Supplementary Information

Potential-bioactive revelation of water-soluble Boc-L-Valine and Imidazole appended metal complexes {M = Co(II), Cu(II) & Zn(II)}: Synthesis, characterization, ct-DNA binding, pBR322 cleavage, SOD mimetic and cytotoxicity studies

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1.Experimental section

1.1. Materials and instrumentation

All reagents, $Co(ClO_4)_2.6H_2O$, $Cu(NO_3)_2.6H_2O$, $Zn(ClO_4)_2.6H_2O$, Boc-L-Valine, imidazole Ethidium bromide(EB) and Tris-(hydroxymethyl)aminomethane (Tris-buffer), were purchased from Sigma Aldrich and ct-DNA was purchased from Sigma Chemicals.co and stored at 4°C. All other solvents were obtained from Merck and used as such without further purification. The interaction studies of DNA were carried out in tris buffer (5.0mM Tris-HCl, 50.0mM NaCl, pH=7.4). Fourier-Transform infrared (FT-IR) spectra were recorded on Perkin Elmer FT-IR spectrometer in the 4000-400 cm^{-1} range with KBr. The NMR (^{1}**H**) was obtained on Bruker Advance NEO 500MHZ spectrometer. High resolution mass spectrum was recoded on Agilent ESI-Q-TQF mass spectrometer. PerkinElmer Lambda 25 was used to record electronic spectra using cuvettes of 1cm path length. Emission spectra were obtained on Shimadzu RF-5301 PC spectrofluorometer. Circular dichroism (CD) measurements were carried on Jasco J-815-CD spectropolarimeter using 1 cm quartz cuvette. Cyclic voltammetry was carried out at CH instrument electro-chemical analyzer. Electrophoretic assays were carried out in Tris-borate-ethylenediaminetetraacetic acid buffer at 50 V cm^{-1} and were visualized using UV Transilluminator.

Molecular docking studies were performed using HEX 8.0 software which is an interactive molecular graphics program for calculating and displaying feasible docking modes of enzymes

and DNA molecules. Visualization of the docked pose was achieved using the Discovery Studio molecular graphics program.

Rigaku Oxford Diffraction XtaLAB Synergy-S and -I (Dual source) (2020) was used for the collection of single X-ray data at 293 K for all the complexes using Mo-Kα radiation (λ = 0.71073 Å). The structures were solved using Olex2 software, refined by using 'SHELXT 2018/2 (Sheldrick, 2018)' and PLATON version 28/11/22.

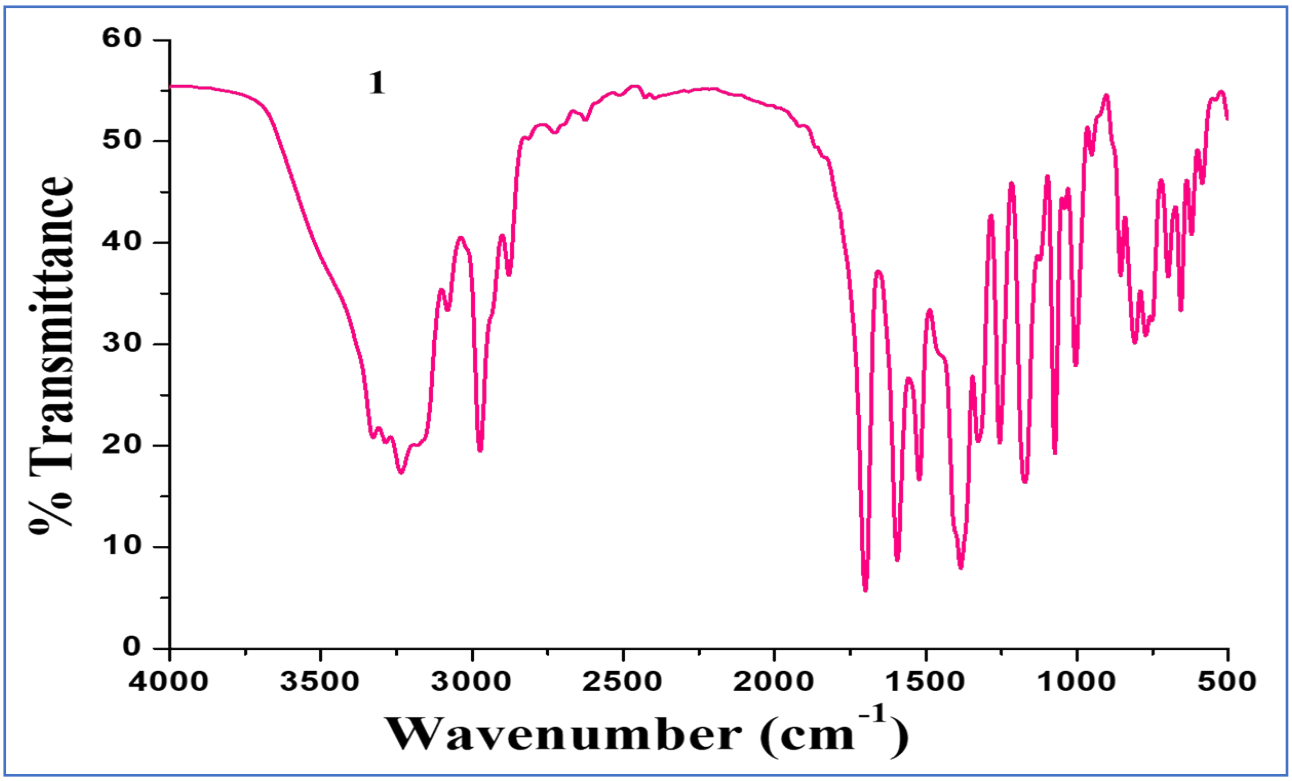
1.2. Synthesis of Complexes (**1-3)**

Complexes (**1-3)** were synthesized by adding respective metal salts $Co(ClO_4)_2.6H_2O$ (0.365 g, 1 mmol), $Cu(NO_3)_2.6H_2O$ (0.241 g, 1 mmol), $Zn(ClO_4)_2.6H_2O$ (0.372 g, 1 mmol) to a mixture of Boc-L-Valine (0.434 g, 2 mmol) and imidazole (0.136 g, 2 mmol) dissolved in methanol/DMF/Et_3N. The respective reaction mixtures were stirred for 6 hours, filtered in beaker, and kept for crystallization. After 15 days crystals suitable for X-ray diffraction were obtained.

Complex $[C_{26}H_{44}CoN_6O_8]$ **1**, MW: 627.60, Yield: 81%, Melting point: 170˚C ; FT-IR(KBr, υ_{max}/cm^{-1}): 32461 υ(–NH), 2976 υ(–CH_2), 1700 υ(–C=O), 1608 υ(–C=N), 558 υ (M−O), 471 υ (M−N) ; ESI-MS(*m/z*): 622, 566, 492, 466, 208 ; UV-vis(1×10^{-4}M,Methanol,λ/nm) : 207, 225-300, 450-650.

Complex $[C_{26}H_{44}CuN_6O_8]$ **2**, MW: 632.22, Yield: 82%, Melting point: 168 ˚C ; FT-IR(KBr, υ_{max}/cm^{-1}): 3274 υ(–NH), 2987 υ(–CH_2), 1700 υ(–C=O), 1614 υ(–C=N), 578 υ(M−O), 480 υ(M−N); ESI-MS(*m/z*): 631, 629, 628, 624, 581, 410, 383, 210; UV-vis(1×10^{-4}M,Methanol,λ/nm) : 207, 225-300, 525-750.

