Synthesis, theoretical investigations, biocidal screening, DNA binding, *in vitro* cytotoxicity and molecular docking of novel Cu(II), Pd(II) and Ag(I) chlorobenzylidene Schiff base: Promising antibiotic and anticancer agents

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S1. Material and instrumental

2-aminophenol, 4-chlorobenzaldehyde, $CuCl_2.2H_2O$ (99.00%), $Pd(OAc)_2$ (99.99%), and $AgNO_3$ (99.50%) were utilized and purchased from Sigma–Aldrich Chemise (Germany). The important reagents for DNA interaction studies, Calf-thymus DNA (CT-DNA), 2-amino-2-hydroxyl methyl-propane-1, 3- diol (Tris) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich. CT-DNA dissolved in Tris-HCl buffer (pH=7.2) which was prepared using deionized water. Tris-HCl buffer (pH=7.2) is used to control the pH of the reaction system. TAE Buffer (Tris-acetate-EDTA) (50X), Top Vision Agarose, ethidium bromide solution (10 mg/mL), 6X DNA Loading Dye and 100 bp DNA Ladder are utilized in gel electrophoresis and purchased from Thermo Fisher Scientific.

The studied compounds were characterized by measuring melting points by using a melting point device (Gallenkamp, UK), IR spectra by using a Shimadzu FTIR-8300 spectrophotometer, ^{1}H NMR, and ^{13}C NMR spectra by using a Bruker Advance DPX-500 spectrometer where the investigated compounds were dissolved in deuterated dimethyl sulfoxide (DMSO) solution. All of the scanning UV–visible spectra in DMF were recorded using Quawell (UV-Vis spectrophotometer Q5000). Elemental analyses were conducted on an elemental analyzer (PerkinElmer 240c) at the main laboratory of Cairo University. Thermogravimetric analysis was conducted with a heating rate 5 °C min^{-1} with DTG 60H Detector. Magnetic susceptibility was measured using a Gouy balance and diamagnetic corrections were executed by Pascal's constants. Molar conductivity measurements were performed utilizing a JENWAY 4510 conductivity meter. Mass spectra were measured using DI analysis Shimadzu Qp-2010 plus at the micro-analytical center at Cairo university. The values of absorbance each complex were determined at various pH levels. The pH levels were calculated utilizing a series of Britton universal buffers. The pH values were measured by using an ADWA AD 1020 and AD 1000 pH meter at 298 K that it is

equipped with a CL-51B combined electrode and calibrated with standard buffers (pH 4.02 and 9.18) before measurements.

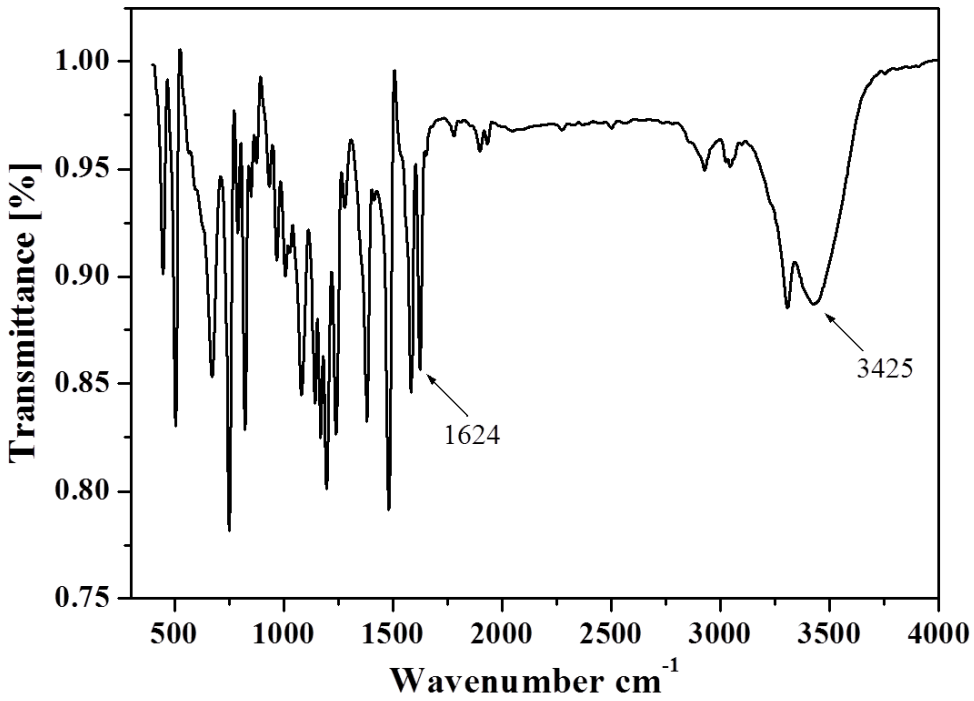

Figure S1: IR spectrum of the ligand HL.

