Supplementary materials

S1

1.1. Chemicals: High quality and purity chemicals purchased from Aldrich and Merck were used directly in this work without any further action.

1.2. Synthesis of $[Ru(VA)_2ClH_2O]$ complex

0.261 g (0.001 M) of ruthenium $RuCl_3.3H_2O$ salt dissolved in 30 ml of ethanol was added dropwise to 30 ml of the ethanol containing 0.3142 g (0.002 M) of violuric acid while stirring. The resulting reaction mixture was subjected to boiling under reflux for one hour during which dark red solids appeared in the flask. The flask containing the hot reaction mixture was allowed to cool to room temperature and then the colored precipitate was filtered and washed with ethanol several times. The filtration crucible containing the precipitate was kept in a dry atmosphere for seven days. The validity of the synthesis and purity of the solid complex present are inferred from the analytical data as follows: Yield 85%, Analytical data for $RuC_8H_6N_6O_9Cl$; Calcd: C, 20.56; H, 1.28, N, 18.01, Ru, 21.64; Found: C, 20.30; H, 1.49; N, 17.98, Ru, 21.84.

Physicochemical measurements, DNA-binding studies, investigations of antiviral activity and method of molecular docking calculations

1.3. Physical measurements

The infrared spectra were recorded using KBr disks in the 4000-200 cm^{-1} range on a Unicam SP200 spectrophotometer. The electronic absorption spectra were obtained in DMF solution with a Shimadzu UV-2450 spectrophotometer. The magnetic moments of the prepared solid complexes were determined at room temperature using Gouy method. Mercury(II) tetrathiocyo cobaltate(II) complex $Hg[Co(SCN)_4]$ was used for the calibration of the Gouy tubes. The specific conductance of the complex was measured using freshly prepared 10^{-3} M solutions in electrochemically DMF at room temperature, using an YSI Model 32 conductance meter. The thermogravimetric measurements were performed using a Shimadzu TG 50-Thermogravimetric analyzer in the 25 - 800 °C range and under an N_2 atmosphere. Elemental analyses were carried out at the Micro analytical Unit of Cairo University. The powder X-ray diffraction spectra of the solid

microcrystalline samples of the Ru(III) complex was performed using Shimadzu 6000 XRD spectrometer. Measurements conditions are 45 kV, 30 μA and Cu Kα radiation with λ= 1.5406 Å at scan range of 2θ = 5-80. The fluorescence emission of the freshly prepared solutions of CT-DNA or HSA was recorded at ambient temperature by using Shimadzu RF-5301PC spectrofluorimeter.

1.4. DNA – binding measurements

UV–visible absorption spectroscopy was used to investigate the binding of isolated violuric acid and its Ru(III) complex complexes to CT-DNA. The stock solution of CT-DNA (6.25×10^{-4} M) was prepared by dissolving in Tris–HCl buffer solution (pH 7.2) and stored at a temperature of less than 4 °C. The stock solutions of violuric acid (6.25×10^{-4} M) and its Ru(III) complex (9×10^{-4} M) were prepared by using DMSO solvent. The binding experiments were carried out with the violuric acid at a concentration of (6.25×10^{-5} M) and its Ru(III) complex at a constant concentration of (9×10^{-5} M) while the CT-DNA quantities were varied (0.662252×10^{-5} to 4.4586×10^{-5} M).

Fluorescence quenching measurements were performed by using fixed amount of CT-DNA (6.25×10^{-5} M) and violuric acid with the varying concentrations from 6.21118×10^{-6} to 6.97674×10^{-5} M, while the Ru(III) complex concentration was varied between 9.28793×10^{-6} and 1.18457×10^{-4} M.

1.5. HSA – binding measurements

Interactions of violuric acid and Ru(III) complex with HSA have been studied by utilizing UV–visible absorption and fluorescence spectral techniques. The HSA solution was prepared by dissolving in Tris–HCl buffer solution (pH 7.2) and the violuric acid Ru(III) complex were dissolved in DMSO solution. The absorption investigation was carried out by gradually increasing the quantity of HAS (6.0241×10^{-6} to 7.30337×10^{-5} M); keeping the fixed quantity (9×10^{-5} M) of violuric acid and its Ru(III) complex. Furthermore the fluorescence quenching experiment was performed by addition of gradually increasing of violuric acid (from 1.2945×10^{-5} to 1.15942×10^{-4} M) and Ru(III) complex of concentration from 9.74026×10^{-6} to 1.10787×10^{-4} M into a fixed concentration (1.64×10^{-5} M) of the HSA solution.

1.6. Viscosity titration measurements

Viscosity experiments were conducted on an Ubbelodhe viscometer, immersed in a water bath maintained at 25.0 ± 0.1 ºC. The flow time was measured with a digital stopwatch and each sample was tested, three times to get an average calculated time. Titrations were performed for the violuric acid and Ru(III) complex (3 – 30 μM), and each compound was introduced into CT-DNA solution (42.5 μM) present in the viscometer. Data were analyzed as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound to CT-DNA, where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA-containing solutions corrected from the flow time of buffer alone (t_0), $\eta = (t - t_0)$ [1].

[1] R. Gaur, R. A. Khan, S. Tabassum, P. Shah, M. I. Siddiqi, L. Mishra, Interaction of a ruthenium(II)–chalcone complex with double stranded DNA: spectroscopic, molecular docking and nuclease properties, J. Photochem. Photobiol. A, 220 (2011) 145–152

1.7. Cytotoxicity

To evaluate the *in vitro* cell viability of the violuric acid and its Ru(III) complex, the 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed as previously described [2] with minor modifications. Briefly, cells were seeded in 96-well plates in DMEM supplemented with 10% fetal bovine serum, and 1% antibiotic antimycotic mixture. After 24 h of cell preparation, the growth medium was aspirated from each well and the cells washed with 1X phosphate buffered saline (PBS). Different concentrations of violuric acid and its Ru(III) complex starting from 100 μM were serially diluted in DMEM then added to cultured cells in 96-well plate in triplicate and incubated for 24 h post treatment to determine the cytotoxic concentration 50 (CC_{50}). The medium was then removed, and the monolayer of cells washed with 1X PBS three times before adding MTT solution (20 μL/well of 5 mg/ml stock solution) and incubated at 37 ˚C for 4 h till formulation of formazan crystals. Crystals were dissolved using a volume of 200 μL of dimethyl sulfoxide (DMSO) and the absorbance measured at λ_{max} 540 nm using an ELISA microplate reader. Finally, the percentage of cytotoxicity compared to the untreated cells was determined. The CC_{50} of violuric acid and its Ru(III) complex was determined from a linear exponential equation.

[2] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol Methods, 65 (1983) 55–63. https://doi.org/10.1016/0022-1759(83)90303-4PMID: 6606682

1.8. Plaque reduction assay

The antiviral activity of violuric acid and its Ru(III) complex was determined by plaque reduction assay [3] with minor modifications. Briefly, Vero-E6 cells were seeded in 6-well culture plates (10^5 cells/ml) and incubated overnight at 37 ˚C under 5% CO_2 condition. Previously titrated SARS-CoV-2 was diluted to optimal virus dilution, which gave countable plaques, and mixed with the safe concentrations of violuric acid and its Ru(III) complex. The mixtures of virus and violuric acid and its Ru(III) complex were incubated for 1 h at room temperature. Growth medium was removed from the 6-well cell culture plates and virus-extract mixtures inoculated in duplicate. After 1 h contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose, 1% antibiotic antimycotic mixture, and 4% bovine serum albumin (BSA) (Sigma, St. Louis, Missouri, USA) were added to the cell monolayer then the plates were incubated at 37 ˚C for 3 days. The cells were fixed using 10% formalin solution for 1 h and the over layer was removed from each fixed well. Fixed cells were stained using 0.1% crystal violet in distilled water. Untreated virus was included in each plate as a control. Finally, plaques were counted and the percentage reduction in virus count recorded as follows:

Viral inhibition (%) = (viral count of untreated cells – viral count of the treated cells/viral count of untreated cells) × 100

[3] F. G. Hayden, R. G. Jr Douglas, R. Simons, Enhancement of activity against influenza viruses by combinations of antiviral agents. Antimicrob Agents Chemother, 18 (1980) 536–541. https://doi.org/10.1128/aac.18.4.536 PMID: 7447417

1.9. Molecular Docking

One of the most effective and efficient methods for determining drug-protein interactions is molecular docking. A molecular modeling investigation using the autodock tools version 1.5.7 was carried out to against four receptors hepatocellular carcinoma (HCC) suppressor (PDB ID: **8HN9**) [4], a dimeric form of MERS-CoV RBD (PDB ID: **7C02**) [5], topoisomerase II DNA (PDB ID: **1DL8**) [6] and cisplatin and

human serum albumin (PDB ID: **7WOK**) [7] . The 3D structures of these receptors were obtained from the protein data bank. The co-crystallized ligand is first separated from the receptor. Water molecules were removed from the receptor to prepare it for docking, after which hydrogen atoms and kollman charges were added, and the ligand was prepared by adding hydrogen atoms and then saved as pdbqt format. The docking capabilities of the programme were validated by redocking the original ligand using autodock version 4.2.6 at the same pocket site of the co-crystallized ligand and comparing the original and redocked ligand, the reference RMSD values for all receptors were about 2.00 Å.The results of the docking were analyzed using UCSF CHIMERA (version 1.16) software packages.

The molecular docking was carried out using Lamarckian genetic algorithm and 100 runs were performed per ligand and the metal complex structures obtained from powder XRD structural analysis were used for docking as discussed in experimental part in supplementary file. Ru(III) complex and violuric acid have not parameterized in the AutoDock force field, the Van der Walls (VdW) parameters for ruthenium were added to gpf file from parameter file AD4_parameters.dat and the parameters were as follows:

atom_par Ru 2.96 0.056 12.000 -0.00110 0.0 0.0 0 -1 -1 4 # Non H-bonding.

[4] DOI**:** https://doi.org/10.15252/embr.202256052.

[5] DOI: https://doi.org/10.1016/j.cell.2020.06.035.

[6] DOI: https://doi.org/10.1124/mol.58.3.649.