Complex $[C_{26}H_{44}ZnN_6O_8]$ **3**, MW: 634.06, Yield: 71%, Melting point: 164 ˚C ; FT-IR(KBr, υ_{max}/cm^{-1}): 3217.0 υ(–NH), 2981 υ(–CH_2), 1700 υ(–C=O), 1603 υ(–C=N), 547 υ(M−O), 483 υ(M−N); **^{1}H-**NMR(500MHz, DMSO, δ, ppm): 8.09(s,1H), 7.2(s,1H), 7.89(d,1H), 7.22(d,1H), 6.23(t,1H), 2.9(m,1H), 2.4(d,6H), 1.21(s,9H), 0.8(d,6H); **^{13}C**-NMR(125 MHz, DMSO, *δ*, ppm): 162, 155, 77.7, 59.9, 40.13, 38, 35, 30, 28, 18 ESI-MS(*m/z*): 639, 436, 358, 249 ; UV-vis(1×10^{-4}M,Methanol,λ/nm) : 207, 225-300.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30
20
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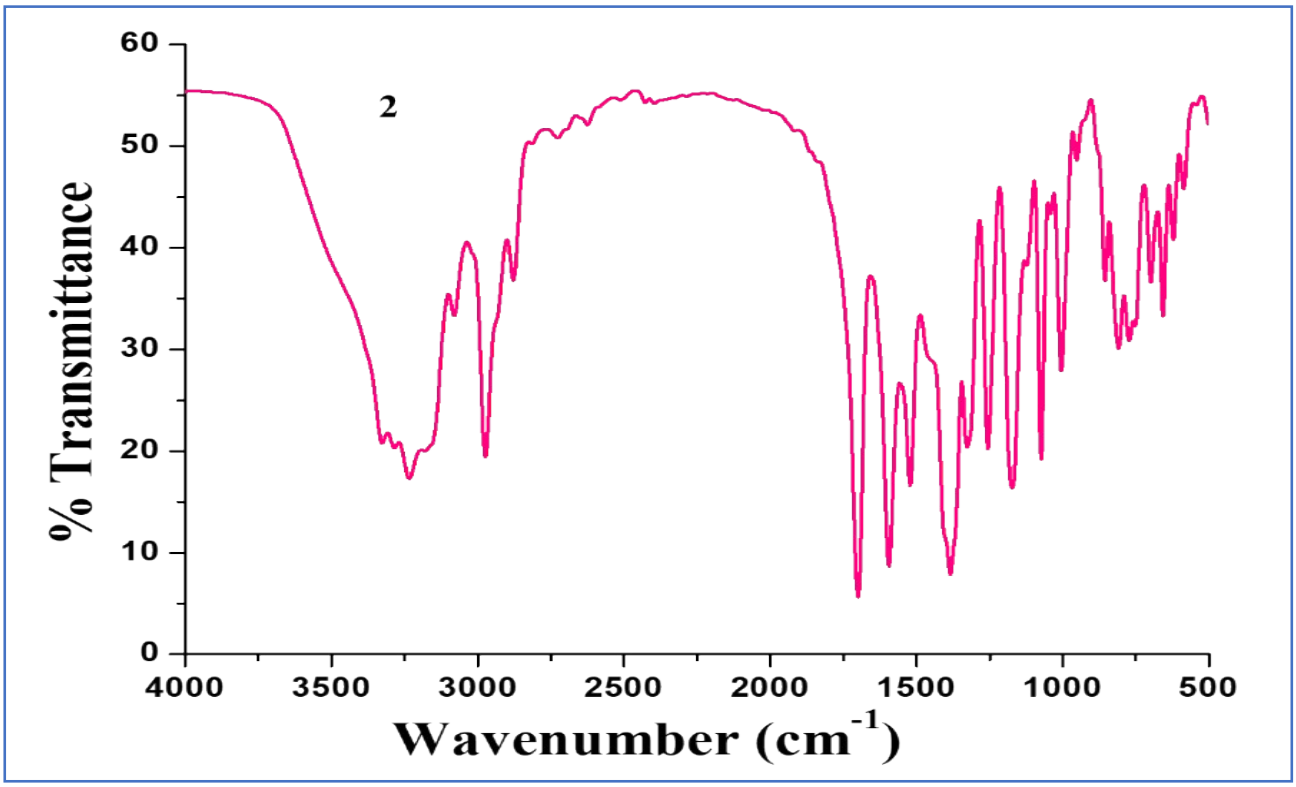
0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)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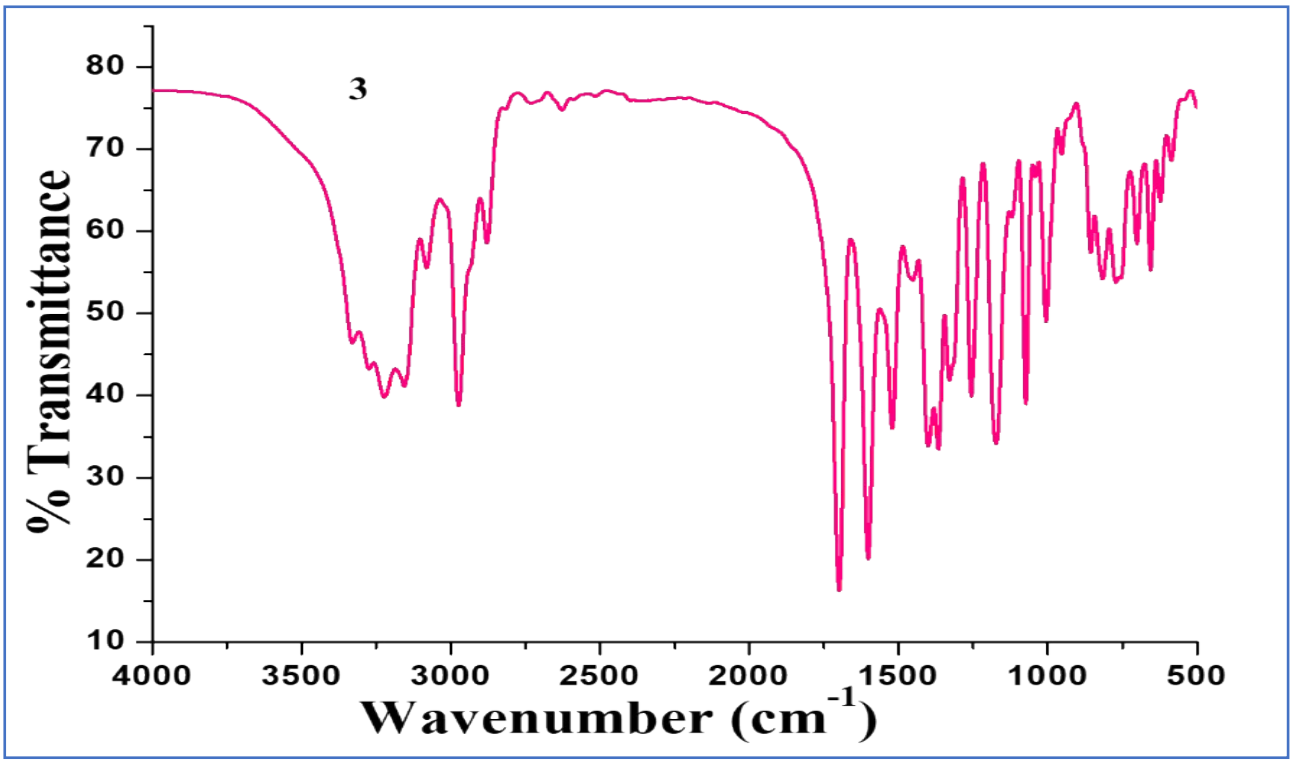
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Wavenumber (cm-1)
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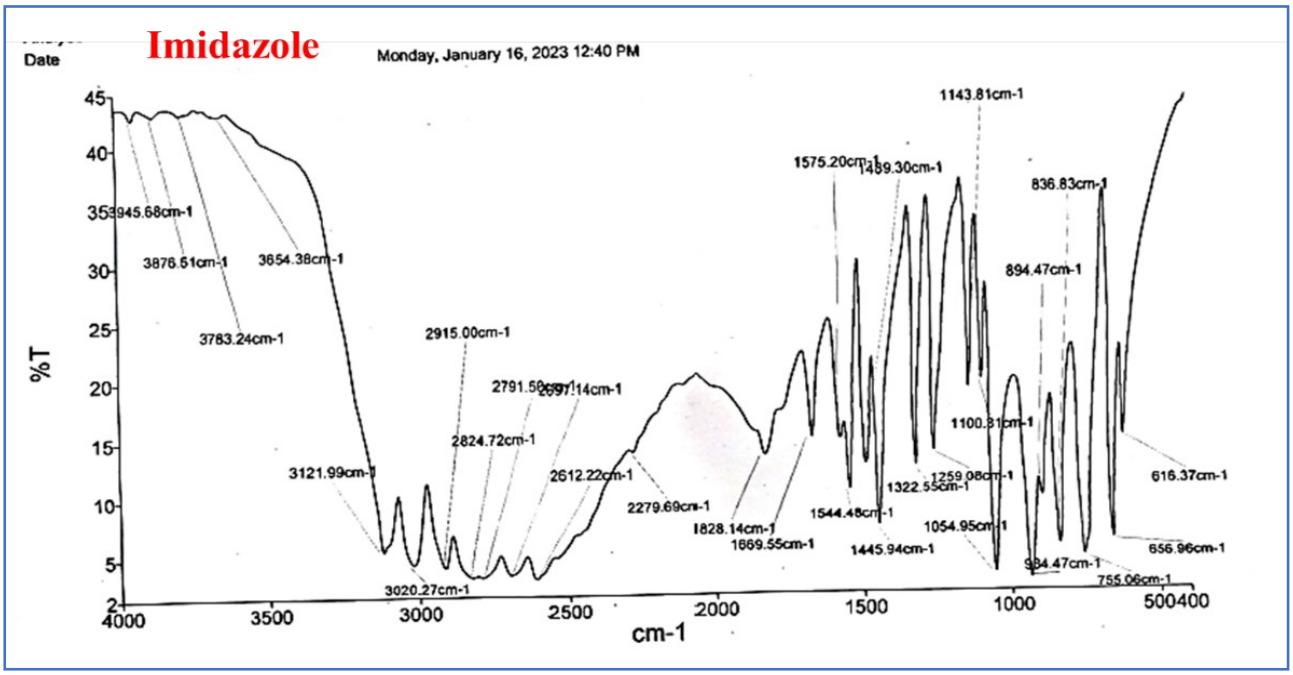
Imidazole
Date
Monday, January 16, 2023 12:40 PM
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2915.00cm-1
2824.72cm-1
3121.99cm-1
2612.22cm-1
2279.69cm-1
1828.14cm-1
1669.55cm-1
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1575.20cm-1
1489.30cm-1
1143.81cm-1
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894.47cm-1
1100.31cm-1
1259.08cm-1
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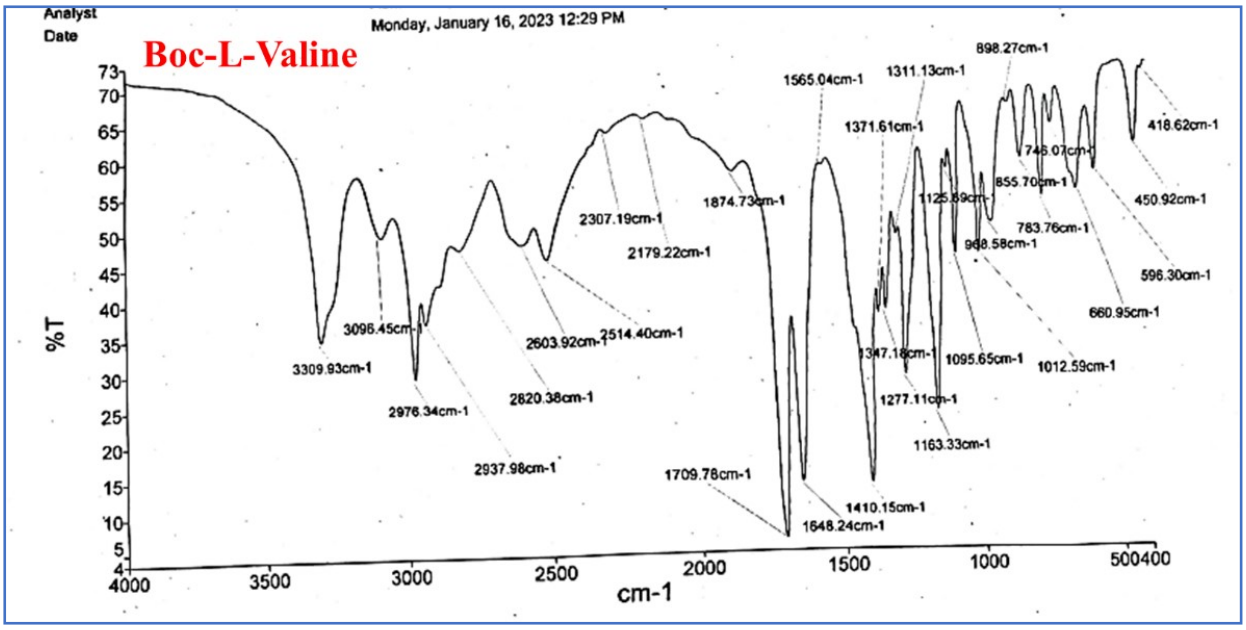
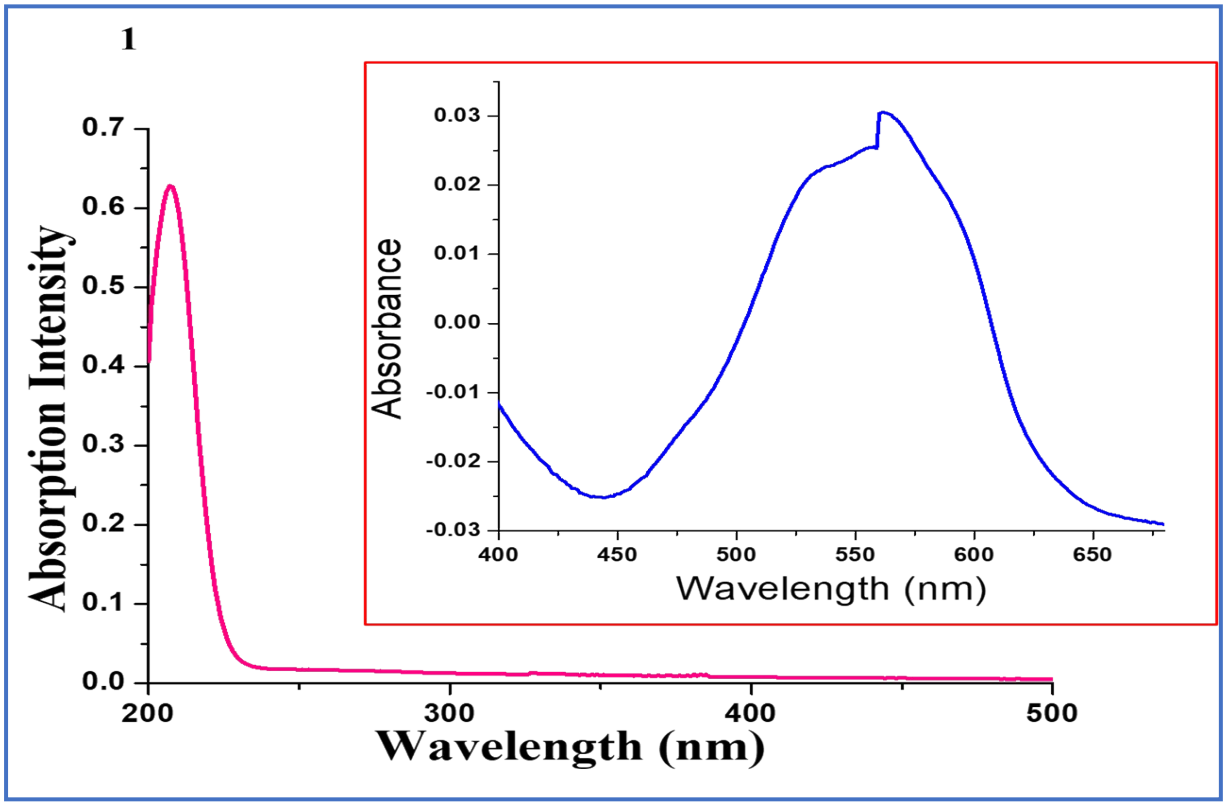


Fig. S1. FT-IR spectra of Complexes (**1-3**) and free ligands.