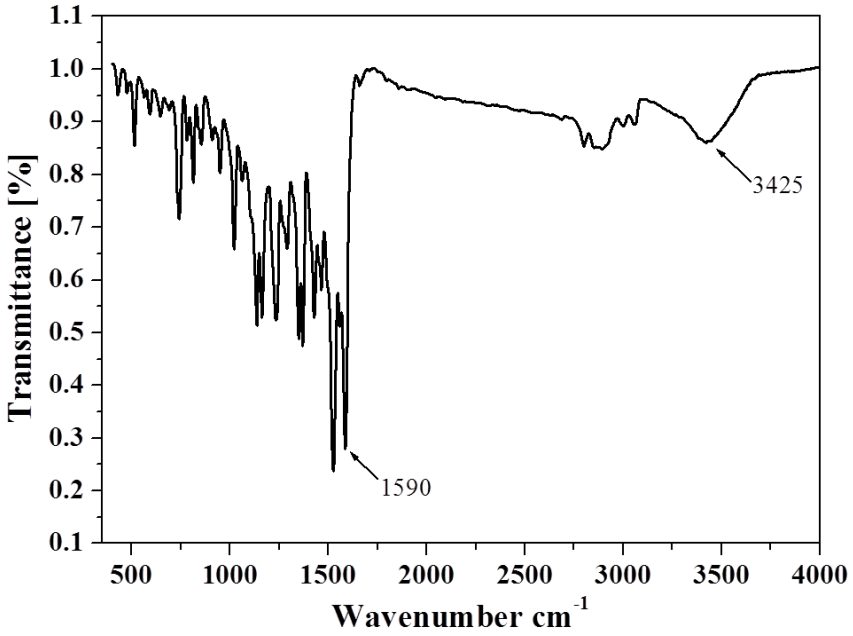

Figure S2: IR spectrum of the PdL complex.

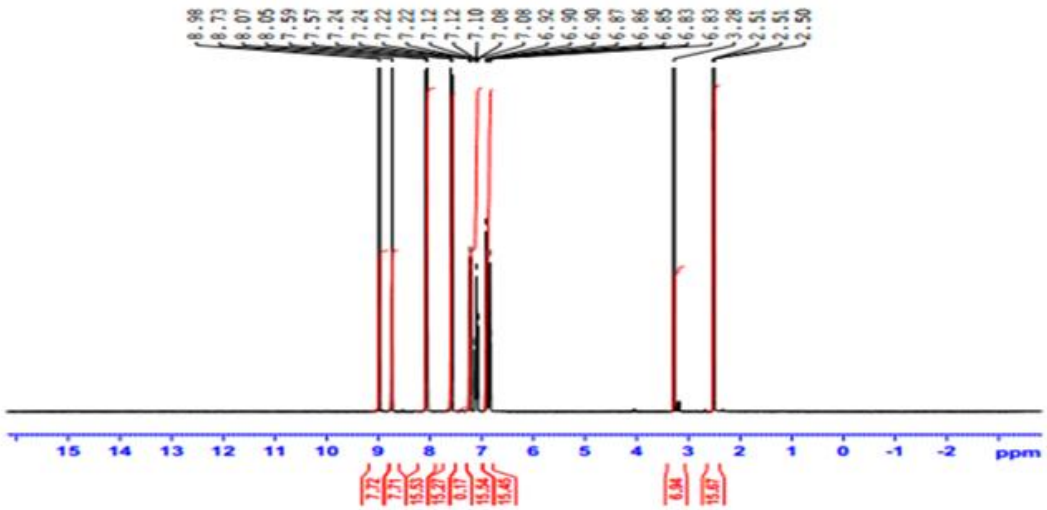

Figure S3: ^{1}H NMR spectrum of the ligand HL.