[7] https://doi.org/10.1016/j.ijbiomac.2022.06.181

Determination of binding energy scores

The binding energy scores were calculated from the values of intermolecular Energy, Final Total Internal Energy, Torsional Free Energy and Unbound System's Energy. For example the binding energy for Ru(III) complex against 7C02 protein was -6.07 kcal/mol and was calculated as follows:

Estimated Free Energy of Binding = -6.07 kcal/mol = (1) + (2) + (3) - (4)

Estimated Inhibition Constant, Ki = 35.61 μM

(1) Final Intermolecular Energy = -6.37 kcal/mol

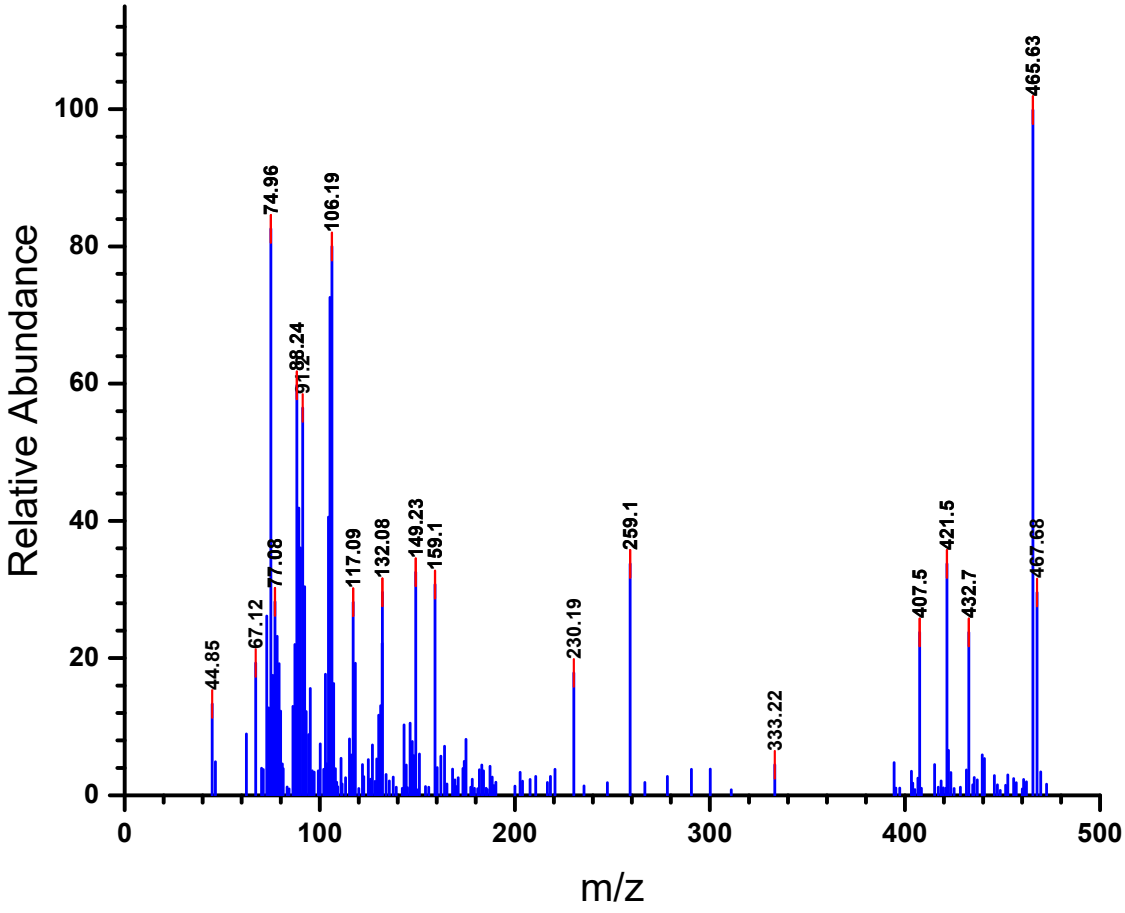
VdW + H-bond + de-solve Energy = -6.24 kcal/mol

Electrostatic Energy = -0.12 kcal/mol

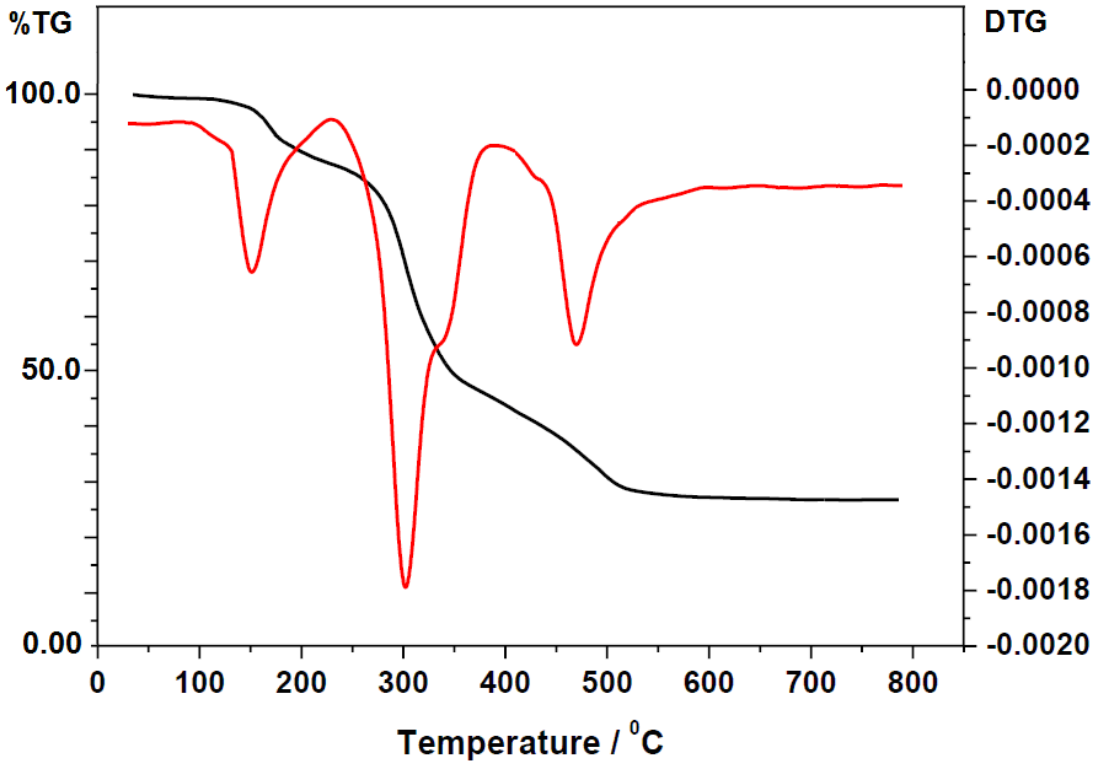
(2) Final Total Internal Energy = -0.07 kcal/mol

(3) Torsional Free Energy = + 0.30 kcal/mol

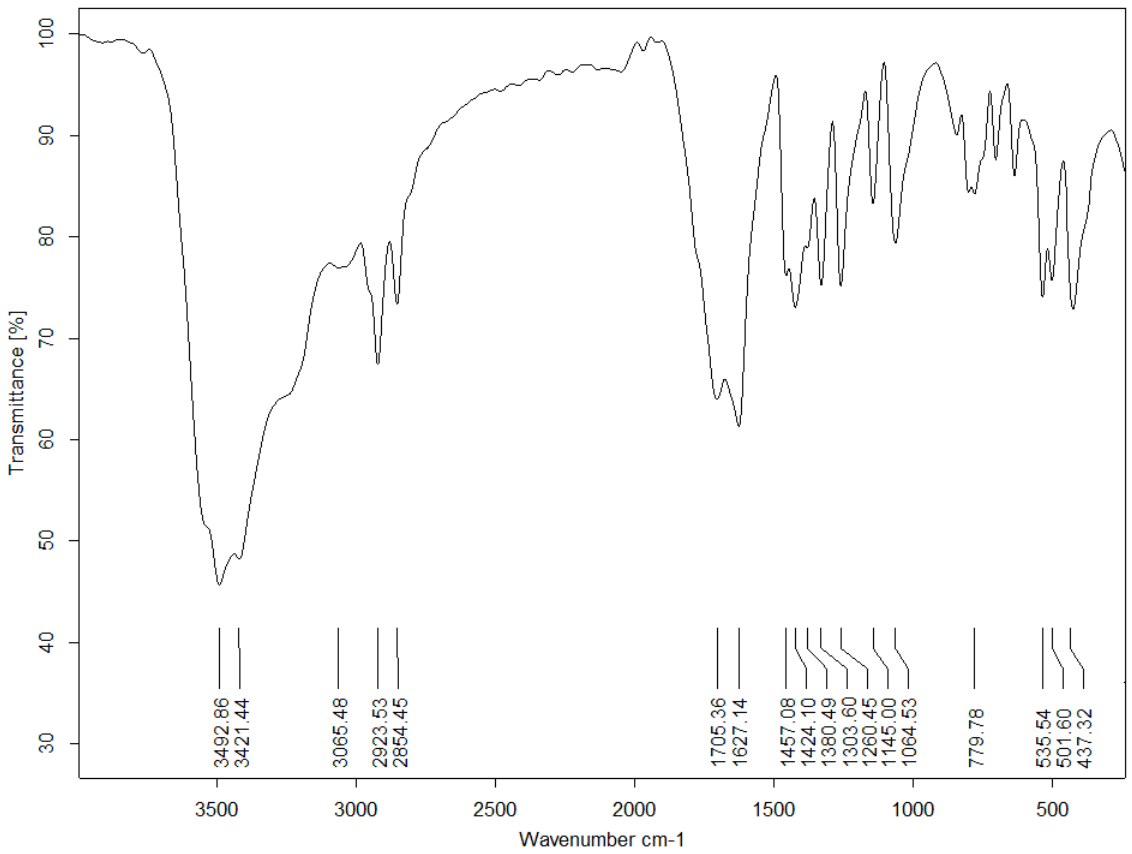
(4) Unbound System's Energy = -0.07 kcal/mol