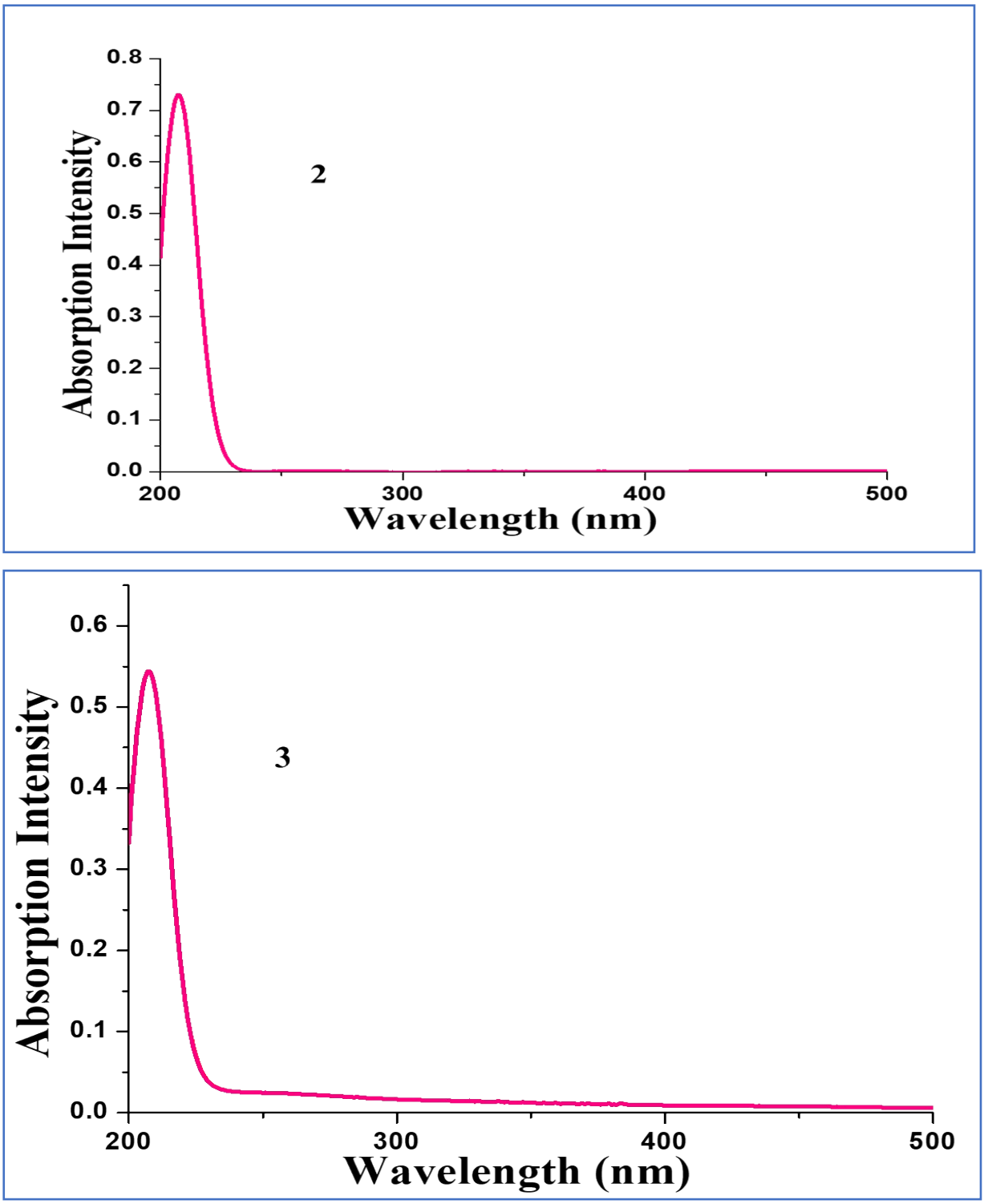


Fig. S2. UV-visible spectra of Complexes (**1-3**).

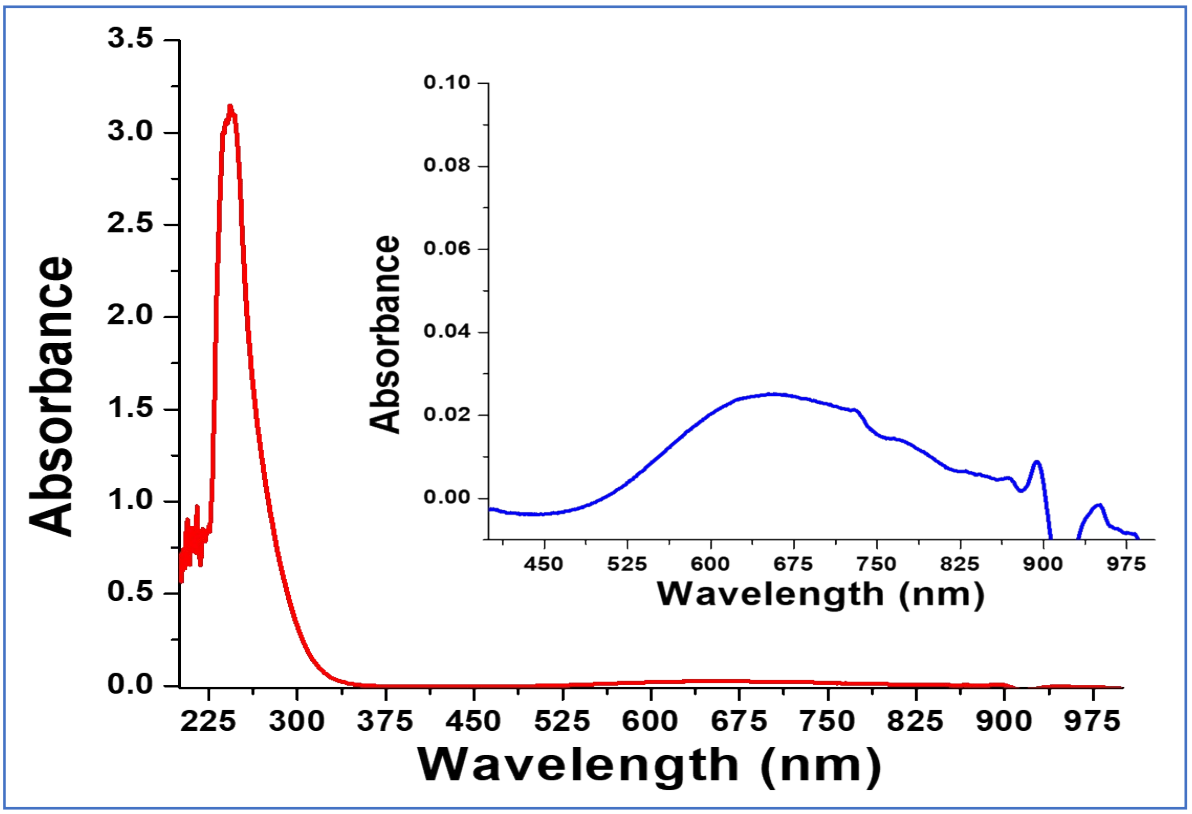


Fig. S3. UV-visible spectra of Complex **2** showing broad d-d band.

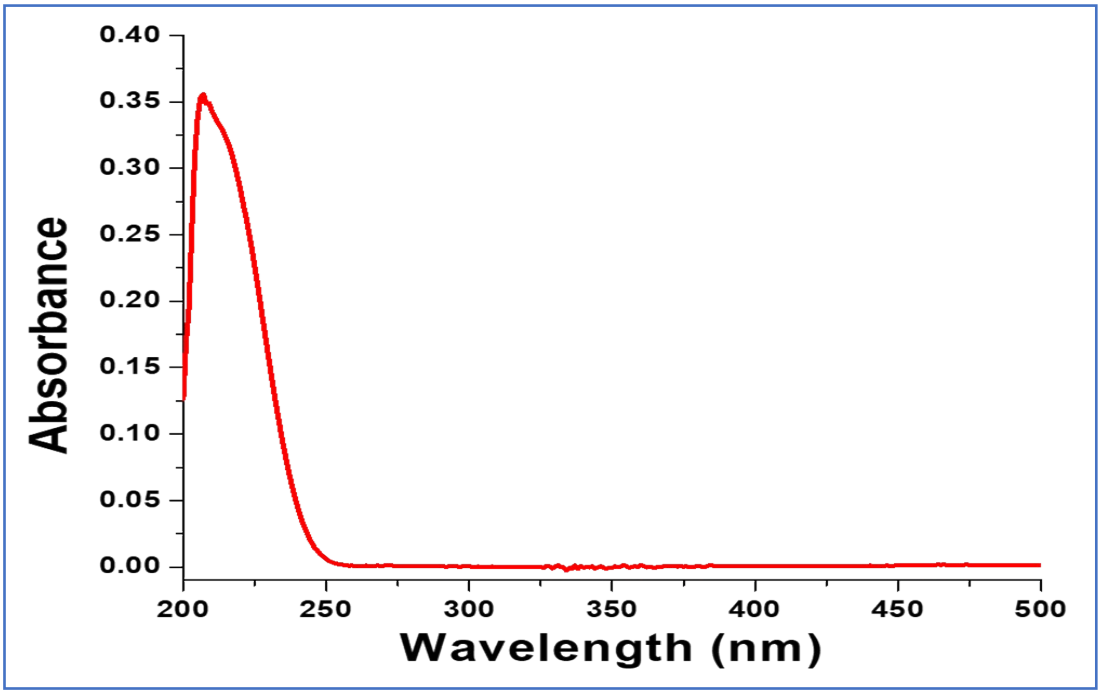


Fig. S4. UV-visible spectra of free Boc-L-Valine.

