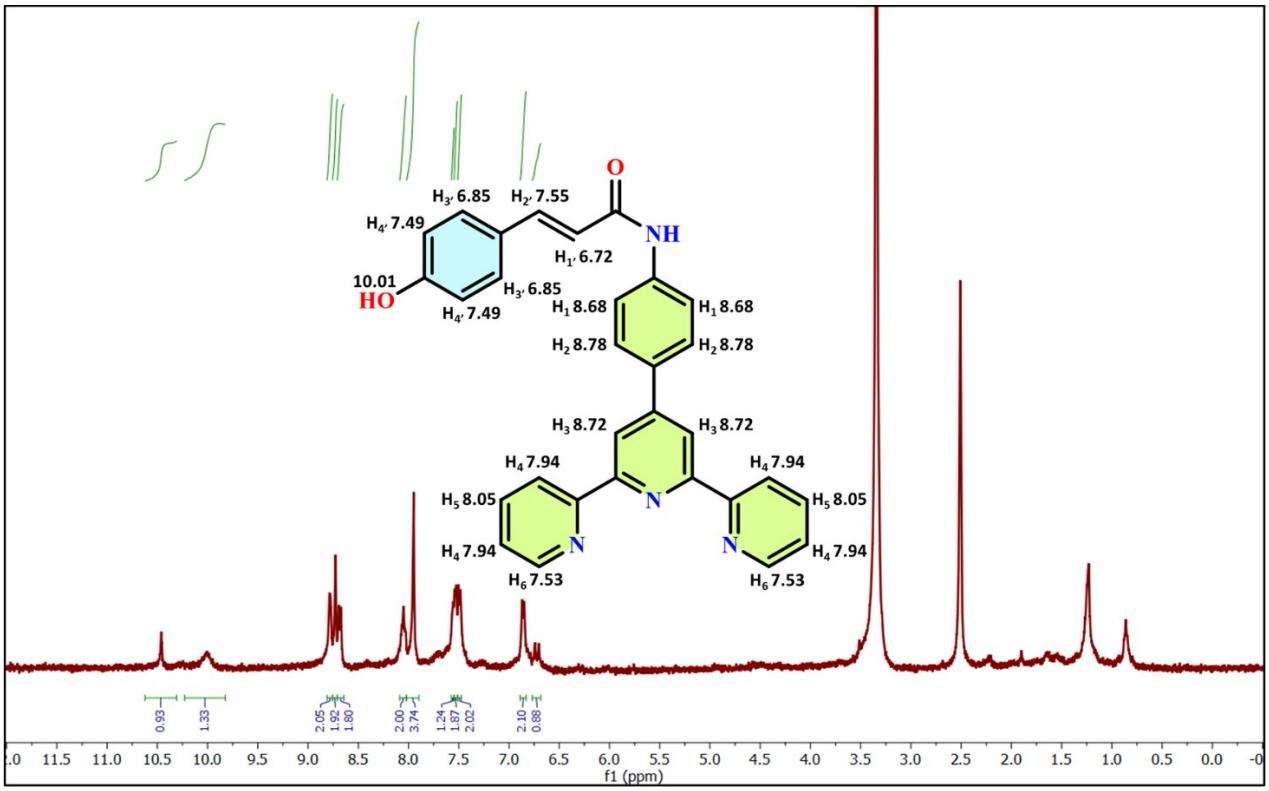


Figure S4. ^{1}H NMR (400 MHz) spectrum of **3** in DMSO – d_6.

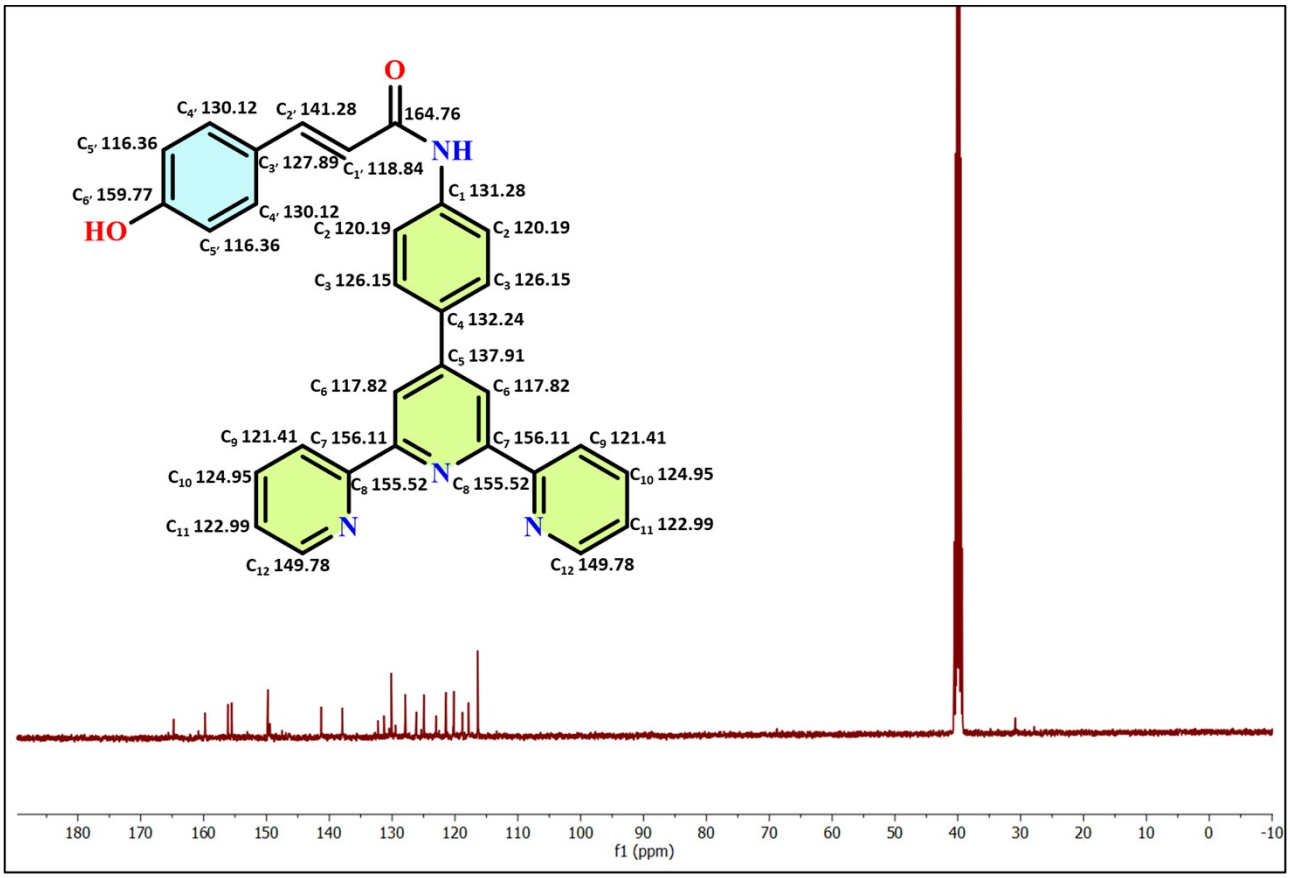


Figure S5. ^{13}C NMR (400 MHz) spectrum of **3** in DMSO – d_6.