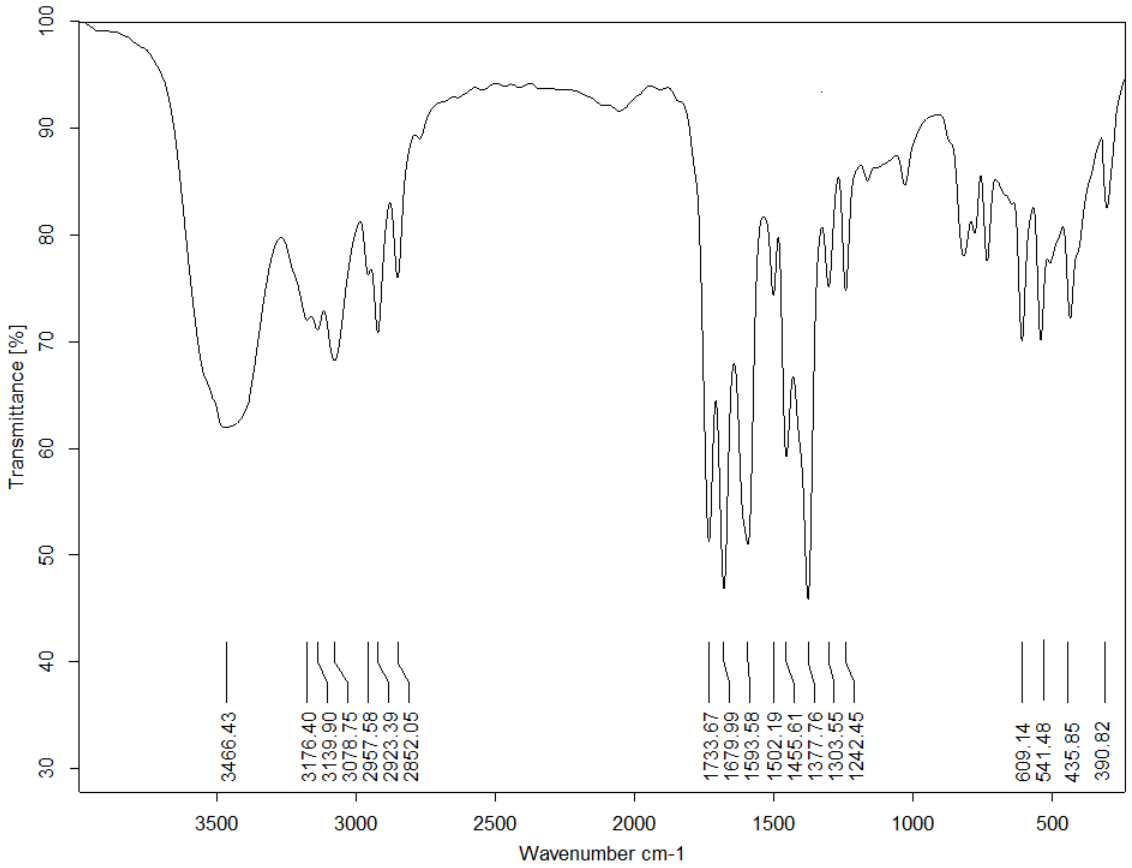
S2: EI-MS spectrum of [Ru(VA)$_2$ ClH$_2$O] complex



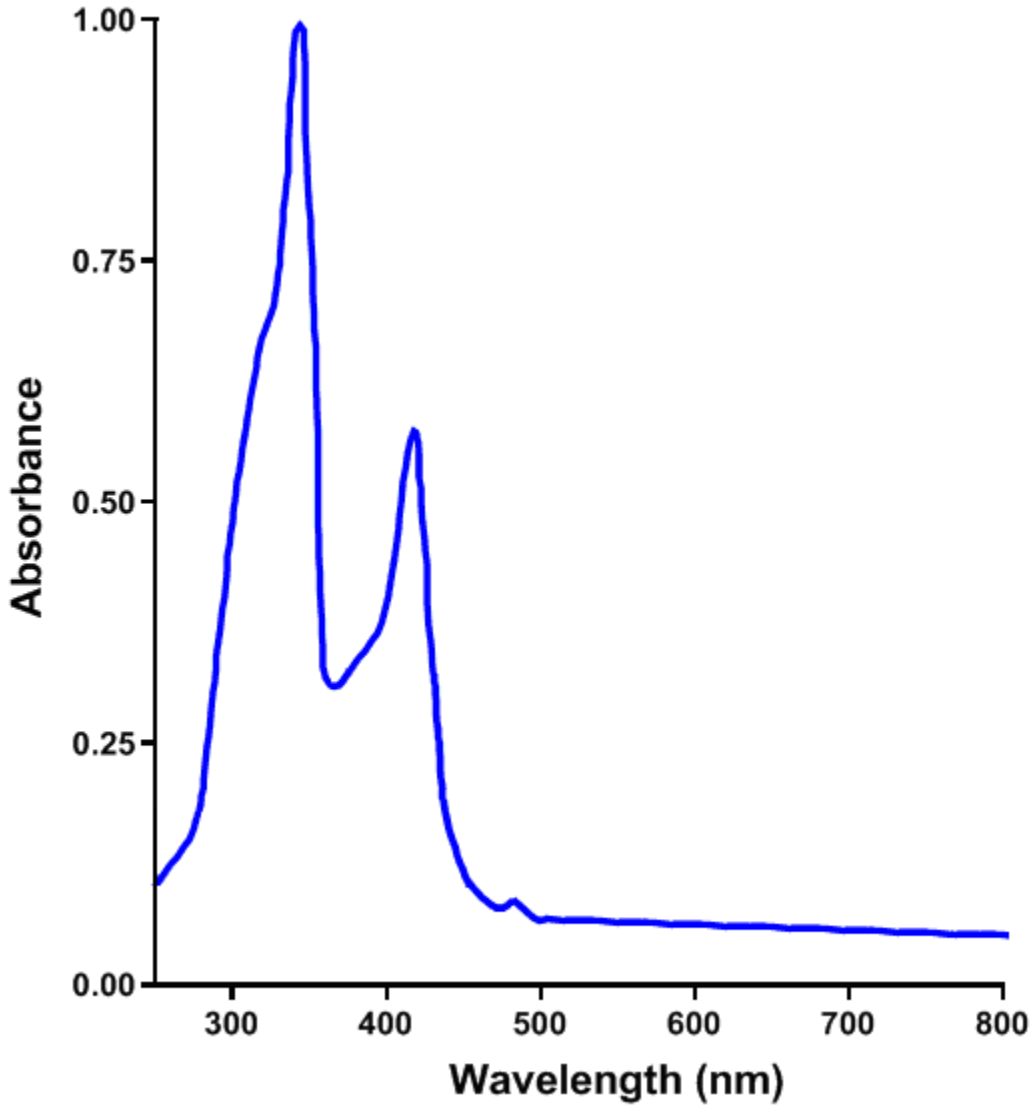
S3: Thermal analysis (TGA and DTG) curve of violurate – based Ru(III) complex



