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DNA Interactions and Biological activities of Co(II), Ni(II), Cu(II) Complexes of of 2-methoxy 5-trifluoromethyl benzenamine: Synthesis, Characterization

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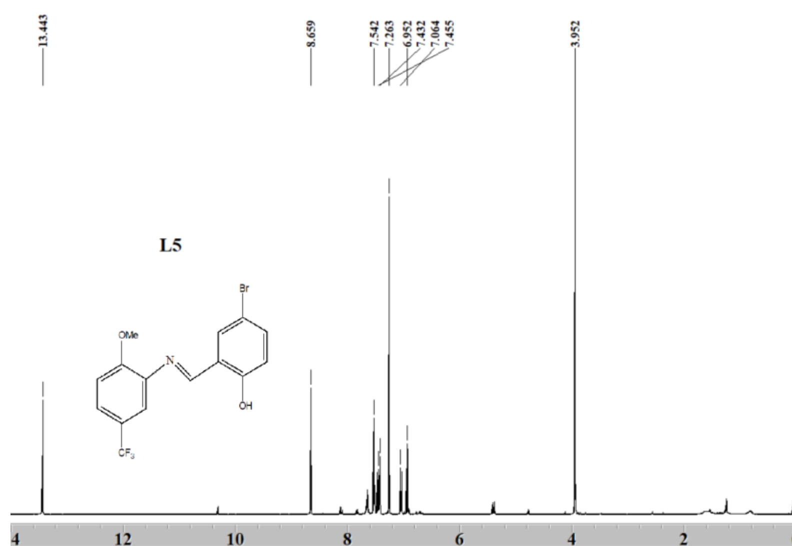


Figure S1. ^{1}HNMR sprectra of Ligand 1.

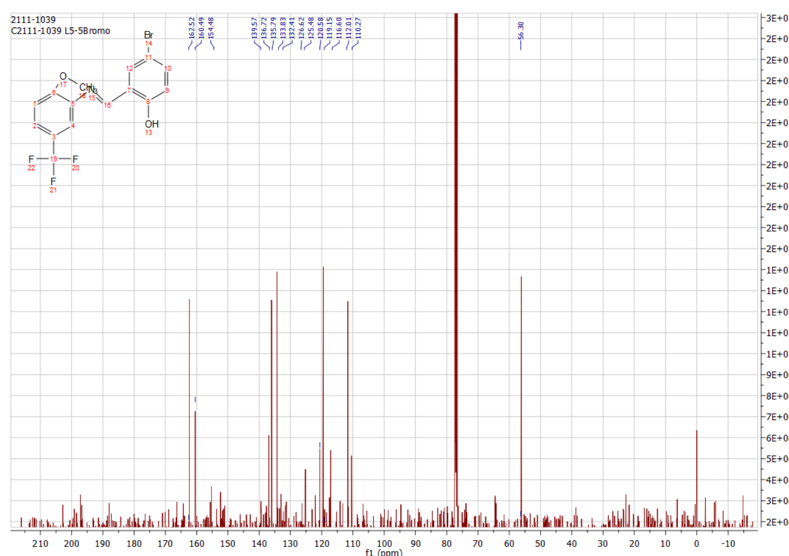


Figure S2. ^{13}CNMR sprectra of Ligand 1.