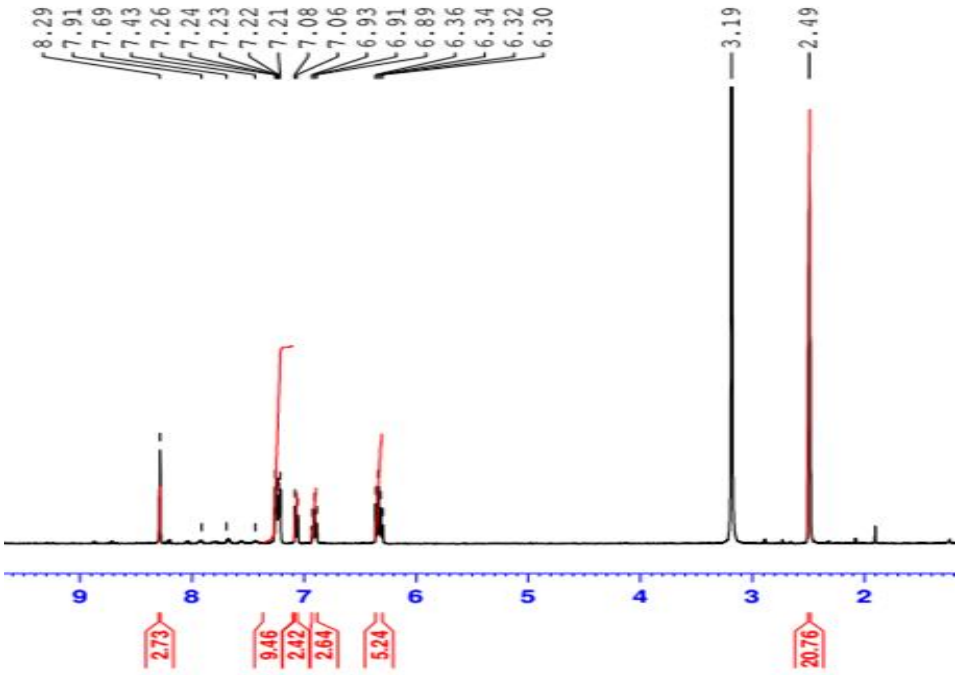

Figure S4: ^{1}H NMR spectrum of the PdL complex.