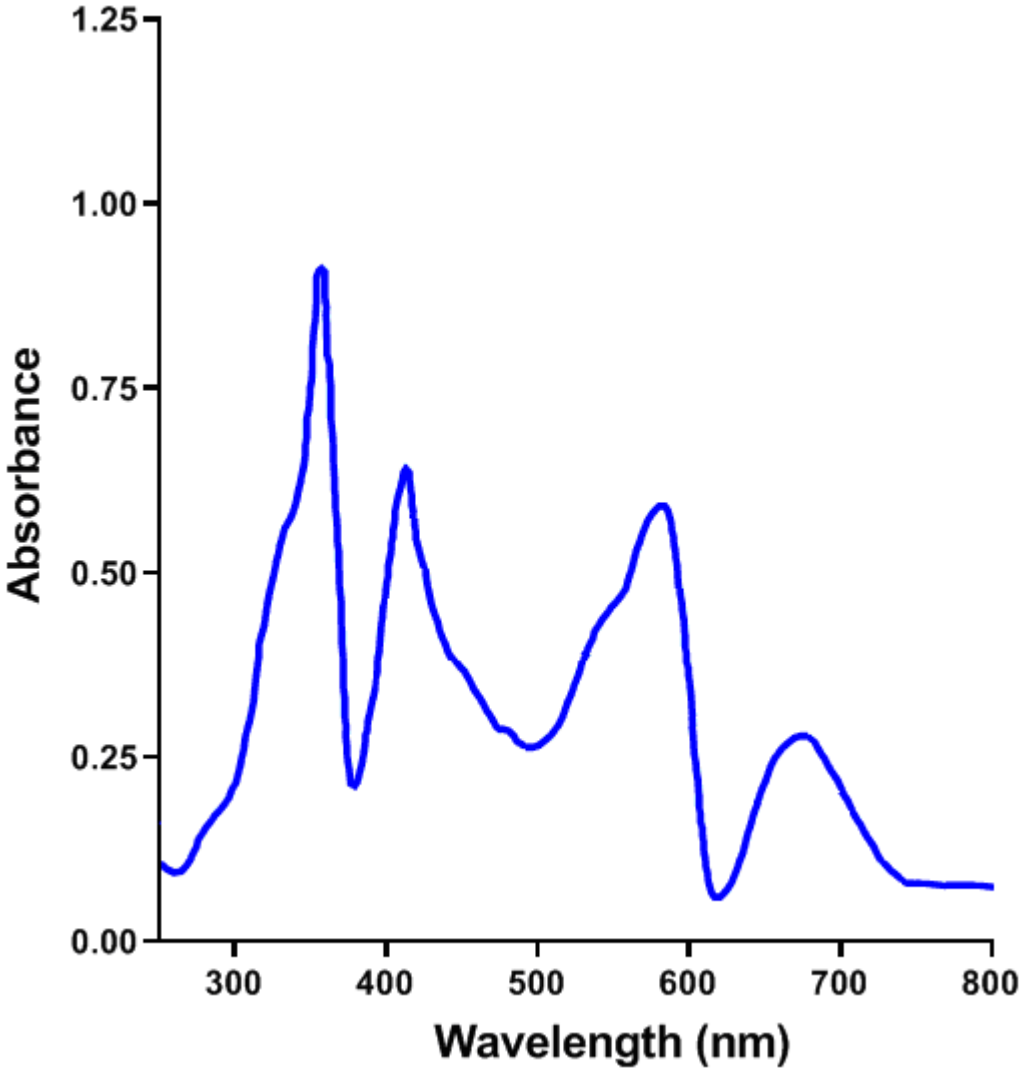
S4: FTIR spectrum of violuric acid



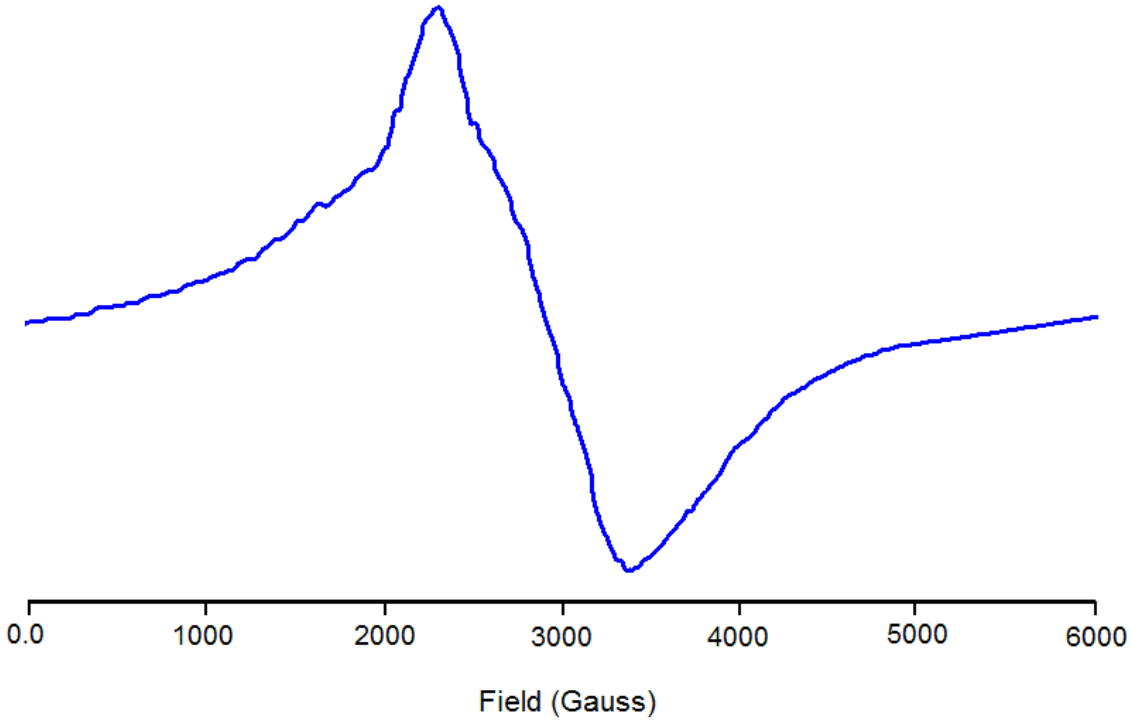
S5: FTIR spectrum of Ru(III) complex



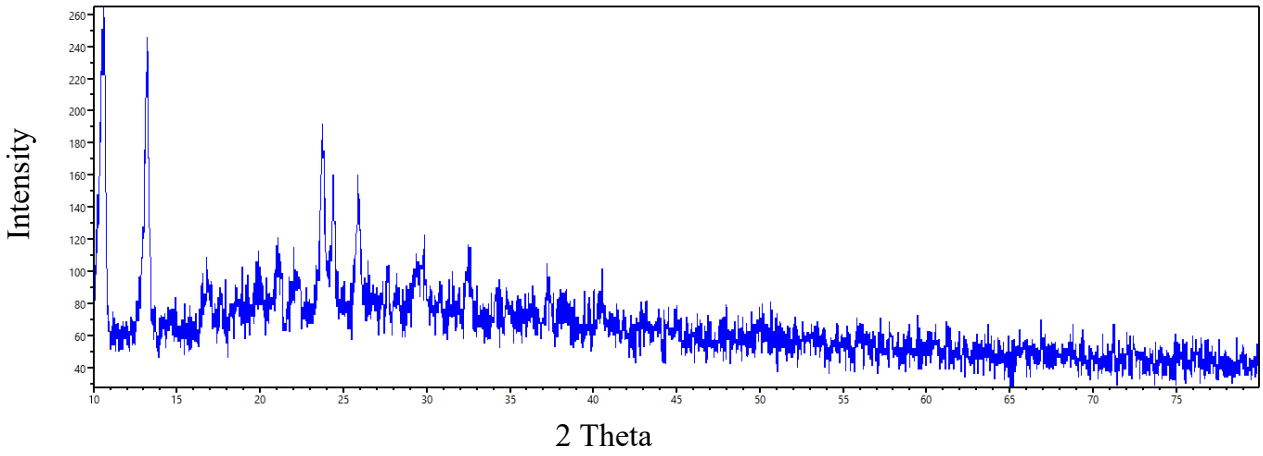
S6: Electronic absorption spectrum of violuric acid



