Planar triazinium cations from vanadyl-mediated ring cyclizations: the thiazole species for efficient nuclear staining and photocyto-toxicity

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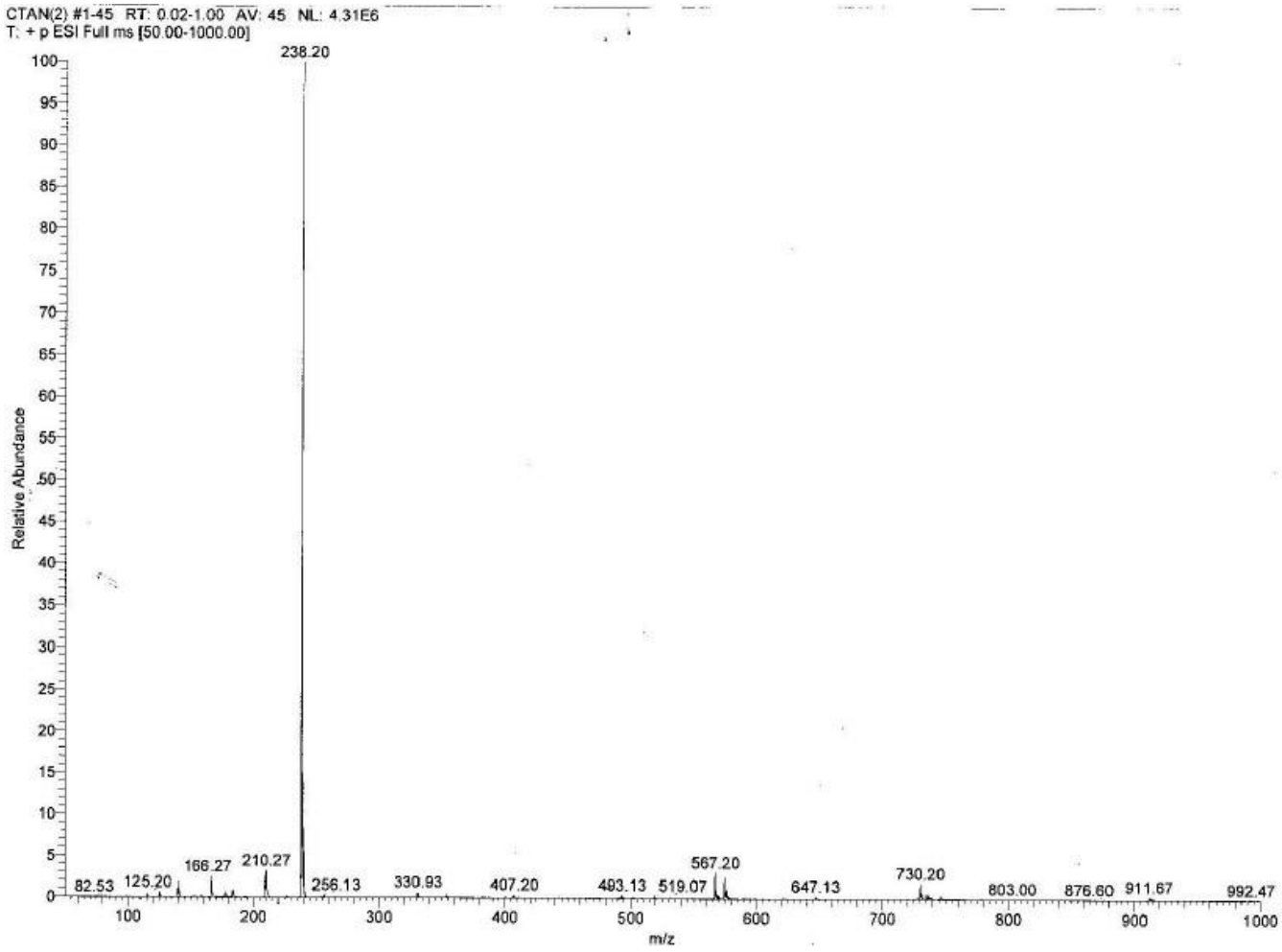
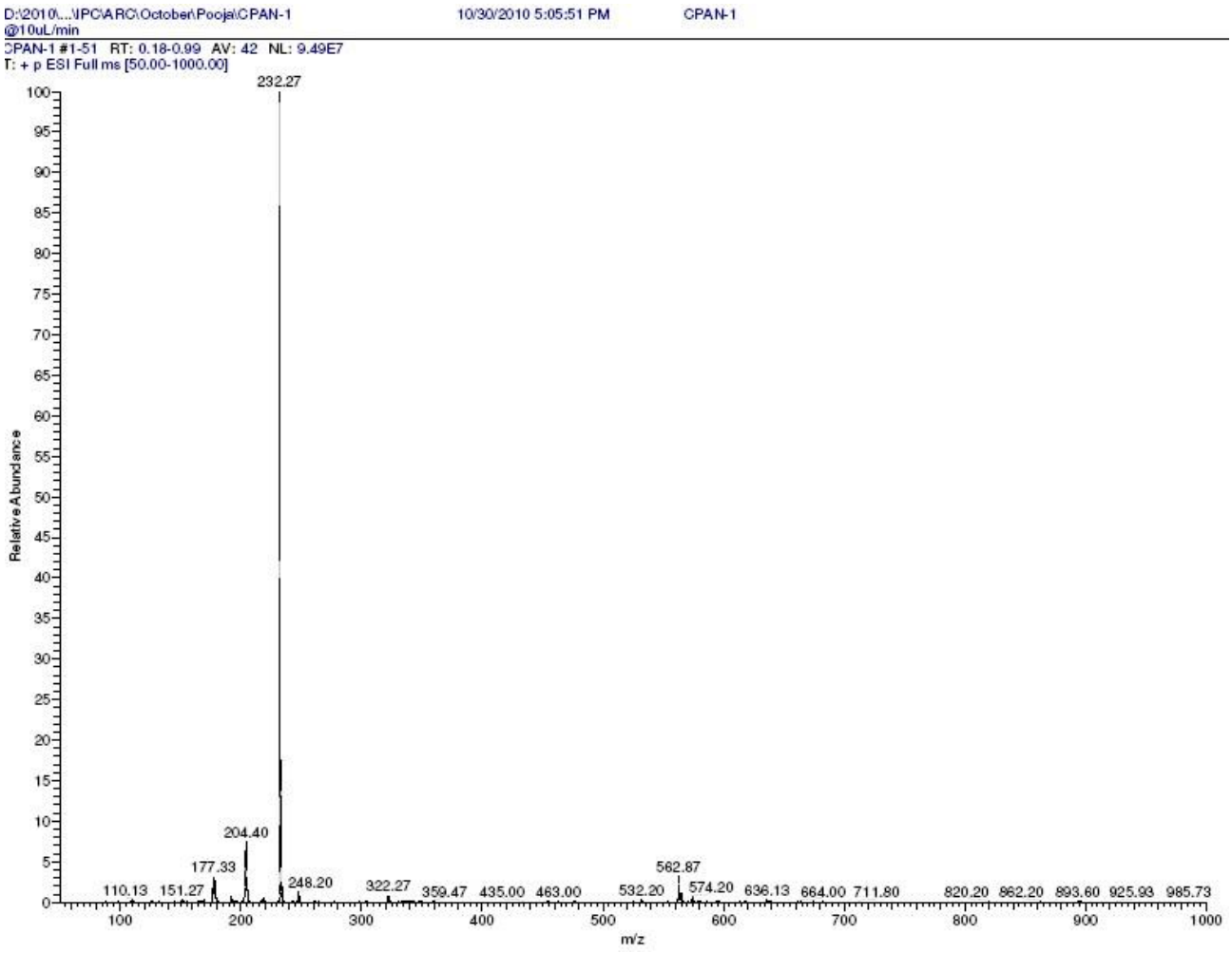
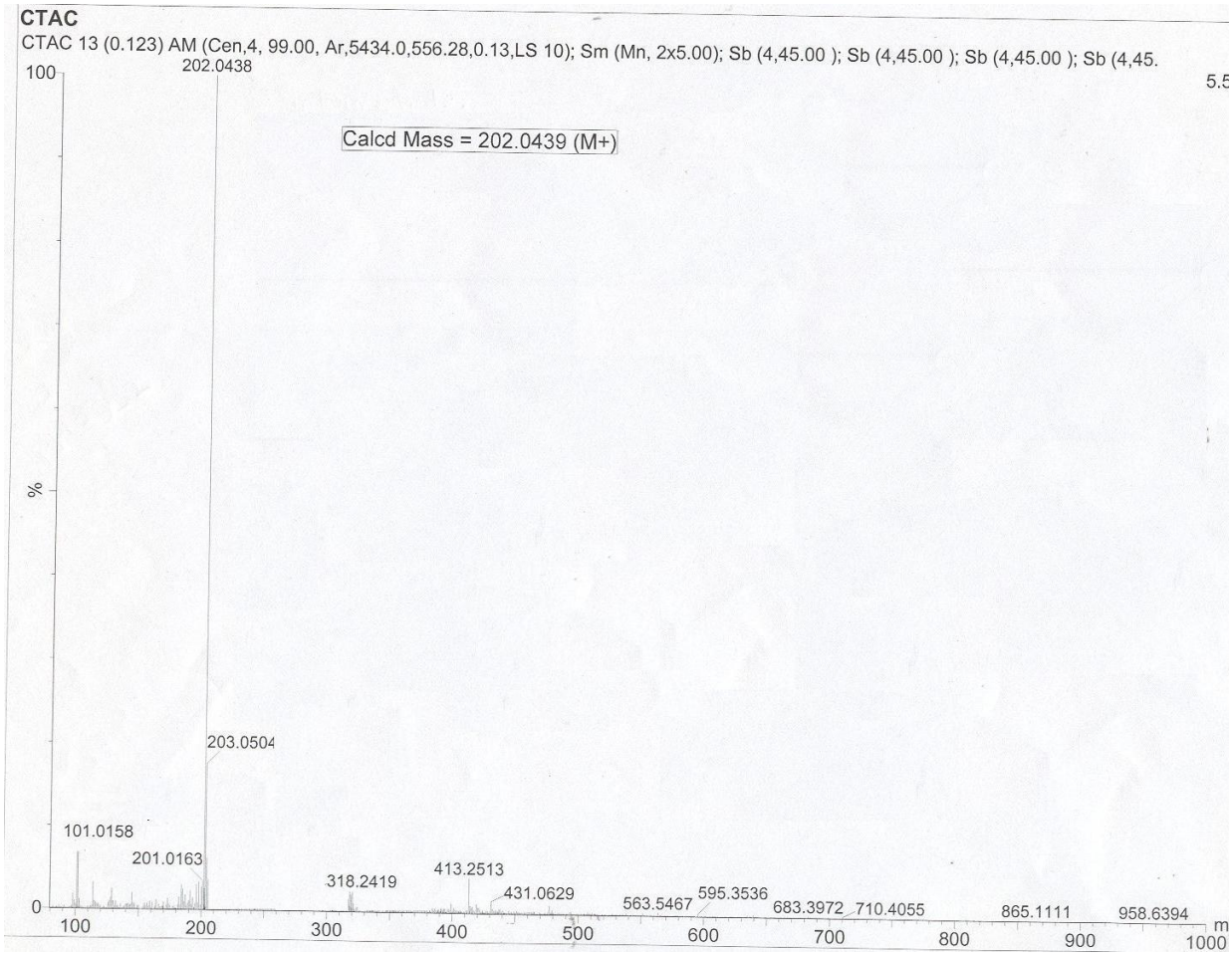
Electronic Supplementary Information (ESI†)



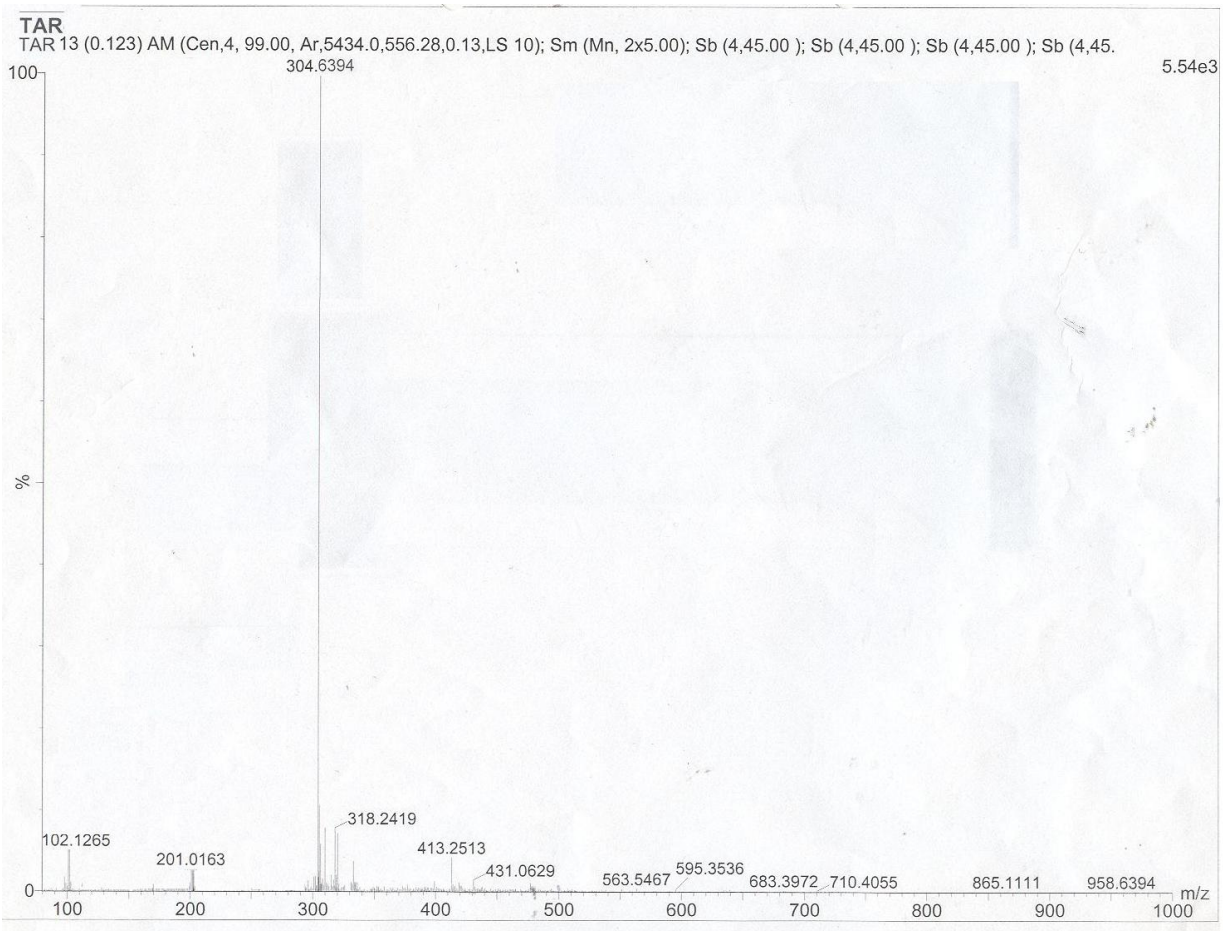
ESI Fig. S1. The ESI-MS spectrum of compound **1** showing the molecular ion peak at 238.20 (m/z) in CH_3CN.