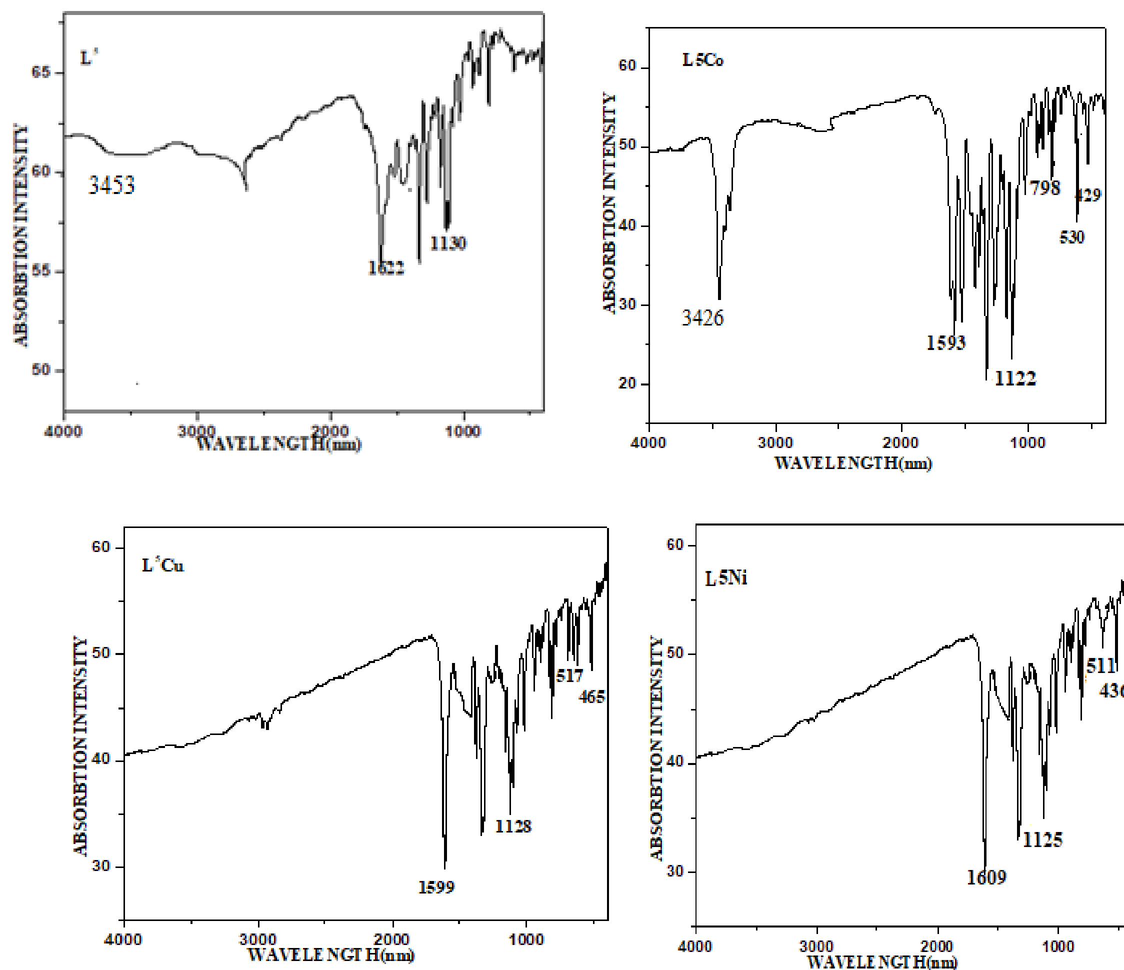


Figure S3: FTIR spectra of the ligands and complexes.