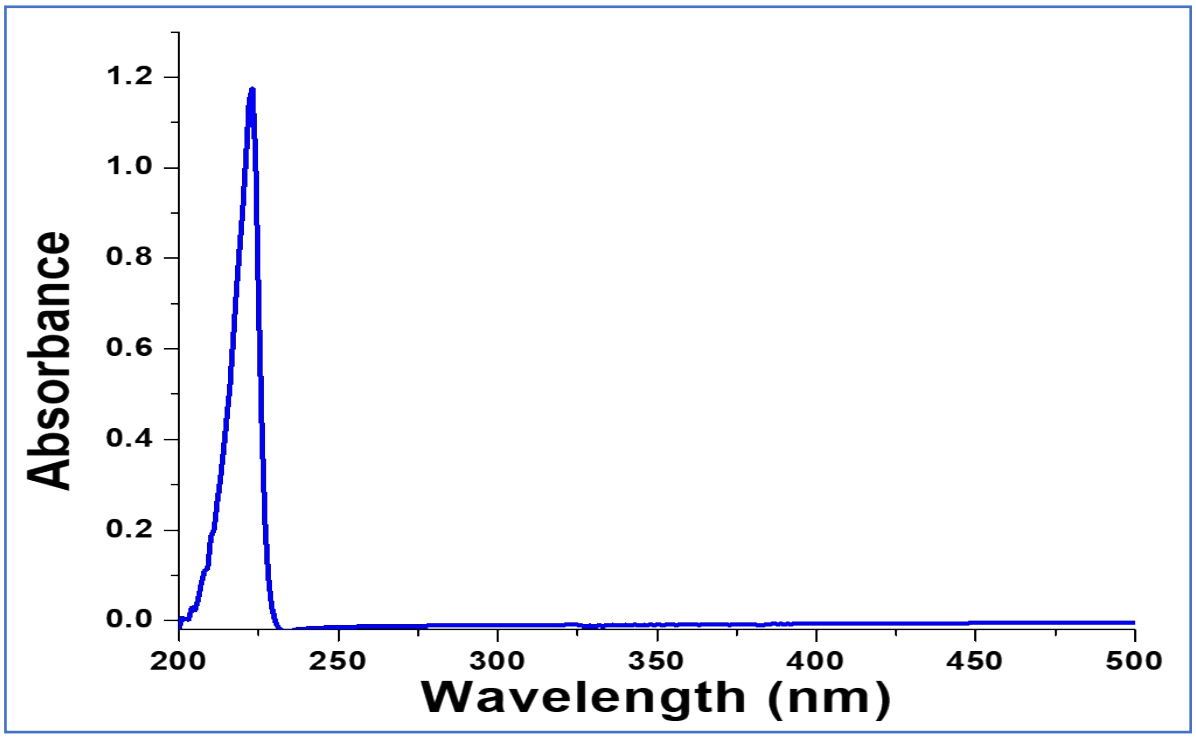
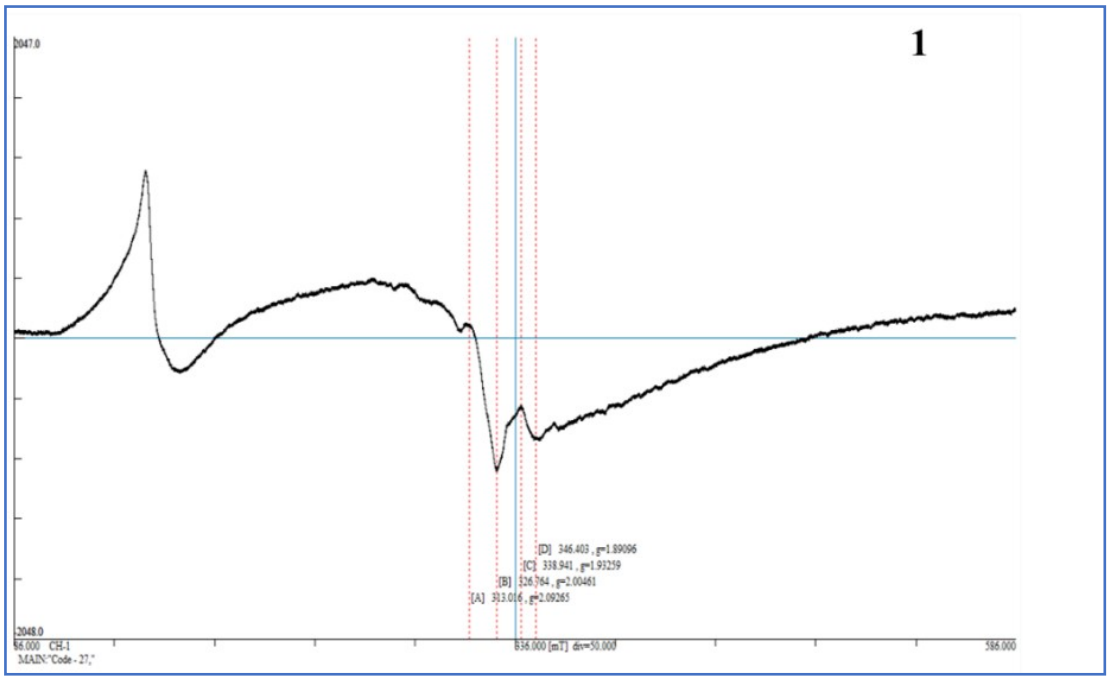


Fig. S5. UV-visible spectra of free Imidazole.



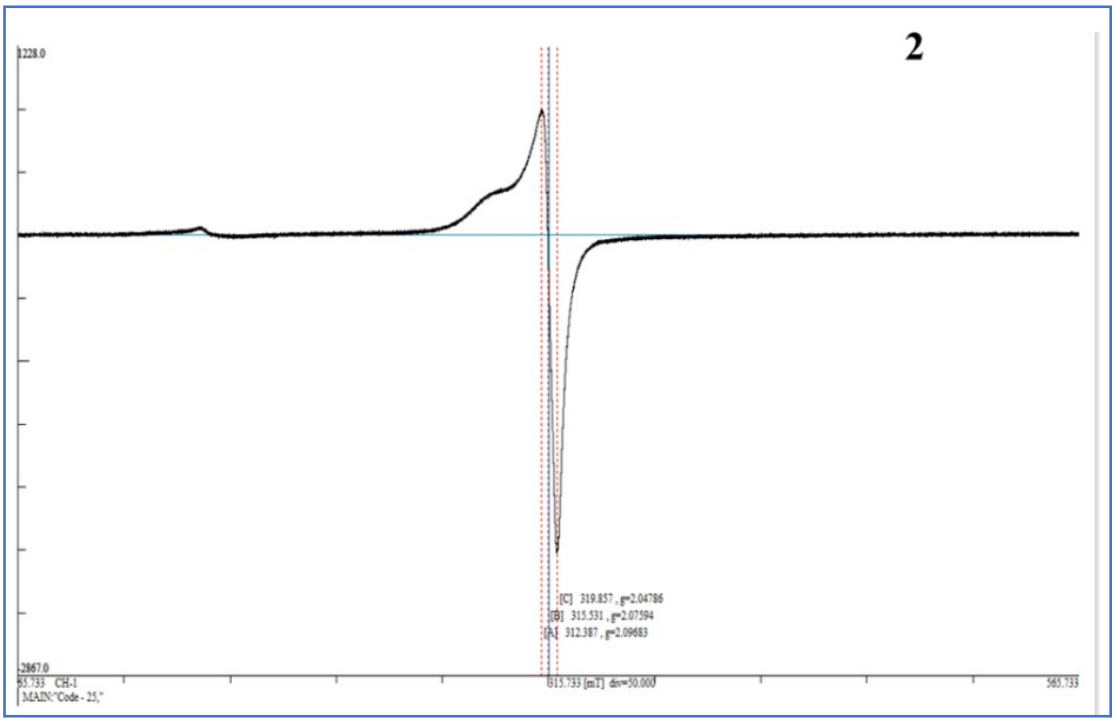
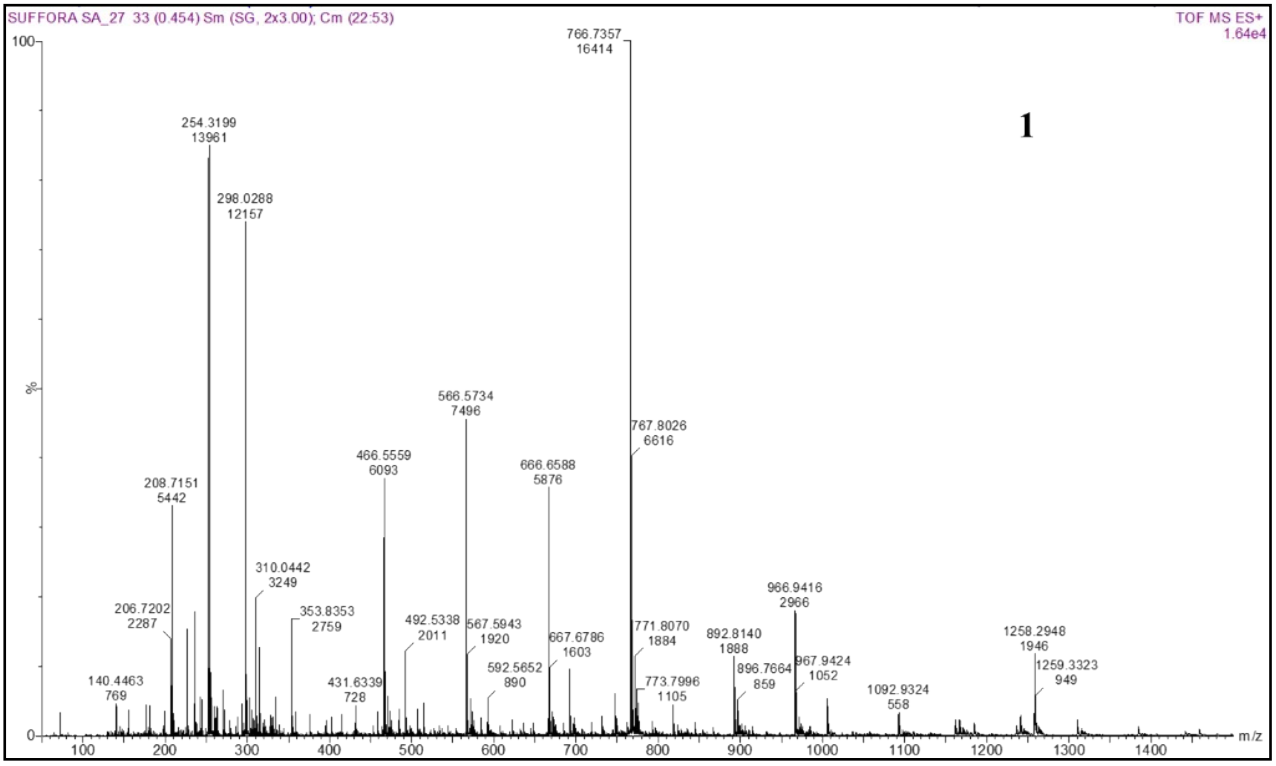
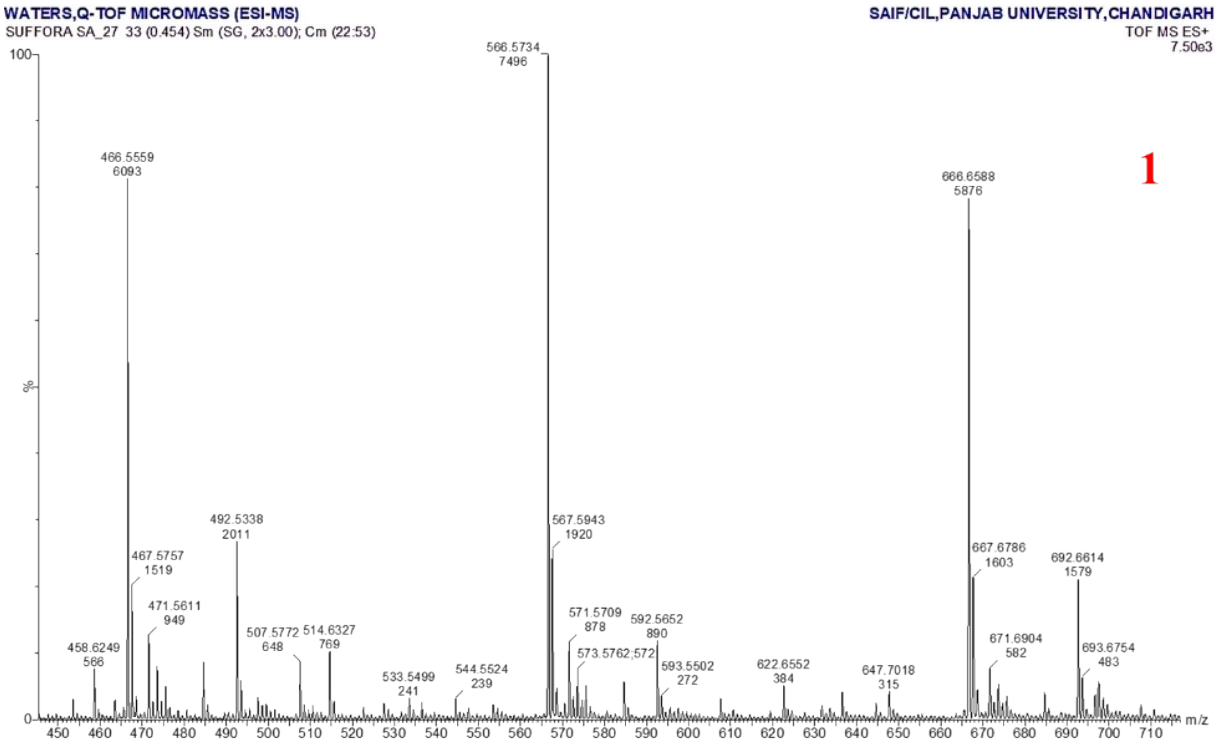


