Spectrophotometric and Computational Insights into 2-Ethylimidazole–3,5-Dinitrobenzoic Acid Complex: BSA Interaction and Antimicrobial Activity

Farha Naaz[a], Ishaat M. Khan[a *], Sonam Shakya[a], Maidul Islam[a,b], Nisat Alam[c], Abdullah Alarifi[d], Xiang Li[e]

[a] Department of Chemistry, Faculty of Science, Aligarh Muslim University, Aligarh (U.P.) – India 202002
[b] Research and Development Cell, Lovely Professional University, Phagwara, Punjab-144411, India
[d]Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
[e]Jiangsu Key Laboratory of Pesticide Science and Department of Chemistry, College of Science, Nanjing Agricultural University, Nanjing, 210095, China.

Table: S1. Gaussian analysis of synthesized CT complex in 4 polar solvents.

Solvent	Area of the curve (A)	Width of the curve (w)	Centre of the curve (x_c)	y_o
Methanol	63.4161 ± 14.725	38.8196 ± 0.18243	239.6856 ± 0.82224	1.7219 ± 0.18204
Ethanol	17.1471 ± 7.49478	37.7472 ± 8.23096	218.9692 ± 0.8546	1.2506 ± 0.10353
DMSO	17.3921 ± 3.53897	24.4567 ± 2.19525	264.6052 ± 0.09732	0.6557 ± 0.07952
ACN	31.8063 ± 1.38499	22.7899 ± 0.59747	203.4427 ± 0.13474	2.2730 ± 0.0253

Table: S2. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for CT Complex. U_{eq} is defined as 1/3 of the trace of the orthogonalized U_{IJ} tensor.

Atom	*x*	*Y*	*z*	U(eq)
O5	-1886.2(19)	-3047.9(15)	-3357.7(11)	57.0(4)
O4	-4098(2)	-2628.0(19)	-7927.3(13)	66.9(4)
O6	-3322.3(18)	-4772.8(17)	-2359.7(12)	59.6(4)
N4	679(2)	-6947.7(19)	1004.6(16)	47.3(4)
N2	-5494(3)	-3252.9(19)	-7825.5(14)	52.6(4)
O2	-10639.3(19)	-5185(2)	-5955.3(14)	77.3(5)
O3	-6666(2)	-3561.6(19)	-8713.0(12)	79.1(5)
N1	-9382(2)	-5350(2)	-5098.4(17)	57.0(5)
N3	-669(2)	-5897.1(19)	-677.4(13)	48.2(4)
O1	-9464(2)	-6026(2)	-4122.7(15)	80.6(5)
C7	-3143(3)	-3956(2)	-3280.5(16)	43.0(4)
C3	-5790(3)	-3685(2)	-6566.8(15)	40.5(4)
C6	-6266(2)	-4666(2)	-4254.4(16)	40.0(4)
C10	-834(3)	-6267(2)	479.4(16)	41.7(4)

C5	-4637(2)	-4062(2)	-4405.5(15)	37.5(4)
C1	-7646(2)	-4712(2)	-5270.1(16)	41.5(4)
C4	-4386(2)	-3566(2)	-5575.1(15)	40.0(4)
C2	-7443(3)	-4219(2)	-6441.3(17)	45.6(5)
C8	1802(3)	-7017(2)	154.1(19)	55.3(6)
C11	-2411(3)	-5944(3)	1059.9(19)	56.6(6)
C9	963(3)	-6362(2)	-895.4(19)	54.6(5)
C12	-2930(3)	-7305(3)	1816(2)	67.8(6)