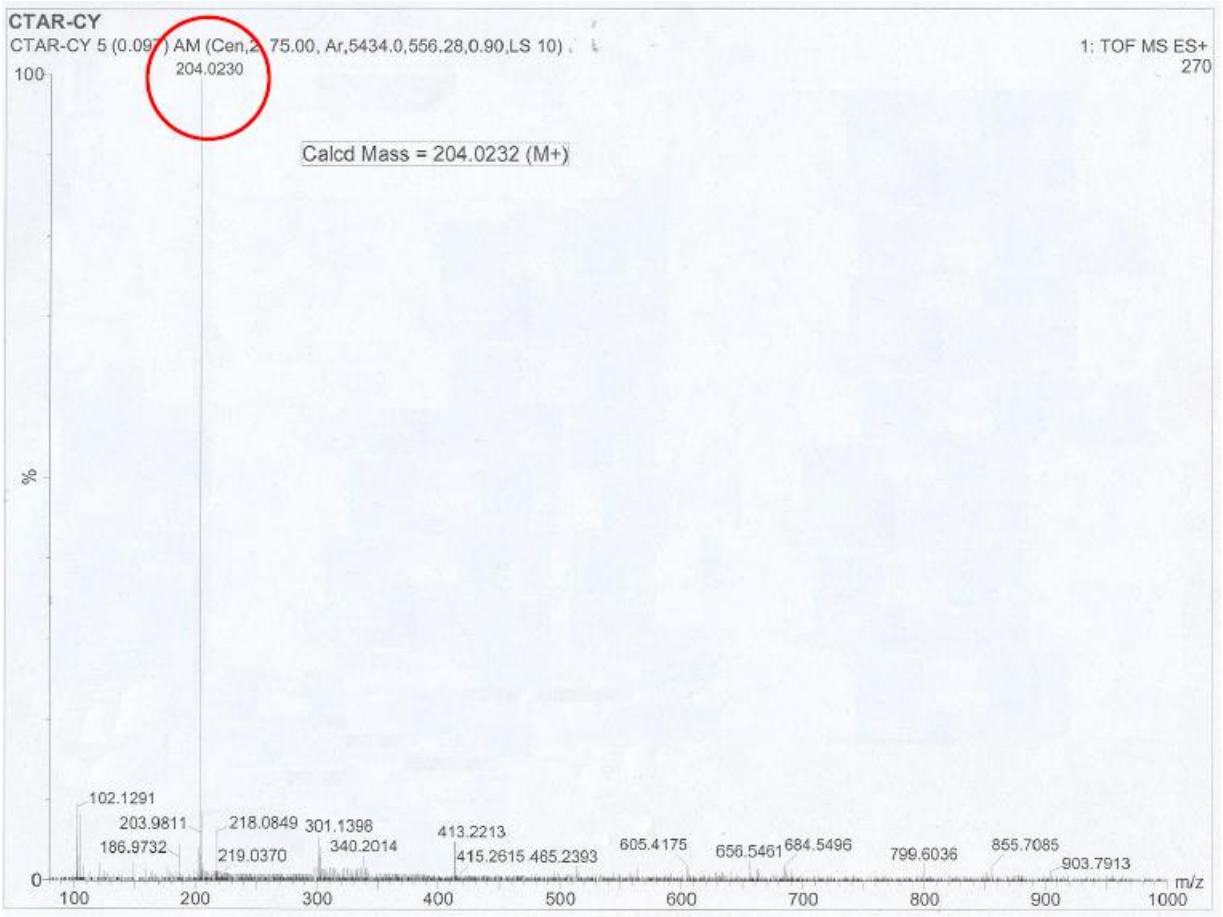
ESI Fig. S2. The ESI-MS spectrum of compound **2** showing the molecular ion peak at 232.27 (m/z) in CH_3CN.



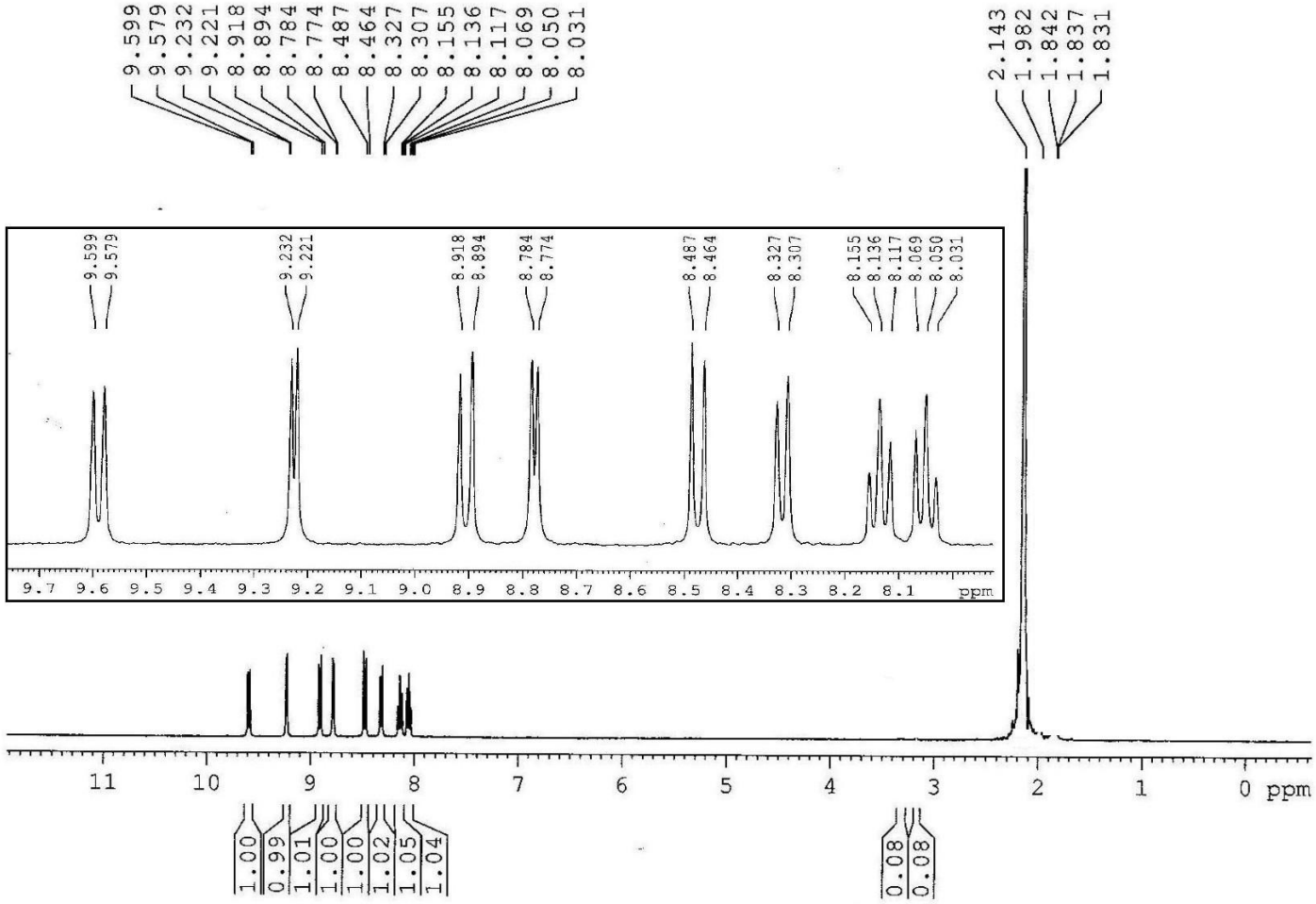
ESI Fig. S3. The ESI-MS spectrum of compound **3** showing the molecular ion peak at 202.04 (m/z) in CH_3CN.



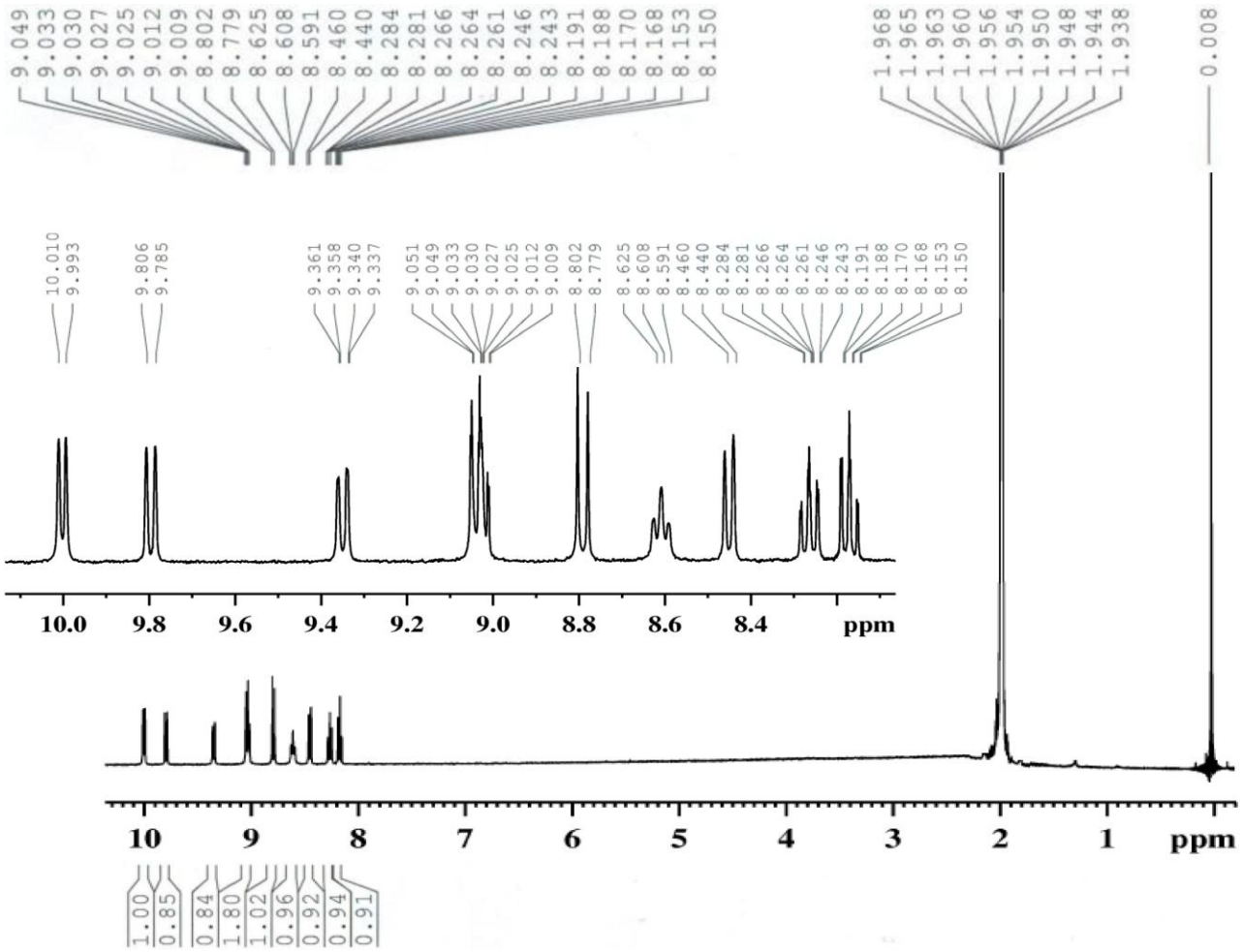
ESI Fig. S4. The ESI-MS spectrum of complex **4** showing the molecular ion peak at (m/z) in 304.63 in MeOH:H_2O (1:1).