Fig. S6. EPR spectra of Complexes **1** and **2.**

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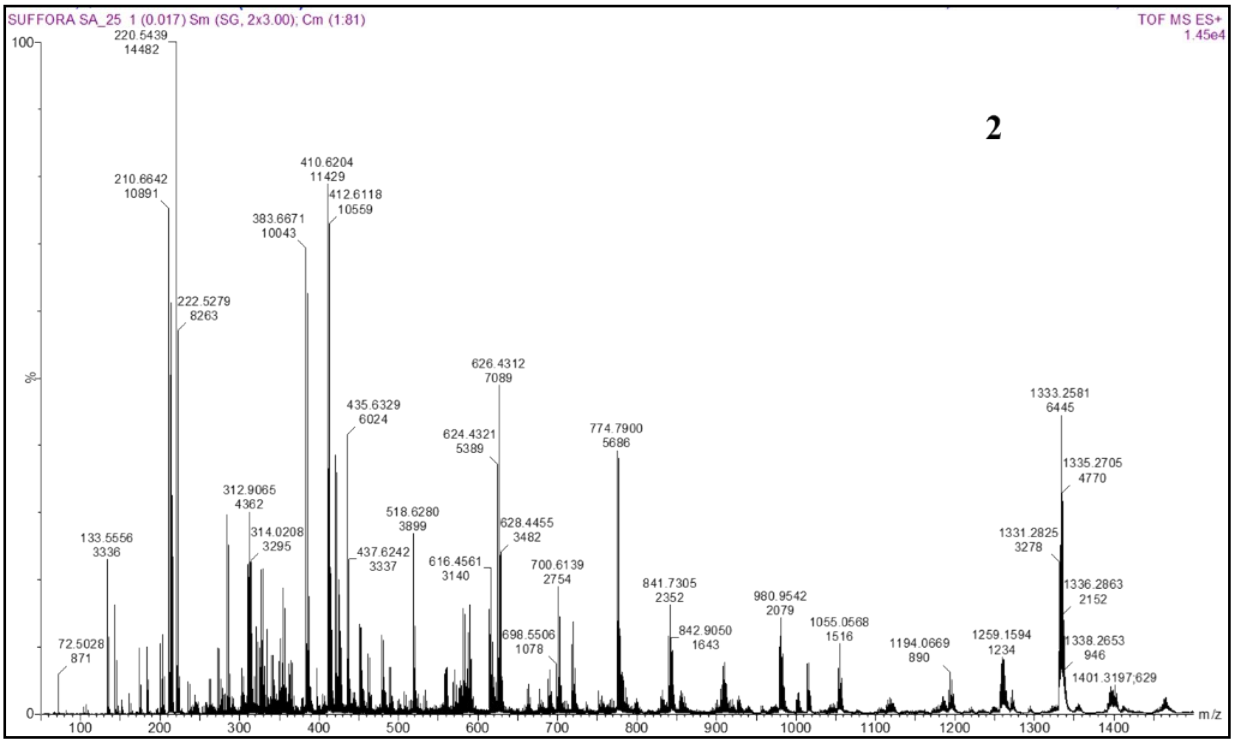
SAIF/CIL,PANJAB UNIVERSITY,CHANDIGARH
TOF MS ES+
7.50e3
1
566.5734 7496
466.5559 6093
666.6588 5876
492.5338 2011
567.5943 1920
467.5757 1519
667.6786 1603
692.6614 1579
471.5611 949
571.5709 878
592.5652 890
671.6904 582
458.6249 566
507.5772 648
514.6327 769
573.5762;572
693.6754 483
593.5502 272
533.5499 241
544.5524 239
622.6552 384
647.7018 315
%
m/z


SUFFORA SA_25 1 (0.017) Sm (SG, 2x3.00); Cm (1:81)
TOF MS ES+
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383.6671 10043
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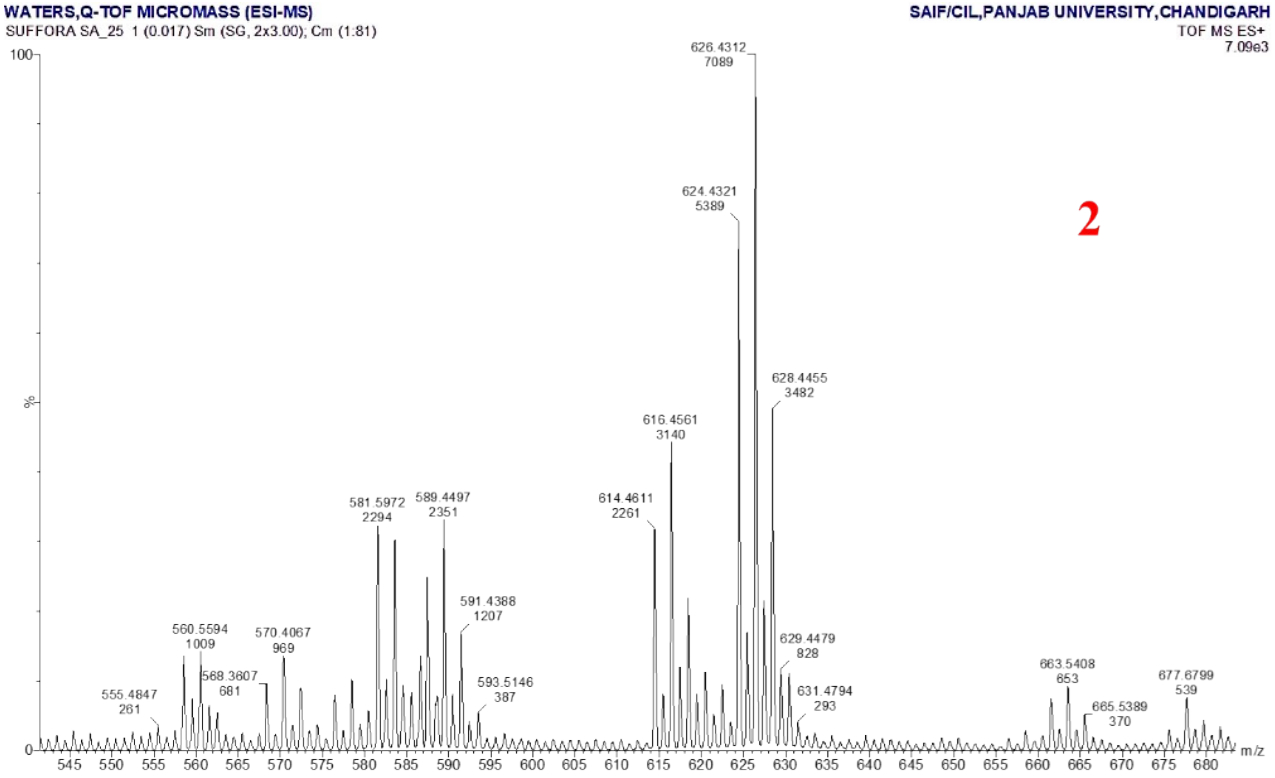
%
m/z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TOF MS ES+
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2
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624.4321 5389
628.4455 3482
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614.4611 2261
581.5972 2294
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560.5594 1009
570.4067 969
588.3607 681
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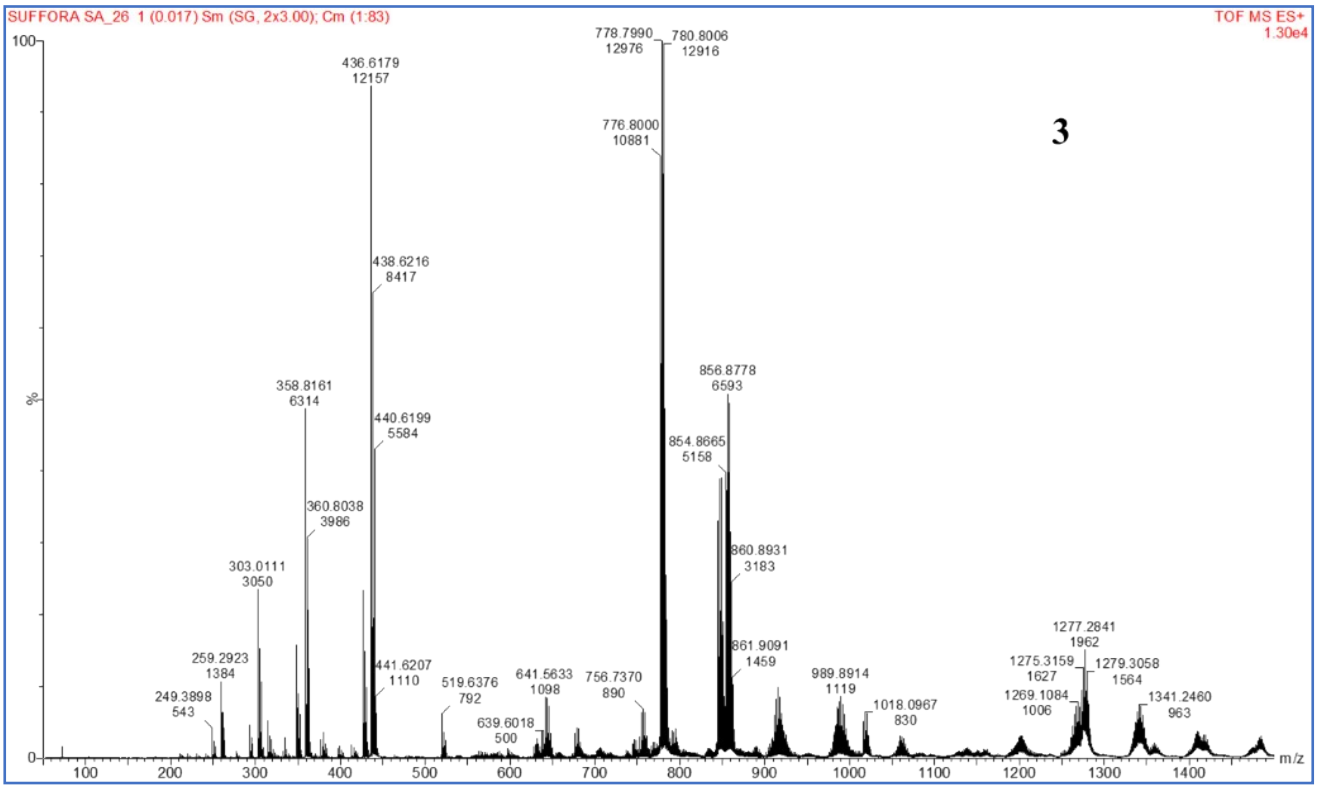
m/z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TOF MS ES+
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776.8000 10881
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856.8778 6593
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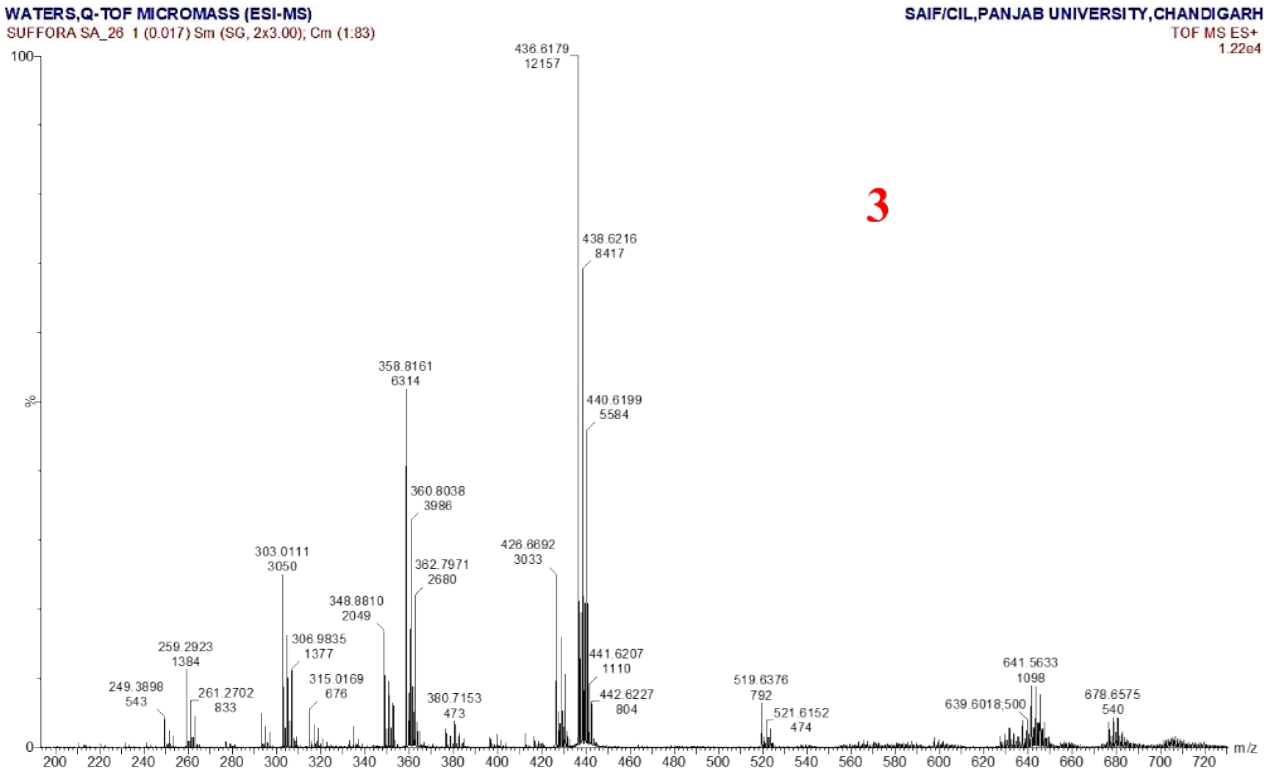