Table: S3. Anisotropic Displacement Parameters ($Å^2×10^3$) for CT Complex. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O5	58.2(9)	65.7(10)	40.2(7)	-18.2(8)	-10.3(6)	0.4(7)
O4	75.4(11)	80.5(11)	48.5(8)	0.0(9)	21.4(8)	3.0(8)
O6	56.1(9)	75.9(10)	39.7(7)	-6.2(7)	-11.2(6)	19.7(7)
N4	51.2(11)	50.0(10)	35.7(8)	6.7(8)	-5.8(8)	3.4(8)
N2	69.1(12)	51.7(11)	34.6(9)	9.7(10)	3.0(9)	0.8(8)
O2	39.9(9)	108.6(14)	75.4(11)	1.7(9)	-11.5(8)	-9.5(10)
O3	102.4(13)	92.8(13)	32.5(7)	-7.6(10)	-14.5(8)	3.2(8)
N1	43.7(11)	68.9(12)	55.5(11)	1.1(9)	1.2(9)	-11.3(10)
N3	51.5(11)	53.3(10)	36.0(8)	4.3(8)	-2.9(7)	9.8(7)
O1	60.4(10)	113.1(14)	69.3(10)	16.7(10)	14.2(8)	11.5(11)
C7	44.6(11)	46.8(11)	32.9(10)	2.5(10)	5.6(8)	1.8(9)
C3	49.8(12)	40.4(11)	29.1(9)	6.5(9)	0.9(8)	0.2(8)
C6	46.0(11)	40.9(10)	31.0(9)	5.3(9)	1.3(8)	-1.0(8)
C10	47.2(11)	40.7(10)	33.8(9)	1.1(9)	-1.9(8)	2.6(9)
C5	41.1(10)	35.5(10)	32.3(9)	2.2(8)	-3.8(7)	-0.2(8)
C1	34.9(10)	44.6(11)	42.7(10)	2.4(9)	0.4(8)	-4.2(9)
C4	42.4(11)	39.6(10)	35.8(9)	2.1(8)	1.3(8)	-3.3(8)
C2	47.0(12)	48.6(12)	35.6(10)	7.3(9)	-8.5(8)	-5.1(9)
C8	47.8(13)	62.3(14)	54.8(12)	7.7(11)	6.7(10)	-0.4(11)
C11	58.2(13)	62.3(14)	48.9(11)	11.9(11)	8.7(10)	3.1(11)
C9	55.2(14)	61.2(13)	48.3(12)	4.4(11)	12.0(10)	6.4(11)
C12	65.8(15)	81.0(17)	58.0(13)	-3.7(14)	14.4(11)	11.3(13)

Table: S4. Bond Lengths for CT Complex.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O5	C7	1.247(2)	N3	C9	1.365(3)
O4	N2	1.213(2)	C7	C5	1.518(2)
O6	C7	1.246(2)	C3	C4	1.381(2)

N4	C10	1.326(2)	C3	C2	1.369(3)
N4	C8	1.367(3)	C6	C5	1.381(2)
N2	O3	1.221(2)	C6	C1	1.383(2)
N2	C3	1.471(2)	C10	C11	1.479(3)
O2	N1	1.220(2)	C5	C4	1.383(2)
N1	O1	1.218(2)	C1	C2	1.374(2)
N1	C1	1.473(2)	C8	C9	1.328(3)
N3	C10	1.322(2)	C11	C12	1.517(3)

Table: S5. Bond Angles for CT Complex.

Atom	**Atom**	**Atom**	**Angle/°**	**Atom**	**Atom**	**Atom**	**Angle/°**
C8	N4	C10	109.23(17)	N3	C10	N4	106.96(17)
O3	N2	O4	123.66(18)	C11	C10	N4	127.52(17)
C3	N2	O4	118.46(17)	C11	C10	N3	125.52(17)
C3	N2	O3	117.88(19)	C6	C5	C7	119.26(16)
O1	N1	O2	124.32(19)	C4	C5	C7	120.96(17)
C1	N1	O2	117.56(19)	C4	C5	C6	119.77(16)
C1	N1	O1	118.11(16)	C6	C1	N1	118.87(17)
C9	N3	C10	109.73(16)	C2	C1	N1	118.78(16)
O6	C7	O5	126.65(16)	C2	C1	C6	122.33(17)
C5	C7	O5	117.29(17)	C5	C4	C3	118.63(17)
C5	C7	O6	116.04(17)	C1	C2	C3	116.79(16)
C4	C3	N2	118.73(17)	C9	C8	N4	107.26(19)
C2	C3	N2	118.12(15)	C12	C11	C10	113.38(17)
C2	C3	C4	123.13(16)	C8	C9	N3	106.82(18)
C1	C6	C5	119.25(16)				

Table: S6. Hydrogen Atom Coordinates ($Å \times 10^4$) and Isotropic Displacement Parameters ($Å^2 \times 10^3$) for CT Complex.