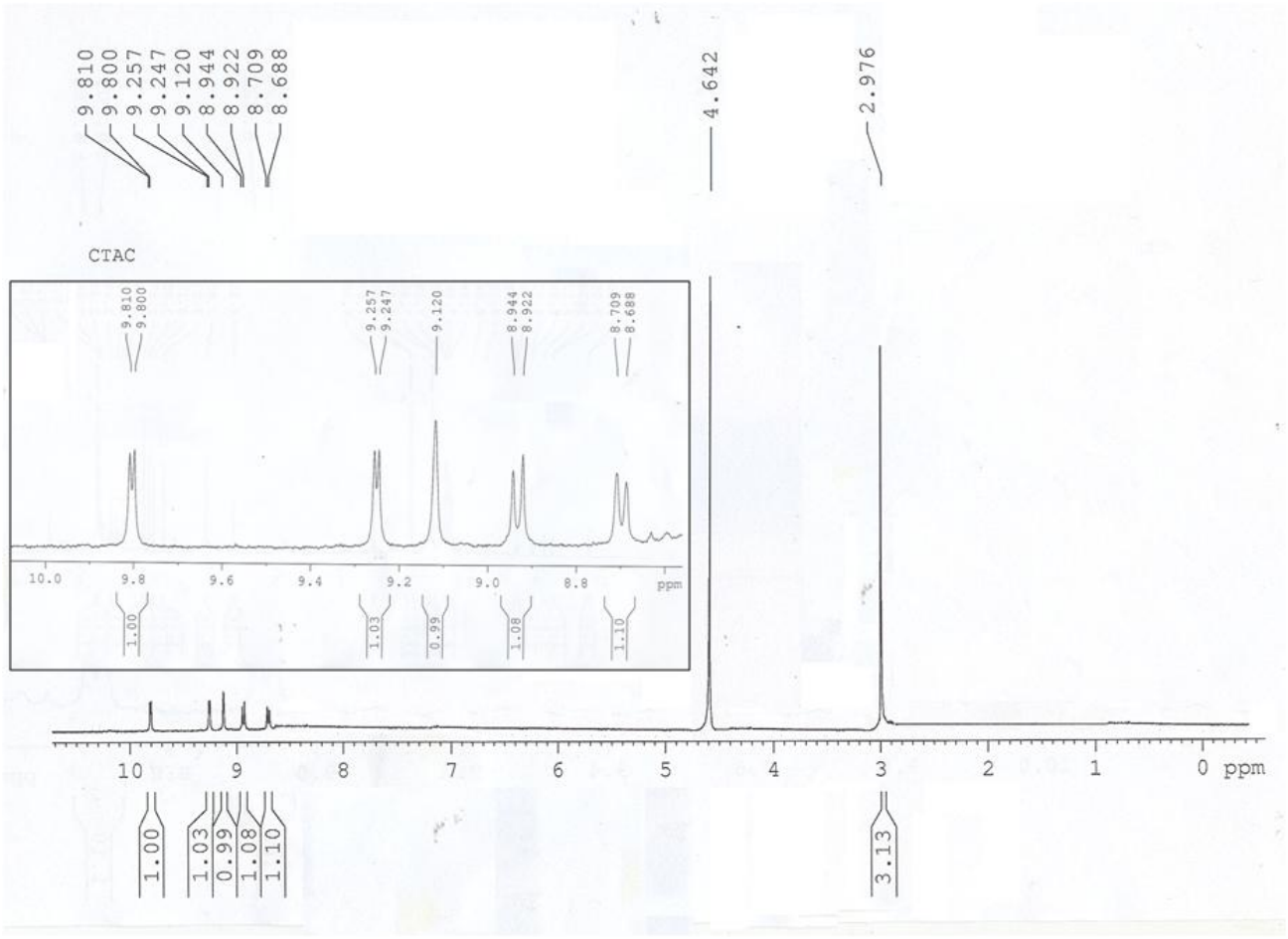
ESI Fig. S5. The ESI-MS spectrum of compound **5** showing the molecular ion peak at (m/z) in 204.02 in MeOH:H_2O (1:1).



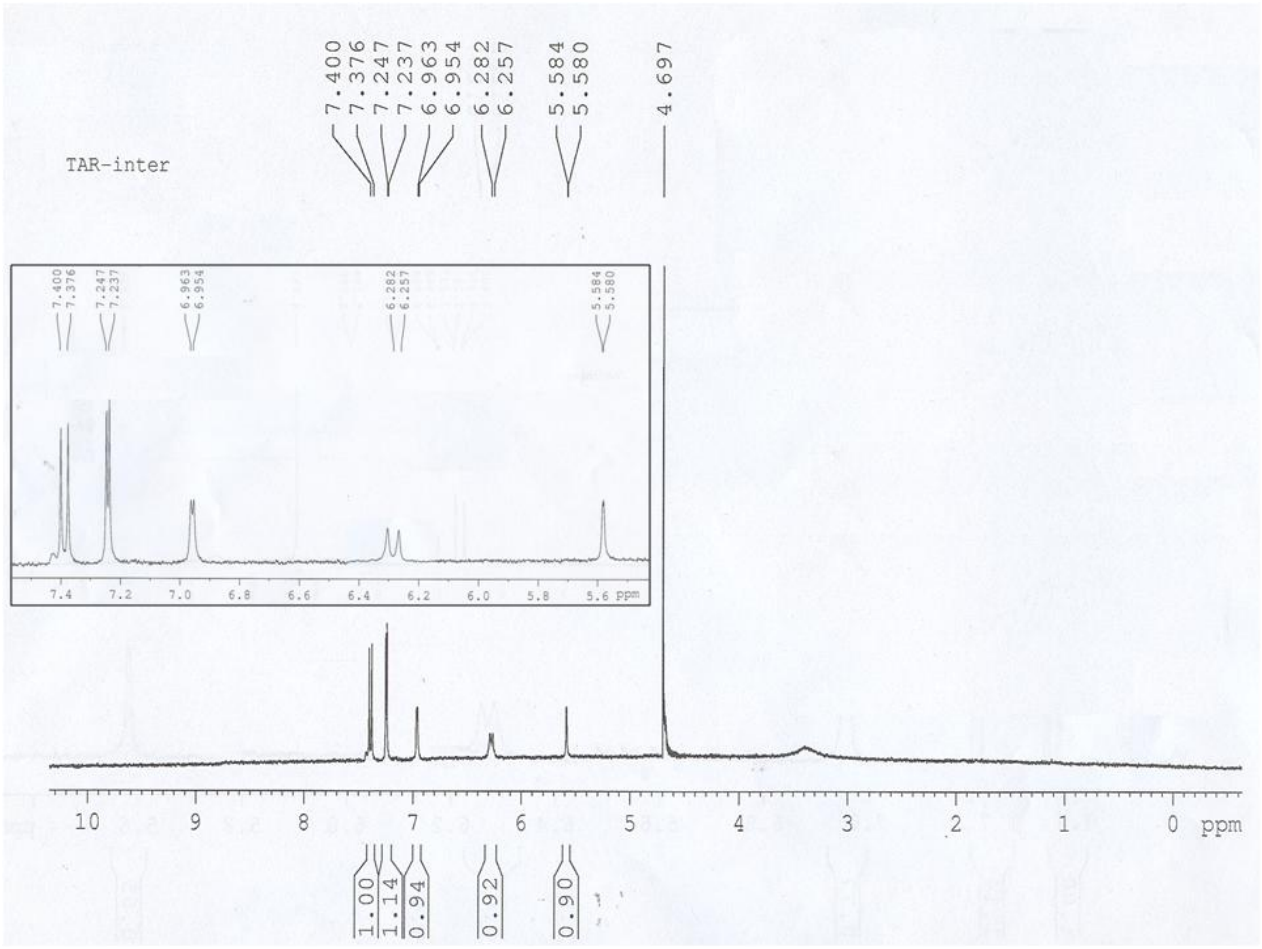
ESI Fig. S6. ^{1}H NMR of **1** in CD_3CN.



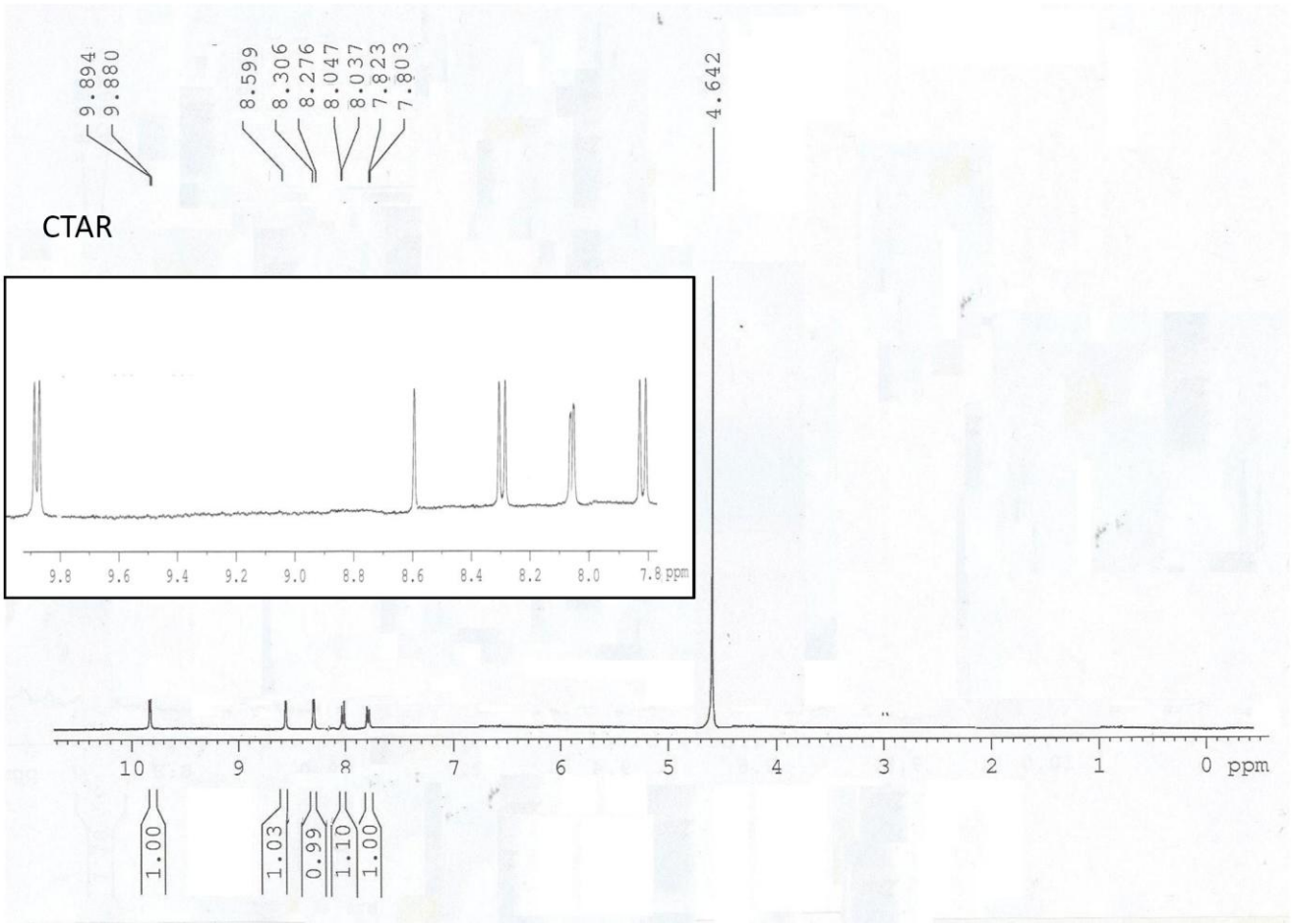
ESI Fig. S7. ^{1}H NMR of **2** in CD_3CN.



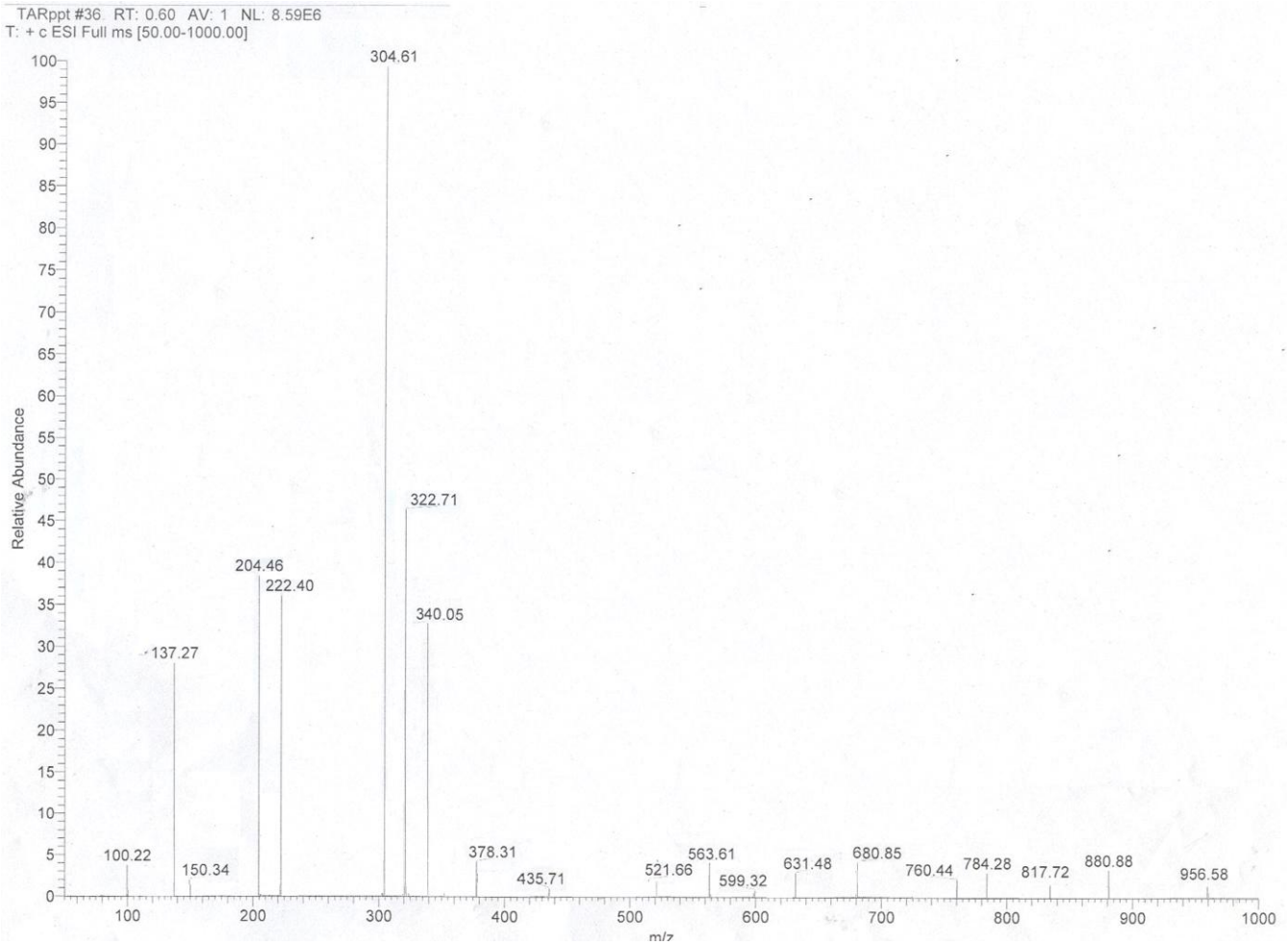
ESI Fig. S8. ^{1}H NMR of **3** in D_2O.