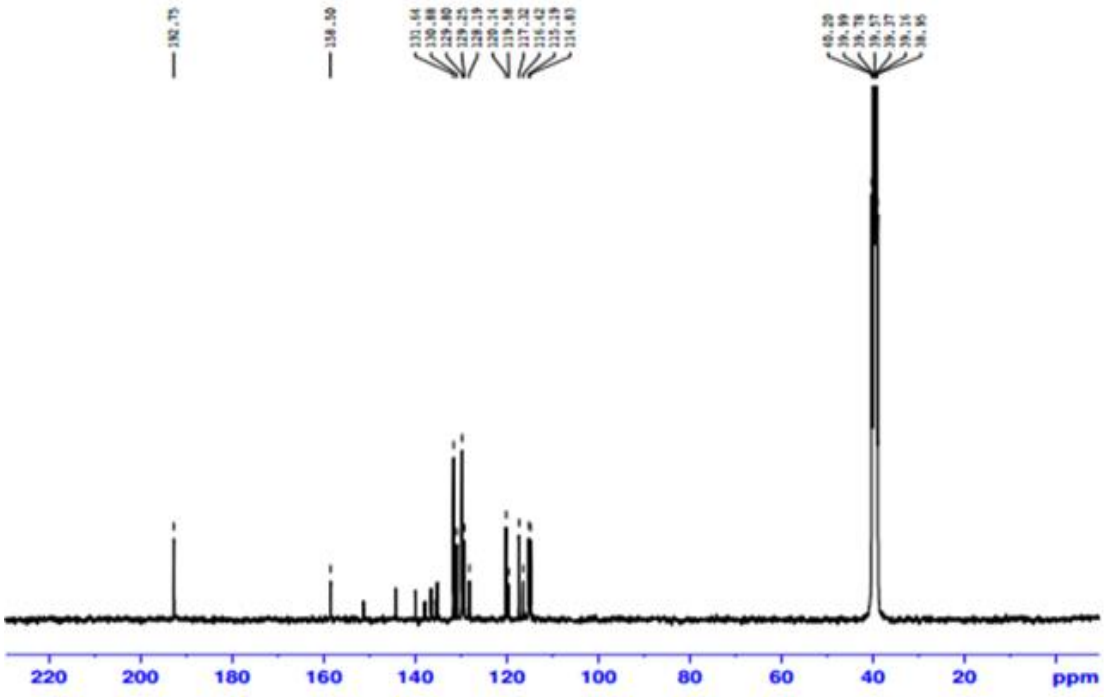

Figure S5: ^{13}C NMR spectrum of the ligand HL.

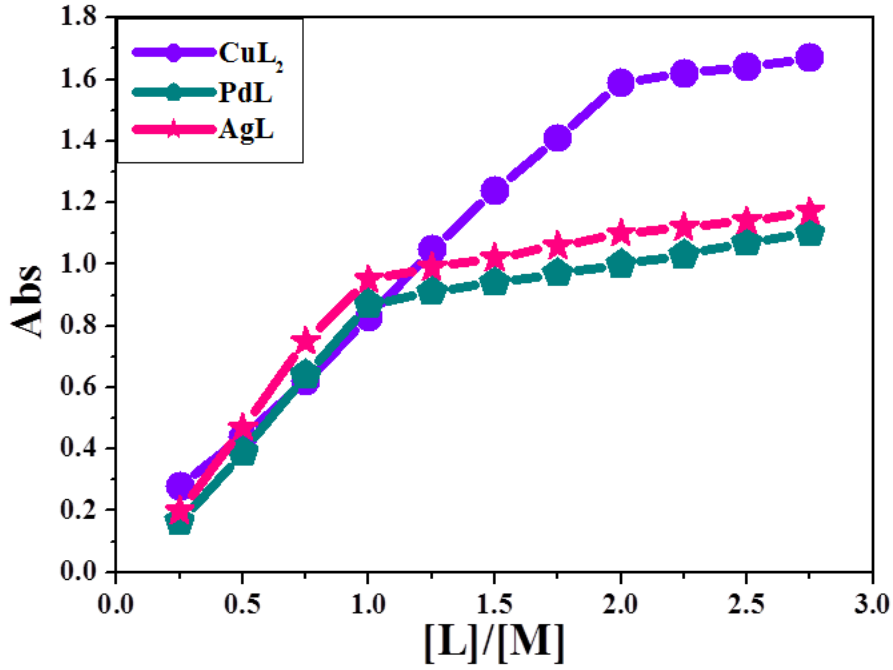

Figure S6: The curve of molar ratio method of the prepared complexes in aqueous-ethanol medium at [M]= [HL] = 10^{-3} M at 298 K.

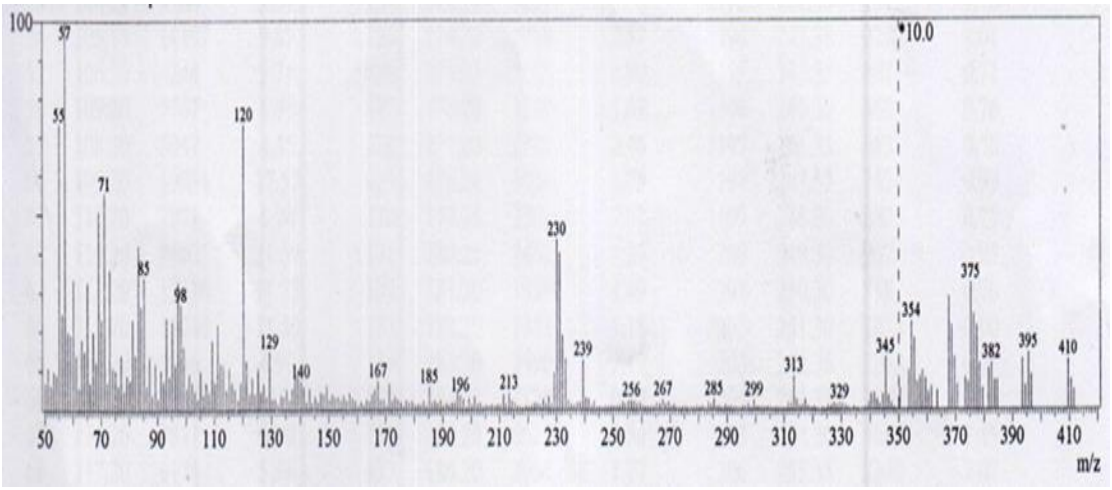

Figure S7: mass spectrum of the PdL complex.