Fig. S7. ESI-MS spectra of Complexes (**1-3).**

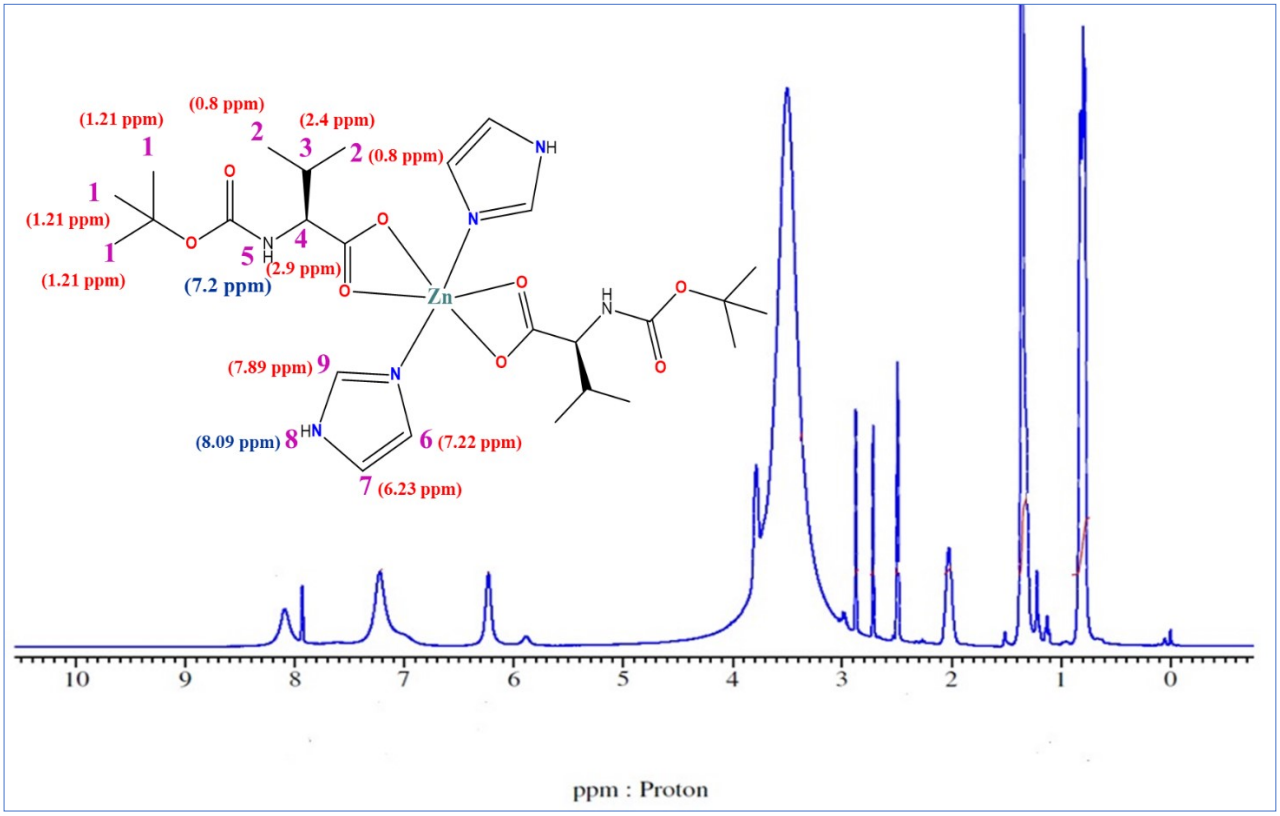

Fig. S8. ^{1}H-NMR spectrum of Complex **3.**

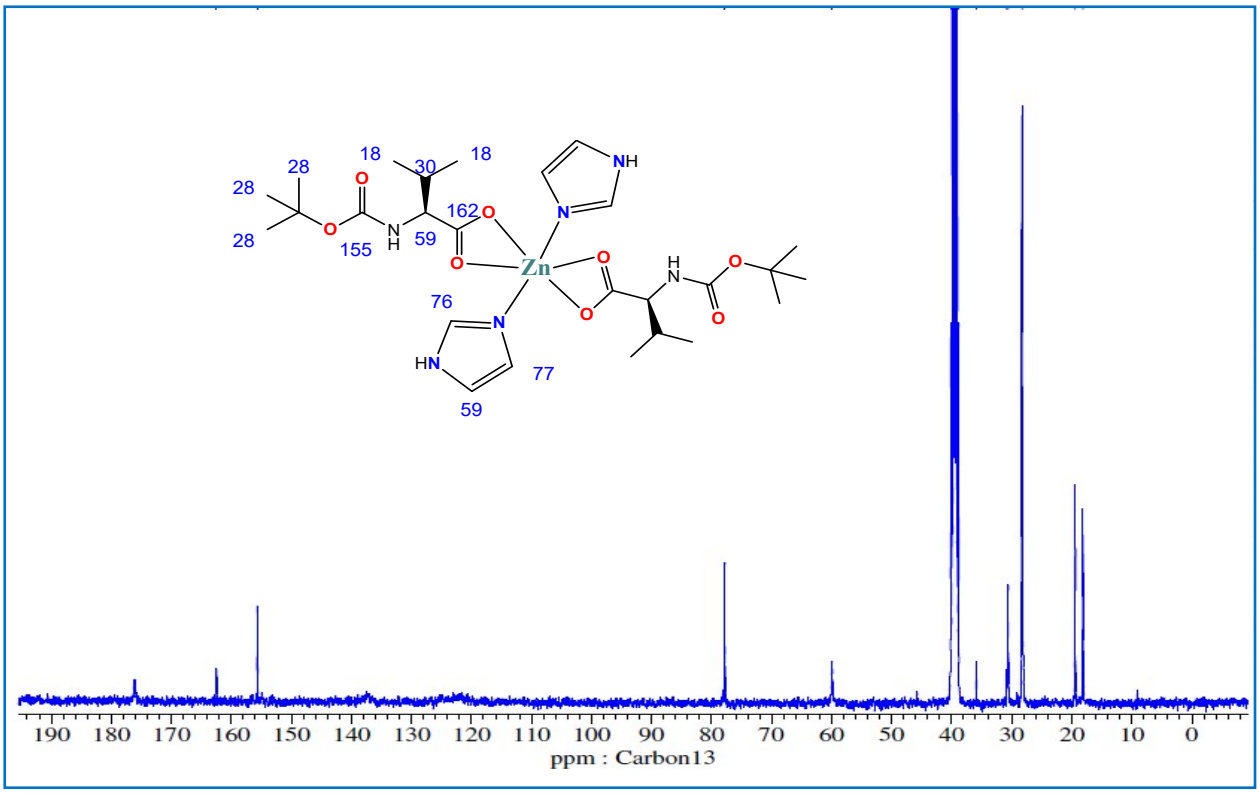

Fig. S9. ^{13}C-NMR spectrum of Complex **3**.

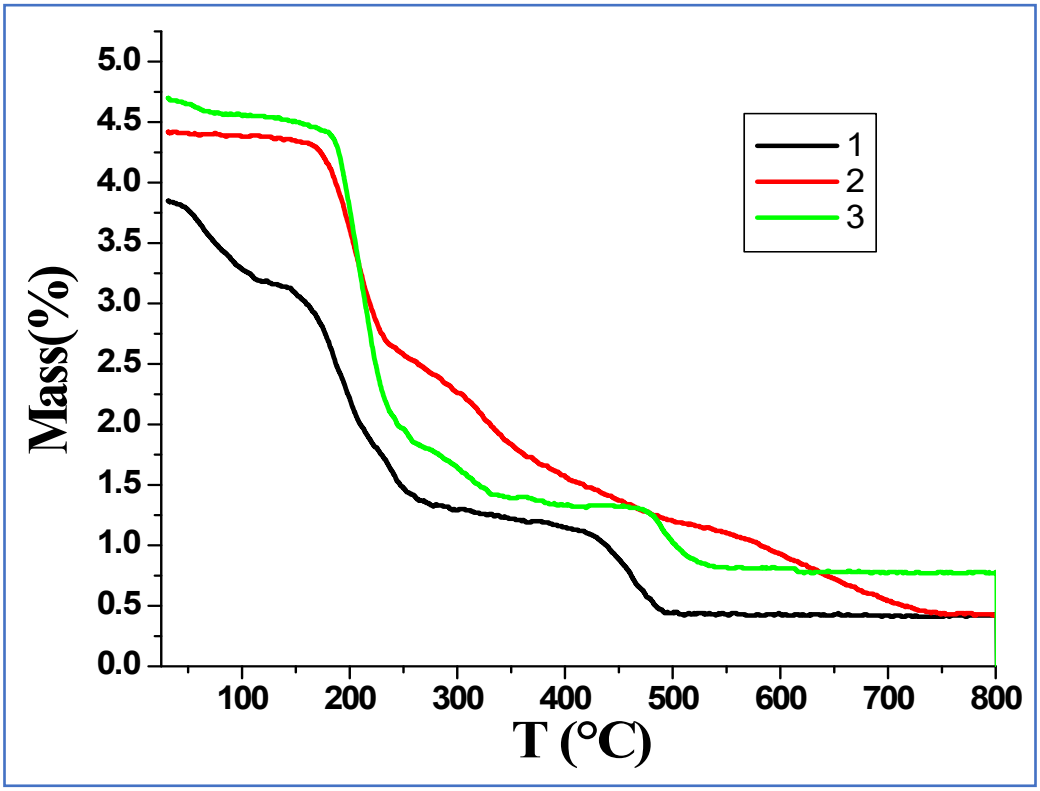

Fig. S10. TGA of Complexes (**1-3**).

Table S1. Crystal and structure refinement data of complexes **(1- 3)**.