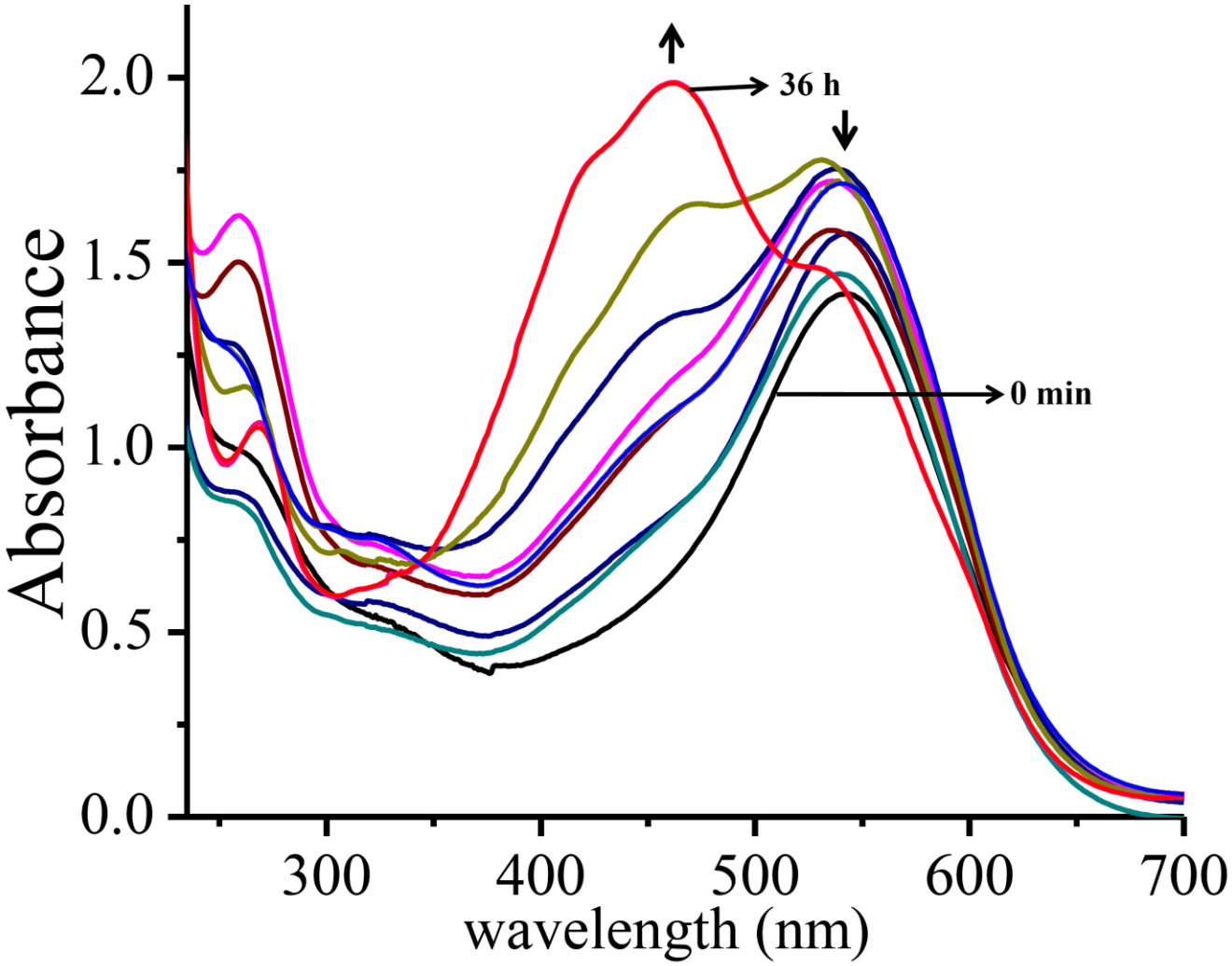
ESI Fig. S9. ^{1}H NMR of **4** in D_2O.



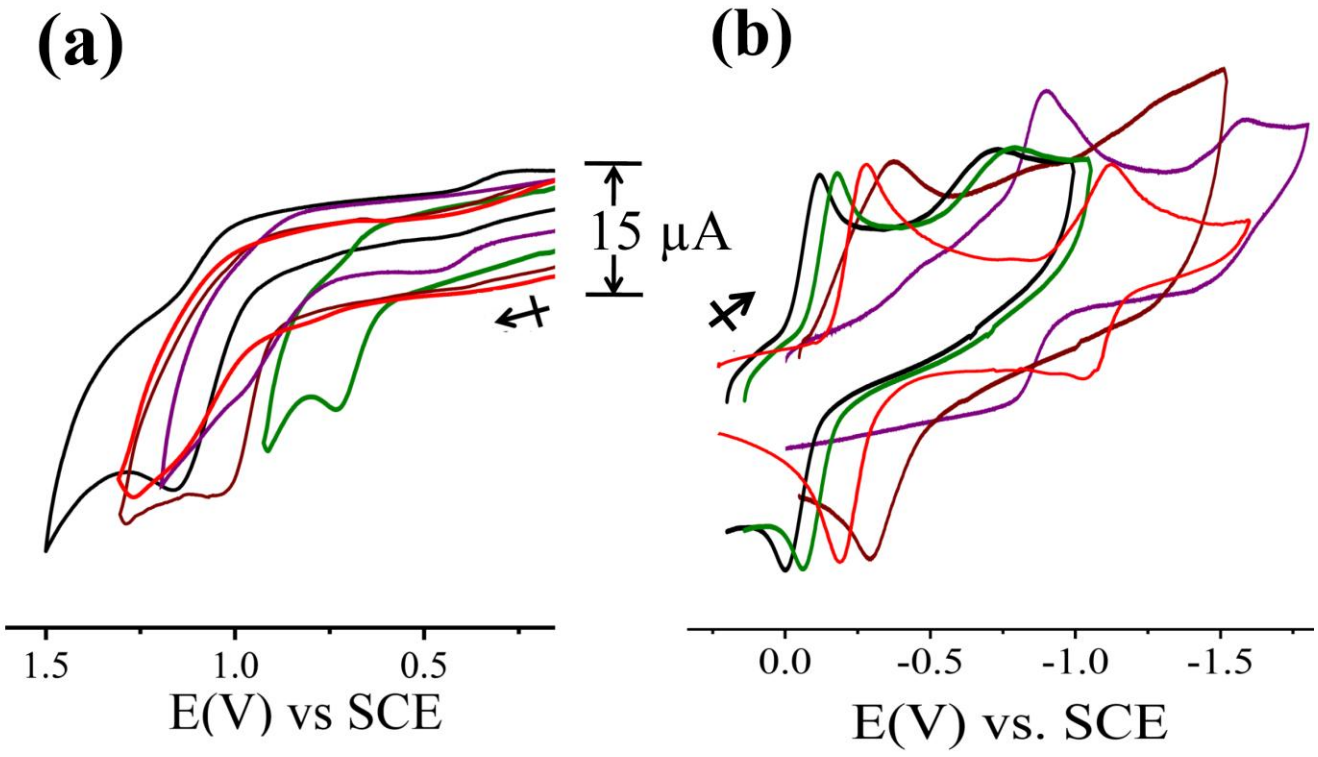
ESI Fig. S10. ^{1}H NMR of **5** in D_2O.



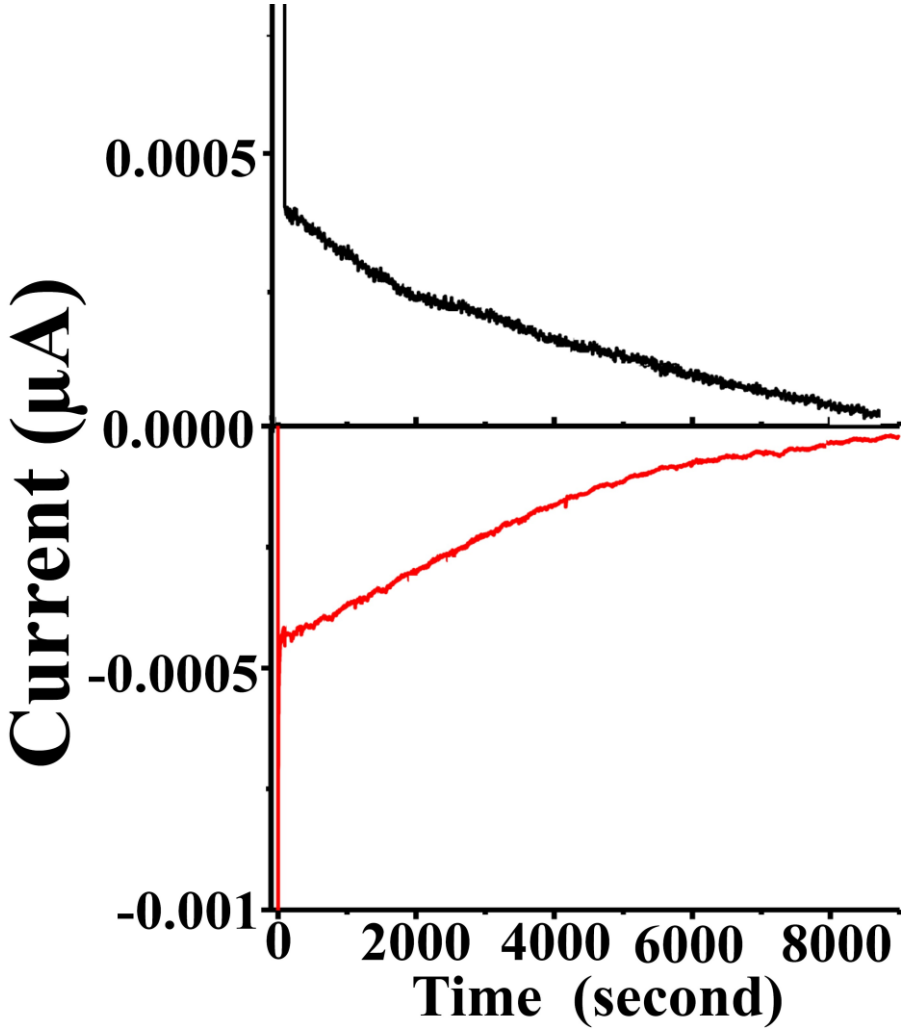
ESI Fig. S11. LCMS of the reaction mixture showing the mass peaks of the intermediates formed.



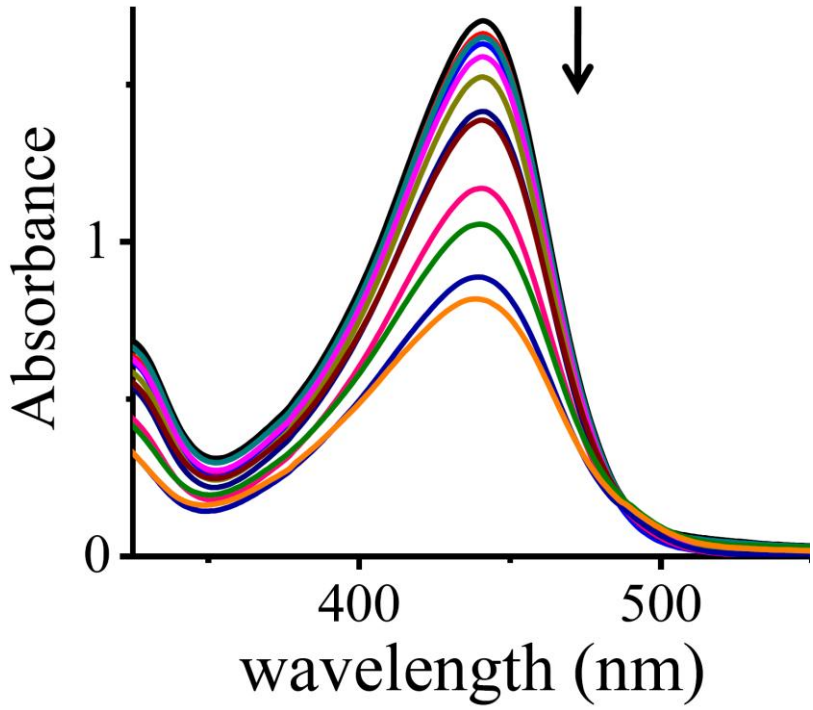
ESI Fig. S12. UV-Visible titration spectra of the reaction mixture showing the conversion of complex **4** to compound **5** with the spectra run at equal interval of 4h.



