Supplementary Information

Repurposing Bilastine antihistamine drug as an anti-cancer metallic drug: Synthesis and Single crystal X-ray structure of Metal -based Bilastine and phen {Co(II), Cu(II) & Zn(II)} tailored anticancer chemotherapeutic agents against resistant cancer cells.

Rijwan[a], Farukh Arjmand[a] and Sartaj Tabassum[a*]

[a]Department of Chemistry, Aligarh Muslim University, Aligarh, UP 202002, India

Email: *tsartaj62@yahoo.com*, Tel: +91 9358255791

1. Experimental section

1.1. *Materials and instrumentation*

All reagents and chemicals, $Cu(NO_3)_2.3H_2O$, $Zn(ClO_4)_2.6H_2O$, $Co(ClO_4)_2.6H_2O$, 1,10-Phenanthroline, Bilastine, ct-DNA, BSA, Ethidium bromide (EB) and Tris-hydroxymethyl)aminomethane (Tris-buffer), were purchased from Sigma Aldrich and Sigma Chemicals.co and stored below 10 ^oC. The other solvents were used in synthesis, purchased from CDH and Merck, and used without further purification. DNA and BSA interaction studies were carried out in tris-Hcl buffer (pH-7.2). Fourier Transformation infrared (FT-IR) spectra were recorded on a Perkin Elmer spectrometer in the 4000-400 cm-1 range with KBr. The NMR spectra were obtained on JEOL JNM-ECZ400S/L1 spectrometer. The high-resolution mass spectrum was recorded on Maldi-TOF Synapt XS HD Mass Spectrometer. Electronic spectra were recorded on Perkin Elmer Lambda 25 with a cuvette path length of 1cm. Shimadzu RF-5301 PC spectrophotometer was used to record Emission spectra. Circular Dichroism measurements were obtained on a J-815-CD spectropolarimeter using a 1cm quartz cuvette. Cyclic Voltammetry was carried out at the CH instrument electrochemical analyzer. The electrophoretic assay was carried out in Tris-borate ethylenediaminetetraacetic acid buffer at 50 V cm-1 and visualized using UV Transilluminator. Molecular Docking studies were performed using HEX 6.0 software and pictured in the discovery studio visualizer. Rigaku

Oxford Diffraction XtaLAB Synergy -S and -I (Dual source) collected single X-ray data at 293 K for all the complexes using Mo-K-alpha radiation (lambda-0;.7103 A). The structure was solved using the software olex2 and refined using 'SHELXT 2018/2 (Sheldrick, 2018)' and the PLATON version.

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

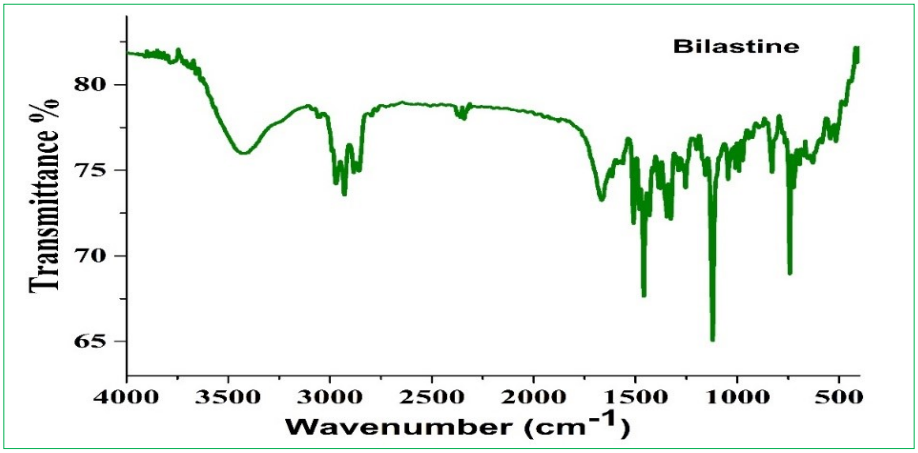
Bilastine (0.463g,1mmol) was soluble in mixture of MeOH and DMF (19:1) and deprotonated by triethylamine, (0.140 ml, 1 mmol) was added methanolic solution of metal salts $Cu(NO_3)_2.3H_2O$ (0.241g, 1mmol), $Zn(ClO_4)_2.6H_2O$ (0.372g, 1mmol), $Co(ClO_4)_2.6H_2O$ (0.365g, 1mmol), and the reaction mixture was stirred for 3 h, followed by the addition of methanolic solution of 1,10-phenanthroline (0.198g, 1mmol). The reaction mixture was further stirred for 3-4 h until the completion of reaction took place (as monitored by TLC). The reaction mixture was filtered, and the product was isolated after drying. The recrystallized product was kept for crystallization for a few days until suitable crystals separated out for single X-ray diffraction. All complexes **1-3** were soluble in dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) (Scheme1).

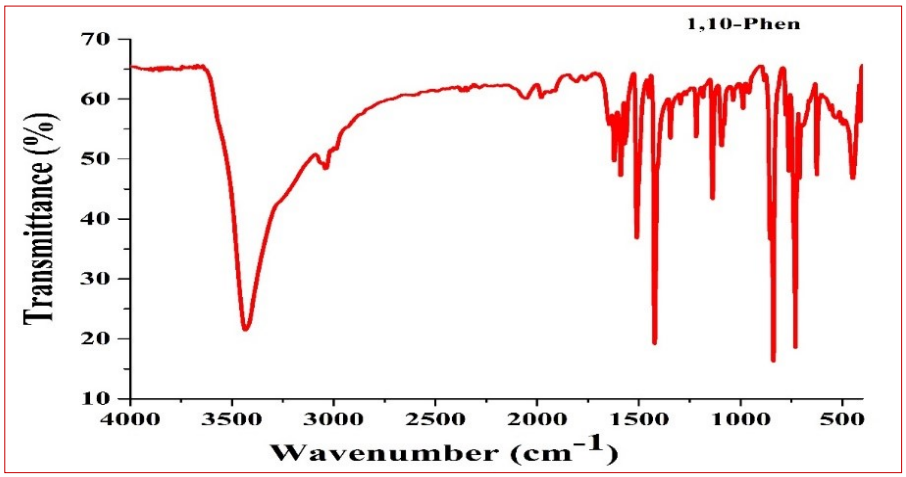
Complex $[C_{52}H_{52}ClCoN_7O_7]$ **1**, MW: 982.39, Yield 85%, m.p.: 220 °C; Anal. calc. (%) C, 63.64; H, 5.34; N, 9.99. Found (%): C, 63.04; H, 5.02; N, 9.59. FT-IR (KBr, υ_{max}/cm^{-1}): 2958 υ(-CH), 2920, 2851 υ(-NH), 1625 υ(-C=O), 1515 υ(-C=N), 1105 υ(-ClO_4 ionic), 510 υ(M-O), 424 υ(M-N); ESI-MS(*m/z*); 881 $[C_{52}H_{52}CoN_7O_3]^+$, 882 $[C_{52}H_{52}CoN_7O_3 + H]^+$; UV-vis ($1x10^{-4}$M, DMSO, λ/nm) : 267, 565. Λ_m: $\Omega^{-1}cm^{-2}mol^{-1}$ = 82 (DMSO).