Identification code	**1**	**2**	**3**
Empirical formula	C_{26} H_{44} Co N_6 O_8	C_{26} H_{44} Cu N_6 O_8	C_{26} H_{44} N_6 O_8 Zn
Formula weight	627.60	632.22	634.06
Temperature/K	293 K	293 K	293 K
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic
Space group	P 2_1 2_1 2 (18)	P 2_1 2_1 2 (18)	P 2_1 2_1 2 (18)
a/Å	9.23250(10)	9.47350(1)	9.1857(2)
b/Å	33.2718(5)	32.9082(6)	33.3813(10)
c/Å	10.5777(2)	10.5219(2)	10.6481(3)
α/°	90	90	90
β/°	90	90	90
γ/°	90	90	90
Volume/Å^3	3249.28(9)	3280.26(9)	3255.86(15)
Z,Z’	4	4	4
ρ_{calc} Mg/m^3	1.283	1.280	1.286
μ/mm^{-1}	0.580	0.718	0.804
F(000)	1332.0	1340.0	1344.0
Radiation	MoKα(λ=0.710)	MoKα(λ=0.710)	MoKα(λ=0.710)
Independent reflections	7061	7101	7061
R factor(%)	3.74	4.04	4.31
wR2	0.0956(7061)	0.0995 (7101)	0.0940 (7061)
S	1.068	1.072	1.051
CCDC	2164581	2164761	2236140

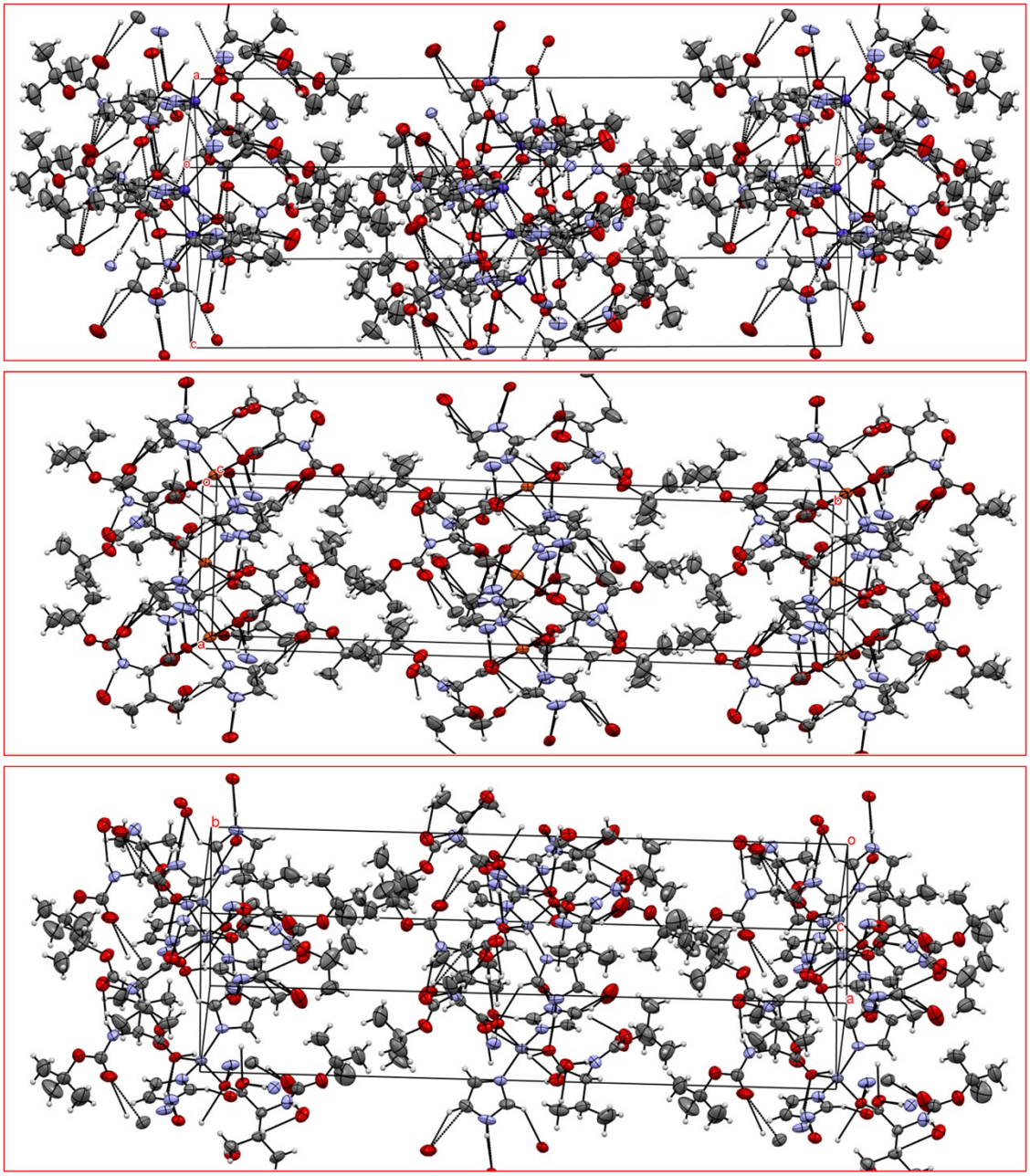

Fig. S11. Packing structures of complexes (**1-3**).

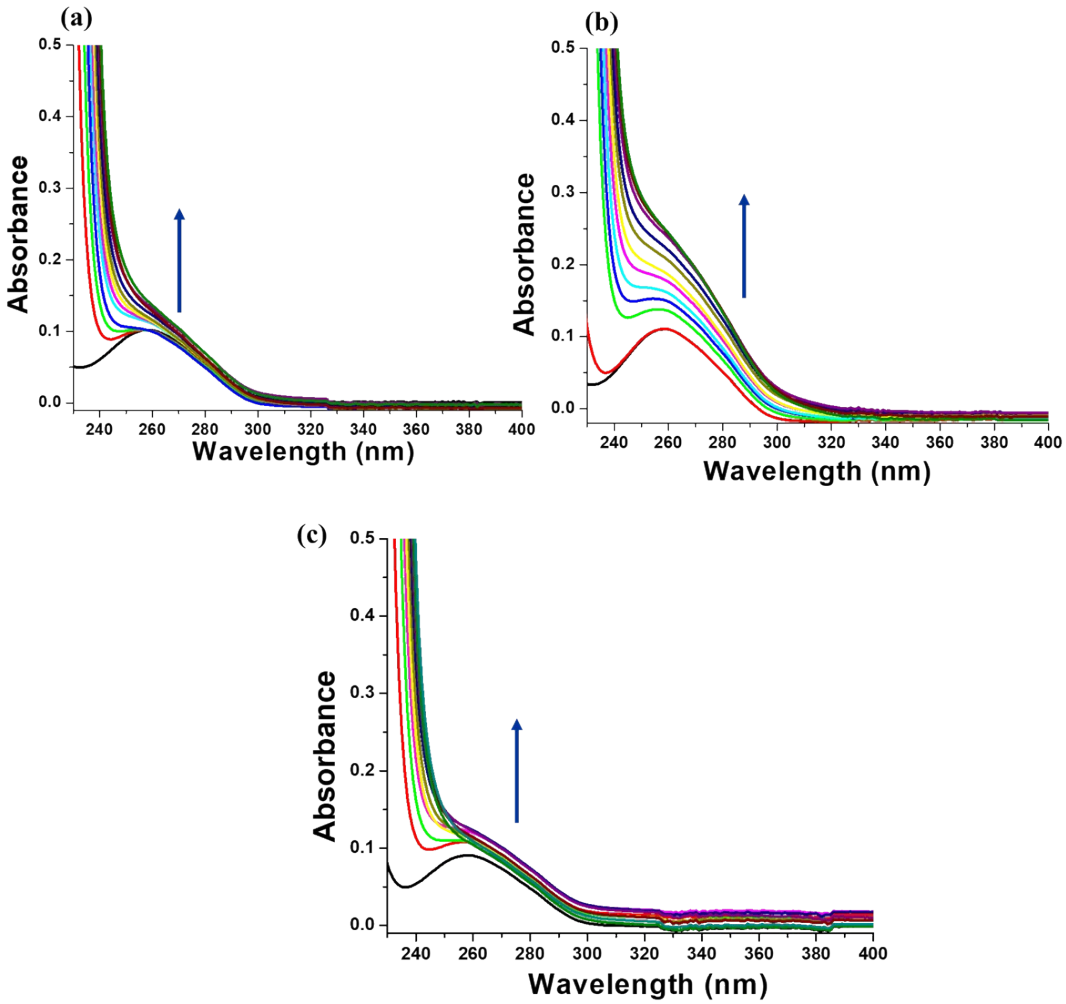

Fig.S12. Titration spectra of a solution of constant ct-DNA concentration with increasing concentration of complexes **1** (a) **2** (b) and **3** (c) in aqueous saline tris HCl buffer.

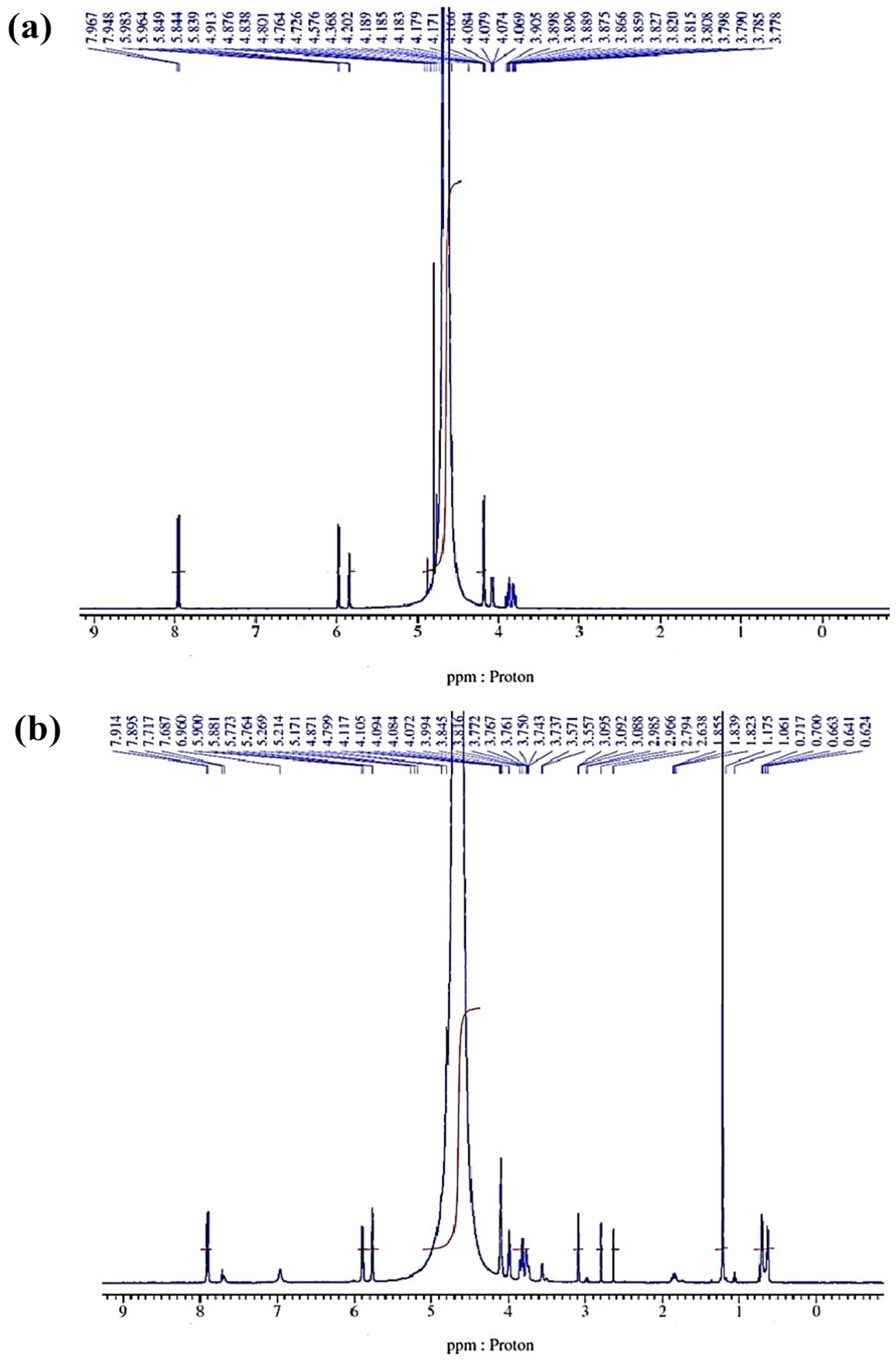

Fig. S13. (a) CMP and (b) CMP + Complex **3**.