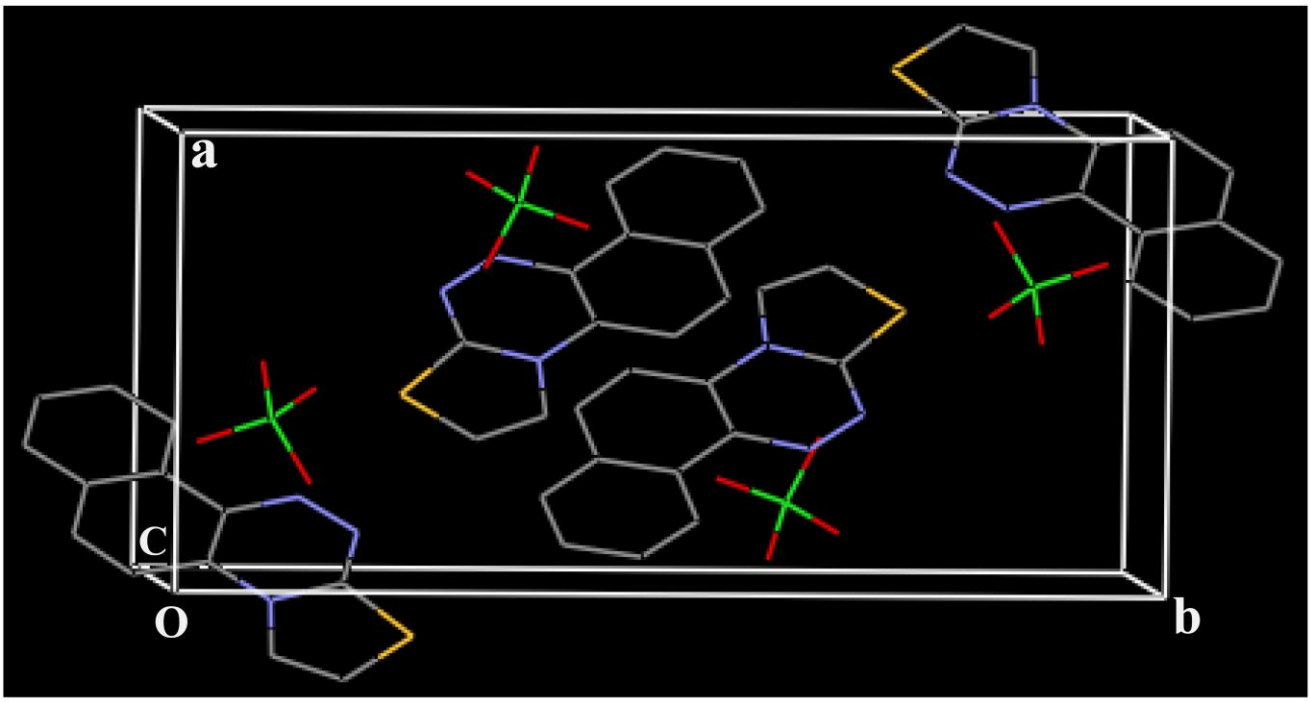
ESI Fig. S13. Cyclic voltammograms of the **1** (—), **2** (—), **3**(—), **4**(—) and **5** (—)showing (a) anodic scans and (b) cathodic scans in DMF at a scan speed of 50 mV s^{-1} and 0.1 TBAP as a supporting electrolyte.



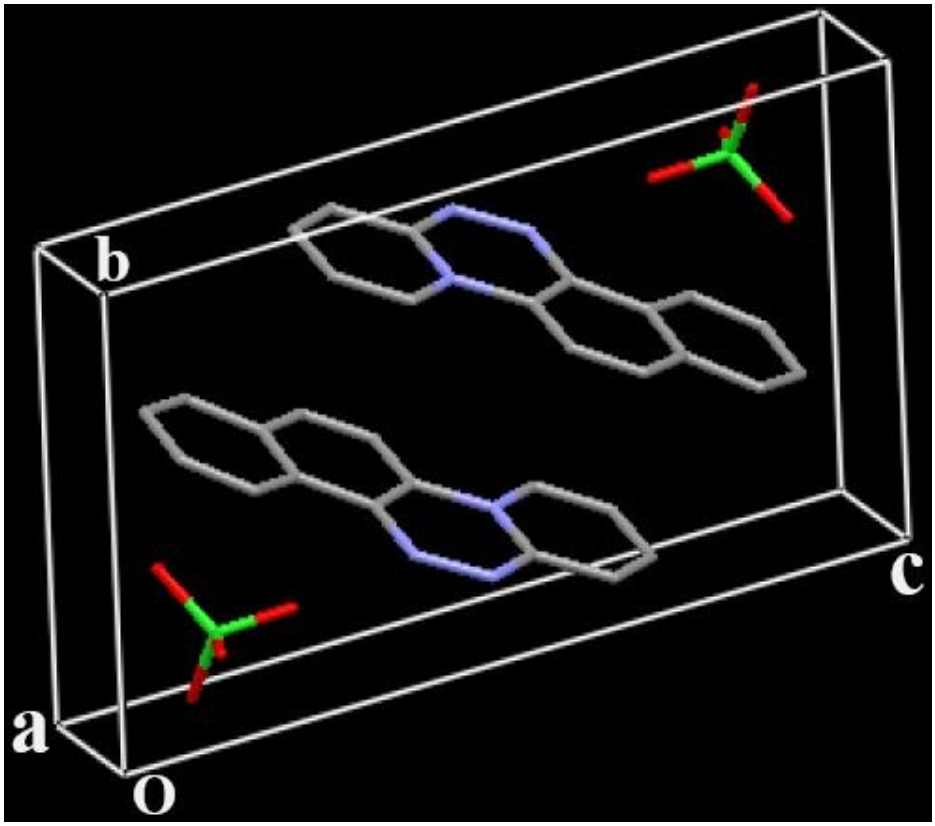
ESI Fig. S14. Chronoamperometry at constant potential of 0.65 V for Ferrocene (—) as standard and –0.5 V for **1** (—) respectively, in DMF and 0.1 M TBAP showing one electron transfer.



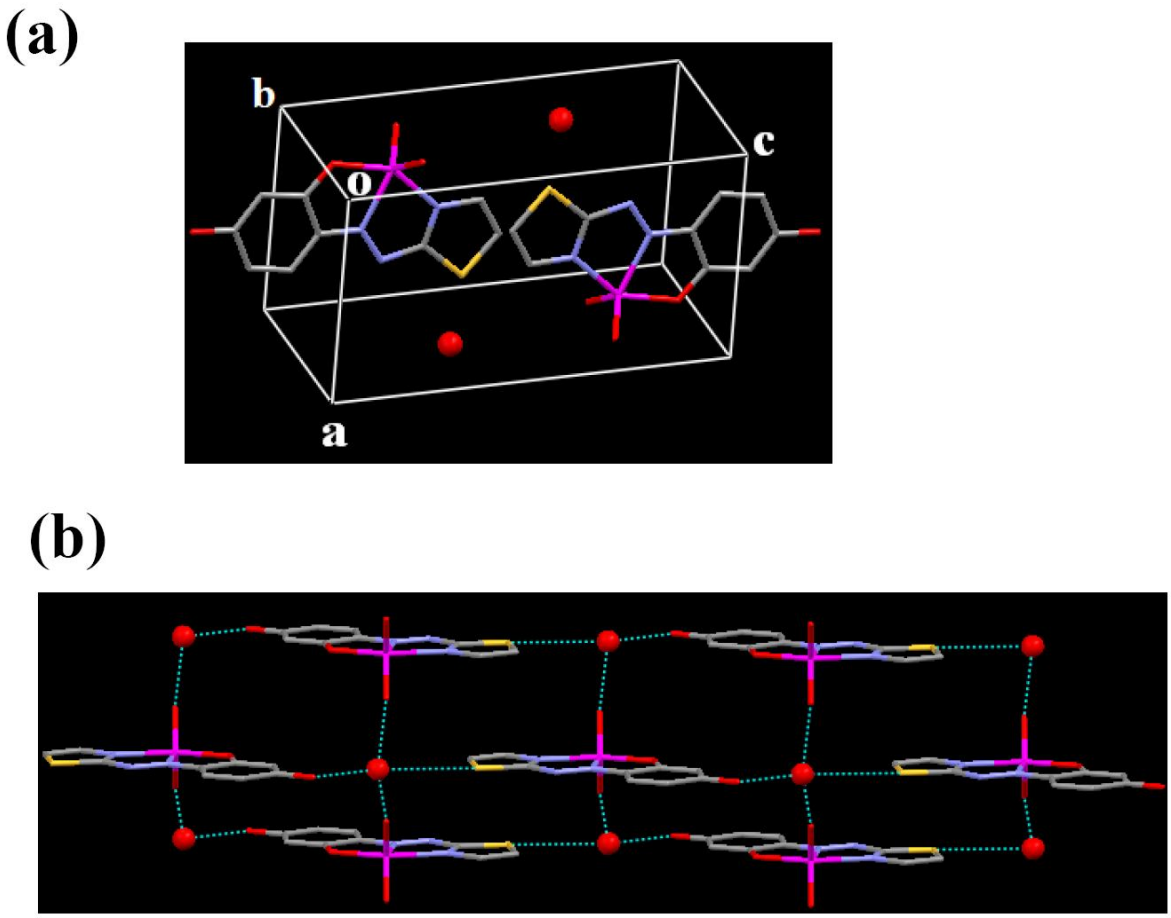
ESI Fig. S15. Titration of compound **1** (100 μM) using sodium dithionite (100 μM).



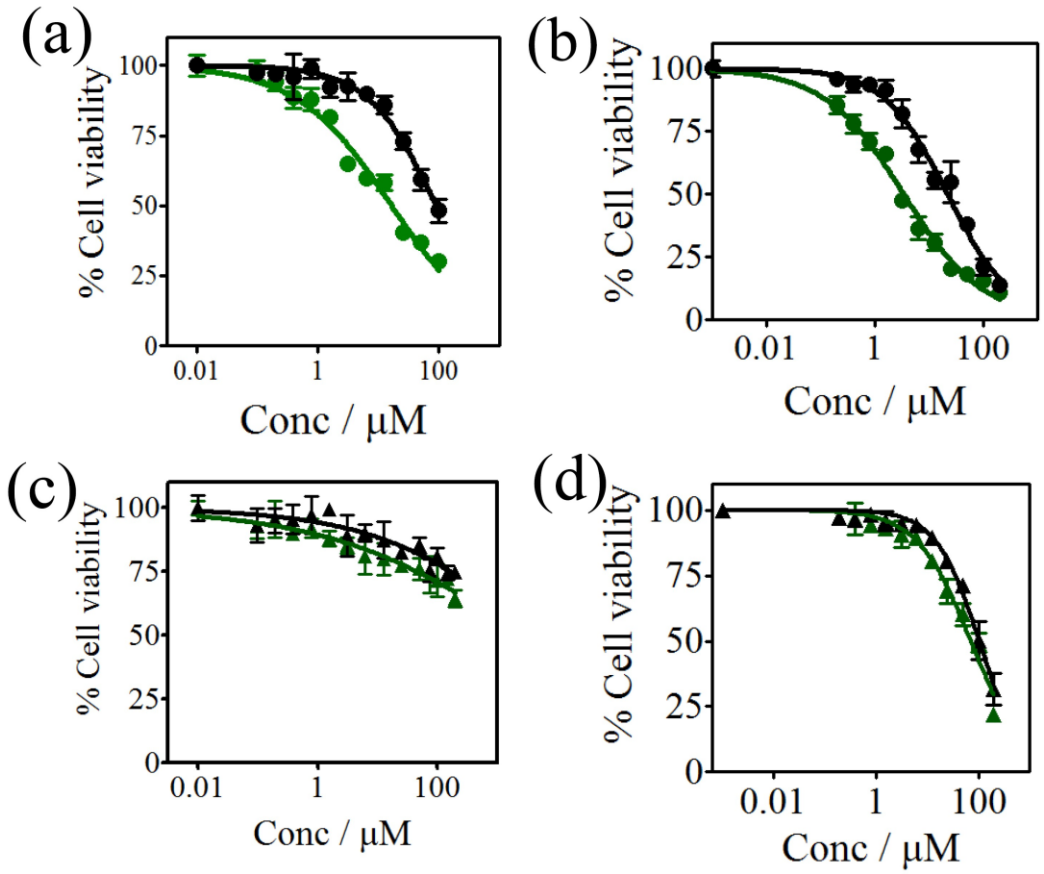
ESI Fig. S16. Unit cell packing diagram of **1**.



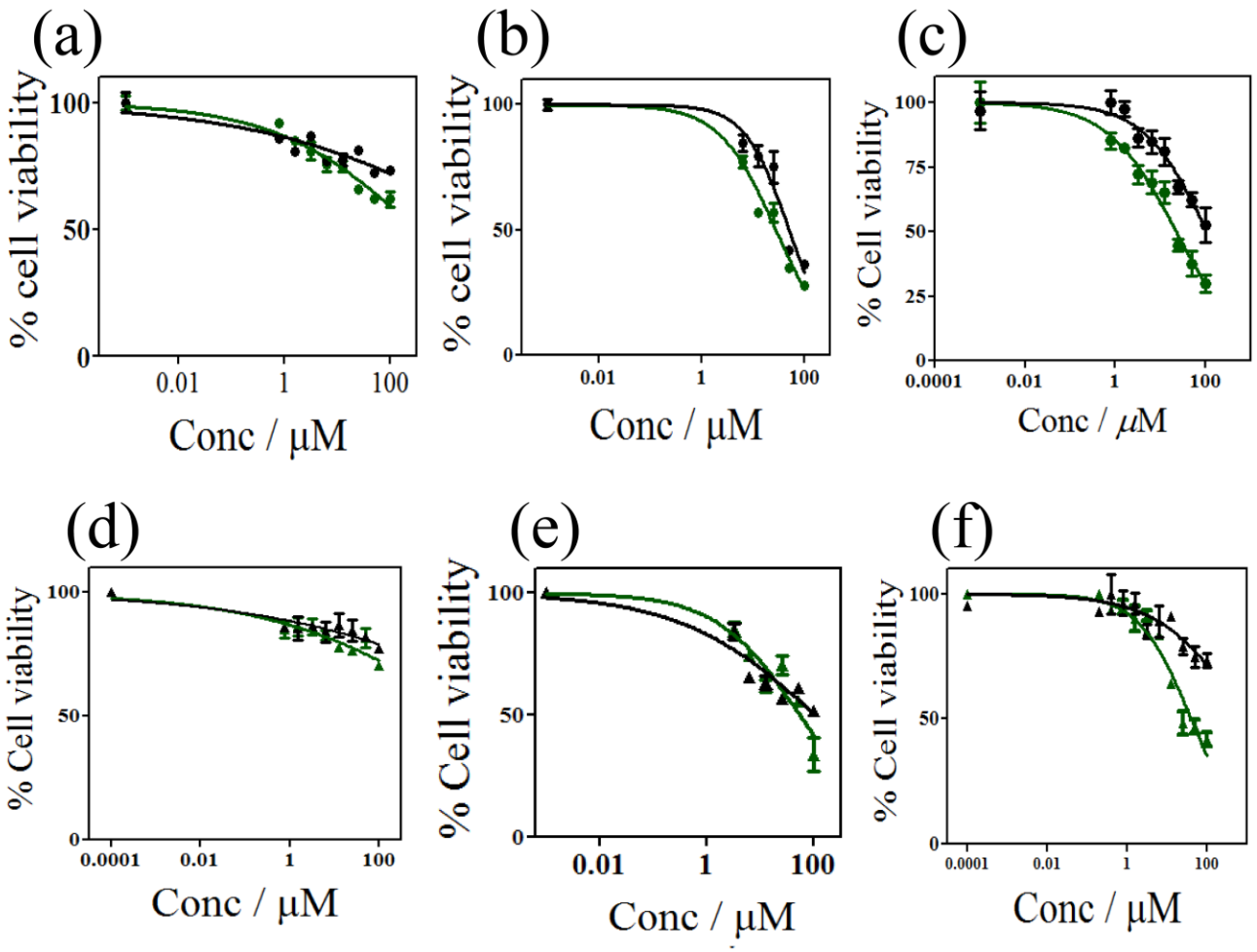
ESI Fig. S17. Unit cell packing diagram of **2**.