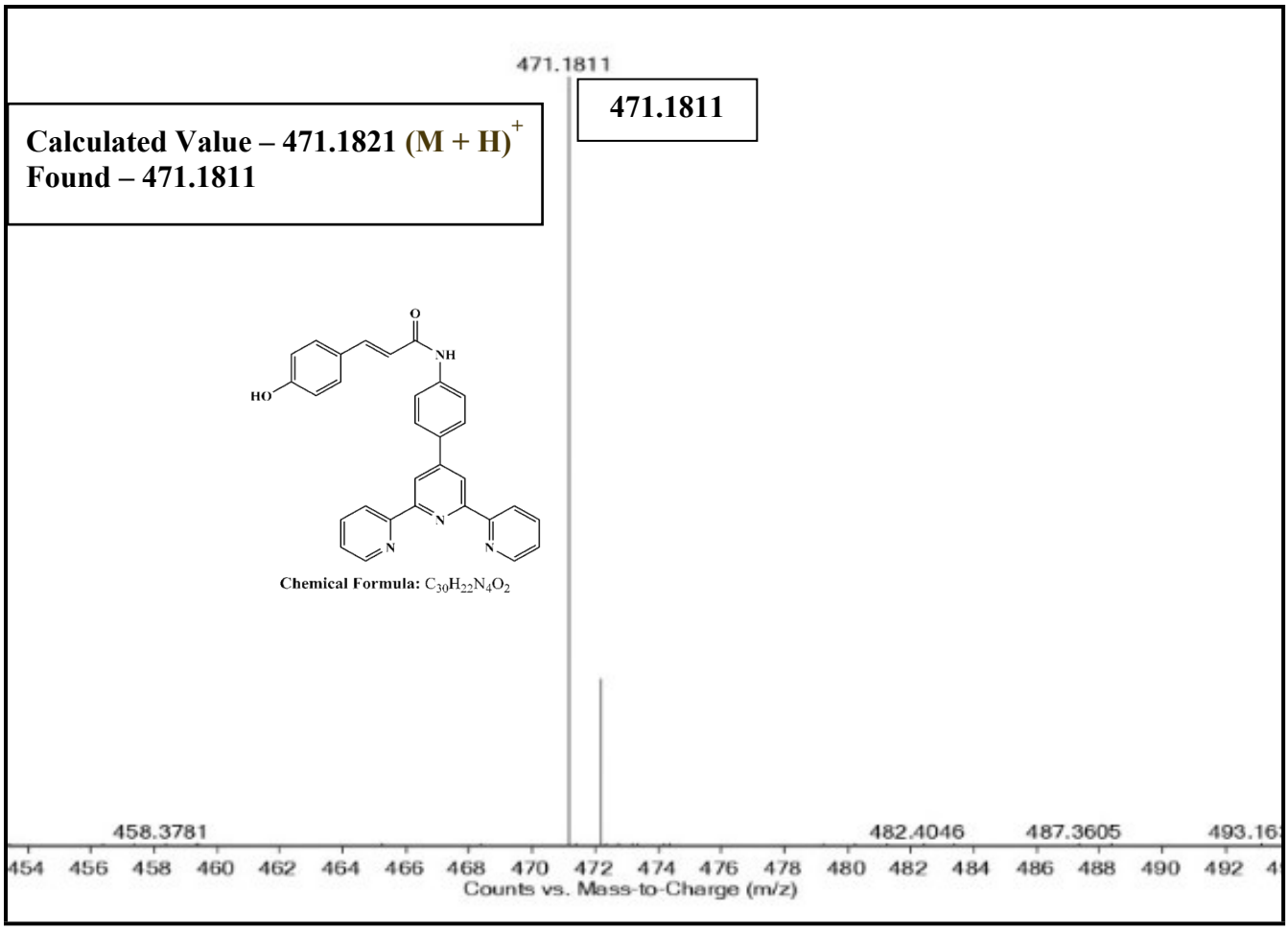


Figure S6. ESI-MS spectrum of 3.

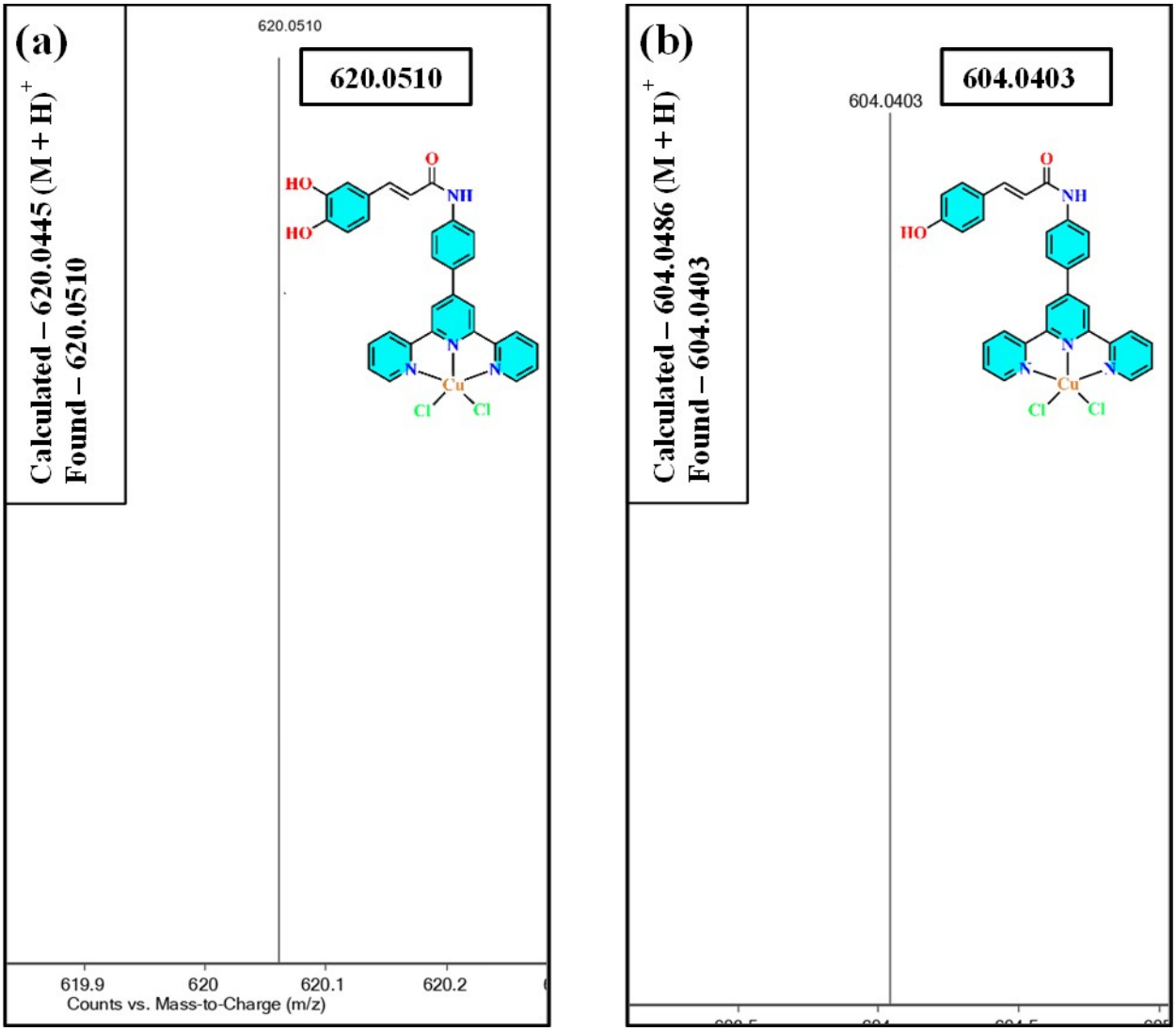


Figure S7. ESI-MS spectra of (a) **4** and (b) **5**.