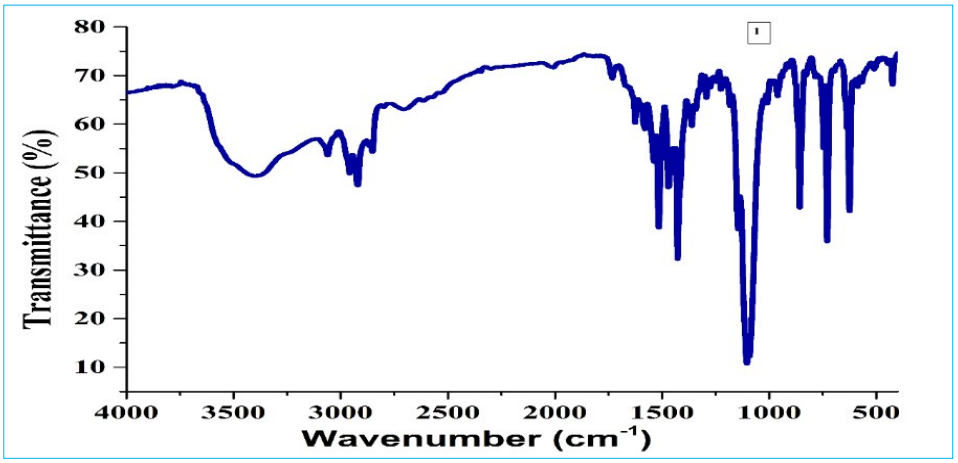
Complex $[C_{52}H_{56}CuN_8O_7]$ **2**, MW: 984.60, yield: 80%, m.p.: 210 °C; Anal. calc. (%) C, 63.43; H, 5.73; N, 11.38. Found (%): C, 63.20; H, 5.72; N, 10.89. FT-IR (KBr, υ_{max}/cm^{-1}): 2971 υ(-CH), 2928, 2850 υ(-NH), 1592 υ(-C=O), 1517 υ(-C=N), 1384 υ(-NO_3 ionic), 486 υ(M-O), 429 υ(M-N) ; ESI-MS(m/z): 983 $[C_{52}H_{56}CuN_8O_7\text{-}H]^+$, 982 $[C_{52}H_{56}CuN_8O_7\text{-}2H]^+$ 965

$[C_{52}H_{53}CuN_8O_7]^+$, 966 $[C_{52}H_{53}CuN_8O_7 + H]^+$, 464 $[C_{27}H_{37}N_3O_3]^+$;UV-vis (1x10^{-4}M, DMSO, λ/nm) : 270, 680. Λ_m: $\Omega^{-1}cm^{-2}mol^{-1}$ = 42 (DMSO).

Complex $[C_{55}H_{59}ClZnN_8O_8]$ **3**, MW: 1060.94, yield: 75%, m.p.: 230 °C; Anal. calc. (%) C, 63.26; H, 5.61; N, 10.56. Found (%): C, 62.87; H, 5.65; N, 10.53. FT-IR (KBr, υ_{max}/cm^{-1}): 2958 υ(-CH), 2926, 2865 υ(-NH), 1624 υ(-C=O), 1517 υ(-C=N), 1103 υ(-ClO_4 ionic), 510 υ(M-O), 423 υ(M-N) ; ^{1}H-NMR (400 MHz, DMSO, δ, ppm): 8.90(d,4H), 8.70(s,4H), 7.90(d,4H), 7.50(t,4H), 7.08-7.16(m,8H), 4.37(t,2H), 3.61(t,4H), 3.30(t,4H), 2.78(s,1H), 2.24 (t,4H), 1.36(s,6H), 0.95(t,4H), 0.79(t,3H). ^{13}C-NMR (100 MHz, DMSO, δ, ppm): 14.90, 26.8, 29, 38, 43.9, 45.7, 53, 61.2, 65.7, 68.36, 110.02, 118.45, 121.09, 121.52, 128.31, 127.3, 134.8, 139.89, 149.10, 142.23, 172.1. ESI-MS(m/z); 988$[C_{52}H_{55}ClZnN_7O_7]^+$, 989 $[C_{52}H_{55}ClZnN_7O_7 + H]^+$, 464 $[C_{27}H_{37}N_3O_3]^+$; UV-vis (1x10^{-4}M, DMSO, λ/nm) : 265, 330. Λ_m: $\Omega^{-1}cm^{-2}mol^{-1}$ = 65 (DMSO).




1,10-Phen
Transmittance (%)
Wavenumber (cm^{-1})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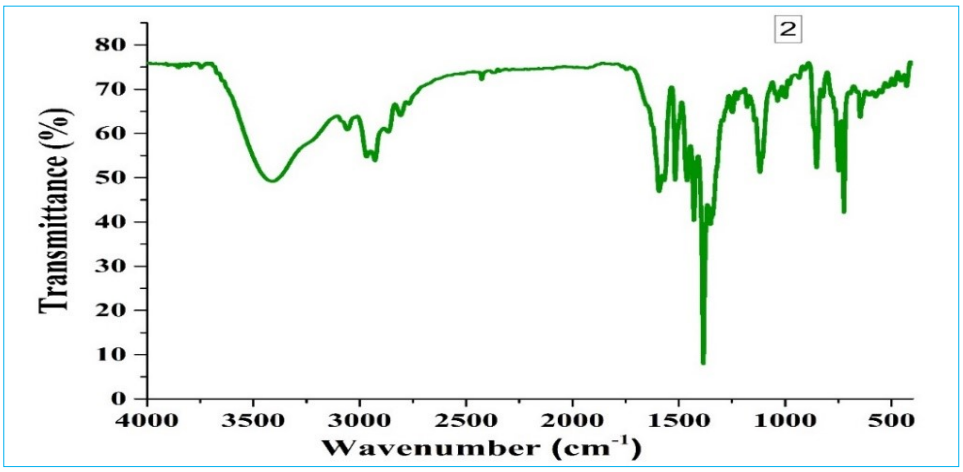
Wavenumber (cm^{-1})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Transmittance (%)
Wavenumber (cm^{-1})