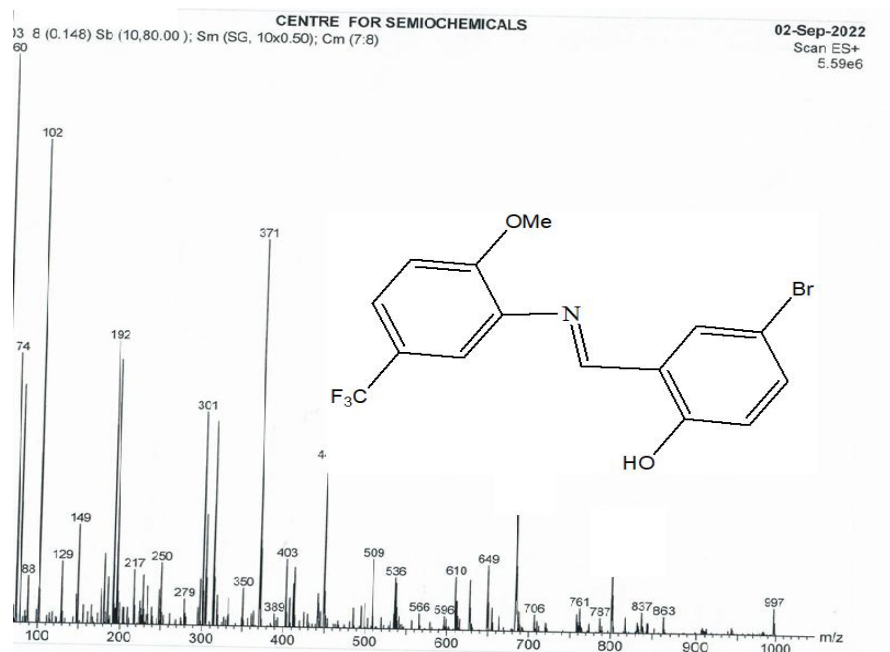


Figure S4: Mass spectrum of HL^1

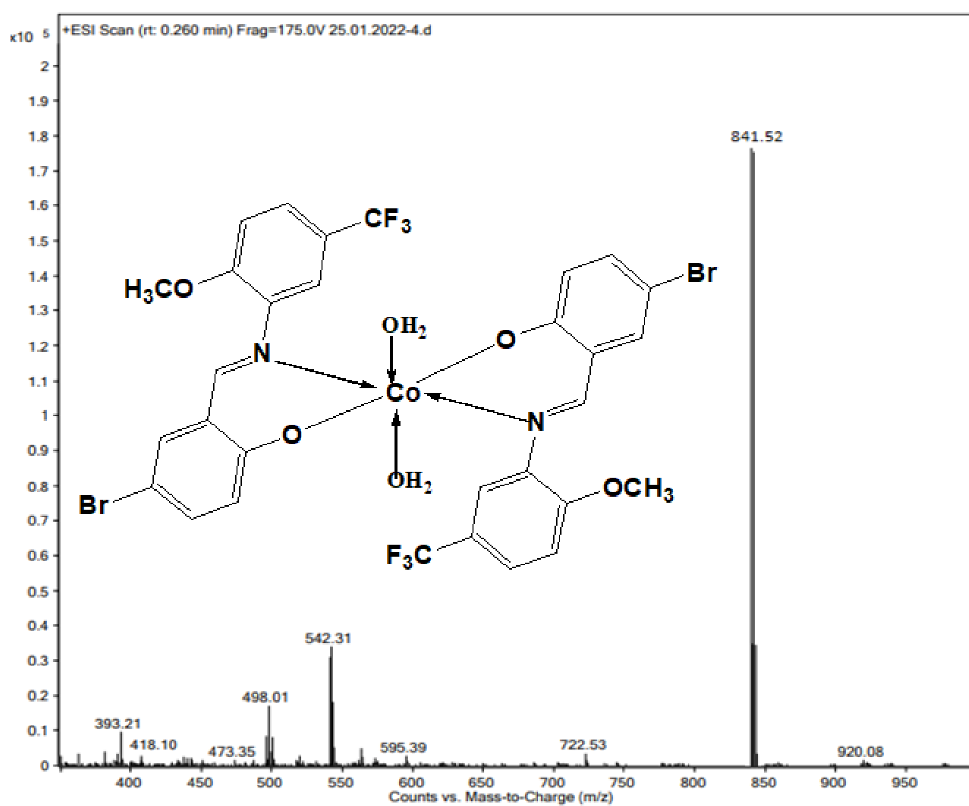


Figure S5: Complex A

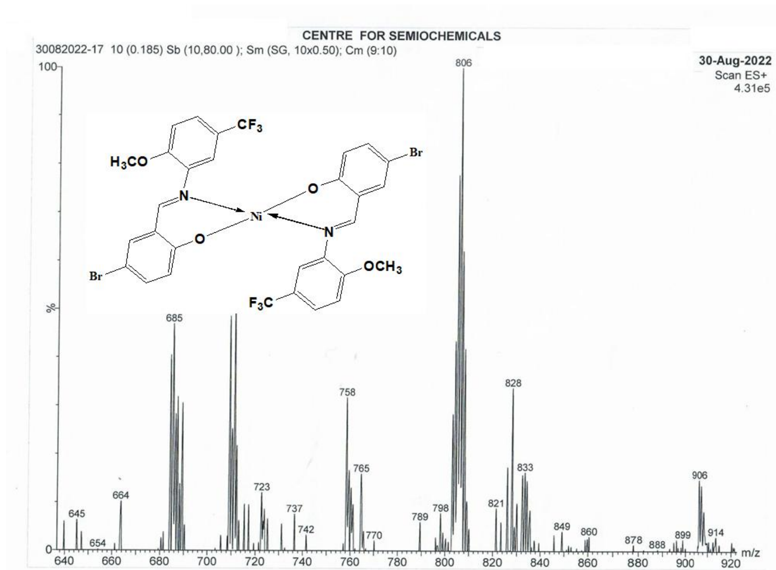


Figure S6: Complex B