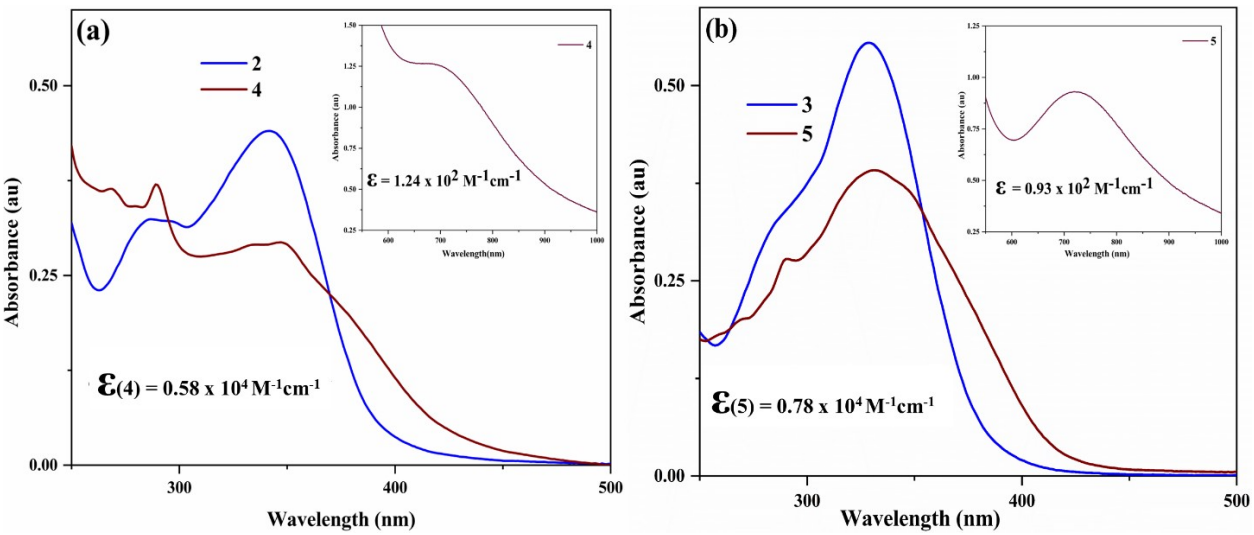


Figure S8. UV - visible spectra of **(a) 2** [50 μM], **4** [50 μM] in Methanol and Characteristic d-d transition spectrum of **4** [10 mM] in DMSO **(Inset)**, **(b) 3** [50 μM], **5** [50 μM] in Methanol and Characteristic dd transition spectrum of **5** [10 mM] in DMSO **(Inset)**.

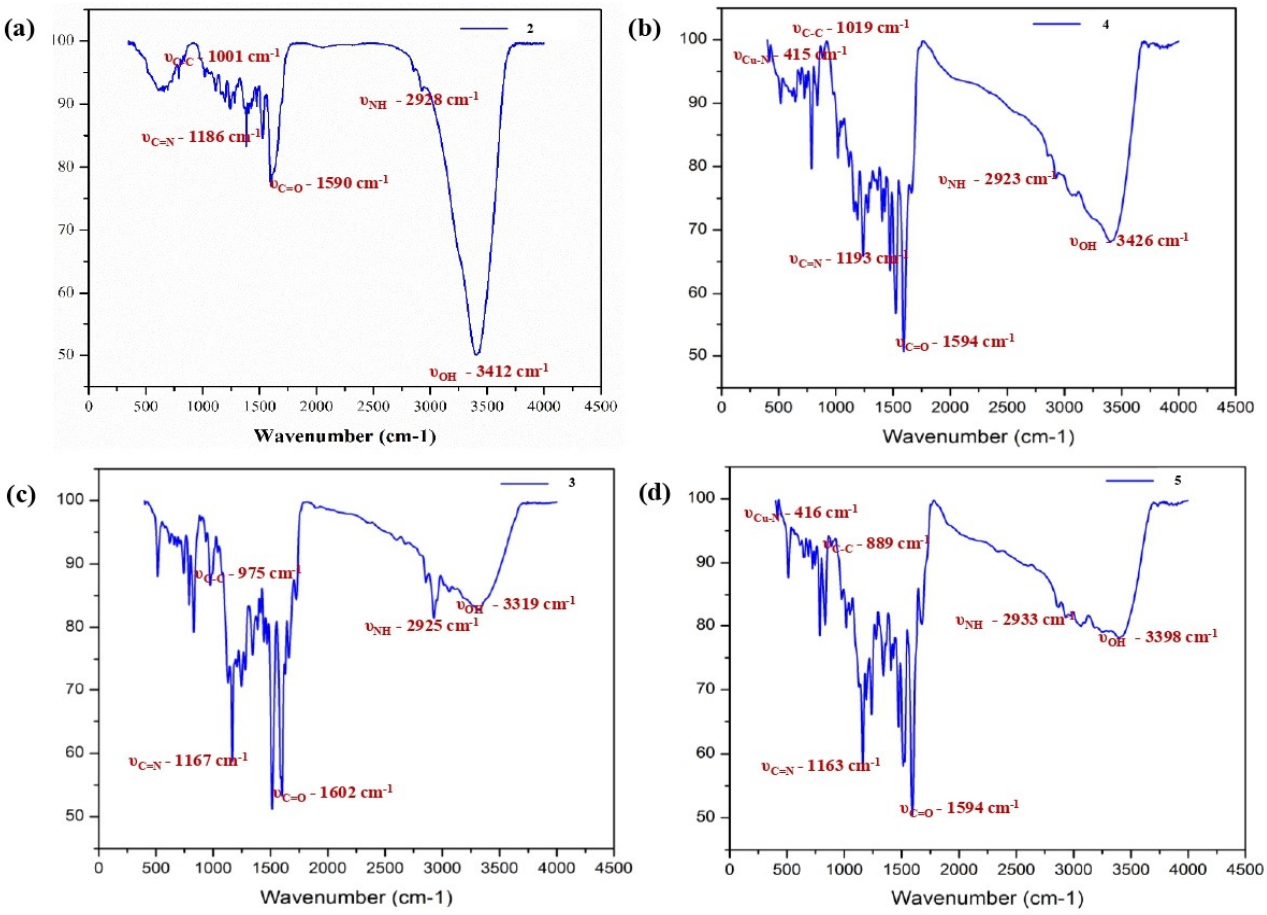


Figure S9. Solid state FT-IR spectra of (a) **2**, (b) **4**, (c) **3**, (d) **5**.