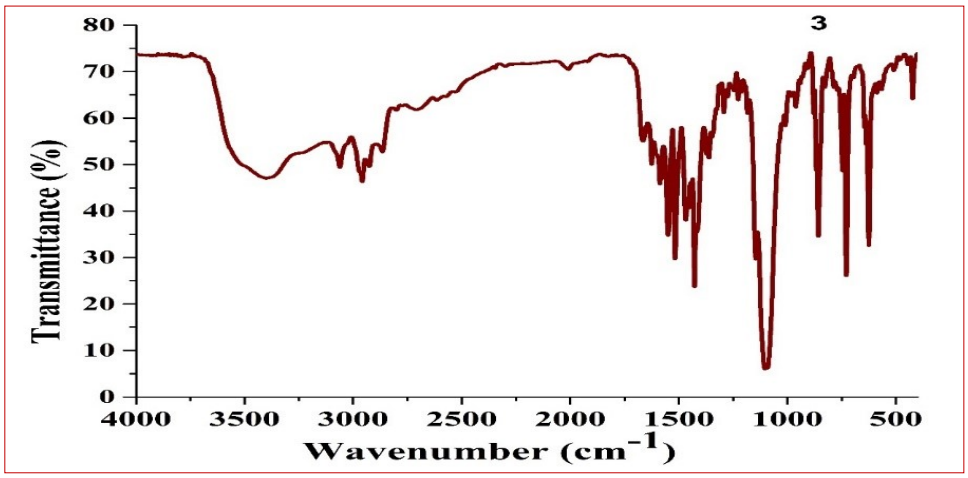


Fig. S1 IR spectra of free ligand and complexes **1-3.**

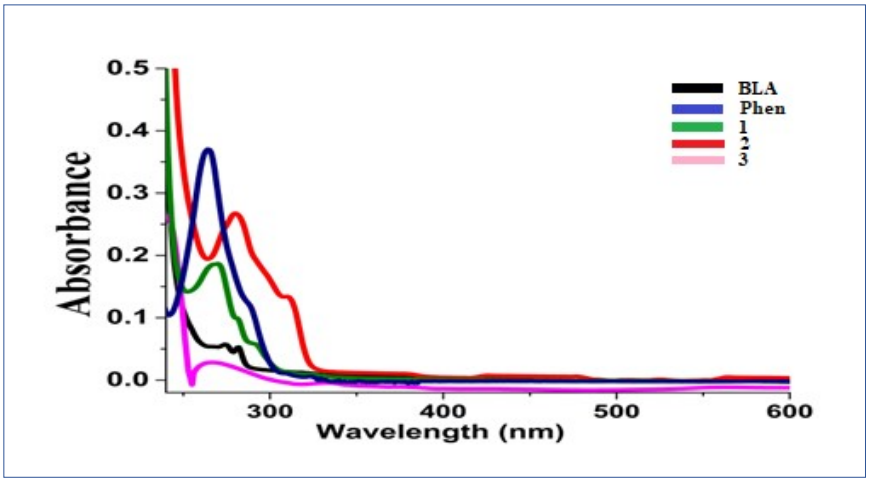
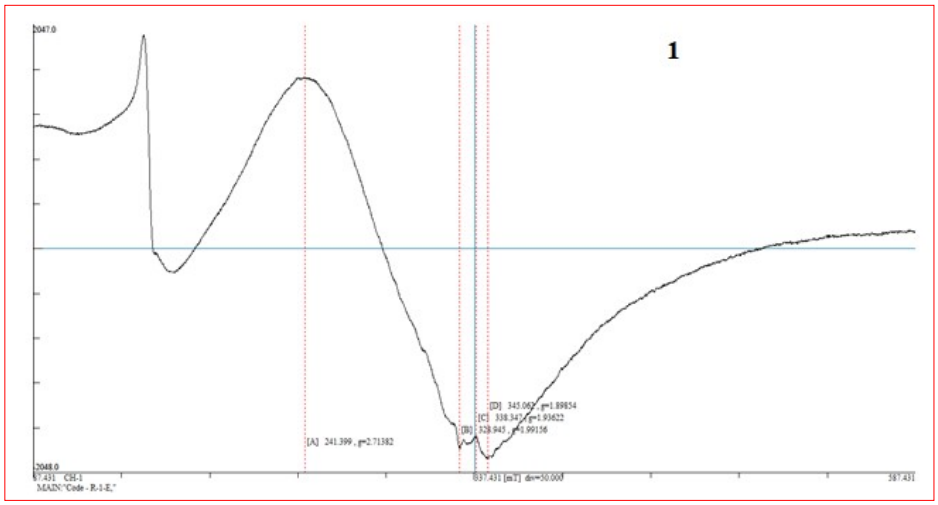


Fig. S2 UV-visible Absorption spectra of bilastine, phenanthroline and complexes **1-3**.



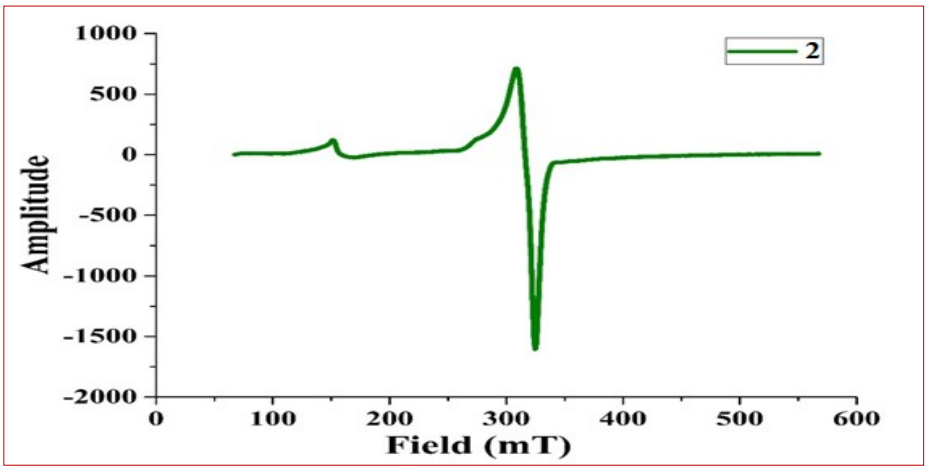


Fig. S3 EPR spectra of Co(II) and Cu (II) complexes **1, 2**.

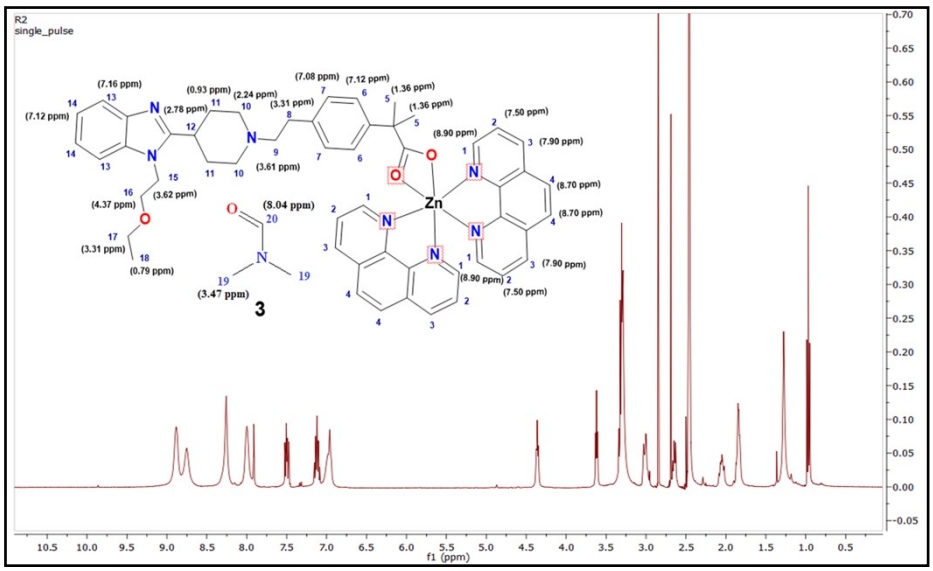


Fig. S4 ^{1}H NMR spectrum of $[BLA(phen)_2Zn(II)]^{+}ClO_4^{-}$ complex **3**.