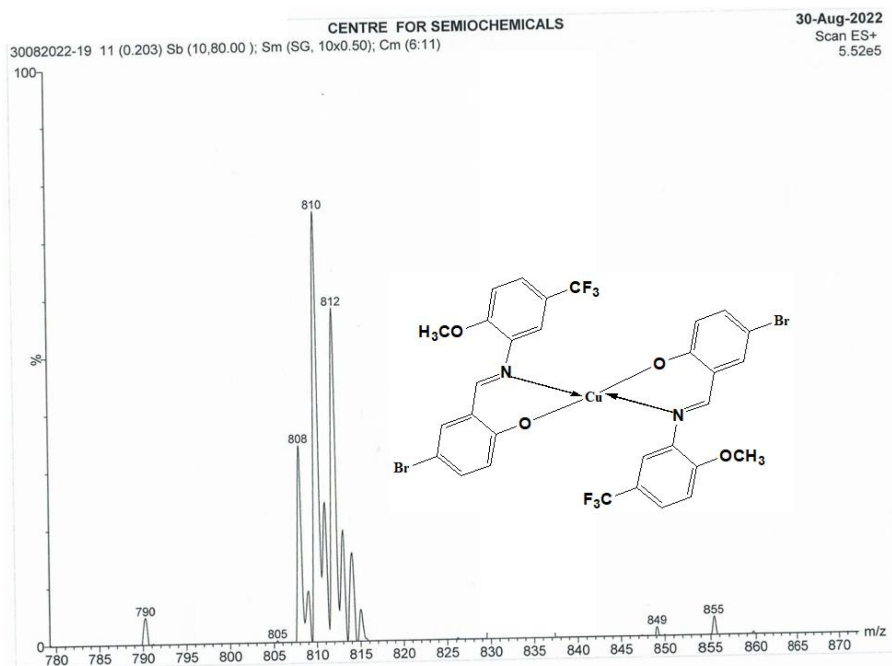


Figure S7: Complex C

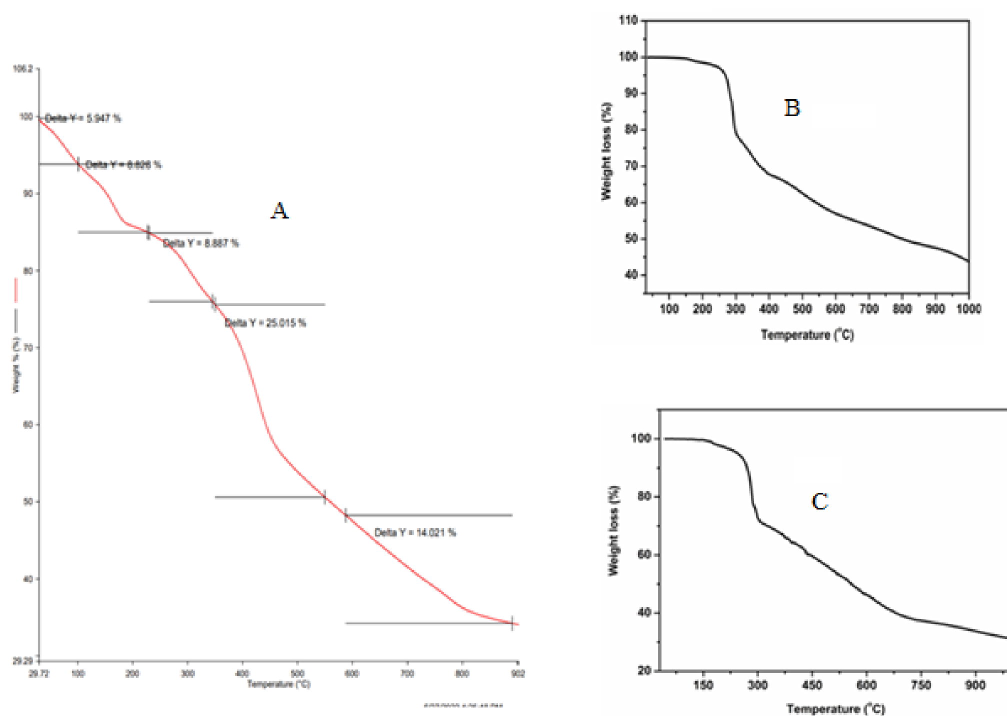


Figure S8: Thermograms of the complexes

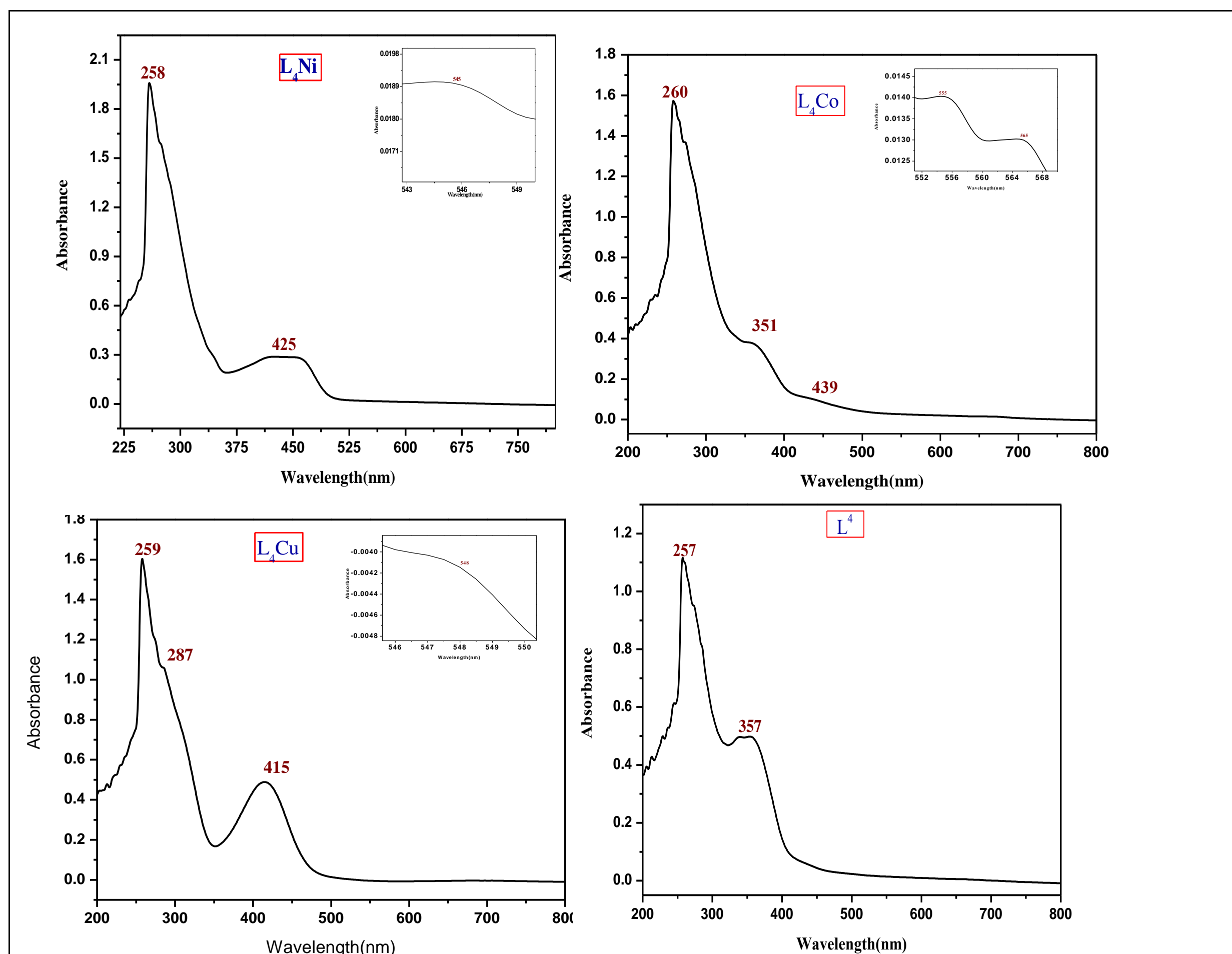