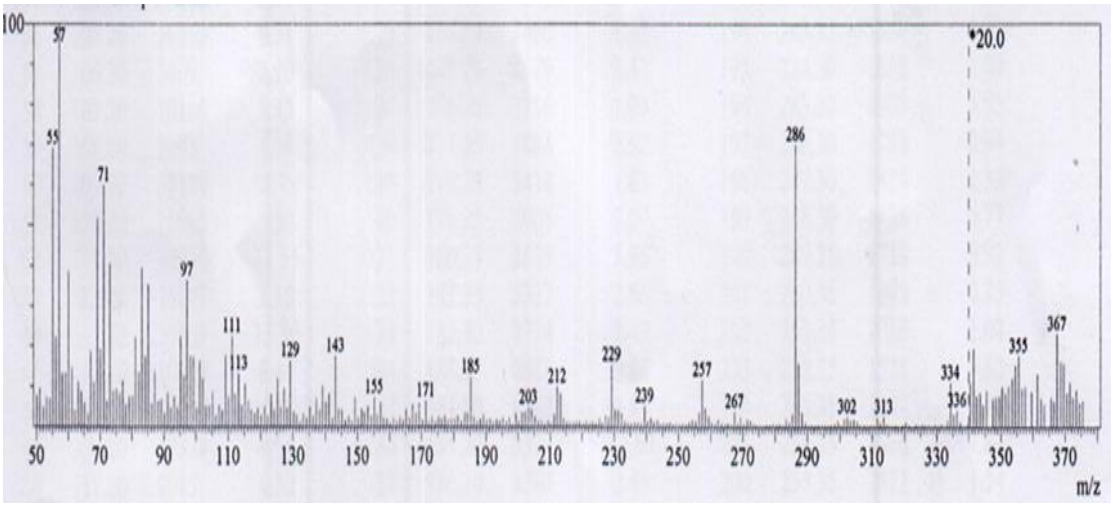

Figure S8: mass spectrum of the AgL complex.