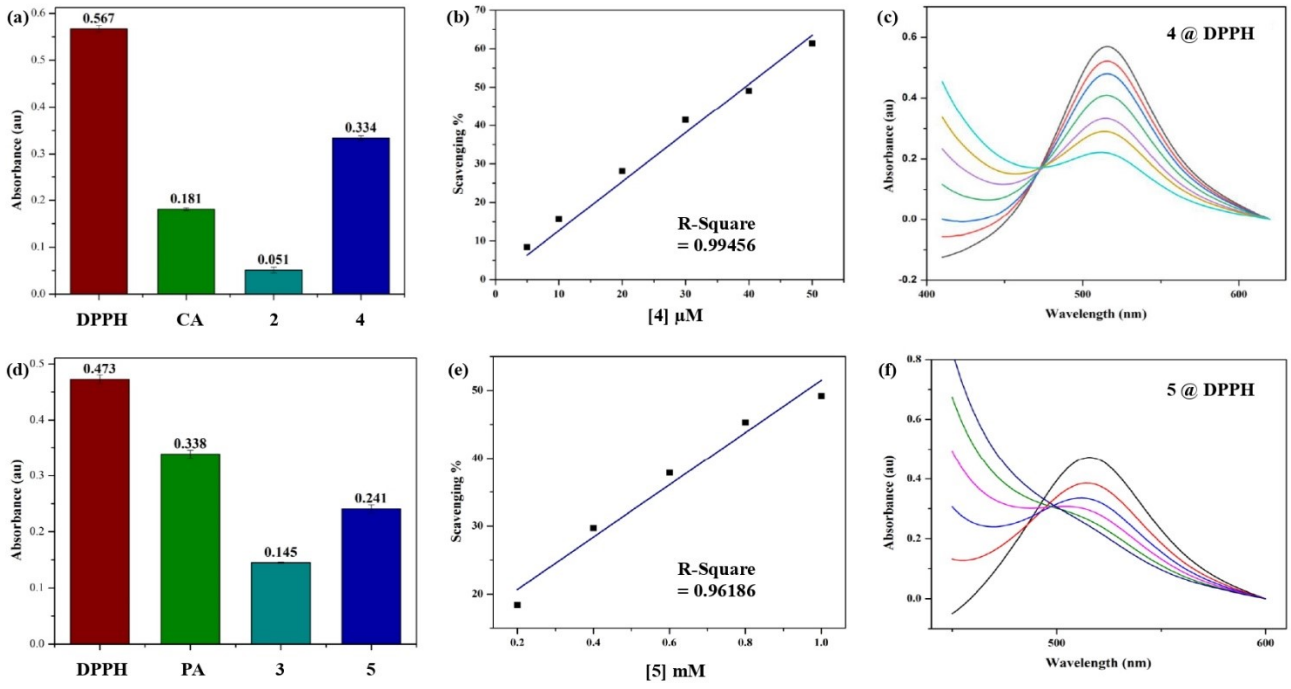


Figure S10. DPPH Radical scavenging assay. (**a**) & (**d**) Graphical representation of DPPH radical scavenging by **CA**, **2**, **4** at 30 µM and **PA**, **3**, **5** at 1 mM. (**b**) & (**e**) Linear regression to determine the IC_{50} values of DPPH scavenging by **4** and **5**. (**c**) & (**f**) UV-Visible absorption spectra for DPPH scavenging by **4** (0 µM - 80 µM) and **5 (**0 mM - 2.5 mM**)**.

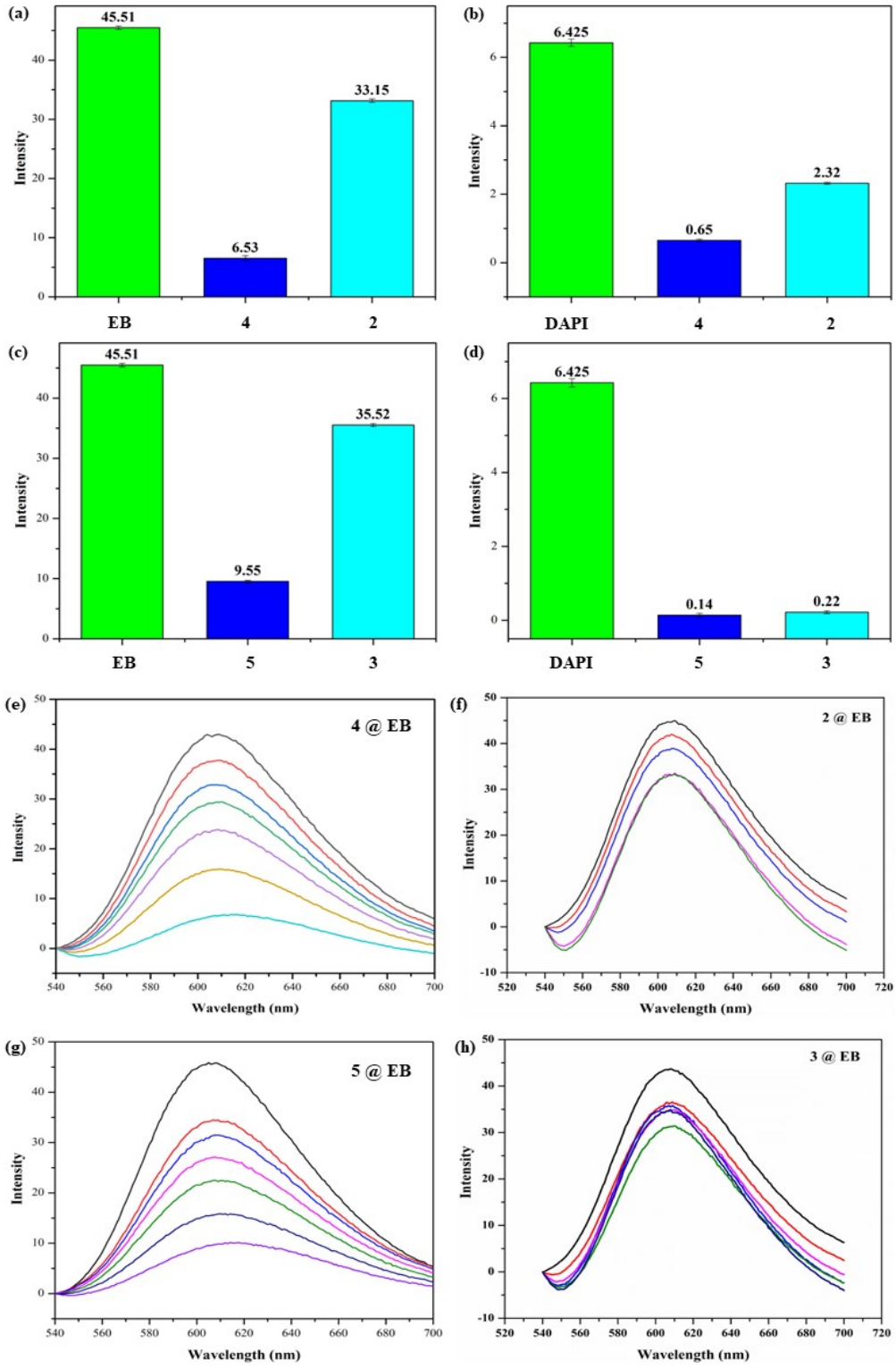


Figure S11. Dye displacement studies towards *ct*-DNA binding to determine the binding fashion. (**a**) & (**c**) Graphical representation of EB ([EB] - 10 μM) displacement by **2**, **4** at 35 μM and **3**, **5**

at 15 µM. (**b**) & (**d**) Graphical representation of DAPI ([DAPI] - 10 nM) displacement by **2**, **4** at 35 µM and **3**, **5** at 15 µM. (**e**) & (**g**) UV-Visible absorption spectra for EB displacement by **4** (0 µM - 50 µM) and **5 (**0 µM - 30 µM**)**. (**f**) & (**h**) UV-Visible absorption spectra for EB displacement by **2** (0 µM - 50 µM) and **3 (**0 µM - 30 µM**)**.