S7: Electronic absorption spectrum Ru(III) complex



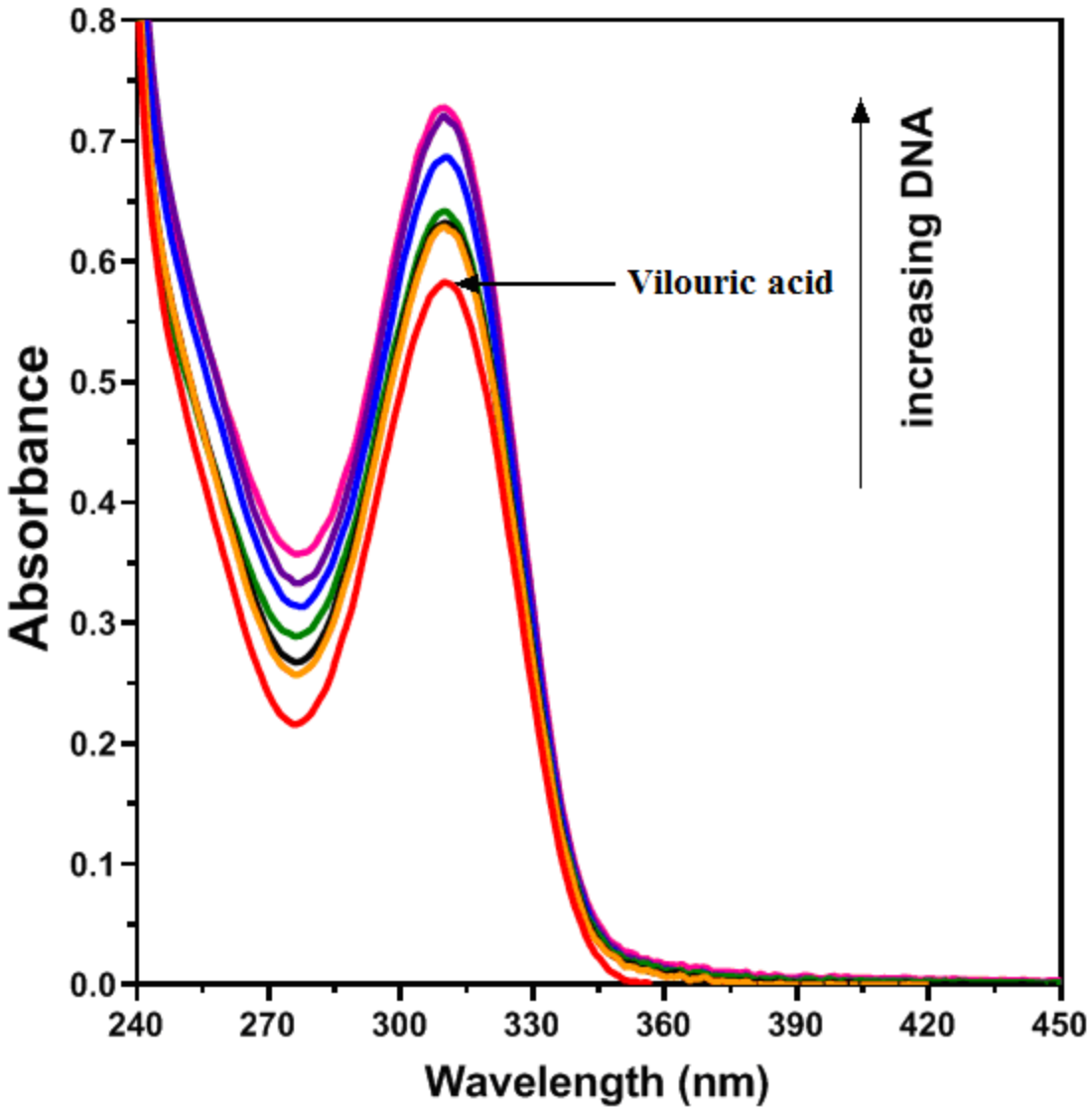
S8: ESR spectrum of Ru(III) complex



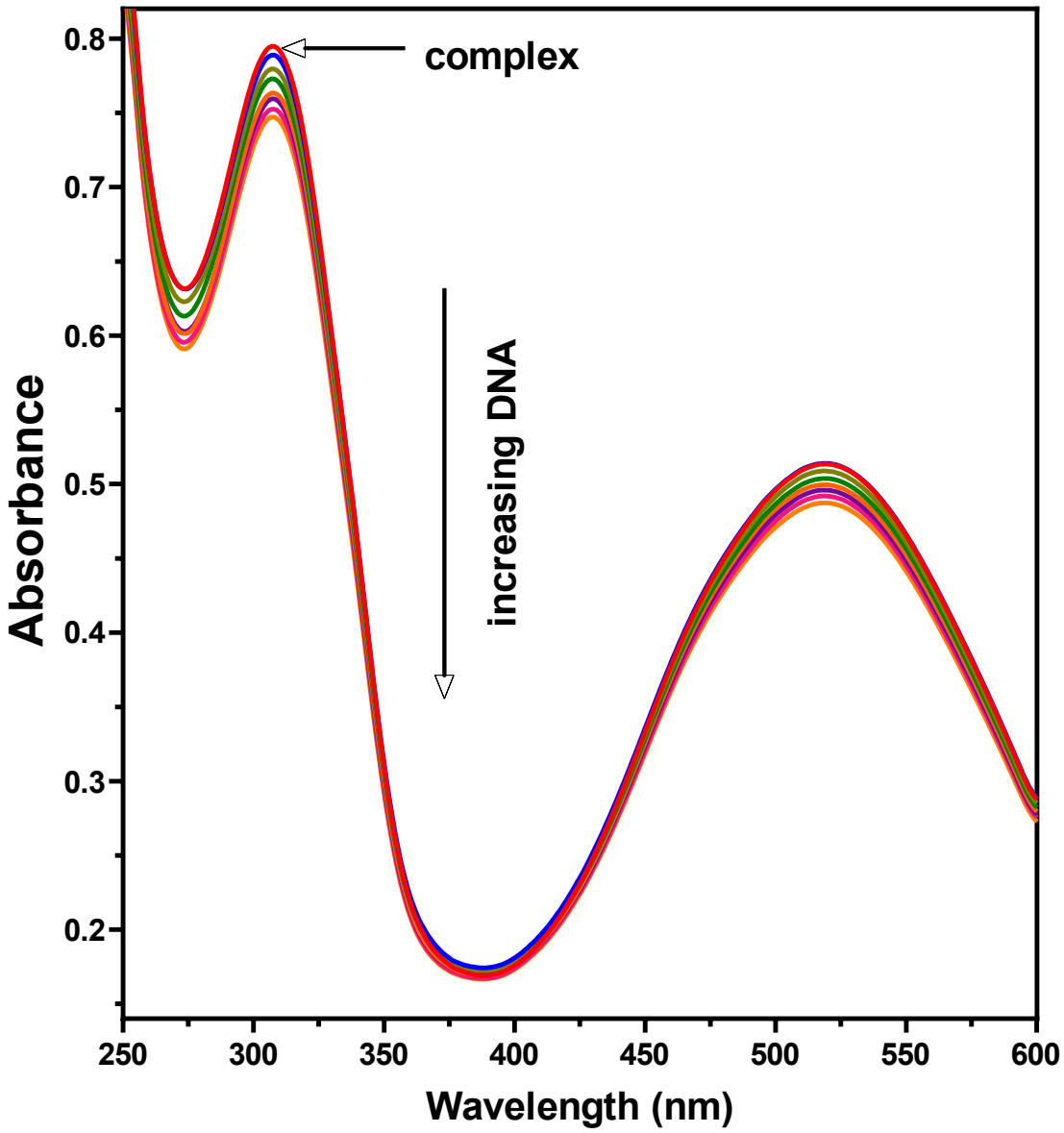
S9: PXRD spectrum of $[Ru(VA)_2\ ClH_2O]$ complex

S10: Crystallographic data of *bis*-(violurate)-based Ru(III) complex

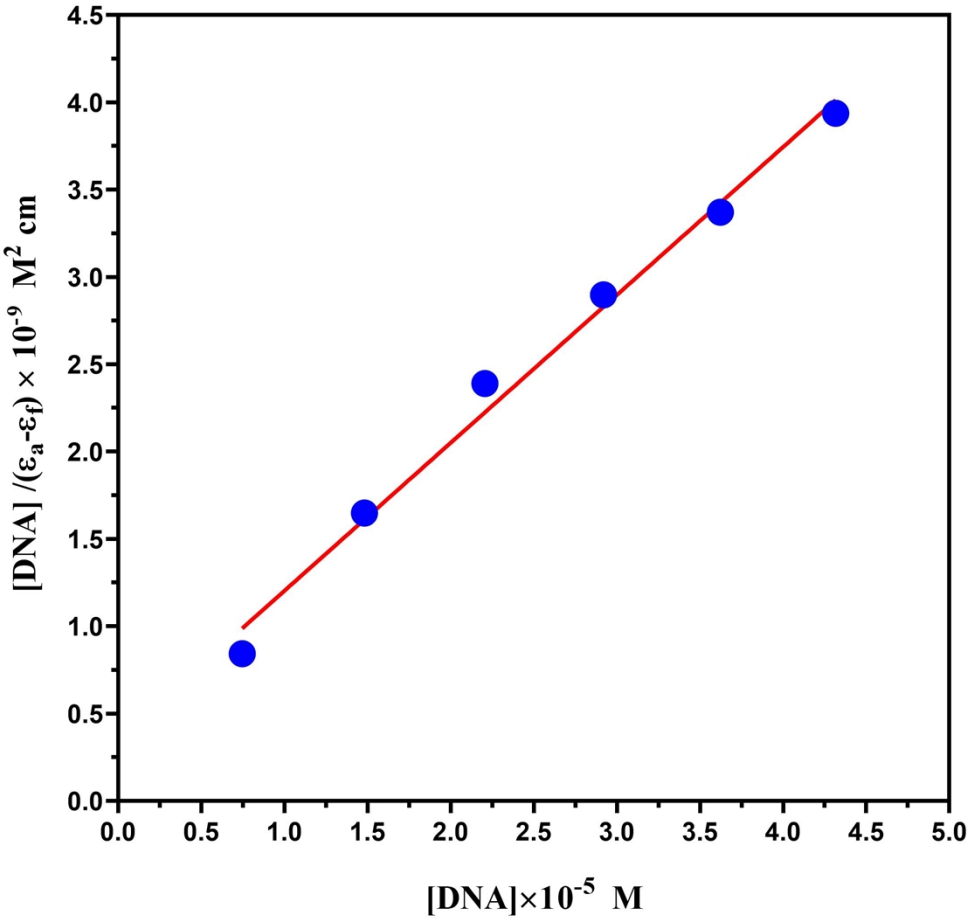
Empirical formula	C_8 H_6 Cl N_6 O_9 Ru
Formula weight	466.69
T (K)	276
λ (Å)	1.5406
Crystal system	Triclinic
Space group	P -1
Centro symmetry	Centric
Space Group Number	2
Z	2
Multiplicity	2
Bravais Lattice	P
Lattice Symbol	tP
Unit cell dimensions:	
a (Å), *b* (Å), *c* (Å)	11.832, 10.309, 7.057
α (°), *β* (°), *γ* (°)	100.360, 103.780, 73.310
Cell volume (Å^3)	795.11
Volume per atom (Å^3)	15.902
Calculated density (g/cm^3)	1.949
θ range for data collection (°)	10.00 – 80.00
Total reflection	945
Rietveld results:	
Rp	11.790
Rwp	14.870
R-Bragg	22.294
R-F	10.679



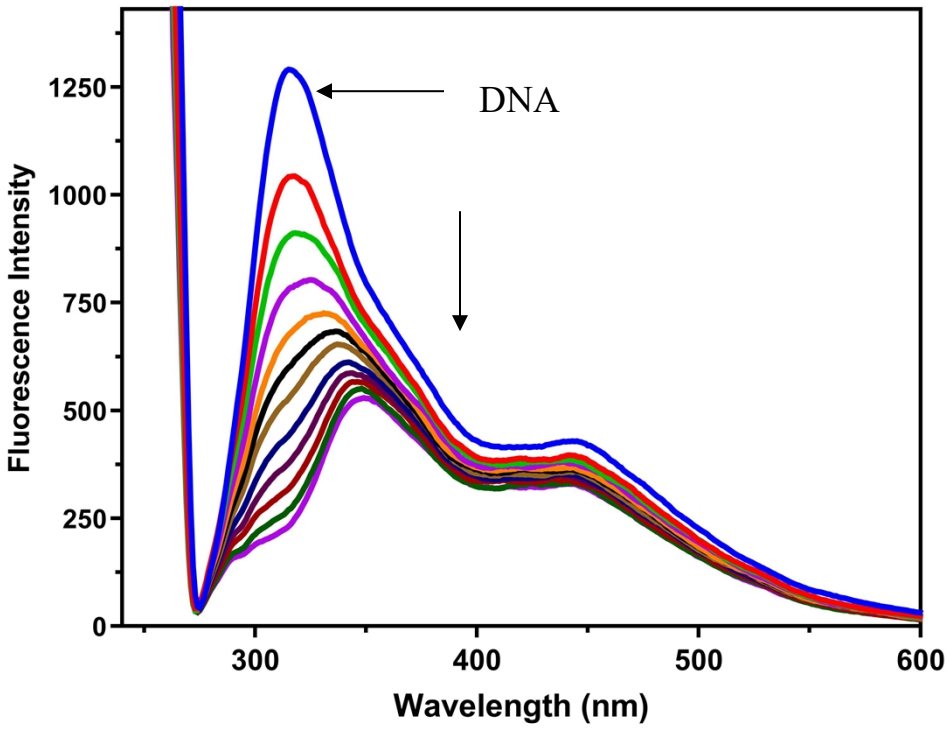
S11: Spectrograph of the spectrophotometeric titration of violuric acid (20 μM) and ct-DNA (5 - 45 μM) in Tris-HCl buffer (pH = 7.2).



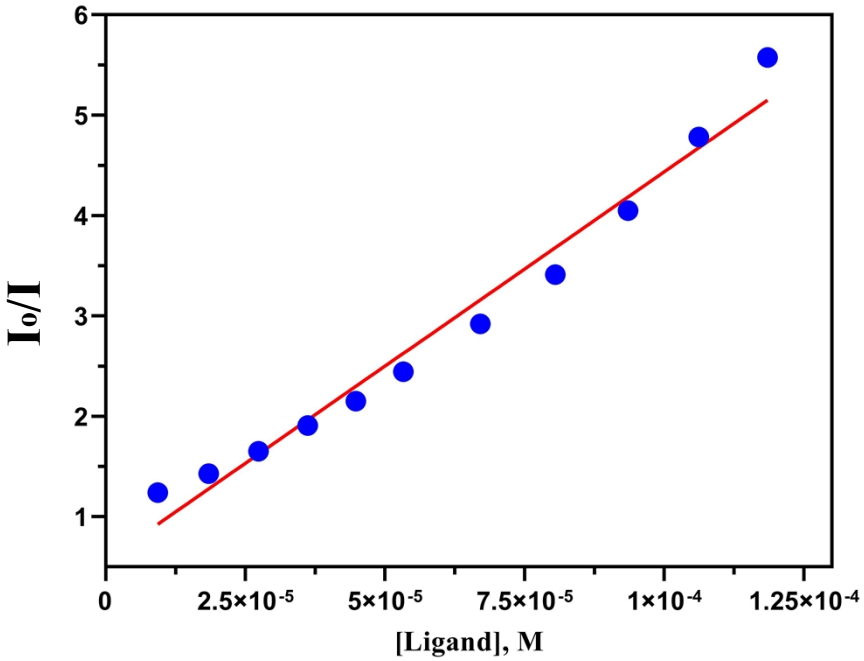
S12: The electronic absorption spectral profile of titration of $[Ru(VA)_2ClH_2O]$ complex by CT-DNA