Atom	***x***	***Y***	***z***	**U(eq)**
H4	-3295(2)	-3161(2)	-5691.0(15)	48.0(5)
H2	-8383(3)	-4247(2)	-7116.4(17)	54.7(6)
H6	-6434(2)	-5037(2)	-3478.3(16)	48.0(5)
H3	-1468(2)	-5433.6(19)	-1211.6(13)	57.9(5)
H8	2943(3)	-7444(2)	286.1(19)	66.3(7)
H11a	-2165(3)	-5039(3)	1602.2(19)	67.9(7)
H11b	-3411(3)	-5686(3)	406.7(19)	67.9(7)
H9	1402(3)	-6242(2)	-1636.6(19)	65.5(7)
H12a	-1940(7)	-7581(12)	2456(9)	101.8(10)

H12b	-3926(13)	-7010(6)	2197(12)	101.8(10)
H12c	-3250(20)	-8186(6)	1275(3)	101.8(10)
H4a	890(30)	-7280(30)	1770(20)	71(7)

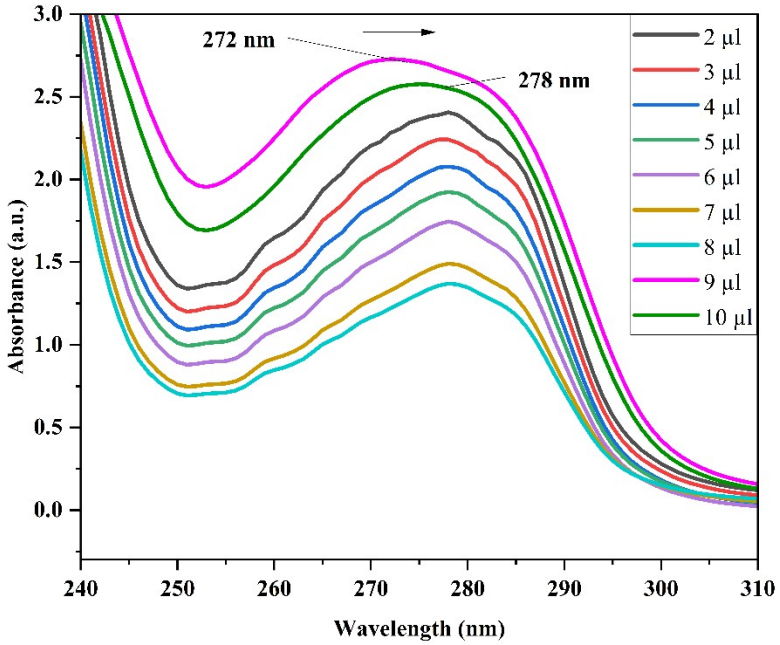

Fig. S1. UV titration spectra displaying shift of 6 nm in the wavelength on the addition of CT Complex in BSA.

Table: S7. Antibacterial activity of CT complex at various concentrations.

S1	Bacterial Strain	D1 (10 µg/mL)	D2 (20 µg/mL)	D3 (40 µg/mL)	D4 (80 µg/mL)	Gentamicin (80 µg/mL)
				Zone of Inhibition (mm)		
1	***Escherichia coli***	9.7±0.69	14.1±1.2	23.5±0.25	24.4±0.95	35.0±0.62
2	***Bacillus subtilis***	15.3±0.57	18.3±0.38	19.2±0.63	26.8±0.54	32.0±1.04
3	***Staphylococus aureus***	8.3±0.54	10.4±0.85	15.6±0.85	16.2±0.87	20.01±0.92

Table S8. Antibacterial activity of donnar at various concentrations.

S1	Bacterial Strain	D1 (10 µg/mL)	D2 (20 µg/mL)	D3 (40 µg/mL)	D4 (80 µg/mL)	Gentamicin (80 µg/mL)
				Zone of Inhibition (mm)		
1	***Escherichia Coli***	6.1±1.34	11.2±0.91	18.4±1.52	19.4±1.12	35.0±0.62

2	***Bacillus Subtilis***	9.3±1.89	13.6±0.91	14.7±1.76	21.9±0.88	32.0±1.04
3	***Staphylococus Aureus***	7.3±0.79	9.2±1.09	15.1±1.91	18.3±0.96	20.01±0.92

Table S9. Antibacterial activity of acceptor at various concentrations.