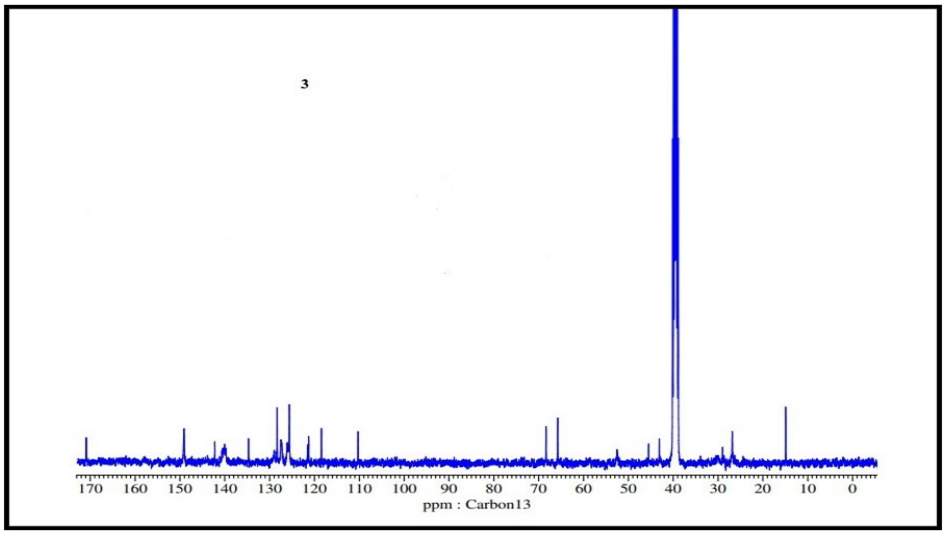
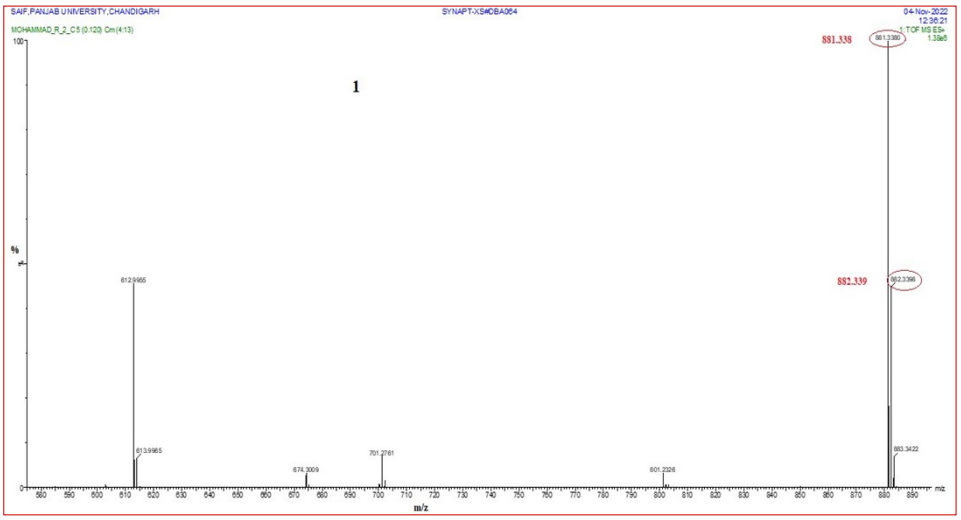


Fig. S5 ^{13}C-NMR spectrum of $[BLA(phen)_2Zn(II)]^{+}ClO_4$ complex **3**.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04-Nov-2022
12:36:21
MOHAMMAD_R_2_C5 (0.120) Cm (4:13)
1: TOF MS ES+
1.33e5
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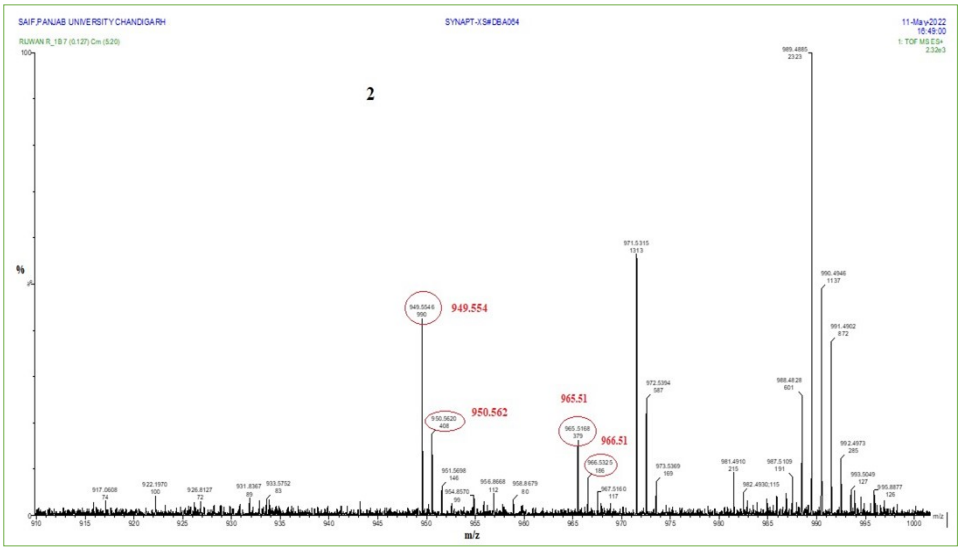
882.339
612.9955
613.9965
674.3009
701.2761
801.2326
883.3422
%
m/z

1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SYNAPT-XS#DBA064
11-May-2022
16:49:00
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2.33e3
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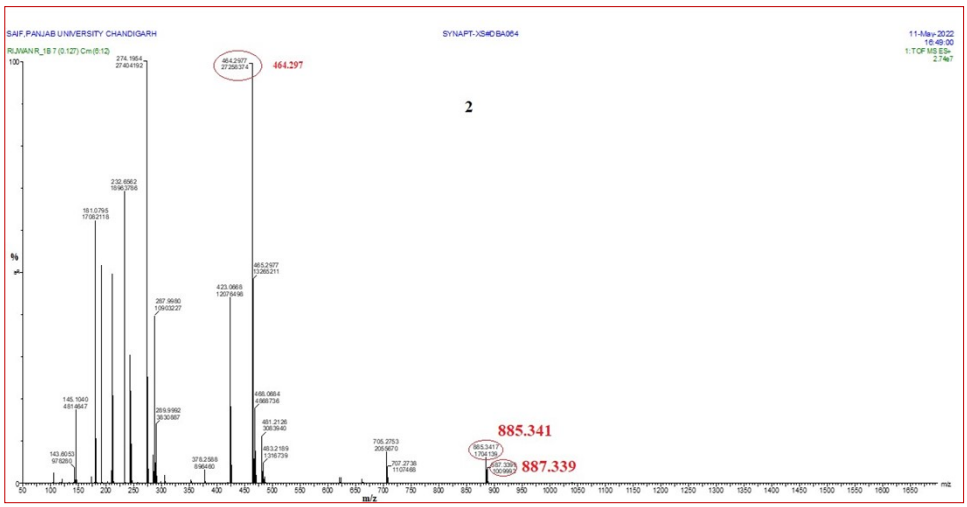
950.562
965.51
966.51
%
m/z

2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16:49:00
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887.339
%
m/z