ESI Fig. S18. (a) Unit cell packing diagram of **4** and (b) 3D network of hydrogen bond present in **4**.



ESI Fig. S19. Photocytotoxicity of compound **1** in (a) and (b) and compound **2** in (c) and (d) in cancer cells on 24 h incubation in dark followed upon photo-irradiation in visible light (400 to 700 nm) for 1 h as determined by MTT assay. Panels (a) and (c) are in HeLa cells and Panels (b) and (d) are in MCF-7 cells. The photo-exposed and dark-treated cells are shown in green and black color symbol, respectively.



ESI Fig. S20. Photocytotoxicity of compound **3** in (a) and (d), compound **4** in (b) and (e) and compound **5** in (c) and (f) in cancer cells on 24 h incubation in dark followed upon photo-irradiation in visible light (400 to 700 nm) for 1 h as determined by MTT assay. Panels (a)-(c) are in HeLa cells and panels (d)-(f) are in MCF-7 cells. The photo-exposed and dark-treated cells are shown in green and black color symbol, respectively.

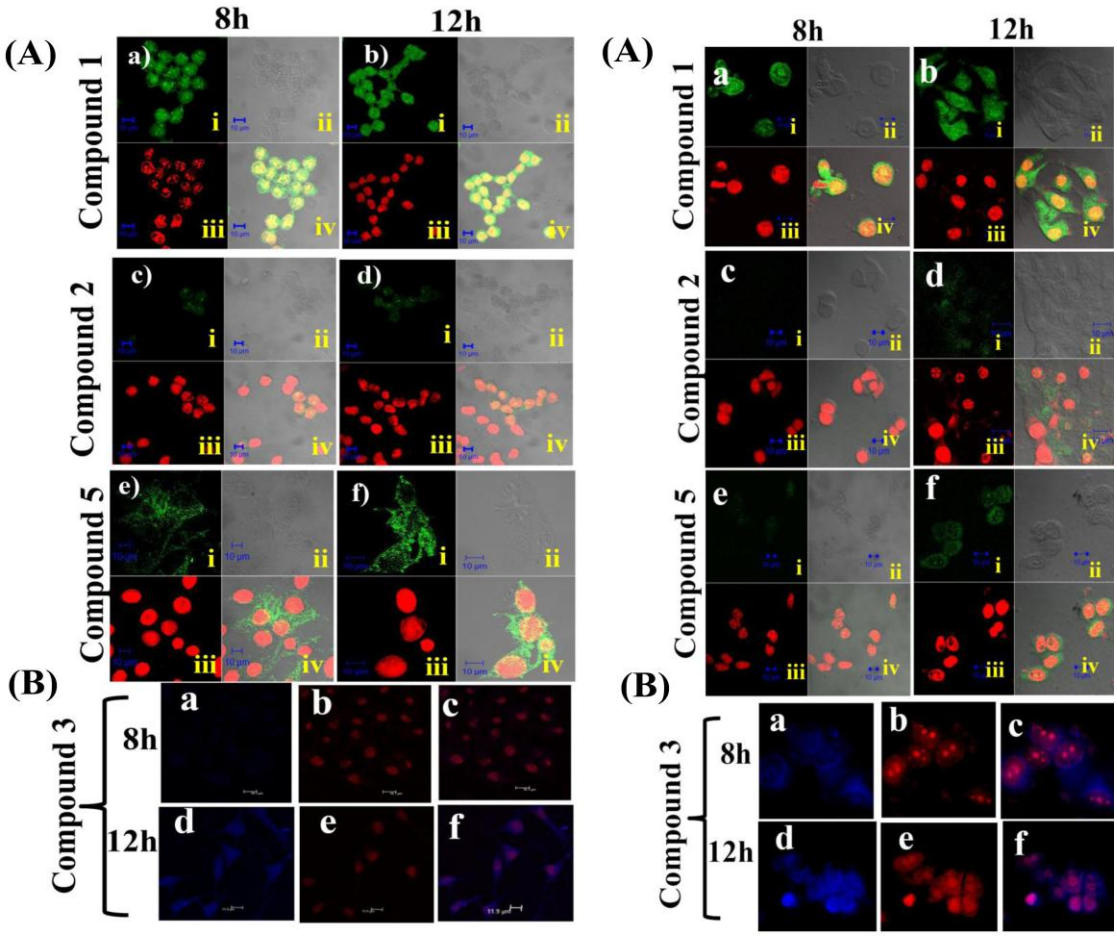


Fig. S21 (A): Panels (a) and (b) are representative images for compound **1**, (c) and (d) for compound **2** and (e) and (f) for compound **5** in HeLa and MCF-7 cells at time point of 8 h and 12 h, respectively, using 20 μM of compounds **1**-**3** and **5** in HeLa cells and 10 μM of compound **1**-**3** and **5** in MCF-7 cells. Panels ((a)-(f))-(i): compounds **1**, **2** and **5** fluorescence. Panels ((a)-(f))-(ii): bright field images. Panels ((a)-(f))-(iii): propidium iodide (PI) staining. Panels ((a)-(f))-(iv): merged images. The uptake of compound **1** in the nucleus increases (evidenced from yellow colour) with time when compared to compound **2** (marginal uptake) in dark. (B): HeLa and MCF-7 cells are treated with 20 μM and 10 μM of **3** at interval of 8 h and 12 h: panels (a),(d): fluorescence of compound **3**, panels (b),(e)**:** fluorescence of PI and panels (c),(f): merged image.

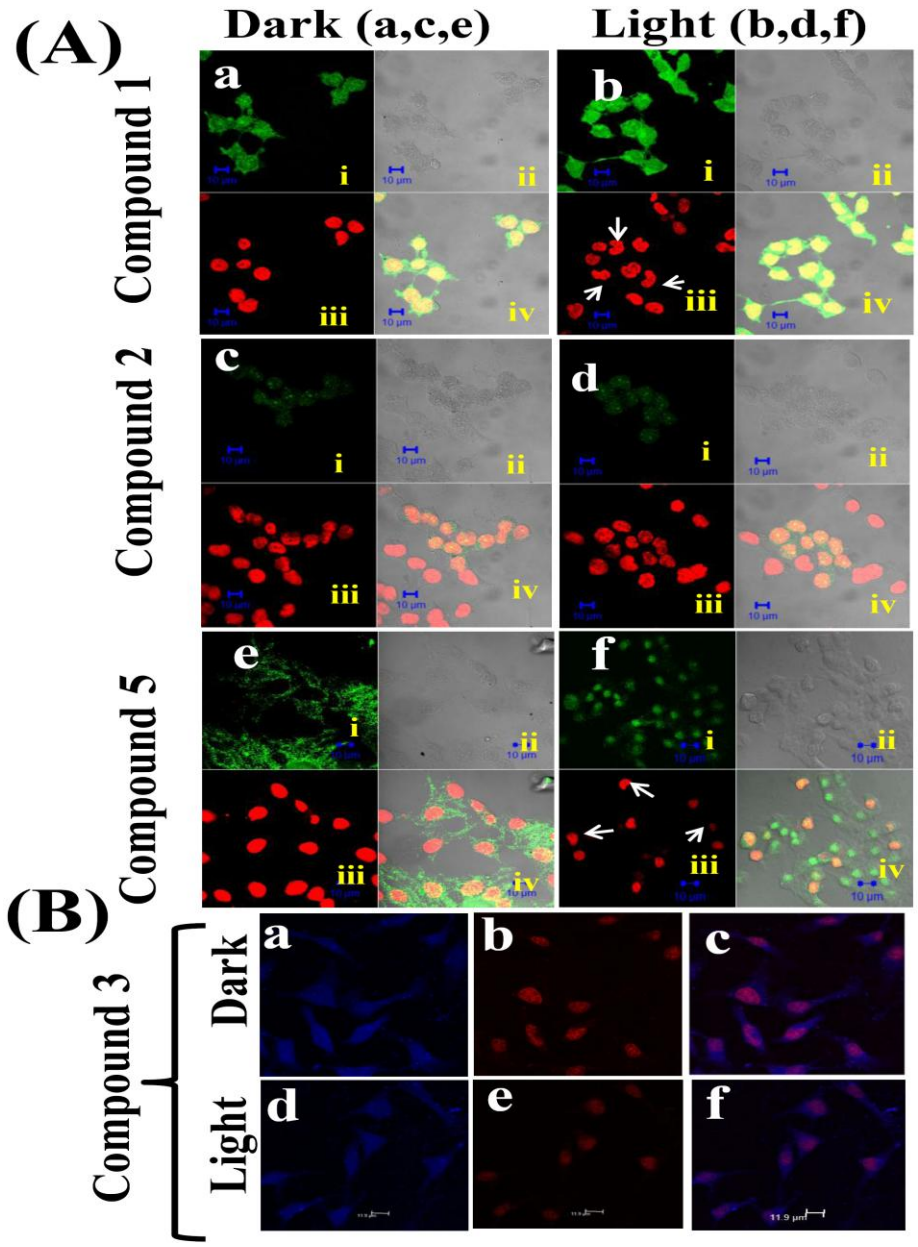


Fig. 22 (A) Confocal images of 20 µM of **1** (a, b), **2** (c, d) and **5** (e, f) in HeLa cells after 12 h incubation in dark followed by light exposure for 1 h. Panels (i)-(iv) are for fluorescence, bright field, PI-staining and merged images, respectively. The PDT effect is shown for **1** and **5** on light expposure (arrows in b(iii) and f(iii)). No apparent PDT effect for **2** showing marginal nuclear uptake. Scale bar in blue is 10 µm. (B) Confocal images for compound **3** (20 µM) in HeLa with (a, d) showing compound fluroscence, (b, e) showing PI-staining and (c, f) for merged image.