S1	Bacterial Strain	D1 (10 µg/mL)	D2 (20 µg/mL)	D3 (40 µg/mL)	D4 (80 µg/mL)	Gentamicin (80 µg/mL)
		Zone of Inhibition (mm)				
1	***Escherichia Coli***	6.9±1.22	10.5±0.98	19.3±1.12	17.2±1.56	35.0±0.62
2	***Bacillus Subtilis***	8.7±1.98	11.3±1.59	15.6±1.94	22.9±1.58	32.0±1.04
3	***Staphylococus Aureus***	8.1±0.94	6.9±0.98	13.2±1.9	19.2±0.97	20.01±0.92

Table S10. Antifungal activity of CT complex at various concentrations.

S1	Fungal Strain	D1 (10 µg/mL)	D2 (20 µg/mL)	D3 (40 µg/mL)	D4 (80 µg/mL)	Nystatin (80 µg/mL)
		Zone of Inhibition (mm)				
1	***Aspergillus niger***	9.1±0.82	17.3±0.65	19.4±0.87	21.2±0.12	24.2±1.483
2	***Candida albicans***	6.9±1.2	16.9±0.41	15.2±0.73	18.6±0.36	22.6±1.140
3	***Fusarium oxysporum***	9.8±0.87	14.3±0.91	16.2±0.90	19.1±0.59	24.6±1.140

Table S11. Antifungal activity of donor at various concentrations.

S1	Fungal Strain	D1 (10 µg/mL)	D2 (20 µg/mL)	D3 (40 µg/mL)	D4 (80 µg/mL)	Nystatin (80 µg/mL)
		Zone of Inhibition (mm)				
1	***Aspergillus Niger***	8.2±1.34	15.3±0.91	19.2±1.18	20.3±1.05	24.2±1.483
2	***Candida Albicans***	7.1±0.91	11.2±0.67	14.9±1.09	17.6±1.71	22.6±1.140
3	***Fusarium Oxysporum***	8.3±1.39	10.1±1.85	9.2±1.71	16.3±1.81	24.6±1.140

Table S12. Antifungal activity of acceptor at various concentrations.

S1	Fungal Strain	D1 (10 µg/mL)	D2 (20 µg/mL)	D3 (40 µg/mL)	D4 (80 µg/mL)	Nystatin (80 µg/mL)
		Zone of Inhibition (mm)				
1	***Aspergillus Niger***	7.3±1.11	14.2±1.45	17.1±0.98	20.1±1.56	24.2±1.483
2	***Candida Albicans***	5.1±1.89	9.5±0.81	15.2±1.89	16.3±0.81	22.6±1.140
3	***Fusarium Oxysporum***	7.3±1.56	11.2±1.74	7.3±1.9	16.9±1.73	24.6±1.140