2

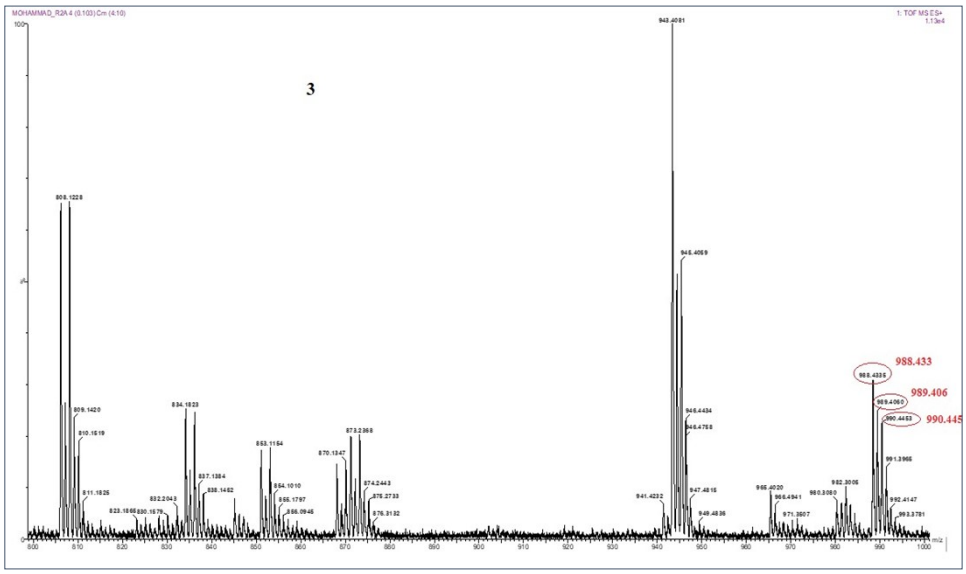
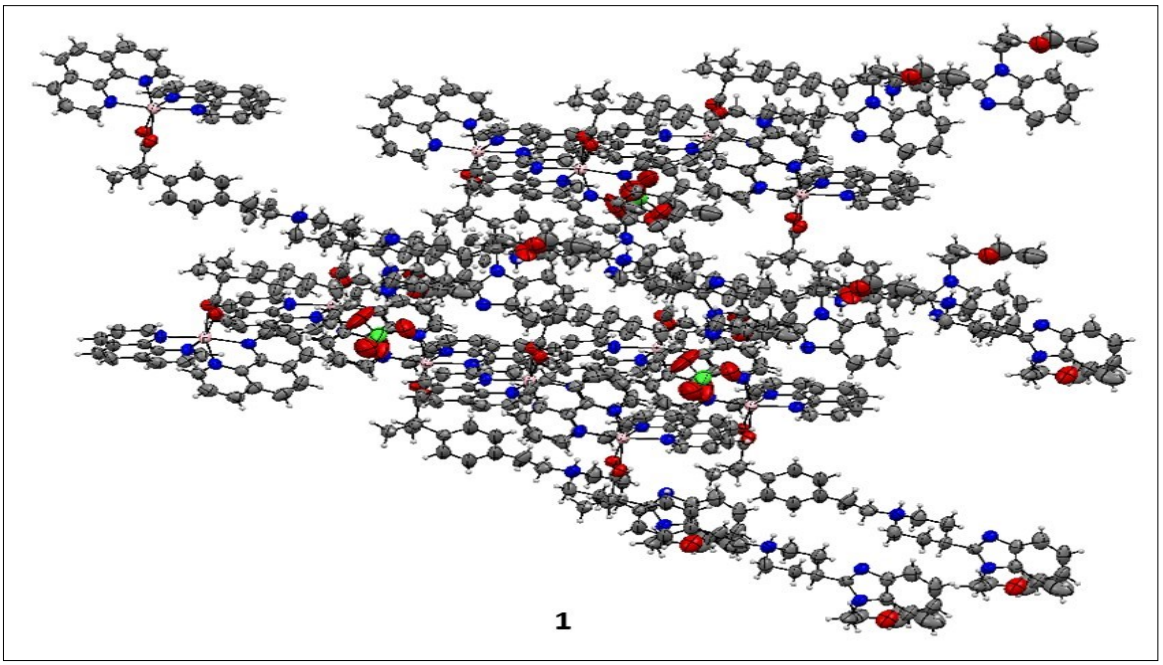
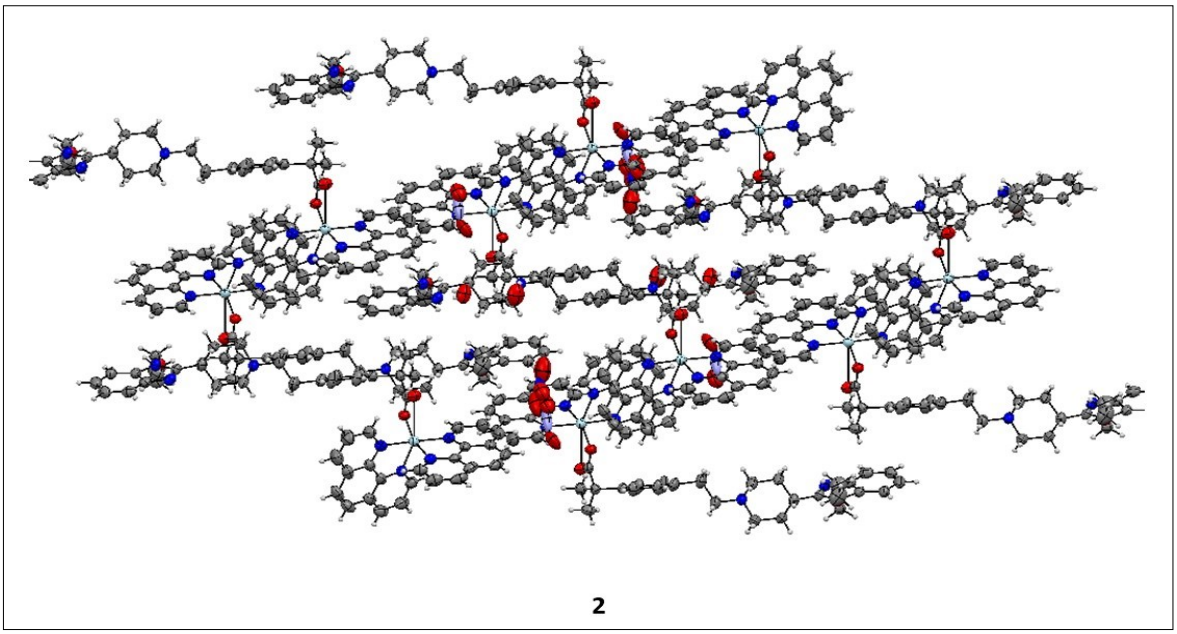