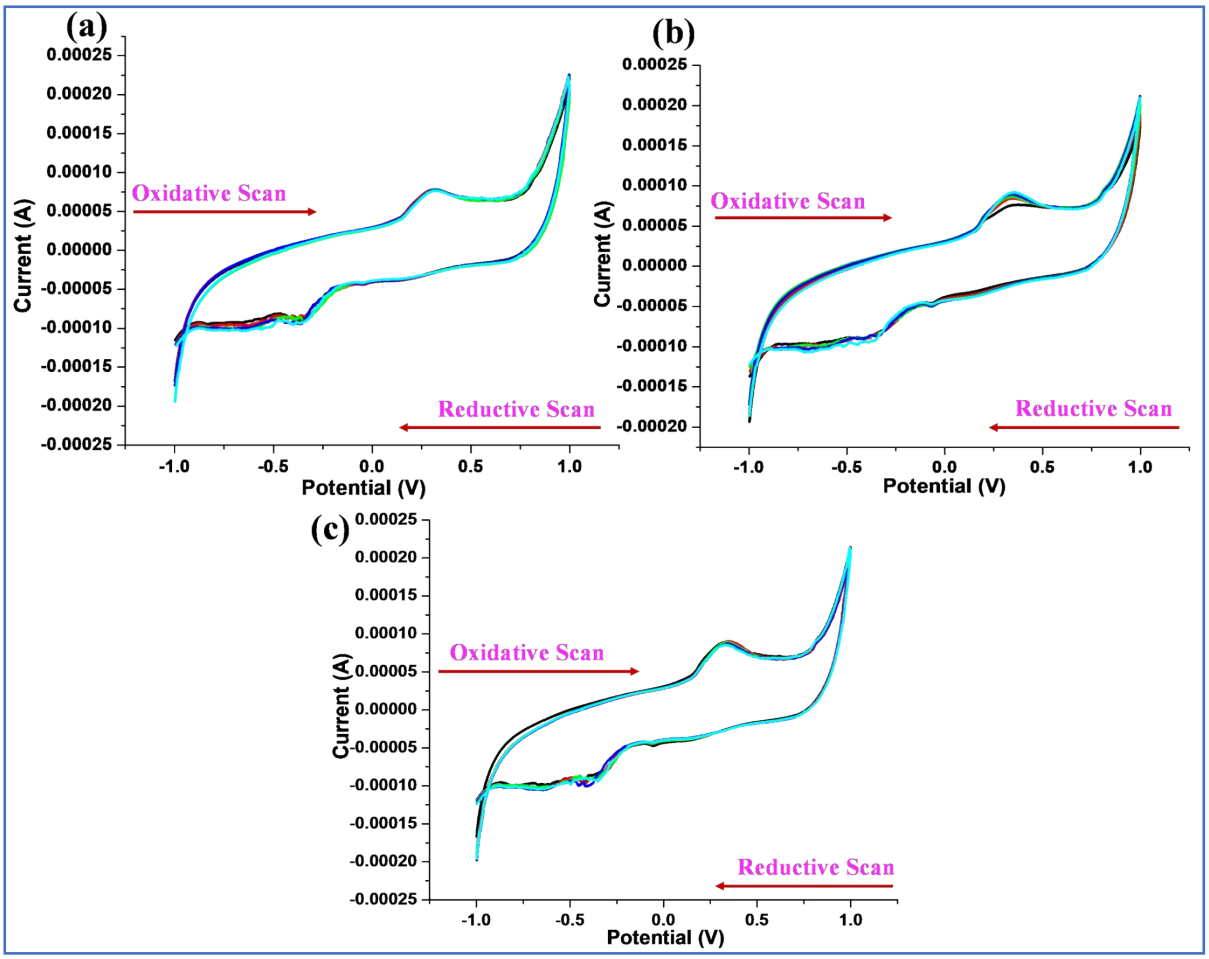

Fig. S14. CV of Complexes **1** (a) **2** (b) and **3** (c) with DNA.

Morphological Studies

In vitro DNA Condensation has long been studied as a potential model for DNA packing *in vivo* and in gene treatments, offering a viable method of non-viral gene delivery. Several compounds have been investigated for their ability to compress DNA, including the polycations, inorganic cations, lipids, polymers, and nanoparticles. Metal complexes are often cationic and this assures their affinity to DNA. Since the metal in the majority of complexes have ligating organic molecules in place, the shape and surface characteristics, such as hydrogen bond donors or acceptors, may be exploited to influence the DNA-metallocomplex interaction. The morphologies and sizes of DNA condensates differ depending on the type of metal complex used. Condensates of ct-DNA with the complexes (**1-3)** were made by evaporating an equimolar mixture in Tris-HCl buffer to examine the morphological changes .

SEM results of condensates displayed the randomization and formation of globular structures, which suggested substantial interaction with biomolecule which was found greater in case of **1**. This could be possibily due to the ability of cobalt complexes to mimic the behaviour of two DNA binding polycations spermidine and spermine which are present in all cells (**Fig. S15**)

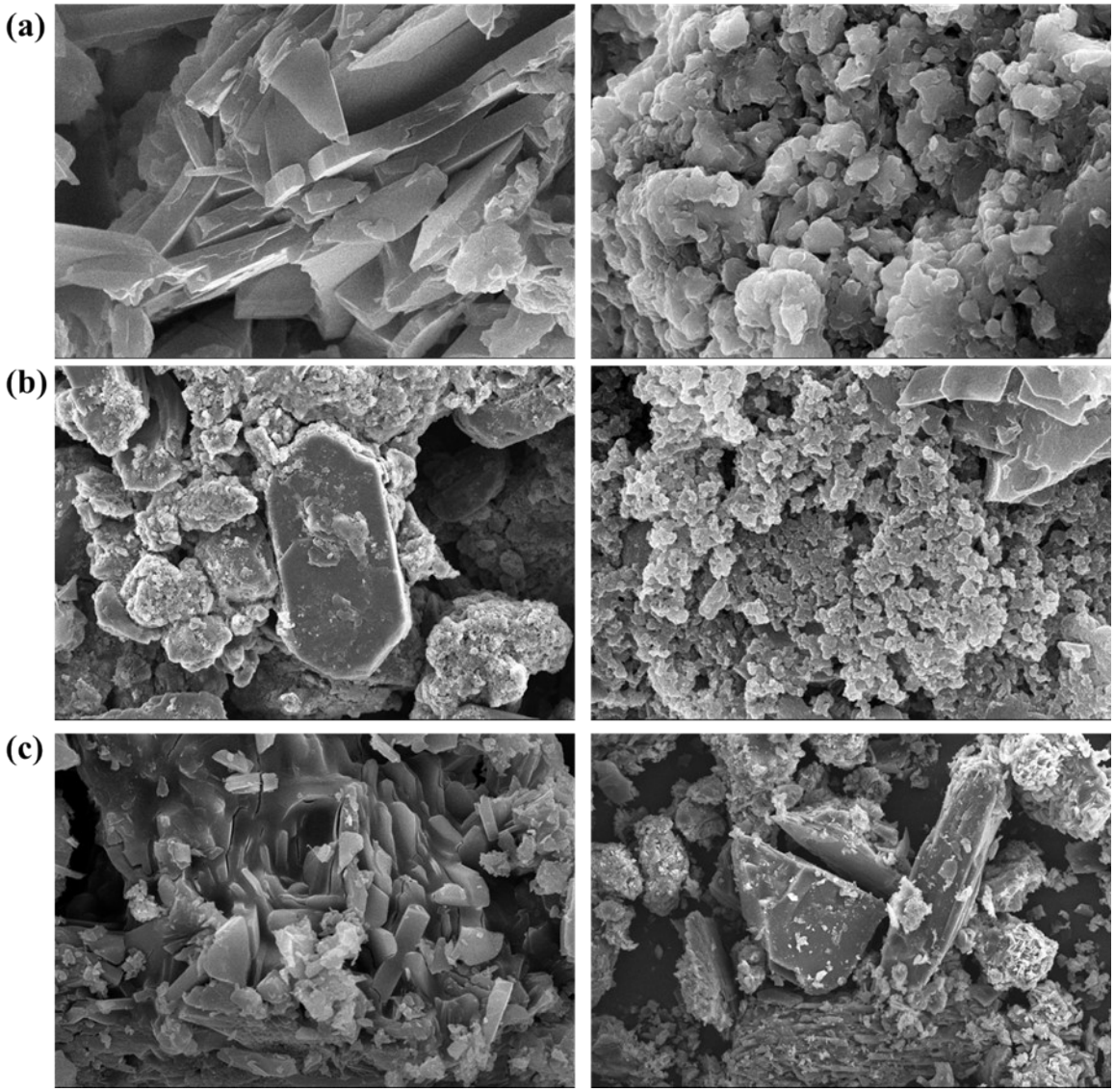

Fig.S15. SEM micrographs showing the morphology ct-DNA with complexes (a) **1** (b) **2** (c) **3.**

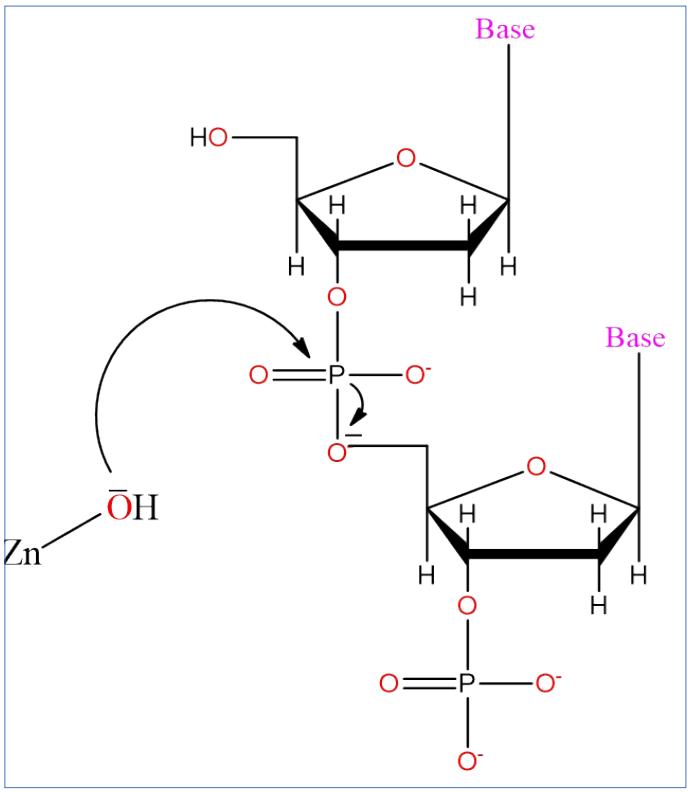

Fig. S16. Proposed mechanism of DNA cleavage by complex **3.**

1.3. UV-visible spectroscopy

PerkinElmer Lambda 25 was used to record electronic spectra using cuvettes of 1cm path length.

1.4. Fluorescence Spectral studies

Emission spectra were obtained on Shimadzu RF-5301 PC spectrofluorometer. The quenching constant (K_{sv}) was evaluated by employing the Stern-Volmer equation (1).

$$I_o / I = 1 + K_{sv} [Q] \qquad (1)$$

Where I_o and I represent fluorescence intensities in the absence and presence of quencher respectively, K_{sv} is the quenching constant and [Q] is the concentration of the quencher.

1.4. Cytotoxicity

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending

on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.,10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was

calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$$[Ti/C] \times 100\ \%$$