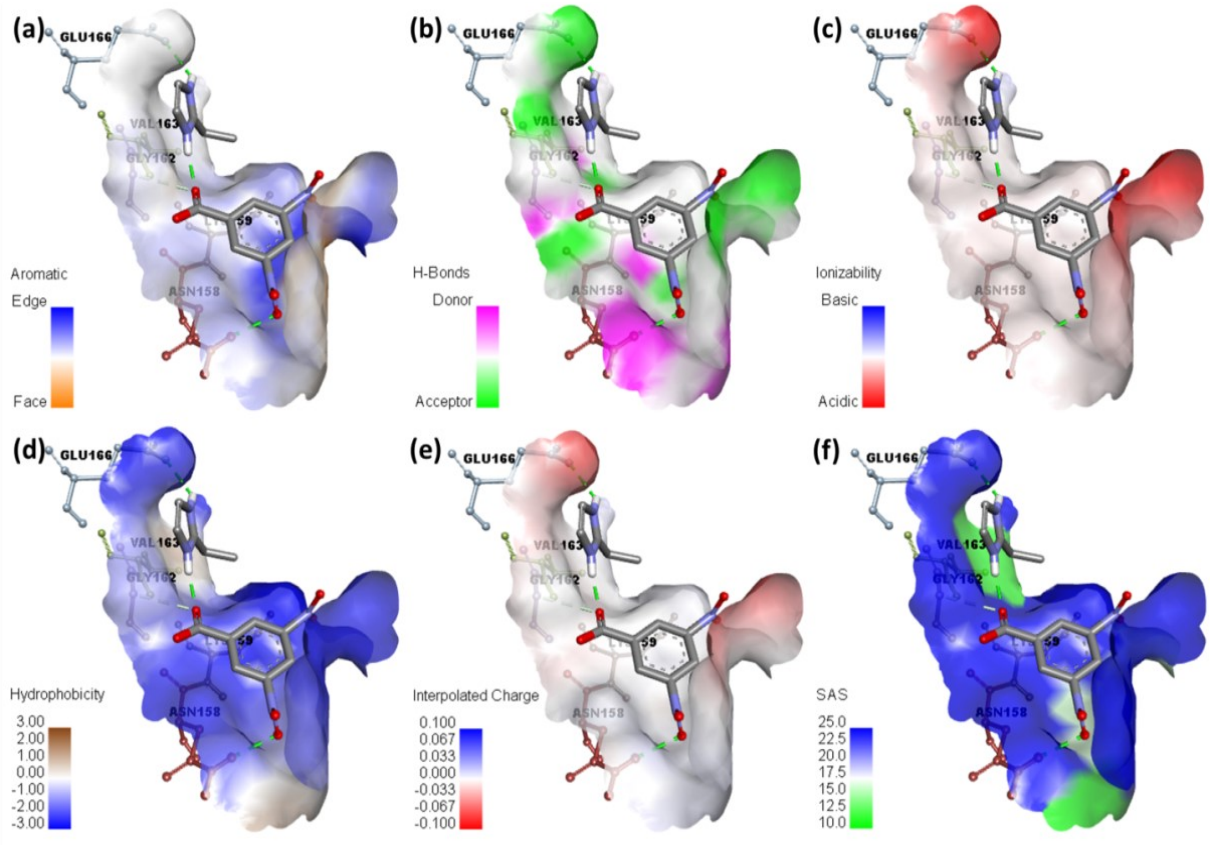

Fig. S2. Representation of (a) aromatic surface, (b) hydrogen binding surface, (c) ionizability surface, (d) hydrophobic surface, (e) interpolated charge surface, and (f) solvent accessible surface at interaction site of CT complex and 3V03.