Fig. S6 ESI-MS spectra of complexes **1-3.**





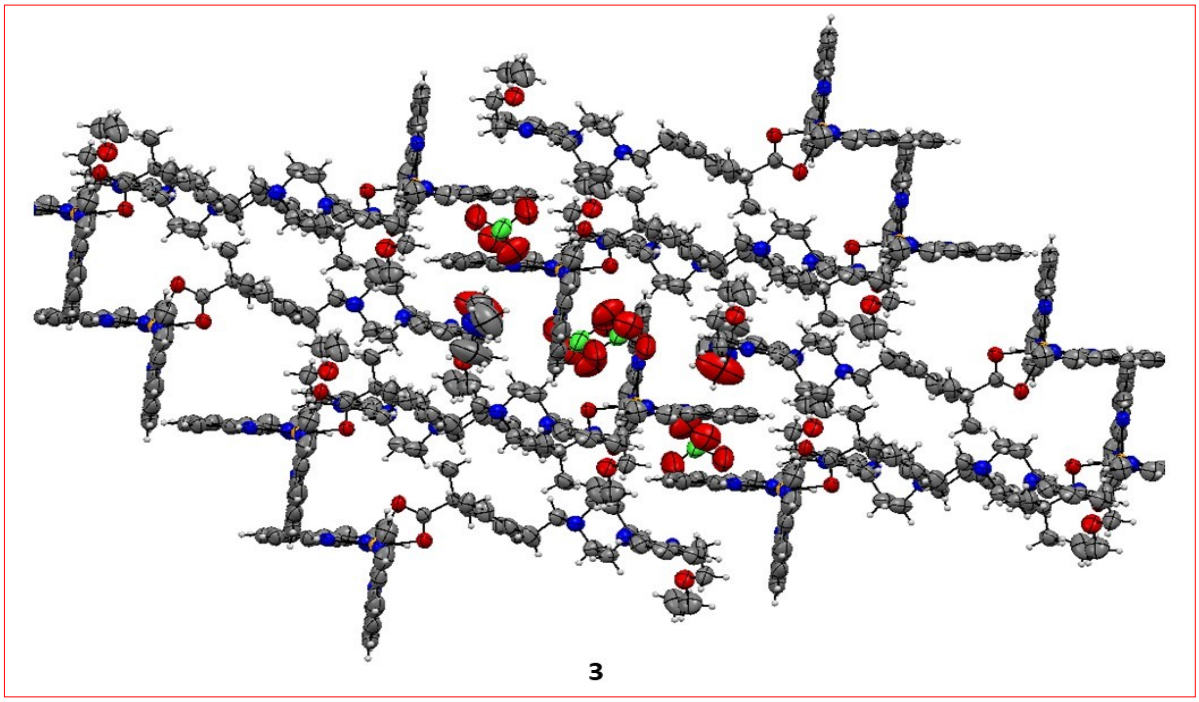


Fig. S7 Packing view of **Bilastine-(Phen)$_2$** based drug candidates (**1-3**).

Table S1 Selected bond length between centre metal atom and ligand in complex **1-3**.

Complex 1	Bond length(Å)	Complex 2	Bond length(Å)	Complex 3	Bond length(Å)
Co01-O004	2.151(5)	Cu1-O28	2.798(2)	Zn01-O004	2.289(3)
Co01-O008	2.150(4)	Cu1-O27	1.961(2)	Zn01-O003	2.100(3)

Co01-N007	2.113(5)	Cu1-N45	2.000(2)	Zn01-N005	2.136(3)
Co01-N005	2.115(7)	Cu1-N59	2.002(3)	Zn01-N006	2.144(3)
Co01-N009	2.132(5)	Cu1-N63	2.199(2)	Zn01-N00A	2.127(3)
Co01-N00K	2.108(7)	Cu1-N49	2.064(2)	Zn01-N00C	2.128(3)

Table S2 Selected bond angels between centre metal atom and ligand in complex **1-3**.

Complex 1	Bond Angle	Complex 2	Bond Angle	Complex 3	Bond Angle
O004-Co01-N005	93.0(2)	O28-Cu1-O27	51.63(6)	O003-Zn01-N004	59.2(1)
O004-Co01-N007	96.1(2)	O28-Cu1-N45	88.91(7)	O003-Zn01-N005	91.4(2)
O004-Co01-O008	60.9(2)	O28-Cu1-N59	91.34(8)	O003-Zn01- N00A	96.7(2)
O004-Co01-N009	156.3(2)	O28-Cu1-N63	155.16(7)	O003-Zn01-N006	161.8(2)
O004-Co01-N00K	94.8(2)	O28-Cu1-N49	107.94(7)	O003-Zn01-N00C	101.2(2)
N005-Co01-N007	95.5(2)	O27-Cu1-N45	91.64(8)	O004-Zn01-N005	93.5(1)
N005-Co01-O008	97.0(2)	O27-Cu1-N59	94.30(8)	O004-Zn01-N005	106.5(1)
N005-Co01-N009	78.8(2)	O27-Cu1-N63	105.79(8)	O004-Zn01- N00A	89.3(1)
N005-Co01-N00K	170.4(2)	O27-Cu1-N49	158.81(8)	O004-Zn01-N00C	155.4(2)
N007-Co01-O008	154.3(2)	N45-Cu1-N59	172.62(9)	N005-Zn01-N006	77.7(2)
N007-Co01-N009	106.7(2)	N45-Cu1-N63	103.44(9)	N005-Zn01-N00A	171.7(2)
N007-Co01-N00K	78.1(2)	N45-Cu1-N49	81.43(9)	N005-Zn01-N00C	102.2(2)
O008-Co01-N009	97.7(2)	N59-Cu1-N63	79.14(9)	N006-Zn01-N00A	94.0(2)
O008-Co01-N00K	91.8(2)	N59-Cu1-N49	91.49(9)	N006-Zn01-N00C	95.4(2)
N009-Co01-N00K	96.0(2)	N63-Cu1-N49	95.31(9)	N00A-Zn01- N00C	78.0(2)

1.3. *DFT studies*

The global reactivity parameters such as electronegativity (χ), hardness (η), global softness (σ), chemical potential (μ), and electrophilicity index (ω) were also calculated[1] and summarized in **Table S3**.

$$\chi = -(E_{HOMO} + E_{LUMO})/2$$

$$\mu = (E_{HOMO} + E_{LUMO})/2$$

$$\eta = -E_{HOMO} + E_{LUMO}$$

$$\sigma = 1/\eta$$

$$\omega = \mu^2/2\eta$$

$$\Delta N_{max} = -\mu/\eta$$

Table S3 complex **1-3**.

Complex	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE(eV)	χ	η	σ	μ	ω	ΔN_{max}
1	-3.488	-2.906	-0.532	-3.197	0.532	3.436	-3.197	17.57	10.986
2	-9.466	-9.953	-0.487	-9.709	0.487	2.053	-3.167	96.23	19.93
3	-2.983	-3.547	-0.564	-3.264	0.564	1.773	3.264	9.44	5.787