Figure S9. Absorption sprectra of Ligands and complexes

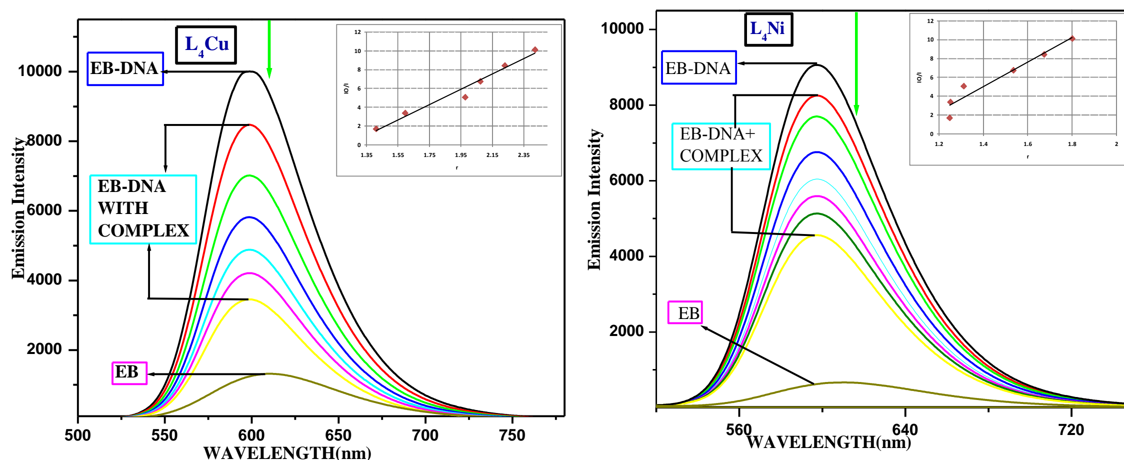


Figure S10: Emission spectra of EB bound to DNA in the absence and presence of complexes (↓) implies the variation of intensity upon