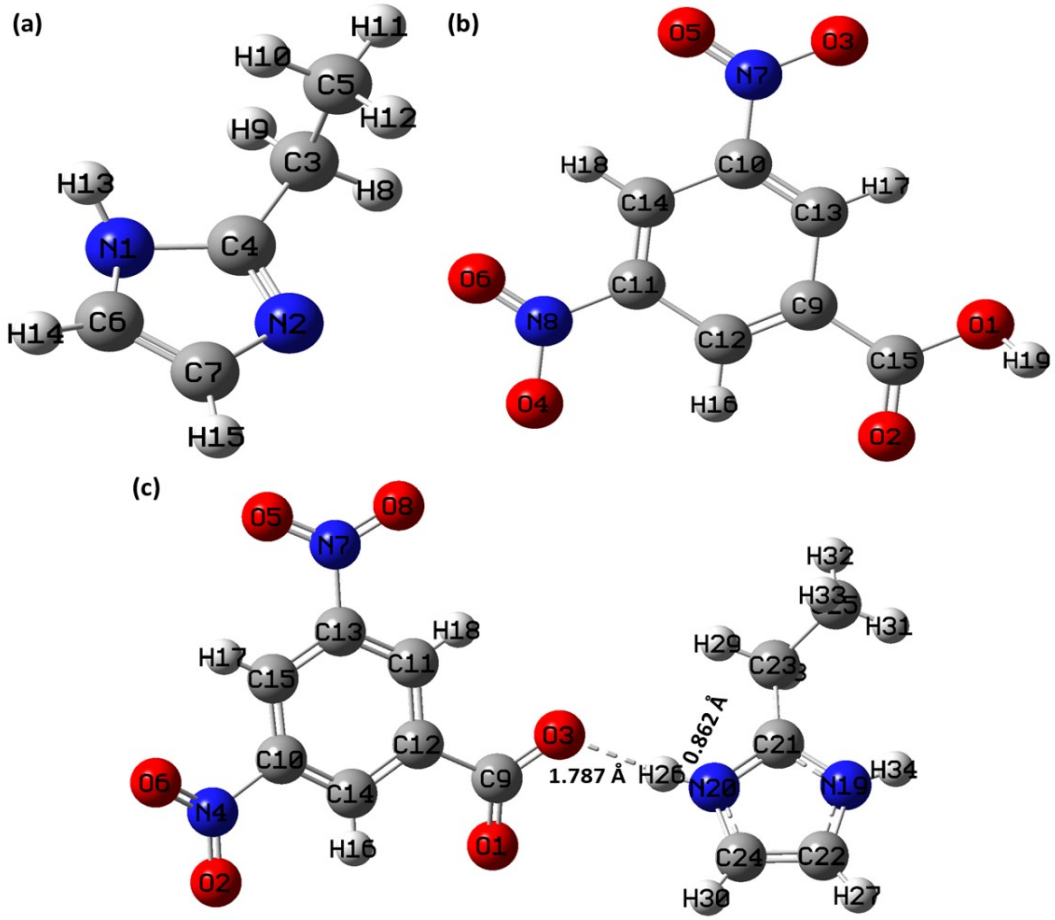

Fig. S3. Optimized structure of (a) 2-EI, (b) 3,5-DNB, and (c) CT complex with Mullikenatom numbering scheme.

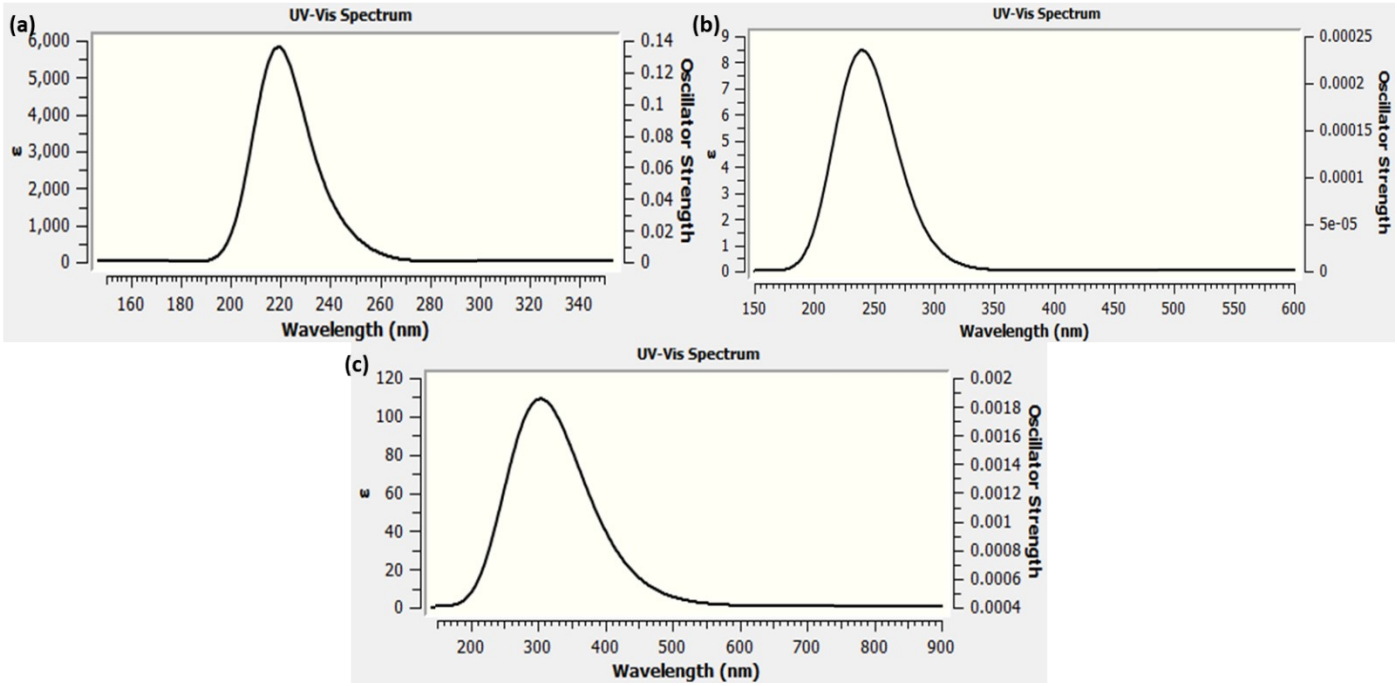

Fig. S4. Simulated UV-Vis spectra of (a) for 2-EI, (b) 3,5-DNB, and (c) synthesized CTC obtained through TD-DFT in gas phase.