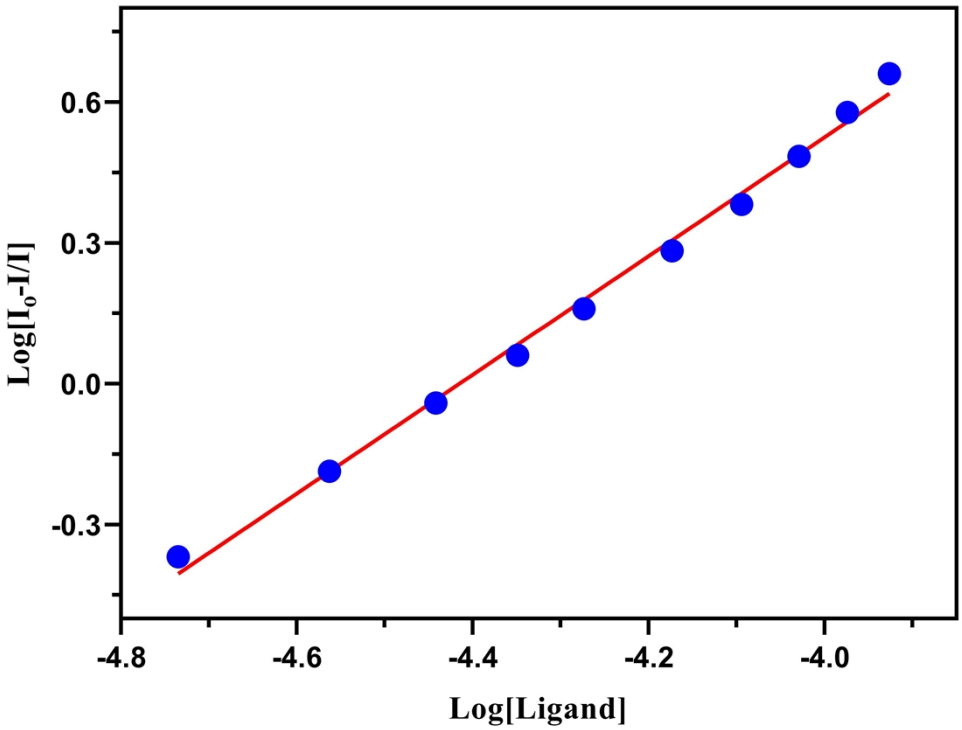
S13: Plot of [DNA]/(ε_a-ε_f) *versus* [DNA] for the DNA binding assay of violuric acid.



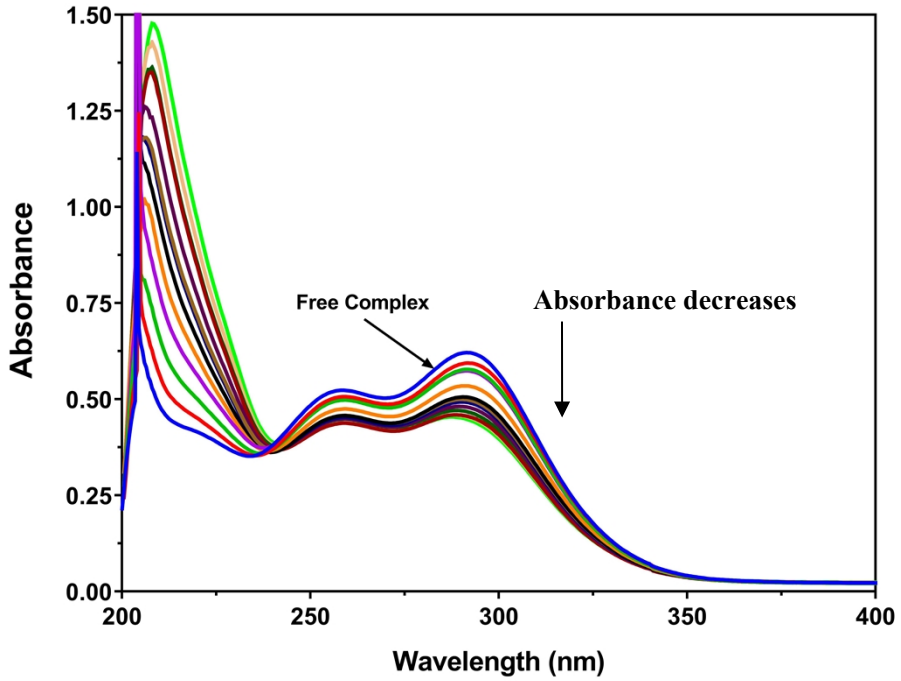
S14: Fluorescence spectral profile of DNA in Tris- HCl buffer with and without of violuric acid



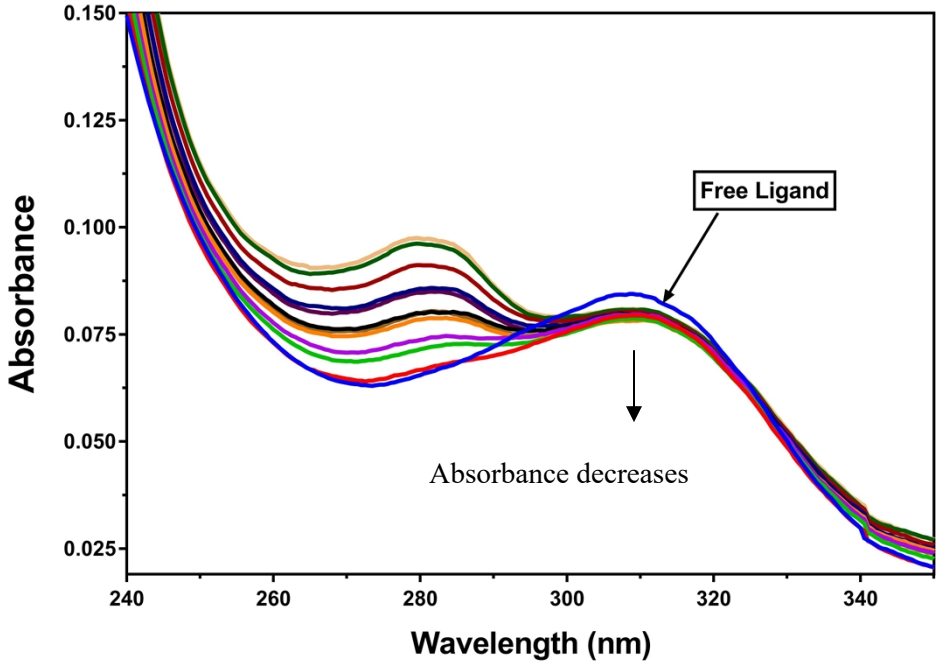
S15: Plot of I_0/I versus [violuric acid]



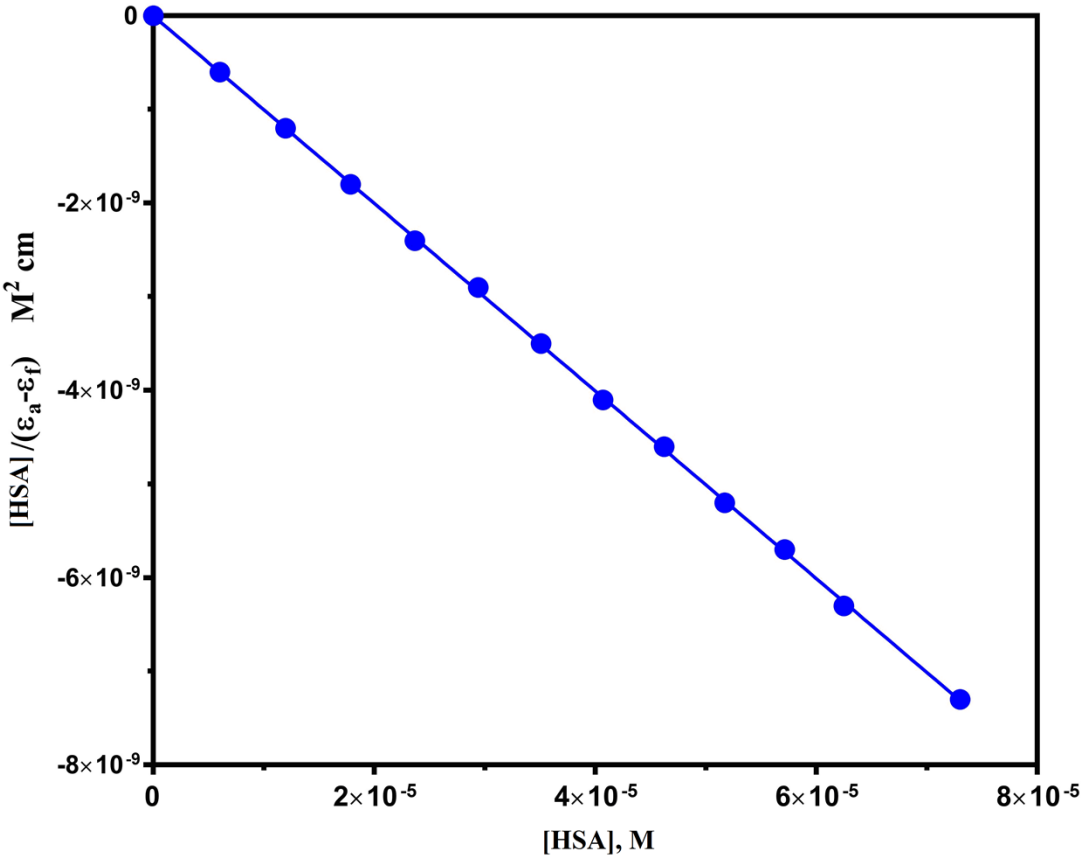
S16: Plot of log[I_o – I/I] versus log[violuric acid]