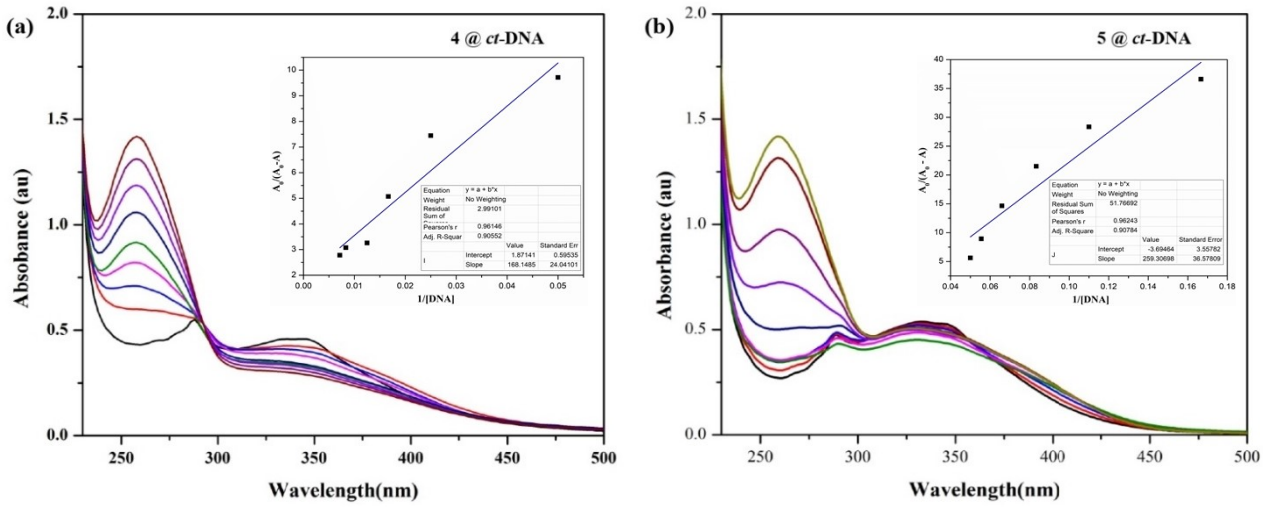


Figure S12. UV-visible absorbance studies towards ct-DNA binding to determine binding fashion. (**a**) & (**b**) UV-visible absorption spectra for ct-DNA binding ([**4**]/[**5**] - 20 µM) by ct-DNA (0 - 200 µM).

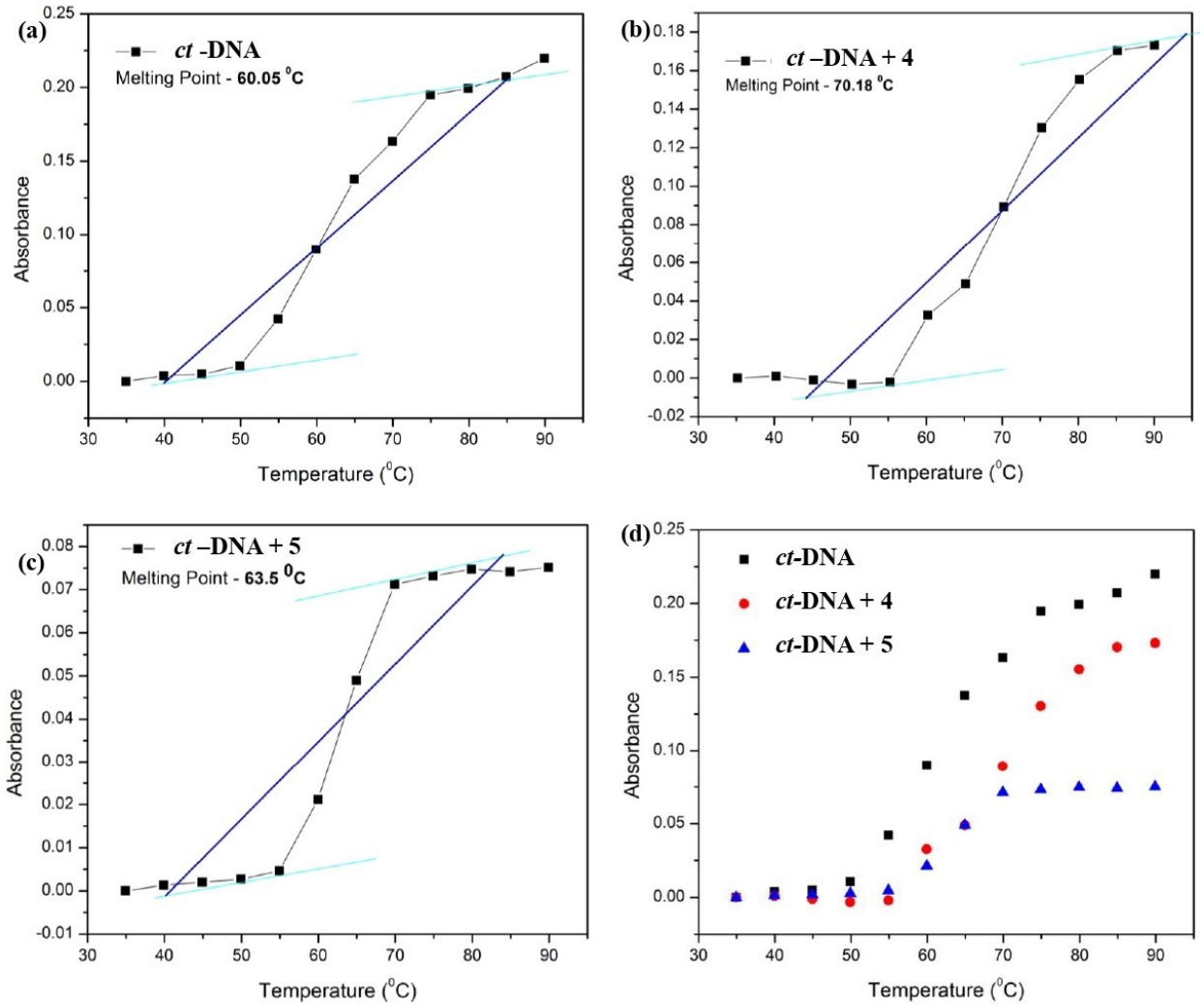


Figure S13. *ct*-DNA melting studies to determine binding fashion. (**a**) The melting profile of *ct*-DNA (100 μM) was studied by a temperature-controlled UV-Visible spectrometer at 260 nm. (**b**) & (**c**) Melting profile of *ct*-DNA (100 μM) in the presence of **4** (10 μM) and **5** (10 μM) at 260 nm. (**d**) combined representation of ct-DNA melting in the presence and absence of **4** & **5**.