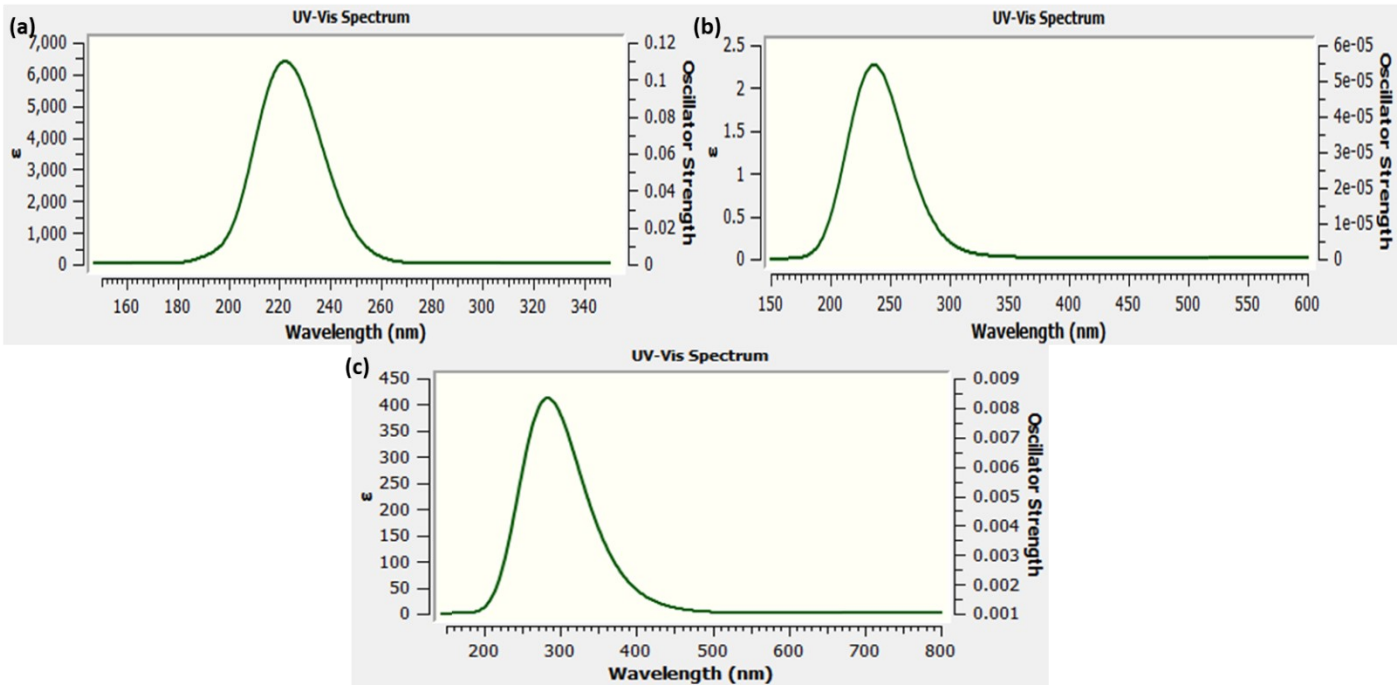

Fig. S5. Simulated UV-Vis spectra of (a) for 2-EI, (b) 3,5-DNB, and (c) synthesized CTC obtained through TD-DFT in DMSO.

Table S13. TD-DFT calculated electronic excitation parameters for the CT complex in DMSO and gas phase, showing the wavelength, energy, oscillator strength, and orbital contributions for the significant excited states.

	Excited State	Wavelength (nm)	Oscillator Strength (f)	Orbital Contribution
Gas phase	S1	272	0.0019	HOMO → LUMO
	S2	247	0.0006	HOMO -1→ LUMO
DMSO	S1	274	0.0082	HOMO → LUMO
	S2	251	0.0018	HOMO -1→ LUMO