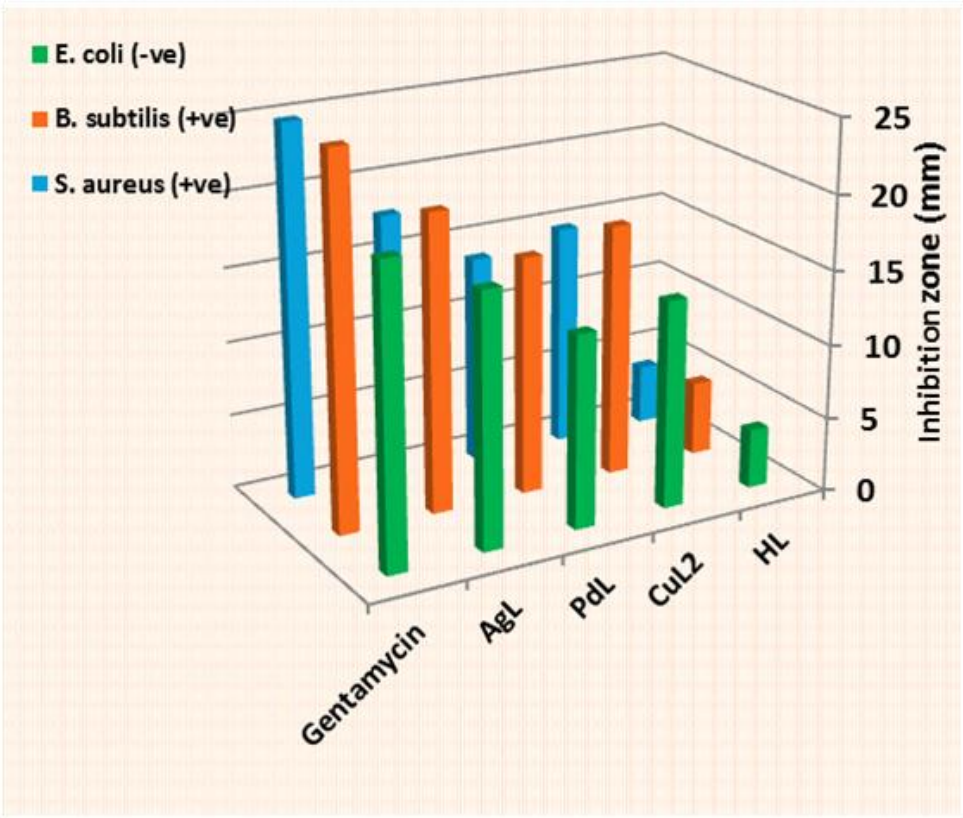

Figure S9: Histogram showing the comparative antibacterial activities of the ligand HL and its imine complexes with concentration 10 mg ml^{-1}.

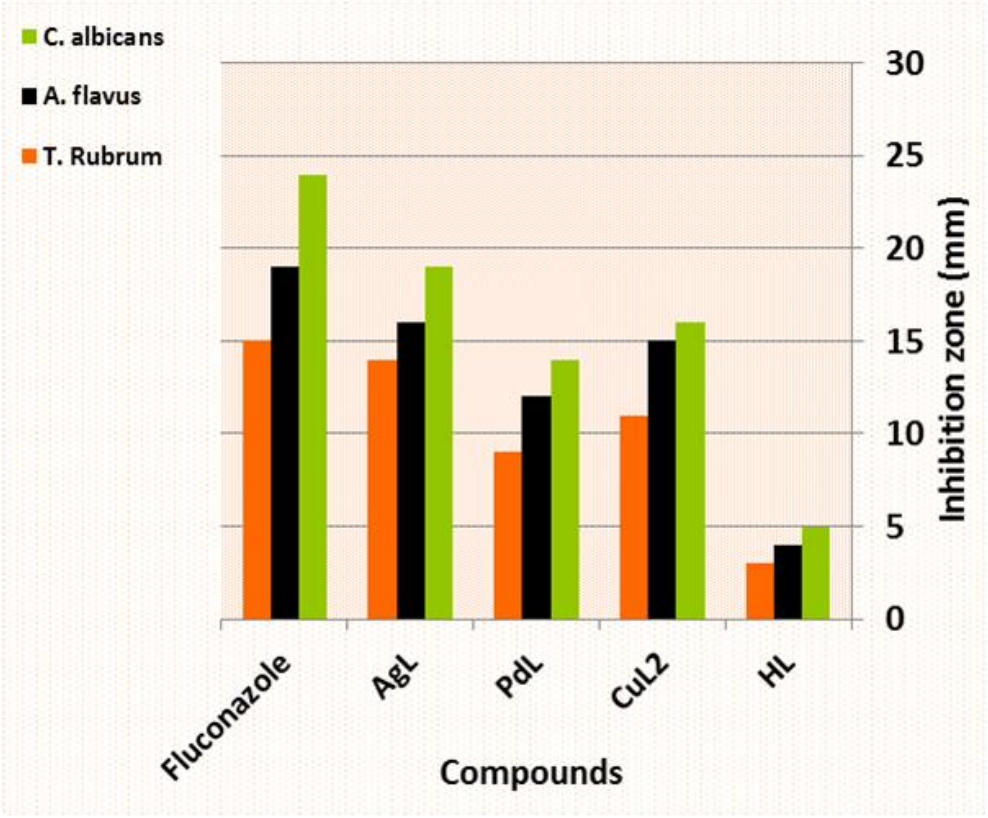

Figure 10: Histogram showing the comparative antifungal activities of the ligand HL and its complexes with concentration 10 mg ml^{-1}.