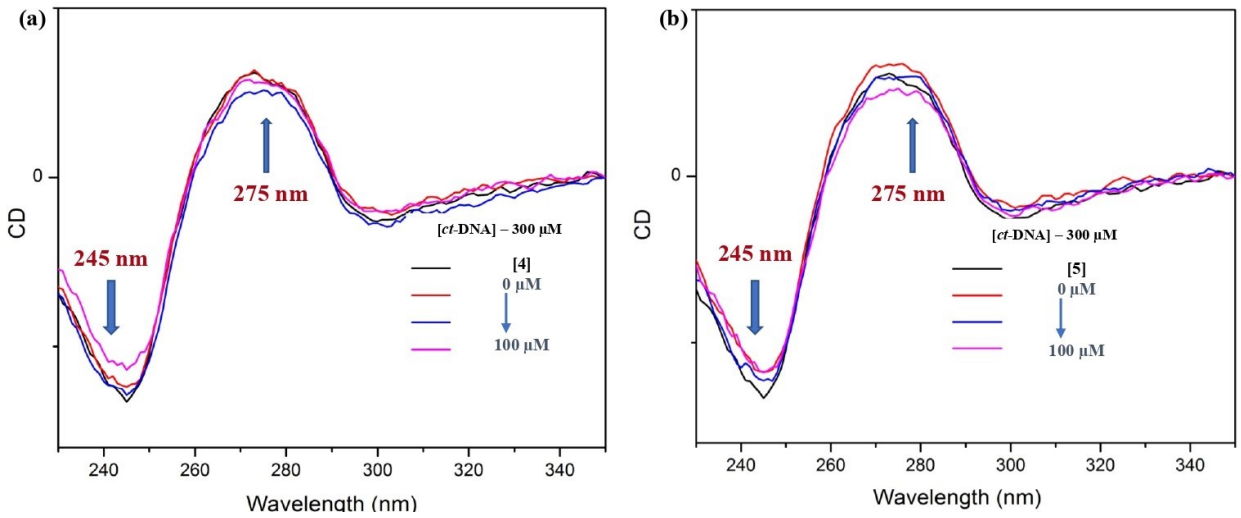


Figure S14. CD spectral analysis to determine *ct*-DNA binding fashion. (**a**) & (**b**) CD spectral representation of *ct*-DNA at 25 ^{0}C (220 nm - 350 nm, ([*ct*-DNA]– 300 µm)) in presence of **4** (0 - 100 µM) and **5** (0 - 100 µM).

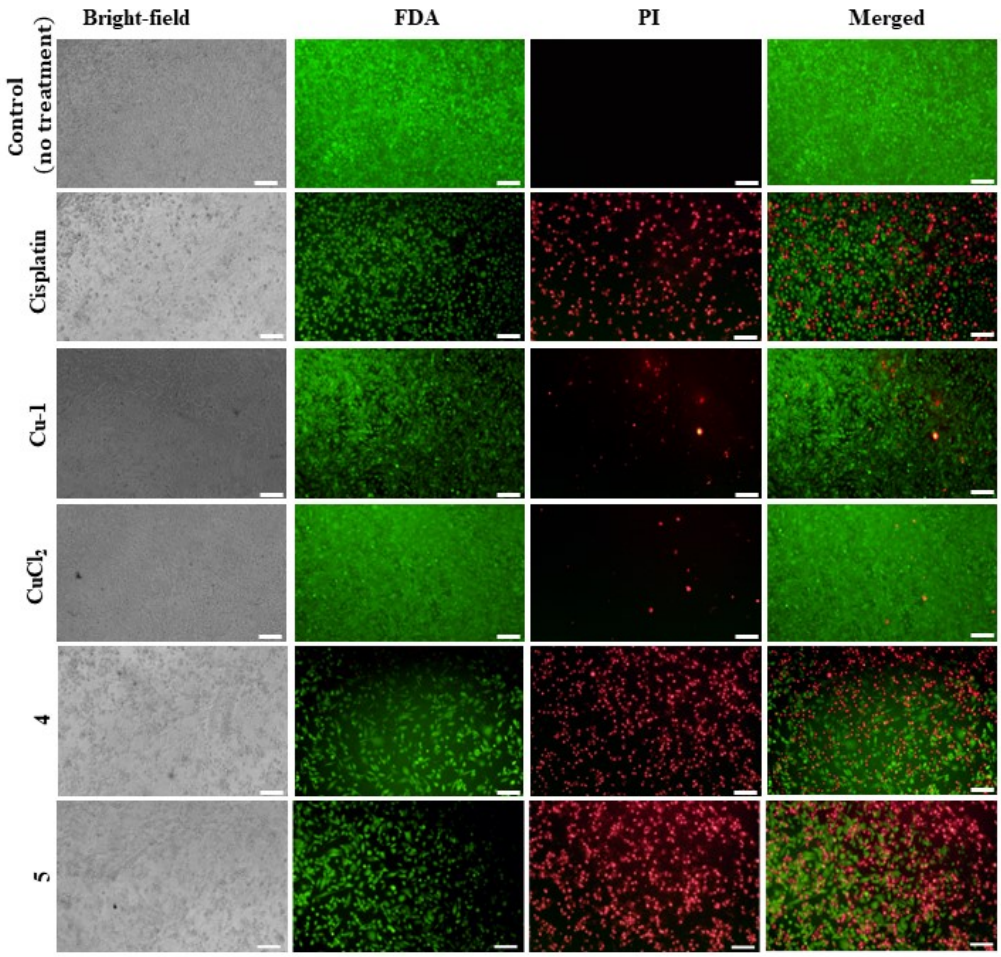


Figure S15. Live–dead imaging of 4T1 cells treated with 3 μM of **4** and **5** for 24 h. Green fluorescence (FDA) corresponds to live cells, red fluorescence (PI) corresponds to dead cells, and merged images for co-localization of live and dead cells. Scale bar: 100 μm.