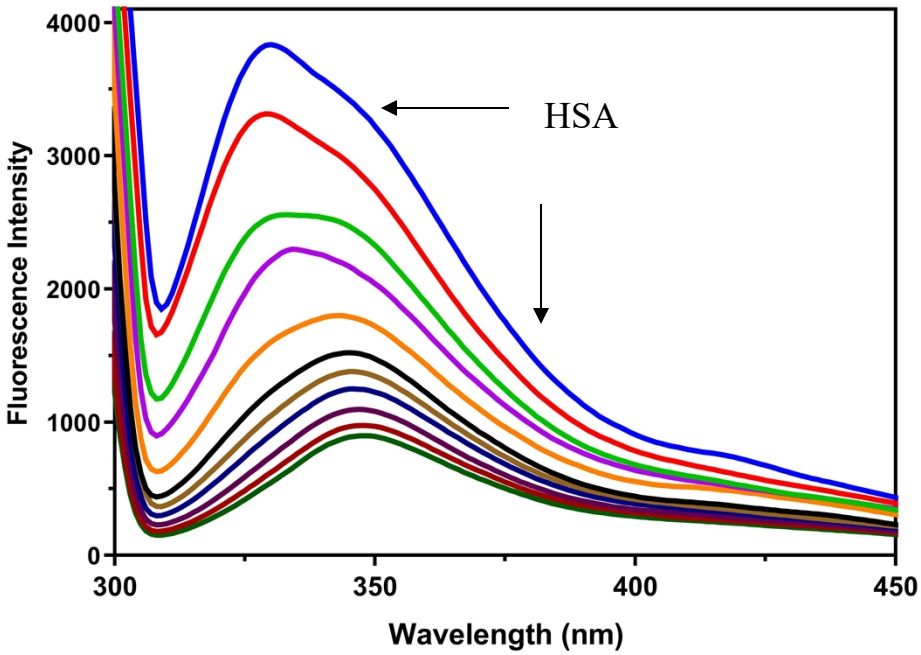
S17: The electronic absorption spectral profile of titration of $[Ru(VA)_2ClH_2O]$ complex by HAS



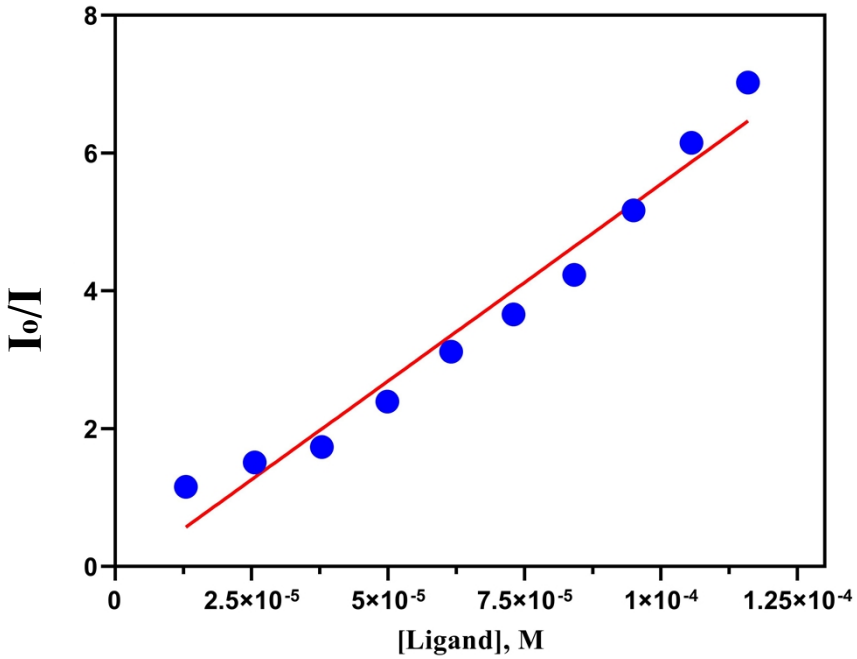
S18: The electronic absorption spectral profile of titration of violuric acid by HSA



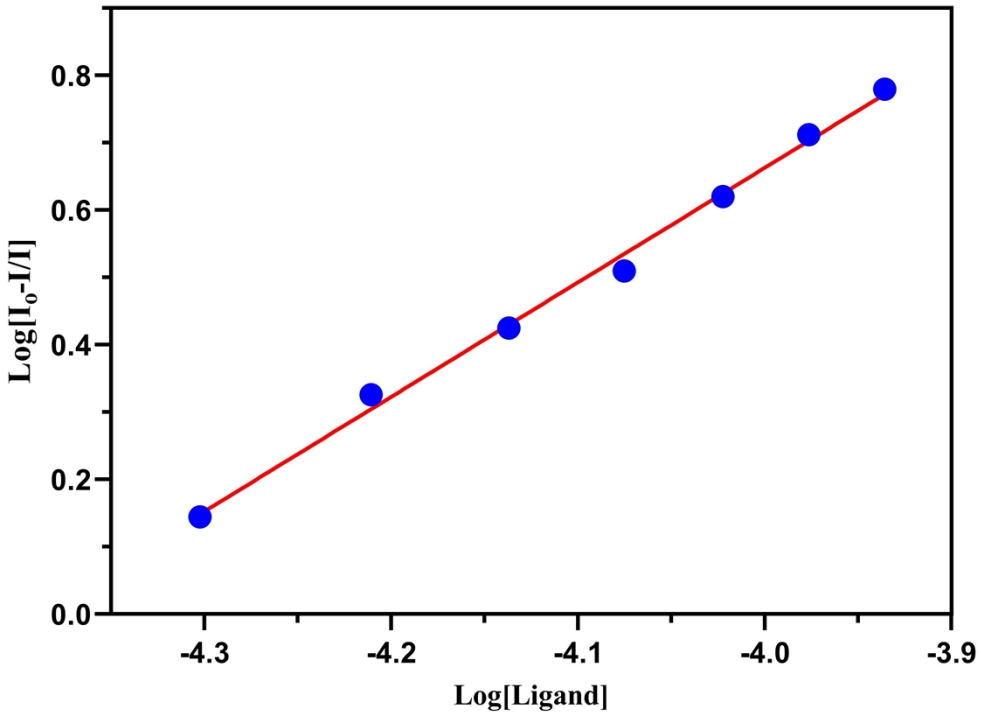
S19: Plot of [HSA]/($\varepsilon_a - \varepsilon_f$) versus [HSA] for the titration of HSA with violuric acid



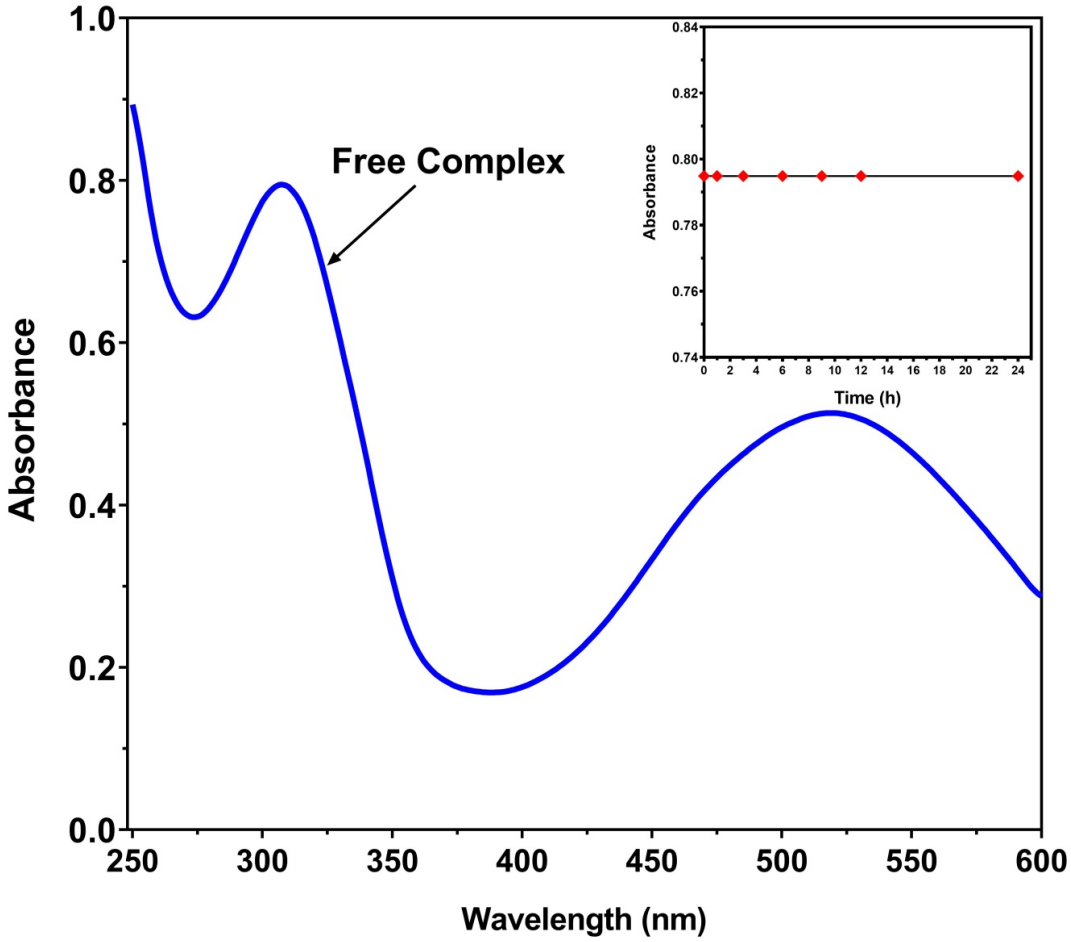
S20: Fluorescence spectral profile of HSA in Tris- HCl buffer with and without of violuric acid