1.4. *Solution stability studies*

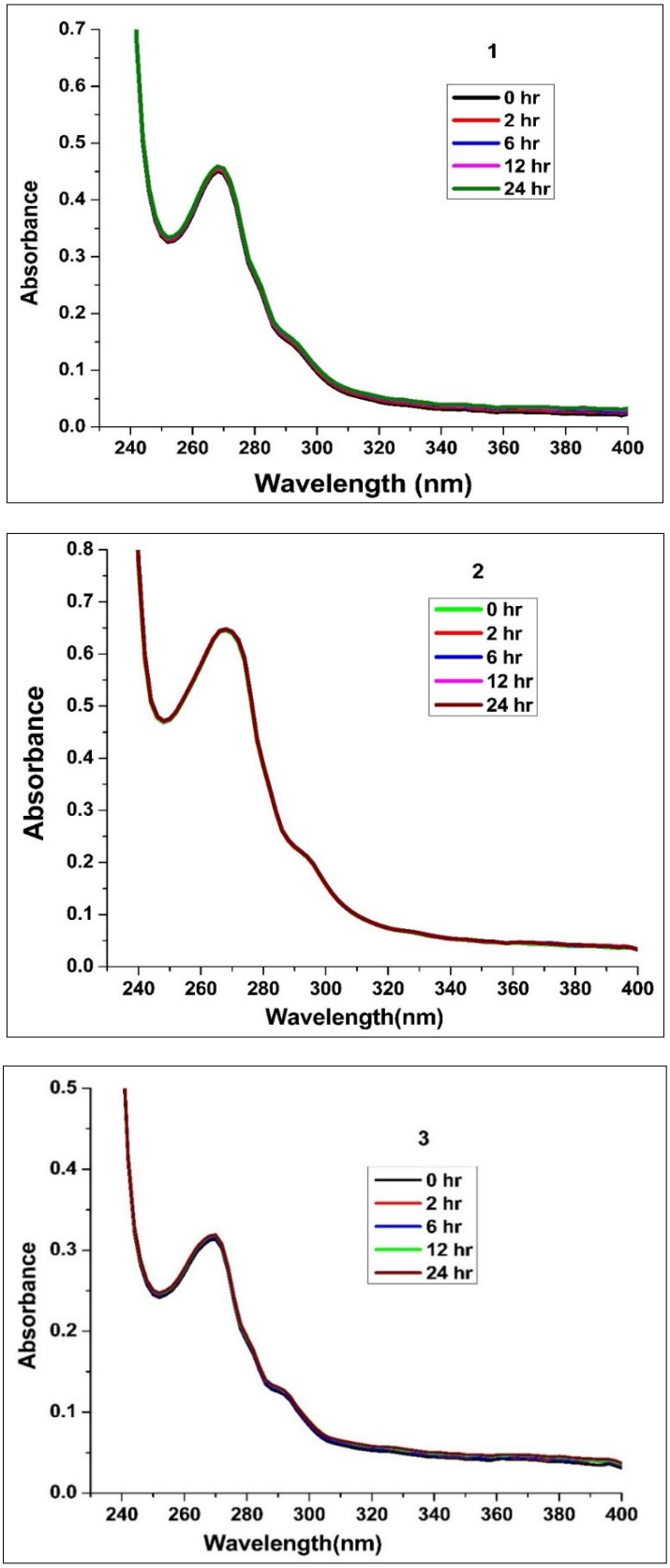


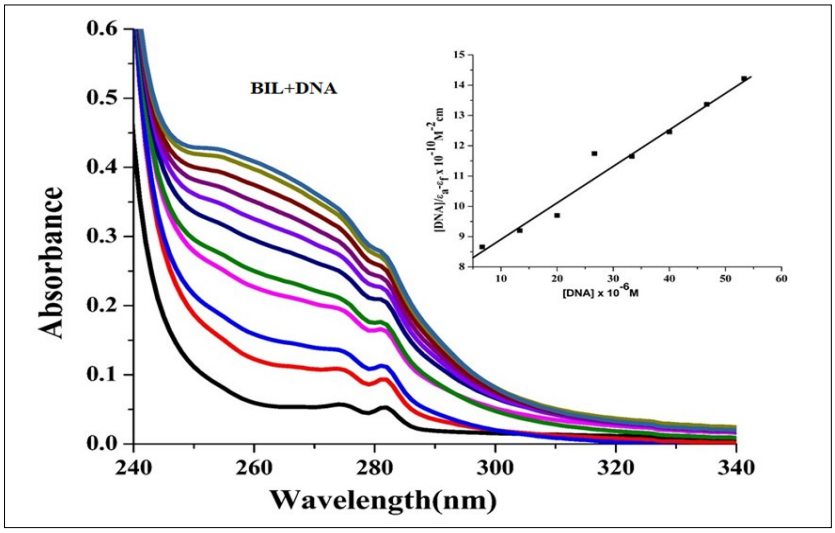
Fig. S 8 UV-visible spectra of **Bilastine-(Phen)$_2$**-based drug candidates (**1-3)** in solvent DMSO at different time intervals (0hr, 2hr, 6hr, 12hr, 24hr).

1.5. *UV-visible spectroscopy*

The intrinsic binding constant K_b was calculated by using Wolfe-Schimer equation[2].

$$[DNA] / (\varepsilon_a-\varepsilon_f)] = [DNA] /(\varepsilon_b-\varepsilon_f)] + 1/ K_b /(\varepsilon_b-\varepsilon_f)$$

Where [DNA] is the ct-DNA concentration and ε_a, ε_f, and ε_b are the apparent (A_{abs}/[complex], free, and bound complex extinction coefficients.



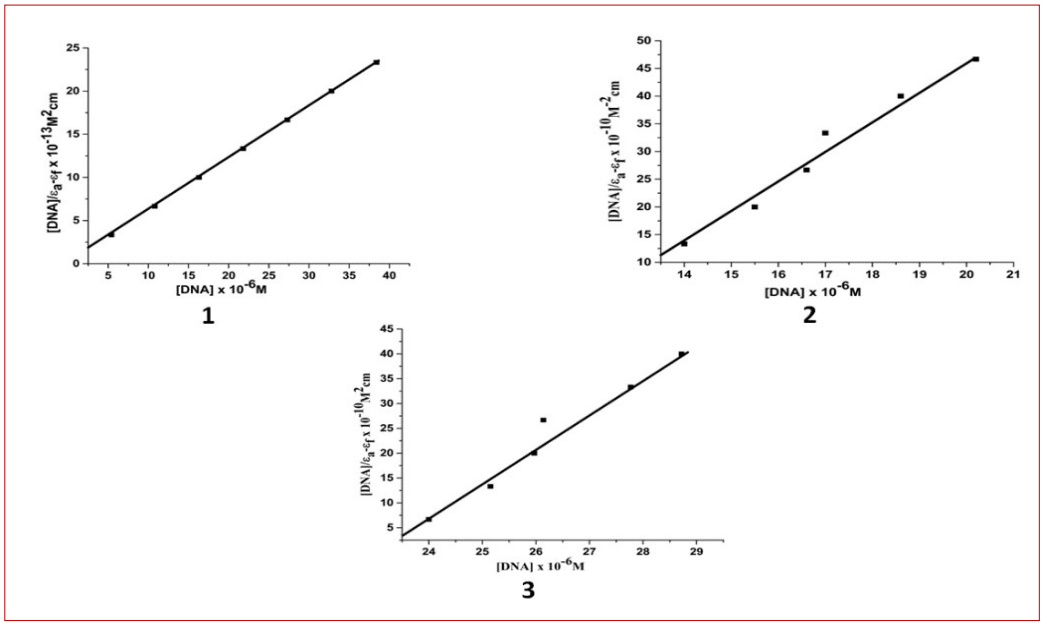


Fig. S9 Absorption spectra of BLA upon increasing concentration of ct-DNA & Plots of [DNA]/ (εa -εf) x 10^{-13} M^2cm vs [DNA] x 10^{-6} M for the titration with complexes **1-3**.