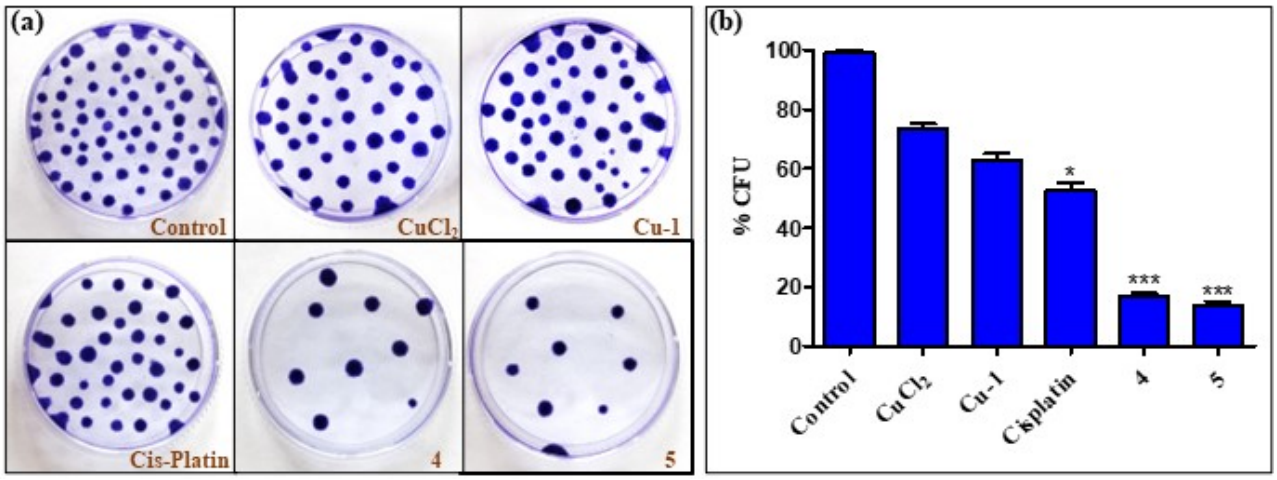


Figure S16. (**a**) Clonogenic assay in 4T1 cells to see the regrowth on post-treatment. (**b**) Graphical depiction of cell viability (%) in the clonogenic assay.

Instrument ID: ARDI067

Sample Name: RGF/4/01/004(Sno:0002)

Vial: 3

Injection Volume : 5.00 uL

Column Name: Kromasil 100 C18,250x4.6mm,5.0µm

Column ID: LC-01316

Result Id: 1193

Project Name: 2024\June_2024\Development\RA

Date Acquired: 12-06-2024 14:39:04 IST

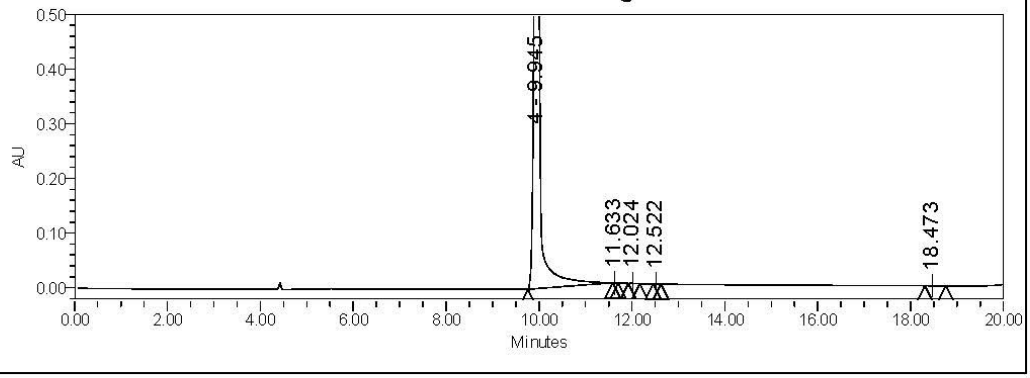
Date Processed: 12-06-2024 16:11:37 IST

Processing Method: 4_PRO

Processed Channel Descr.: PDA 340.0 nm (DAD: Spectrum)

Acq. Method Set: RGF_067_MTH_01

Zoomed-Scaled Chromatogram



Peak Results

	RT	Area	Height (µV)	% Area	Int Type	RT Ratio	Name
1	9.945	9987979	1358710	99.95	BB	1.00	4
2	11.633	548	149	0.01	BB	1.17	
3	12.024	1548	193	0.02	BB	1.21	
4	12.522	664	135	0.01	BB	1.26	
5	18.473	2569	237	0.03	BB	1.86	
Sum		9993307					

Figure S17. HPLC traces of **4**.

Instrument ID: ARDI067

Sample Name: RGF/5/01/002(Sno:0003)

Vial: 4

Injection Volume : 5.00 uL

Column Name: Kromasil 100 C18,250x4.6mm,5.0µm

Column ID: LC-01316

Result Id: 1198

Project Name: 2024\June_2024\Development\RA

Date Acquired: 12-06-2024 15:01:11 IST

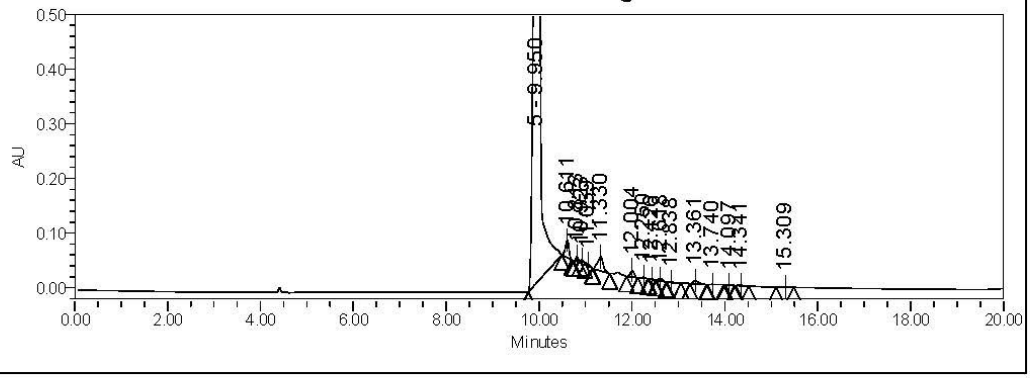
Date Processed: 12-06-2024 16:14:45 IST

Processing Method: 5_PRO

Processed Channel Descr.: PDA 340.0 nm (DAD: Spectrum)

Acq. Method Set: RGF_067_MTH_01

Zoomed-Scaled Chromatogram



Peak Results

	RT	Area	Height (µV)	% Area	Int Type	RT Ratio	Name
1	9.950	17528388	2519869	96.46	BB	1.00	5
2	10.611	169128	32196	0.93	BB	1.07	
3	10.813	24382	6941	0.13	BB	1.09	
4	10.923	10501	3132	0.06	BB	1.10	
5	11.059	15401	2964	0.08	BB	1.11	
6	11.330	215725	25575	1.19	BB	1.14	
7	12.004	71342	12855	0.39	BB	1.21	
8	12.250	13099	2175	0.07	BB	1.23	
9	12.426	4912	1106	0.03	BB	1.25	
10	12.618	28850	5133	0.16	BB	1.27	
11	12.838	9207	1513	0.05	BB	1.29	

Peak Results

	RT	Area	Height (µV)	% Area	Int Type	RT Ratio	Name
12	13.361	50823	6315	0.28	BB	1.34	
13	13.740	4680	518	0.03	BB	1.38	
14	14.097	2730	391	0.02	BB	1.42	
15	14.341	7613	922	0.04	BB	1.44	
16	15.309	14816	1374	0.08	BB	1.54	
Sum		18171597					

Figure S18. HPLC traces of **5**.