incremental addition the complex concentrations (10, 20, 30, 40, 50, 60). **Inset**: linear plot to calculate the k_{sv} values.

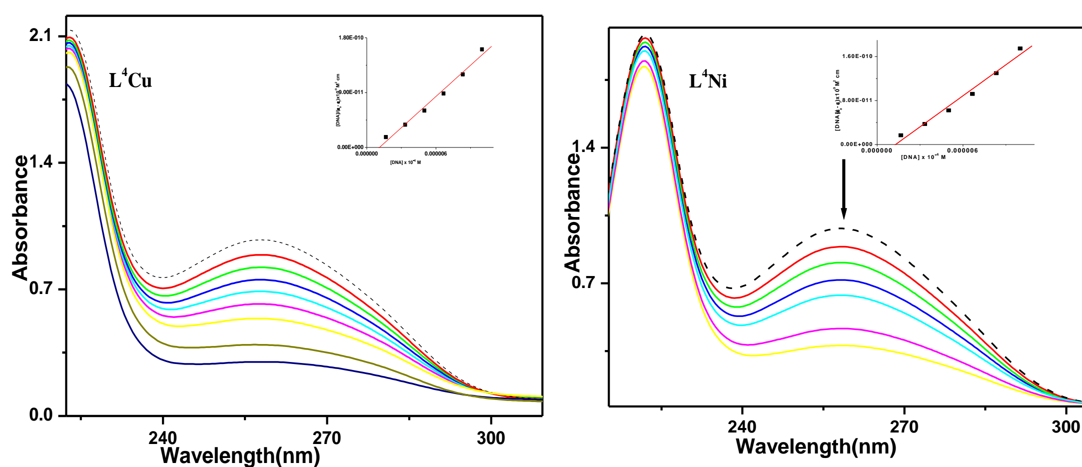
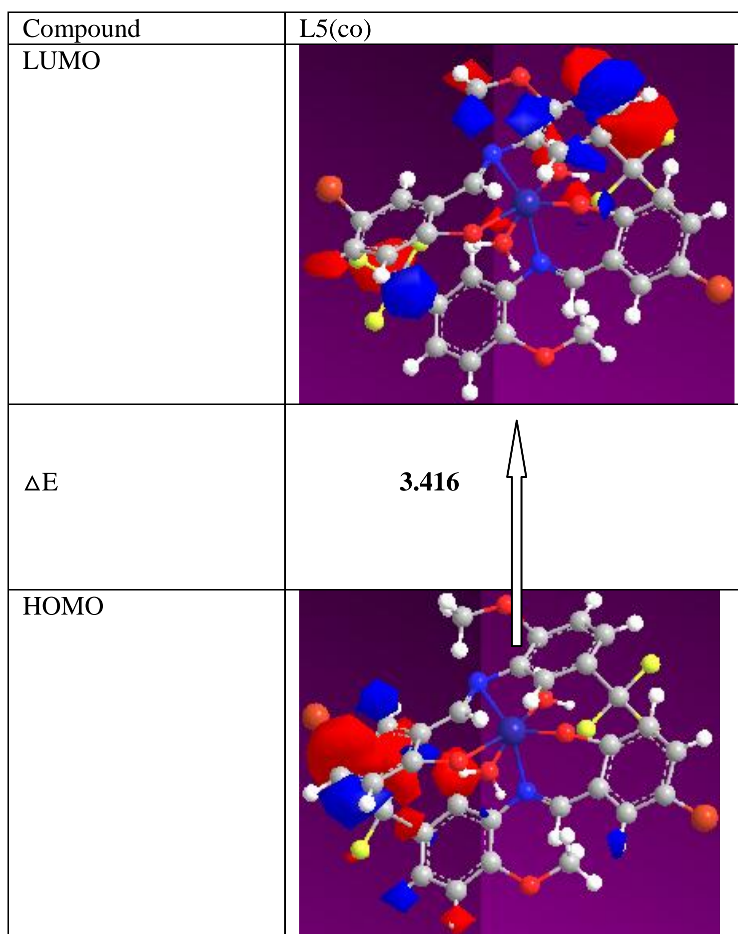