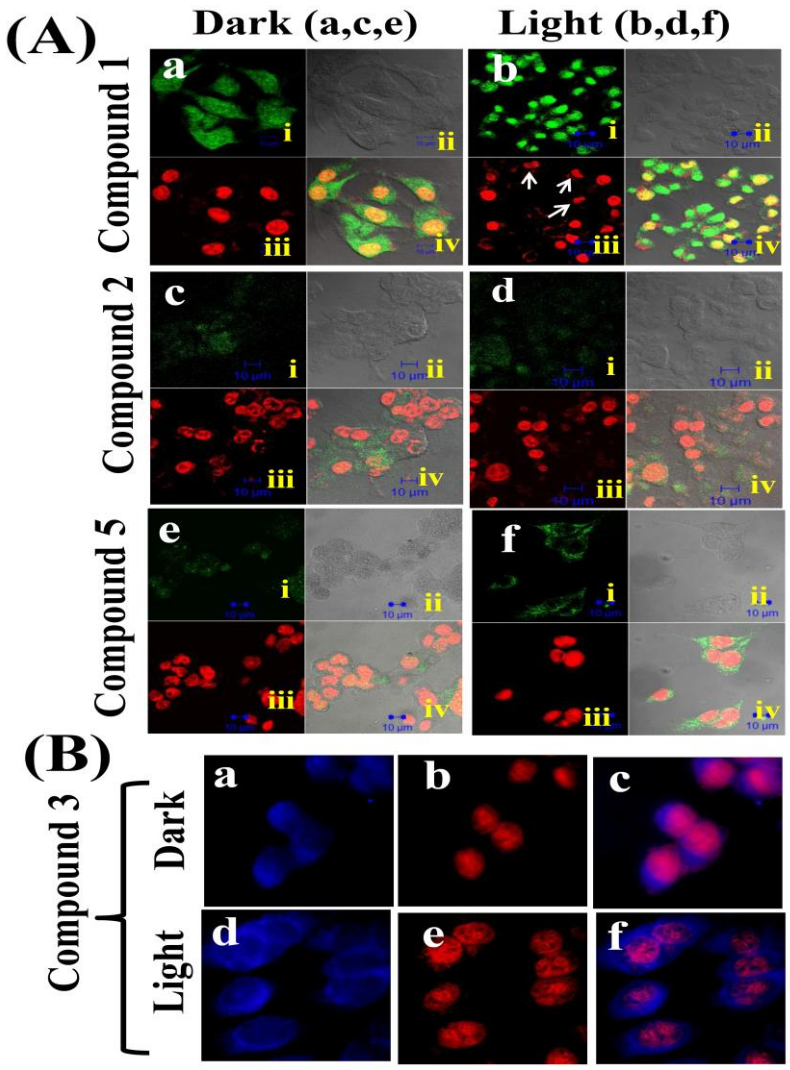
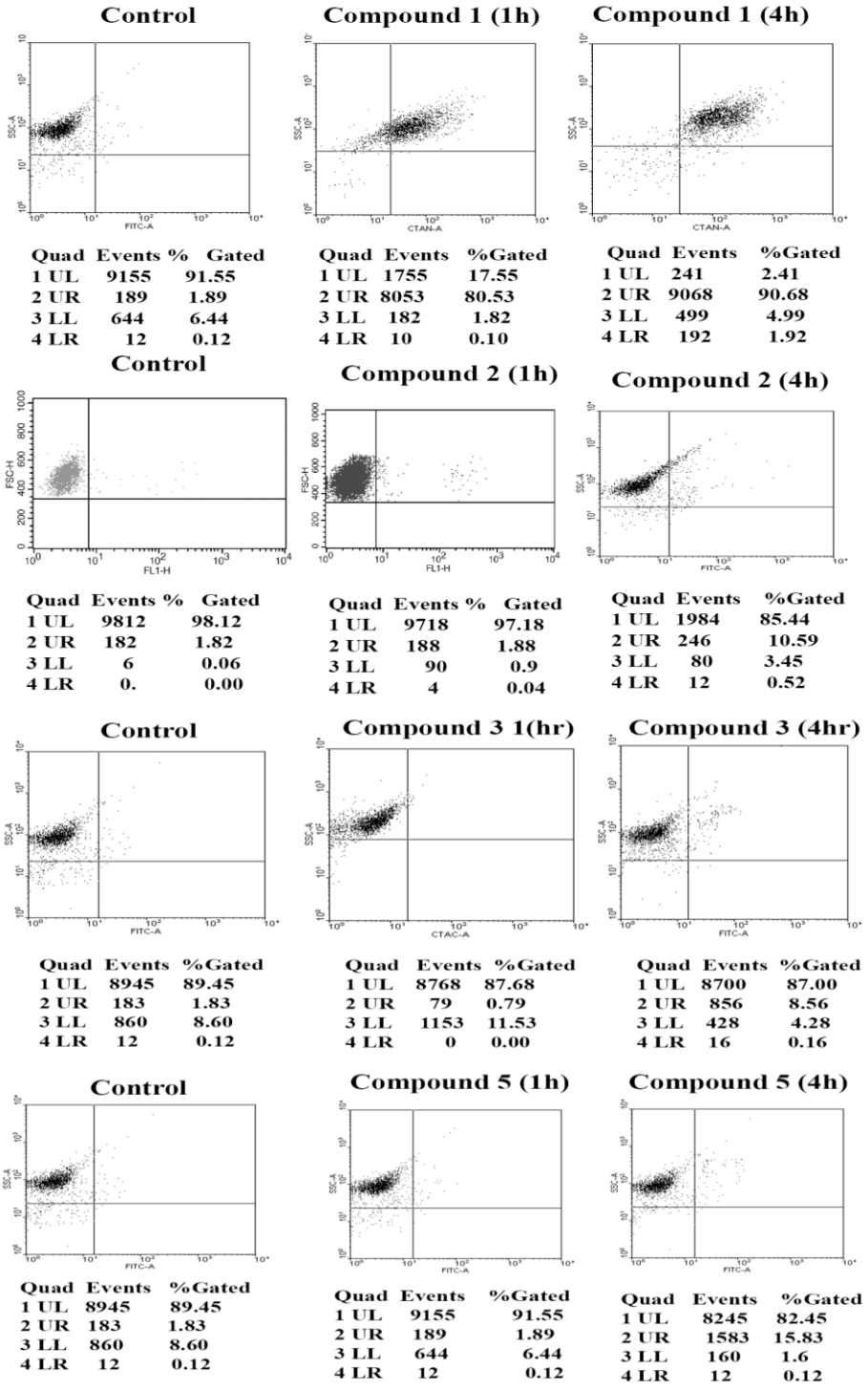
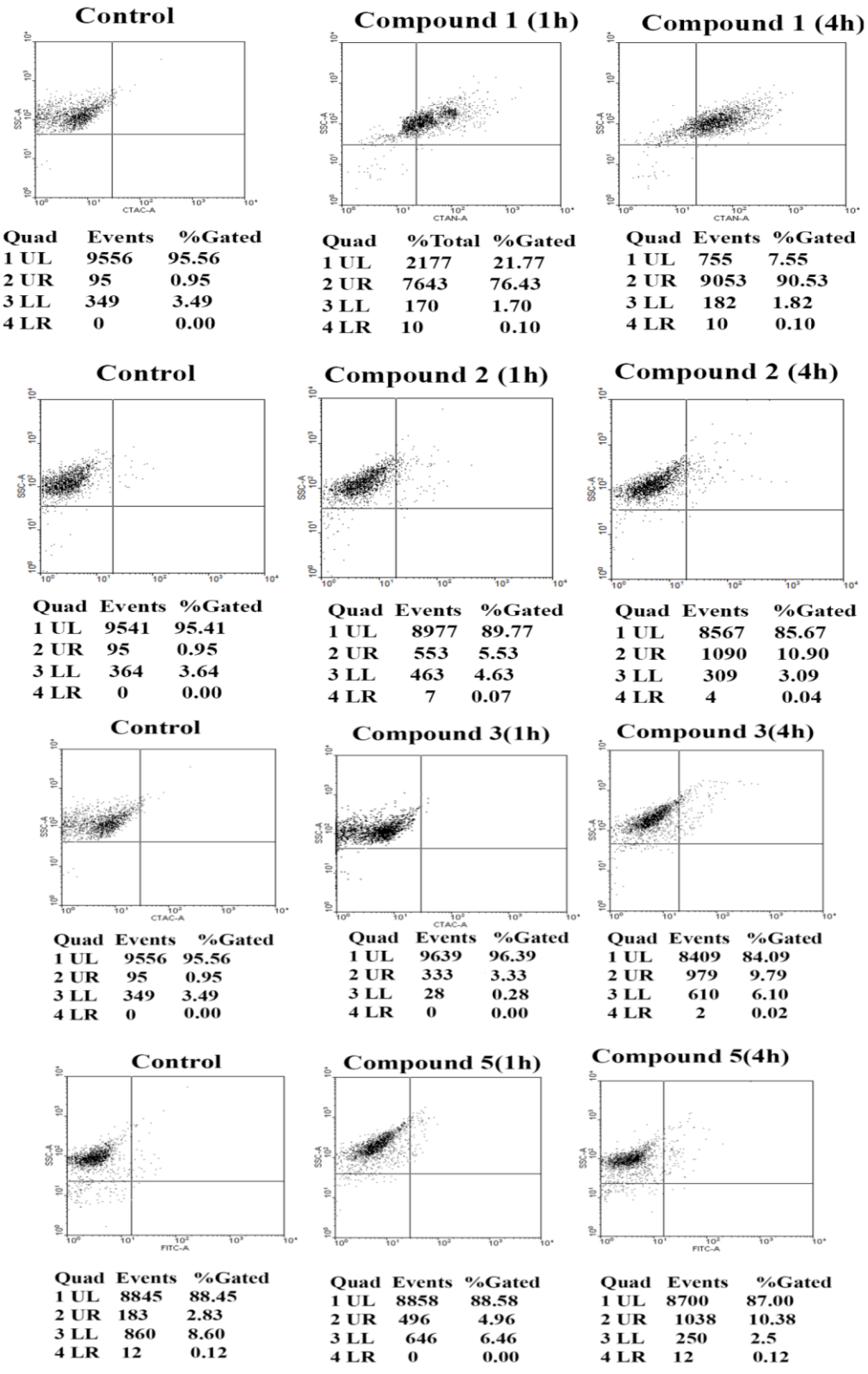


Fig. 23 (A) Confocal images of 20 μM **1** (a, b), **2** (c, d) and **5** (e, f) in MCF-7 cells after 12 h incubation in dark followed by 1h light-exposure . Panels (i)-(iv) are compound fluorescence, bright field, PI-staining and merged image, respectively. The PDT effect is visible for **1** and **5** in light (arrows in b(iii) and f(iii)). No apparent PDT effect for **2** which shows marginal nuclear uptake. Scale bar in blue is 10 μm. Confocal images for compound **3** (20 μM) in HeLa with (a, d) showing compound fluroscence, (b, e) showing PI-staining (c, f) for merged image.



ESI Fig. S24. Flow cytometric analysis (FACS) done for compounds **1-3** and **5** (20 μM) in HeLa cells at different incubation time of 1h and 4h.



Quad	Events	%Gated
1 UL	9556	95.56
2 UR	95	0.95
3 LL	349	3.49
4 LR	0	0.00

Quad	%Total	%Gated
1 UL	2177	21.77
2 UR	7643	76.43
3 LL	170	1.70
4 LR	10	0.10

Quad	Events	%Gated
1 UL	755	7.55
2 UR	9053	90.53
3 LL	182	1.82
4 LR	10	0.10

Quad	Events	%Gated
1 UL	9541	95.41
2 UR	95	0.95
3 LL	364	3.64
4 LR	0	0.00

Quad	Events	%Gated
1 UL	8977	89.77
2 UR	553	5.53
3 LL	463	4.63
4 LR	7	0.07

Quad	Events	%Gated
1 UL	8567	85.67
2 UR	1090	10.90
3 LL	309	3.09
4 LR	4	0.04

Quad	Events	%Gated
1 UL	9556	95.56
2 UR	95	0.95
3 LL	349	3.49
4 LR	0	0.00

Quad	Events	%Gated
1 UL	9639	96.39
2 UR	333	3.33
3 LL	28	0.28
4 LR	0	0.00

Quad	Events	%Gated
1 UL	8409	84.09
2 UR	979	9.79
3 LL	610	6.10
4 LR	2	0.02

Quad	Events	%Gated
1 UL	8845	88.45
2 UR	183	2.83
3 LL	860	8.60
4 LR	12	0.12

Quad	Events	%Gated
1 UL	8858	88.58
2 UR	496	4.96
3 LL	646	6.46
4 LR	0	0.00

Quad	Events	%Gated
1 UL	8700	87.00
2 UR	1038	10.38
3 LL	250	2.5
4 LR	12	0.12

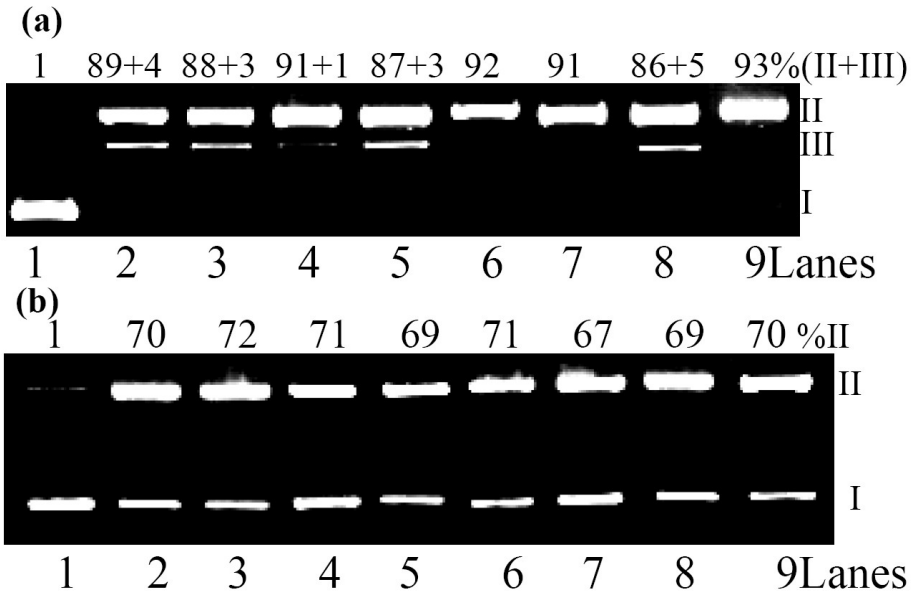
ESI Fig. S25. Flow cytometric analysis (FACS) done for compounds **1-3** and **5** (10 μM) in MCF-7 cells at different incubation time of 1h and 4h.

ESI Fig. S26. Cleavage of SC pUC19 DNA (0.2 μg, 30 μM) by compounds **1** - **5** (20 μM) **in argon** medium in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing 0.1% DMF in visible light of different wavelengths for an exposure time of 2 h. Detailed reaction conditions are given below in a tabular form. Form **I**, **II** and **III** are the SC, NC and linear forms of plasmid DNA.