Empower 3 SOFTWARE

Instrument ID: ARDI067

Sample Name: RGF/Cu-1/02/001(Sno:0002)

Vial: 2

Injection Volume : 5.00 uL

Column Name: Kromasil 100 C18,250x4.6mm,5.0µm

Column ID: LC-01316

Result Id: 1188

Project Name: 2024\June_2024\Development\RA

Date Acquired: 12-06-2024 14:16:58 IST

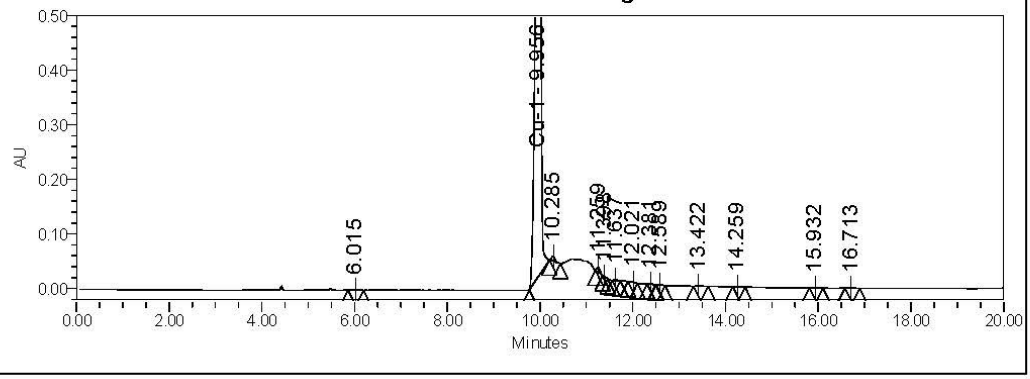
Date Processed: 12-06-2024 16:07:16 IST

Processing Method: Cu-1_PRO

Processed Channel Descr.: PDA 340.0 nm (DAD: Spectrum)

Acq. Method Set: RGF_067_MTH_01

Zoomed-Scaled Chromatogram



Peak Results

	RT	Area	Height (µV)	% Area	Int Type	RT Ratio	Name
1	6.015	962	114	0.01	BB	0.60	
2	9.956	11129299	1792639	98.69	BB	1.00	Cu-1
3	10.285	48402	9343	0.43	BB	1.03	
4	11.259	42480	9419	0.38	BB	1.13	
5	11.398	3314	1029	0.03	BB	1.14	
6	11.637	13124	2636	0.12	BB	1.17	
7	12.021	4976	800	0.04	BB	1.21	
8	12.381	6908	1306	0.06	BB	1.24	
9	12.589	6833	1309	0.06	BB	1.26	
10	13.422	7107	952	0.06	BB	1.35	
11	14.259	3033	374	0.03	BB	1.43	

Peak Results

	RT	Area	Height (µV)	% Area	Int Type	RT Ratio	Name
12	15.932	3557	429	0.03	BB	1.60	
13	16.713	7325	872	0.06	BB	1.68	
Sum		11277321					

Figure S19. HPLC traces of **Cu-1**.

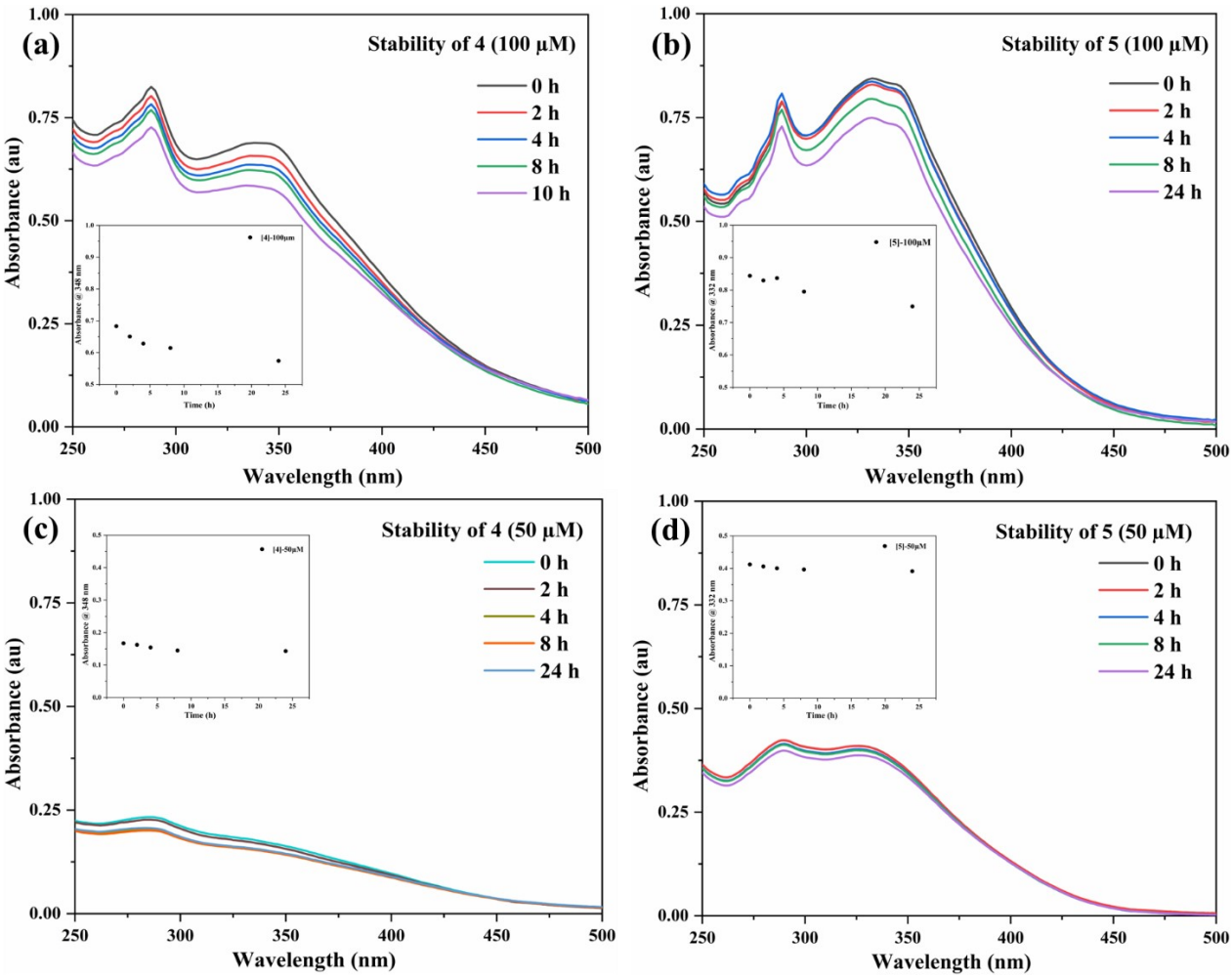


Figure S20. Stability of **4** & **5** in Tris-EDTA buffer (pH 7.8) (**a**) Stability of **4** (100 µM) in TE buffer (0.1% DMSO) over 0 to 24h (**b**) Stability of **5** (100 µM) in TE buffer (0.1% DMSO) over 0 to 24h. (**c**) Stability of **4** (50 µM) in TE buffer (0.1% DMSO) over 0 to 24h (**d**) Stability of **5** (50 µM) in TE buffer (0.1% DMSO) over 0 to 24h. (**Insets**) Change in absorbance with time at respective λ_{max} of **4** & **5** at 100 µM and 50 µM concentrations.