Figure S11: The absence and presence of CT-DNA in the UV-Vis absorption spectra of complexes **(B,C)** are represented by dashed and solid lines, respectively. Arrow (↓) represents **(↓)** implies hypochromism upon increasing DNA concentrations (20, 40, 60,…). **Inset**: linear plot to calculate the k_b values.



Compound	L5(co)
LUMO	
∆E	**3.416**
HOMO	

Figure S12: Homo Lumo structures and the energy difference representation of complex **A**.

Experimental :

Kinetic Studies:

The Coats-Redfern integral method [1,2] is used to figure out the kinetic parameters from the TGA data of metal complexes that have been analysed. The following equation was used to figure out the activation energy (E), frequency factor (A), activation entropy (S), enthalpy (H), and free energy change (G):

$$log\left[\frac{log(W_\infty/(W_\infty - W))}{T^2}\right] = log\left[\frac{AR}{\varphi E^*}\left(1-\frac{2RT}{E^*}\right)\right] - \frac{E^*}{2.303RT}$$

Where $W\infty$ --- the mass loss at the completion of the decomposition reaction,
W --- the cluster loss up to temperature T,
R --- the general gas constant and
ϕ --- the heating rate.

Since $1 - 2RT/E^* \approx \mathbf{1}$, a ploting a graph between the equation (1) vs 1/T would gives a straight line, from the slope and the Arrhenius constant E* can been computed.

A can be resolved from the graph intercept.

Entropy of activation (ΔS*), enthalpy of activation (ΔH*), and free energy change of activation (ΔG*) were all equalised by:

$$\Delta S^* = 2.303 R log\frac{Ah}{K_B T} \quad \textbf{(2)}$$

$$\Delta H^* = E^* - RT \quad \textbf{(3)}$$

$$\Delta G^* = \Delta H^* - T\Delta S^* \quad \textbf{(4)}$$

Where, K_B and h are the 'Boltzmann' and 'Planck constants' respectively.

Binding characteristics:

Electronic absorption titration studies:

DNA and metal complexes have been observed to engage in a physicochemical interaction. Tris-HCl/NaCl and DMSO were used to assess the purity of the CT-DNA to be utilised in titrations to determine how the metal complexes and CT-DNA bind. Using an extinction coefficient of $6600 m1cm^{-1}$ at 260nm, the quantity of CT-DNA was calculated [3]. For DNA binding tests, the concentration of the metal complex was maintained at 10 **μ**M, whilst the concentration of CT-DNA was varied from 0 to 10 mM to determine how binding interactions occurred. In order to get clean spectra, the test solution was allowed to incubate for 4–5 minutes after each aliquot addition. By observing the changes in absorbance of complexes, it was simple to determine their intrinsic binding constant values (kb) using the Wolfe-Shimmer equation:

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

Where, [DNA] is concentration of DNA, εb , εf are apparent absorption coefficients for bound and free DNA.

Fluorescence quenching study *:-*

Fluorescence is a physical-chemical process that involves dimming fluorescent molecules and determining how biomolecules and complexes adhere. The fluorescence of the fluorophore weakens as it interacts with complexes. Since EB is a fluorescent tag, it possesses broad, low-intensity peaks that become more intense upon binding to biomolecules. As increasing amounts of complex (0–60M) were added to the EB-DNA adduct, the EB-DNA adduct's intensity decreased gradually. This is due to the fact that the complex molecule balances the EB. The titrations were performed at wavelengths ranging from 520 to 700 nm. The slope of the straight line in the Stern-Volmer equation was used to calculate the Ksv quenching constant.

$$\mathbf{I_0 / I = 1 + K_{sv}\, r}$$

Where: I_0 – emission intenstity of EB – DNA in absence of quencher, I – emission intenstity of EB – DNA in presence of quencher, r = [ML]/(DNA)