Lane No	λ/nm	Reaction conditions	Lane No	λ/nm	Reaction conditions
1	458 nm	DNA control	12	514 nm	DNA + **1**
2	458 nm	DNA + **1**	13	514 nm	DNA + **2**
3	458 nm	DNA + **2**	14	514 nm	DNA + **3**
4	458 nm	DNA + **3**	15	514 nm	DNA + **4**
5	458 nm	DNA + **4**	16	514 nm	DNA + **5**
6	458 nm	DNA + **5**	17	647 nm	DNA + **1**
7	488 nm	DNA + **1**	18	647 nm	DNA + **2**
8	488 nm	DNA + **2**	19	647 nm	DNA + **3**
9	488 nm	DNA + **3**	20	647 nm	DNA + **4**
10	488 nm	DNA + **4**	21	647 nm	DNA + **5**
11	488 nm	DNA + **5**			

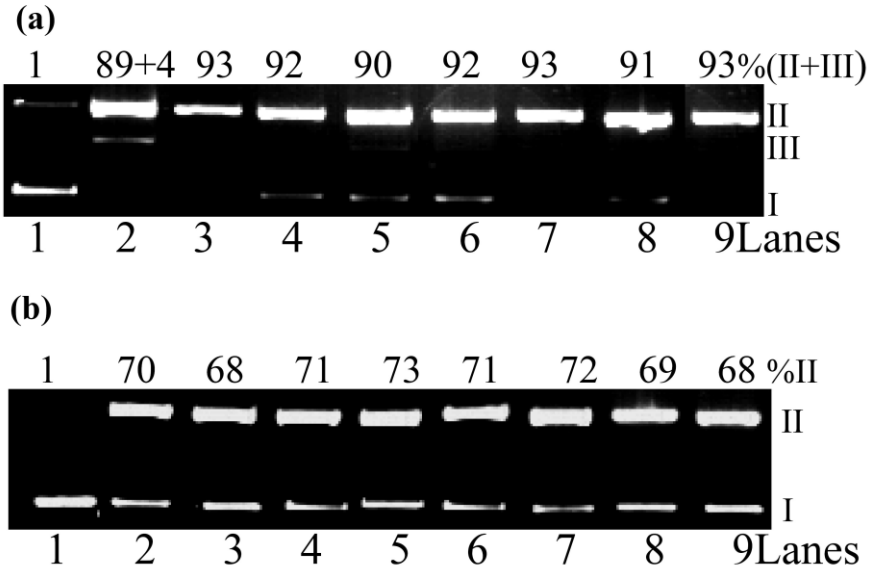
ESI Fig. S27. Cleavage of SC pUC19 DNA (0.2 μg, 30 μM) by compound **1** - **5** (20 μM) **in air** medium in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing 0.1% DMF in visible light of different wavelength for an exposure time of 2 h. Detailed reaction conditions are given below in a tabular form. Form **I**, **II** and **III** are the SC, NC and linear forms of plasmid DNA.

Lane No	λ/nm	Reaction conditions	Lane No	λ/nm	Reaction conditions
1	458 nm	DNA control	12	514 nm	DNA + **1**
2	458 nm	DNA + **1**	13	514 nm	DNA + **2**
3	458 nm	DNA + **2**	14	514 nm	DNA + **3**
4	458 nm	DNA + **3**	15	514 nm	DNA + **4**
5	458 nm	DNA + **4**	16	514 nm	DNA + **5**
6	458 nm	DNA + **5**	17	647 nm	DNA + **1**
7	488 nm	DNA + **1**	18	647 nm	DNA + **2**
8	488 nm	DNA + **2**	19	647 nm	DNA + **3**
9	488 nm	DNA + **3**	20	647 nm	DNA + **4**
10	488 nm	DNA + **4**	21	647 nm	DNA + **5**
11	488 nm	DNA + **5**			



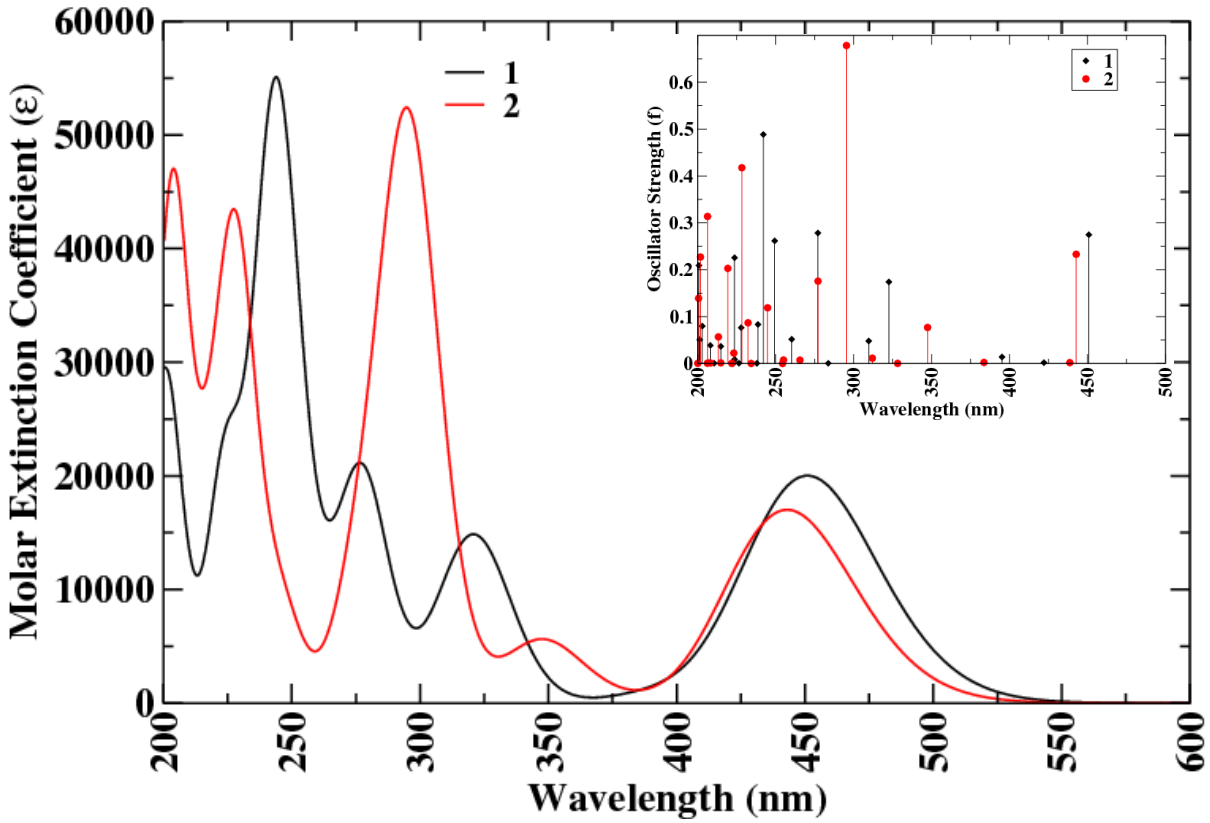
ESI Fig. S28. Gel electrophoresis diagram showing the photocleavage of SC pUC19 DNA (0.2 µg, 30 µM) at 458 nm by **1** (20 µM) in (a) and **2** (20 µM) in (b) in argon medium in the presence of different additives for 1 h exposure time in 50 mM Tris-HCl / NaCl buffer (pH, 7.2). Detail conditions are given below in a tabular form. Form **I**, **II** and **III** are the SC, NC and linear forms of plasmid DNA.

Lane No.	Reaction condition
1	DNA
2	DNA + Compound
3	DNA + Compound + D_2O
4	DNA + Compound + TEMP (1 mmol)
5	DNA + Compound + DABCO (1 mmol)
6	DNA + Compound + KI (1 mmol)
7	DNA + Compound + DMSO (6 µl)
8	DNA + Compound + Catalase (6 units)
9	DNA + Compound + SOD (6 units)



ESI Fig. S29. Gel electrophoresis diagram displaying the photocleavage of SC pUC19 DNA (0.2 µg, 30 µM) at 458 nm by **1** (20 µM) in (a) and **2** (20 µM) in (b) in the presence of different additives for 1 h exposure time in 50 mM Tris-HCl / NaCl buffer (pH, 7.2). Detail conditions are given below in a tabular form. Form **I**, **II** and **III** are the SC, NC and linear forms of plasmid DNA.

Lane No.	Reaction condition
1	DNA
2	DNA + Compound
3	DNA + Compound + D_2O
4	DNA + Compound + TEMP (1 mM)
5	DNA + Compound + DABCO (1 mM)
6	DNA + Compound + KI (1 mM)
7	DNA + Compound + DMSO (6 μL)
8	DNA + Compound + Catalase (6 units)
9	DNA + Compound + SOD (6 units)



ESI Fig. S30. Simulated UV-Visible spectra for **1** and **2** in aqueous solution. Inset: The oscillator strength for singlet-singlet transitions.

Table S1. Selected TDDFT singlet transitions depicted in Figure S23 are shown for both **1** and **2**. These TDDFT excitations are calculated from the PCM optimized ground state geometries of **1** and **2**

	Energy (eV)	Wavelength (nm)	Oscillator strength	Major contributions
I	2.749	451	0.2747	HOMO→LUMO (96%)
II	3.842	323	0.1739	HOMO-3→LUMO (91%)
				HOMO→LUMO+2 (5%)
III	4.474	277	0.2783	HOMO-1→LUMO+1 (67%)
				HOMO→LUMO+2 (25%)
IV	5.121	242	0.4886	HOMO-5→LUMO (33%)
				HOMO→LUMO+2 (35%)
I	2.800	443	0.2330	HOMO→LUMO (96%)
II	3.568	347	0.0768	HOMO→LUMO+1 (83%)
				HOMO-1→LUMO (12%)
III	4.196	295	0.6783	HOMO-1→LUMO+1 (67%)
				HOMO-3→LUMO (19%)
IV	5.429	228	0.4176	HOMO→LUMO+3 (38%)
				HOMO-4→LUMO+1 (15%)

Table S2. B3LYP/6-311+G**/PCM optimized geometries of **1** and **2**.

No.	Cartesian coordinates			
1 (S_0)	S	3.94257	-0.77690	0.00000
	N	0.16100	-1.70961	-0.00022
	N	1.46008	-1.84418	-0.00026
	N	1.72914	0.53092	-0.00009
	C	-3.85856	1.00762	0.00024
	H	-4.30792	1.99377	0.00031
	C	-4.64609	-0.12466	0.00025
	H	-5.72567	-0.03541	0.00034
	C	-4.04997	-1.40010	0.00016
	H	-4.67679	-2.28382	0.00018
	C	-2.67481	-1.53900	0.00005
	H	-2.22046	-2.52022	-0.00002
	C	-1.85194	-0.39690	0.00002
	C	-2.44964	0.89555	0.00013
	C	-1.61859	2.05405	0.00011
	H	-2.09236	3.02876	0.00017
	C	-0.24958	1.98215	0.00005
	H	0.34096	2.88647	0.00003
	C	0.35708	0.71316	-0.00003
	C	-0.40847	-0.48234	-0.00009
	C	2.21686	-0.74816	-0.00019
	C	3.96499	0.95442	-0.00016
	H	4.90268	1.48696	-0.00024
	C	2.72965	1.49885	0.00000
	H	2.48122	2.54592	-0.00003
1 (S_1)	S	3.96429	-0.79013	-0.00024
	N	0.13386	-1.73936	-0.00010
	N	1.47524	-1.84263	-0.00017
	N	1.74877	0.53778	-0.00008
	C	-3.88279	0.99947	0.00022
	H	-4.34268	1.98073	0.00028
	C	-4.67173	-0.14179	0.00021
	H	-5.75026	-0.05421	0.00027
	C	-4.06379	-1.39657	0.00013
	H	-4.66627	-2.29605	0.00013
	C	-2.65934	-1.51138	0.00006
	H	-2.19499	-2.48761	0.00000
	C	-1.83998	-0.36529	0.00006
	C	-2.44706	0.91595	0.00014
	C	-1.64844	2.07827	0.00015
	H	-2.11202	3.05585	0.00020
	C	-0.26312	1.97250	0.00007
	H	0.33476	2.87367	0.00007

	C	0.34803	0.70639	-0.00001
	C	-0.40408	-0.47962	-0.00002
	C	2.21621	-0.76505	-0.00016
	C	3.99198	0.95193	-0.00018
	H	4.92837	1.48535	-0.00021
	C	2.74990	1.48932	-0.00009
	H	2.51051	2.53977	-0.00003
2 (S_0)	N	0.11856	-1.79086	-0.00022
	N	1.38752	-1.98948	-0.00015
	N	1.82294	0.37651	-0.00005
	C	-3.77236	1.10739	-0.00003
	H	-4.17940	2.11168	-0.00006
	C	-4.60528	0.00968	0.00006
	H	-5.68031	0.14321	0.00008
	C	-4.06243	-1.29133	0.00012
	H	-4.72658	-2.14741	0.00021
	C	-2.69608	-1.48882	0.00008
	H	-2.28346	-2.48810	0.00012
	C	-1.82419	-0.38026	-0.00001
	C	-2.36861	0.93527	-0.00004
	C	-1.48418	2.04952	-0.00002
	H	-1.90760	3.04714	-0.00006
	C	-0.11972	1.91003	0.00006
	H	0.49478	2.79652	0.00028
	C	0.43696	0.61518	0.00000
	C	-0.38981	-0.52496	-0.00006
	C	2.25710	-0.93708	-0.00003
	C	3.63131	-1.21969	0.00012
	H	3.91621	-2.26250	0.00012
	C	4.54938	-0.19782	0.00020
	H	5.61050	-0.40858	0.00041
	C	4.08740	1.13011	0.00001
	H	4.77633	1.96335	-0.00006
	C	2.74599	1.39468	-0.00011
	H	2.36354	2.40003	-0.00029
2 (S_1)	N	0.08463	-1.81432	0.00005
	N	1.40169	-1.98692	0.00012
	N	1.84004	0.38100	-0.00000
	C	-3.79046	1.09932	0.00003
	H	-4.21074	2.09805	0.00004
	C	-4.63089	-0.01122	0.00001
	H	-5.70426	0.12555	0.00000
	C	-4.08136	-1.28958	-0.00001
	H	-4.72203	-2.16200	-0.00002
	C	-2.68350	-1.46241	-0.00000
	H	-2.25798	-2.45601	-0.00001

	C	-1.81419	-0.34828	0.00002
	C	-2.36440	0.95545	0.00003
	C	-1.50975	2.07623	0.00005
	H	-1.92377	3.07588	0.00008
	C	-0.13057	1.90240	0.00005
	H	0.49192	2.78493	0.00009
	C	0.42822	0.61432	0.00002
	C	-0.38856	-0.52664	0.00002
	C	2.25138	-0.95784	0.00010
	C	3.64551	-1.22577	0.00015
	H	3.93825	-2.26665	0.00025
	C	4.55962	-0.20592	0.00007
	H	5.62032	-0.42194	0.00012
	C	4.10692	1.13366	-0.00007
	H	4.79744	1.96470	-0.00016
	C	2.76223	1.38966	-0.00011
	H	2.38280	2.39775	-0.00022