1.6. *Fluorescence spectral studies*

The quenching constant K_{sv} was calculated by using Stern-Volmer equation[2].

$$I_o/I = 1+K_{sv}[Q]$$

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

1.7. *Cyclic Voltammetry*

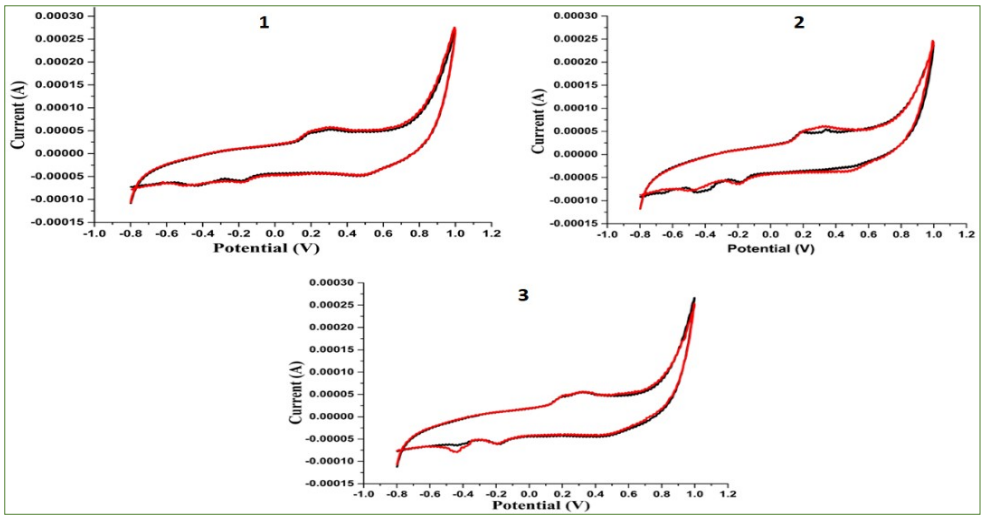
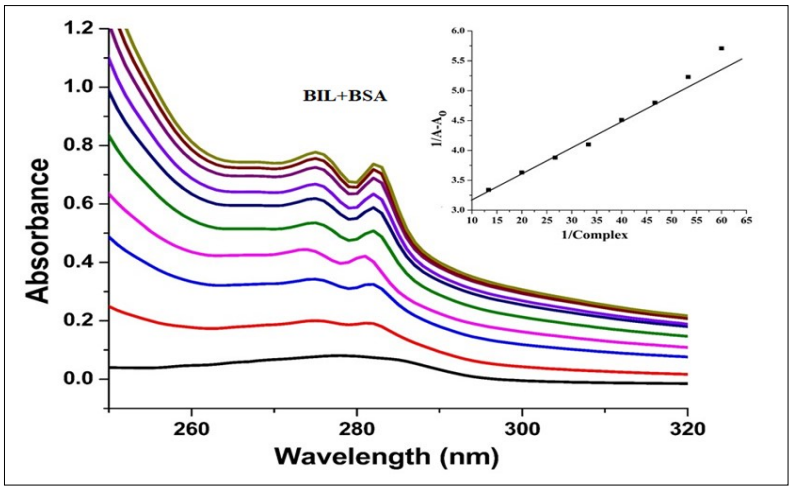


Fig. S10 Voltametric peak current and potentials of complexes **1-3** (black) in absence and presence of ct-DNA (red).

1.8. *Protein binding studies*



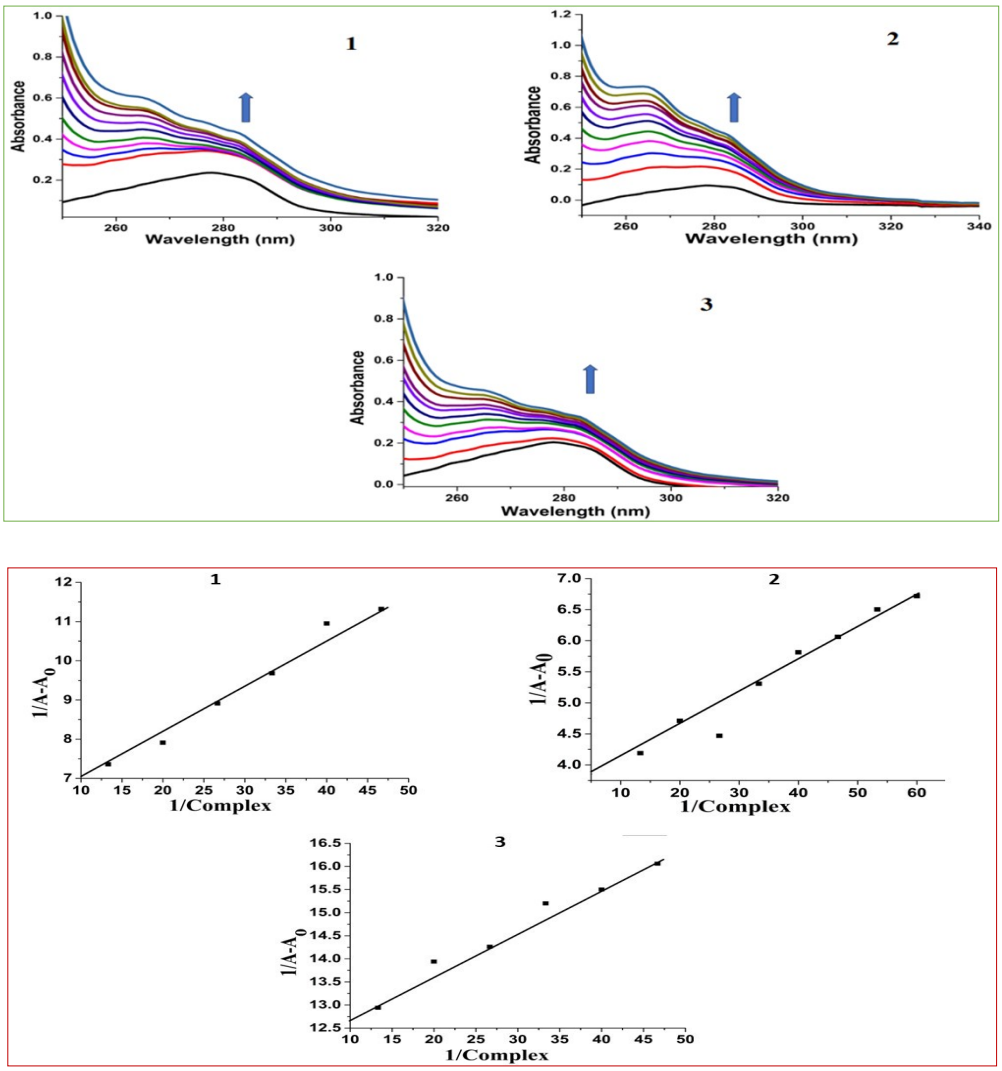


Fig. S11 Absorption spectra of BLA and complexes **1-3** in absence and presence of BSA, with increasing concentration of complex in tris -HCl buffer**.**

1.9. *Cytotoxicity*

The cell lines were grown in appropriate medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, 5000 cells/well were inoculated into 96 well microtiter plates in 100 µL. 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[3].

Experimental drugs were solubilized in DMSO 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)[4] 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:

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

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