DNA cleavage studies by Agarose gel electrophoresis:

The DNA cleavage activity of H_2O_2 (Oxidatively) and UVlight (Photolytically) for ligand and its metal complexes with pBR322 DNA at pH 7.2 in Tris–HCl buffer solution was determined using Agarose gel electrophoresis [4], a typical separation technique. The wells were prepared by agarose solution in to which DNA, 0.25% bromophenol blue peroxide loading dye, the samples were added, which were then diluted to a total volume of 16L with TAE buffer and incubated at 37 °C for 2 hours. Wells were loaded with samples. After 1 hour of electrophoresis at 75V in TAE buffer medium. To agarose, the staining agent was ethidium bromide.

The bands were visualised using a UV transilluminator, and the resulting gel was shot using the BIO-RAD Gel documentation system.

Biological assay:

Anti-oxidant activity:

The scavenging activity of synthesised metal complexes (A-C) was evaluated using the stable 2,2- Diphenyl-2-picryl-hydrazyl (DPPH) free radical according to the modified Blois method [5]. In DMSO solution, a DPPH(0.3 M) solution was produced by dissolving DPPH. Due to the odd electron, a series of dilutions (20-100 µM) of metal complex(A-C) stock solutions were prepared using DMSO. About 1 ml of DPPH solution (0.3 M) was added to 3 ml of prepared metal complexes (A-C) with variable concentrations (20-µ100 M) and incubated at room temperature for 40 minutes in the dark. The absorbance of these substances was then measured at 517 nm [6] On ultraviolet-visible spectrophotometer In terms of IC_{50} values (50% Inhibitory Concentration), the antioxidant activity of metal complexes was determined. The IC_{50} values for each complex were also calculated. The IC_{50} is the concentration required to achieve 50% of the maximum scavenging activity. Utilizing regression lines, the IC_{50} values of all substances were determined. The % of scavenging activity was computed using the formula:

DPPH scavenging%= $(A_o - A_{sample}/A_o) * 100$

Where, Ao is the absorbance of the control, Asample is the absorbance of the sample.

Anti-Microbial activity:

All prepared compounds were tested for antimicrobial activity (i) Gram-positive bacteria, such as B. cereus and Bacillus subtilis, (ii) Gram-negative bacteria, such as Escherichia coli, K. Pneumoniae and (iii) fungal strains, such as A.niger, C.albicans in comparison to the standard antibiotics Gentamycin sulphate and Nystatin. The concentration of each test chemical used to generate the stock solution was 500 µg/1 mL of DMSO. 6 mm sterile Whatman filter paper discs were placed on culture impregnated agar plates and incubated at 37°C for 24 hours. During incubation, the diffusion of the test solution greatly hindered the growth of the injected bacteria. Later, the radial growth was utilised to determine the size of the inhibitory zone. Tracking antimicrobial resistance is possible by comparing the efficiency of various drugs. Minimum Inhibitory Concentration values were determined.

Anticancer activity:

The objective of a cytotoxicity assay is to measure the toxicity of chemotherapeutic medicines (synthetic chemicals) acting on living cells. By inhibiting the $-NH_2$ groups on the DNA base pairs, anti-tumor dicines crosslink the two strands of the double helix and stop gene replication and transcription. The anticancer medication Cis-Platin destroys the DNA within an oncocyte, inducing apoptosis, or "programmed cell death." Compared to other methods, the MTT assay (3-(4,5-dimethylthiazole)-2,5-diphenyltetraazolium bromide) is vastly superior. It is commonly used to measure cell viability and cytotoxicity because it is easy to use, safe, and dependable. KB3 and MCF-7 cell lines were tested against the produced chemicals. The stock solutions of materials were created by dissolving them in DMSO solution, and a portion of these solutions were used to treat cancer cells at a range of concentrations (20-100 M). Cisplatin is believed to serve as a positive control, while just loading the culture medium is assumed to serve as a negative control. Each well received 20 L of the stock MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Continue the incubation process for 3 hours at 37 °C while wrapped in Al foil until purple formazan precipitates. As a result of the reduction of MTT by mitochondrial succinate dehydrogenase, purple formazan precipitates. The absorbance [7,8]. of the product was measured after dissolving it in DMSO. IC_{50} values were calculated by graphing the percentage viability of cells vs concentrations on a logarithmic graph [9,10]. Data is presented as the average of three measurements.

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