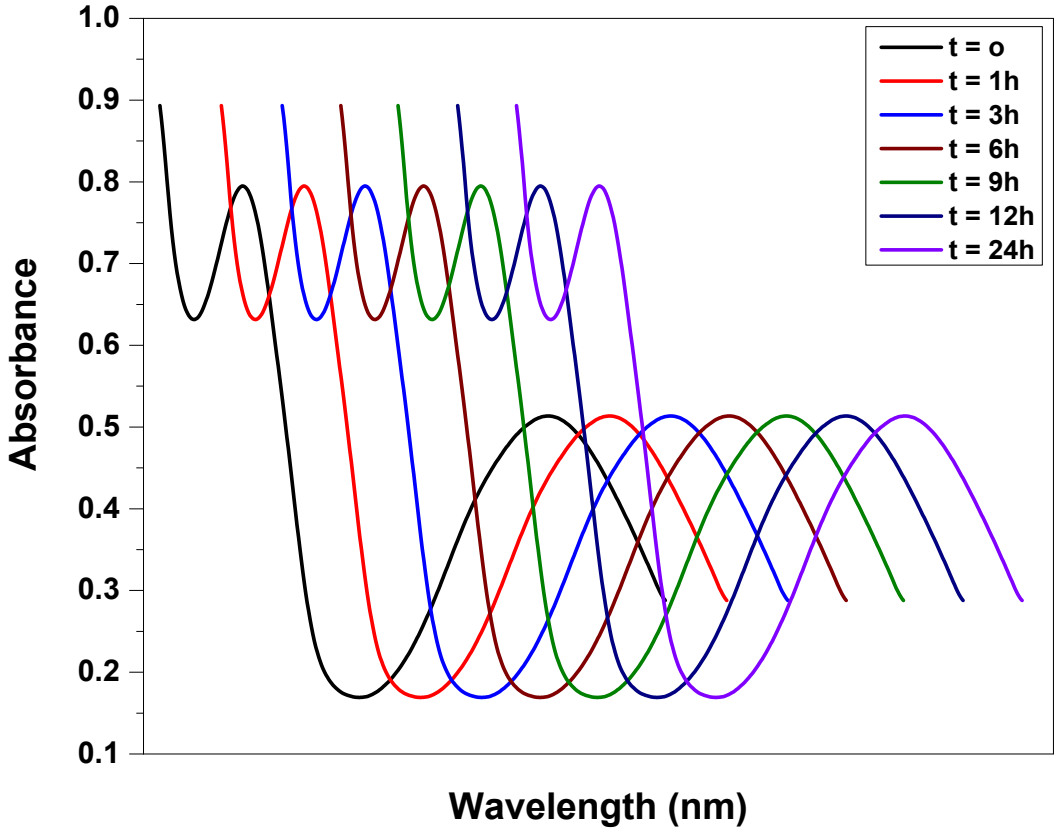
S21: Plot of I_o/I versus [violuric acid]



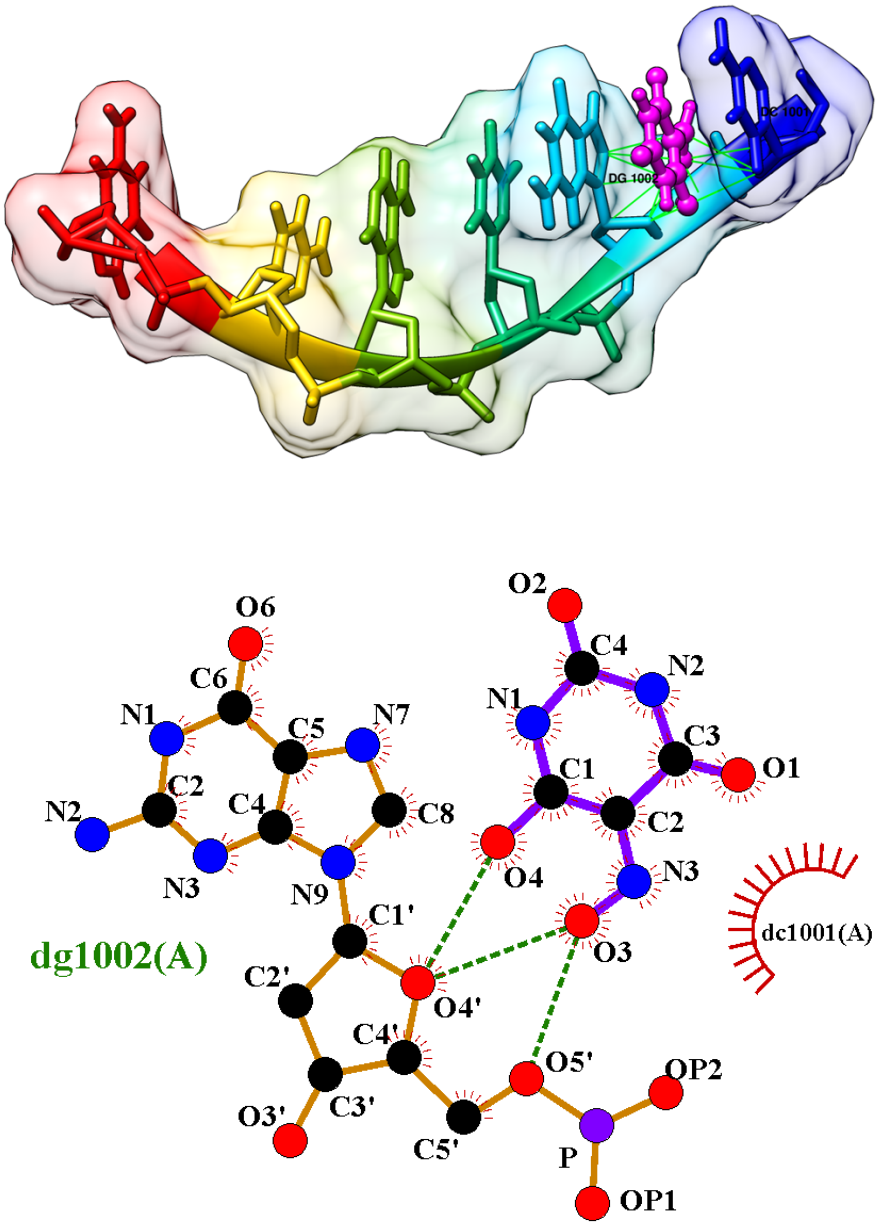
S22: Plot of log[I_o – I/I] versus log[violuric acid]



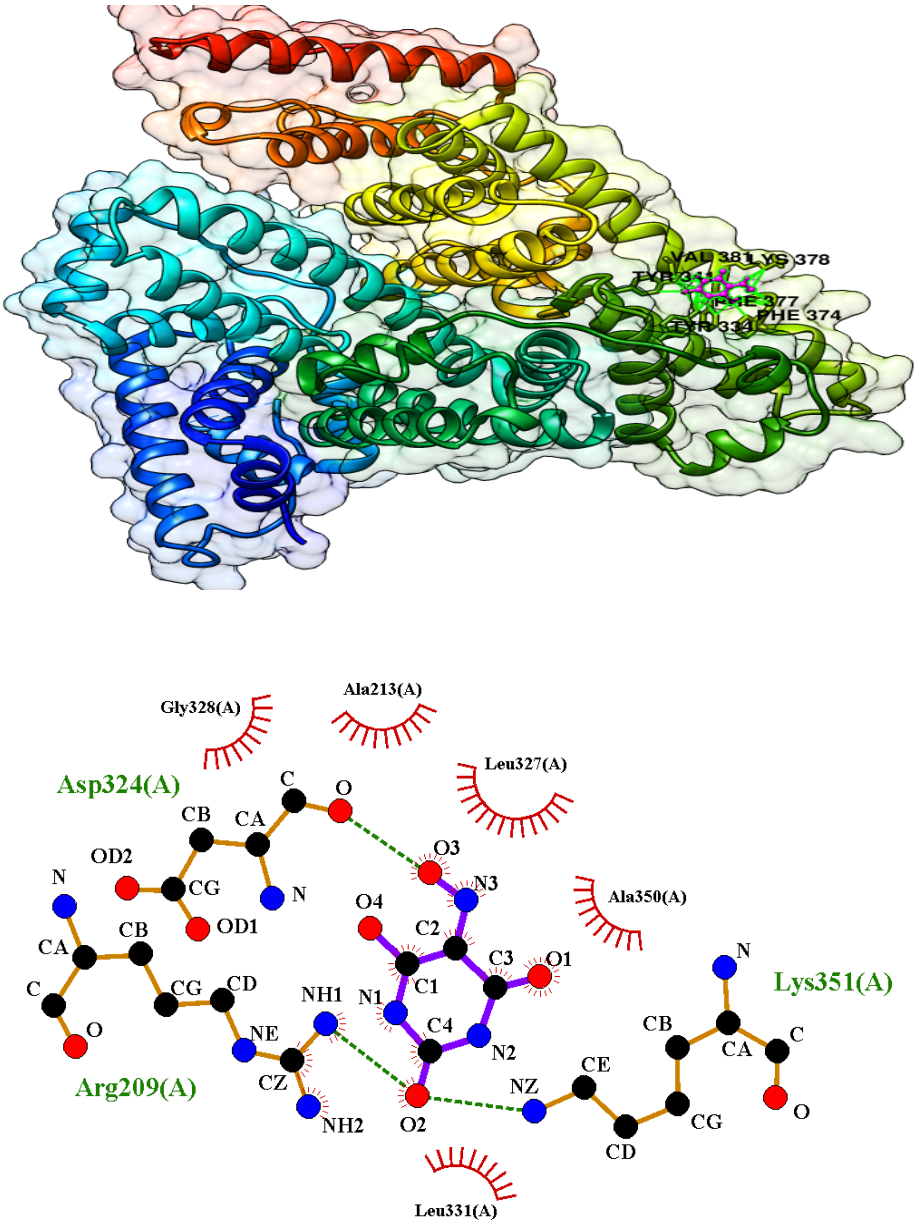
S23: The electronic absorption spectral profile of Ru(III) complex in tris-HCl buffer solution at 307 and 550 nm



S24: The electronic absorption spectral profile of Ru(III) complex in tris-HCl buffer solution at 307 and 550 nm over time periods ranging from zero minutes to 24 hours



S25: 2D and 3D diagrams show the interaction between violuric acid and active sites of **1DL8** protein. Hydrogen bonds are shown as a green line, while amino acid residues that participate in H-bonding and hydrophobic interactions are marked with green and black fonts, respectively.



S26: 2D and 3D diagram show the interaction between violuric acid and active sites of **7WOK** protein. Hydrogen bonds are shown as a green line, while amino acid residues that participate in H-bonding and hydrophobic interactions are marked with green and black fonts, respectively.