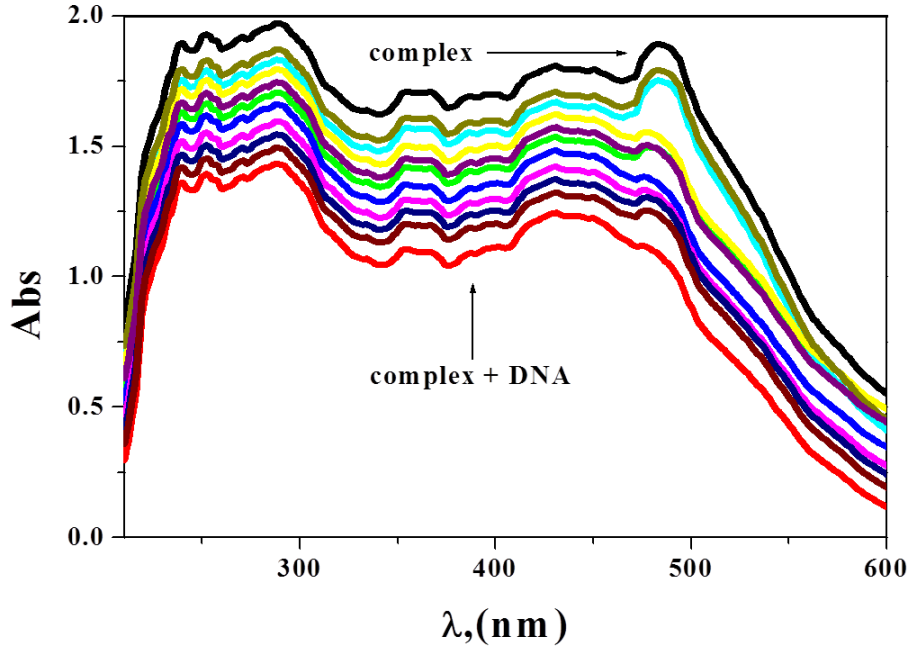

Figure S11: Electronic absorption scans for binding DNA with CuL_2 complex (10^{-3} M) in 0.01 M tris buffer (pH=7.2, 298 K) with CT-DNA (0–100 μM, from bottom to top).

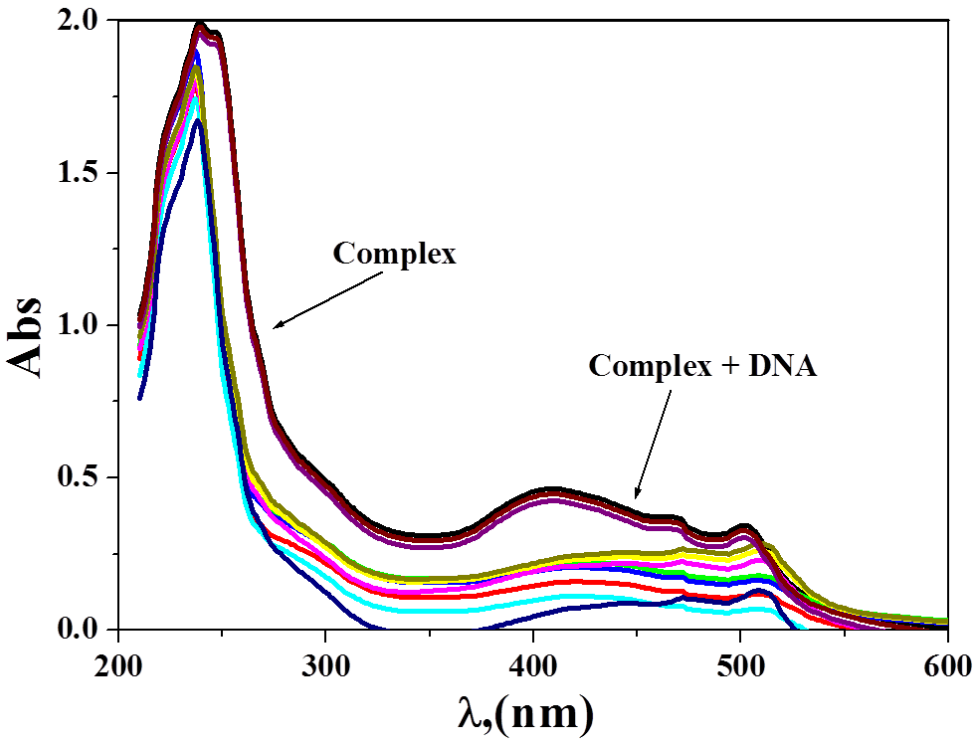

Figure S12: Electronic absorption scans for binding DNA with AgL complex (10-3 M) in 0.01 M tris buffer (pH=7.2, 298 K) with CT-DNA (0–100 μM, from bottom to top).

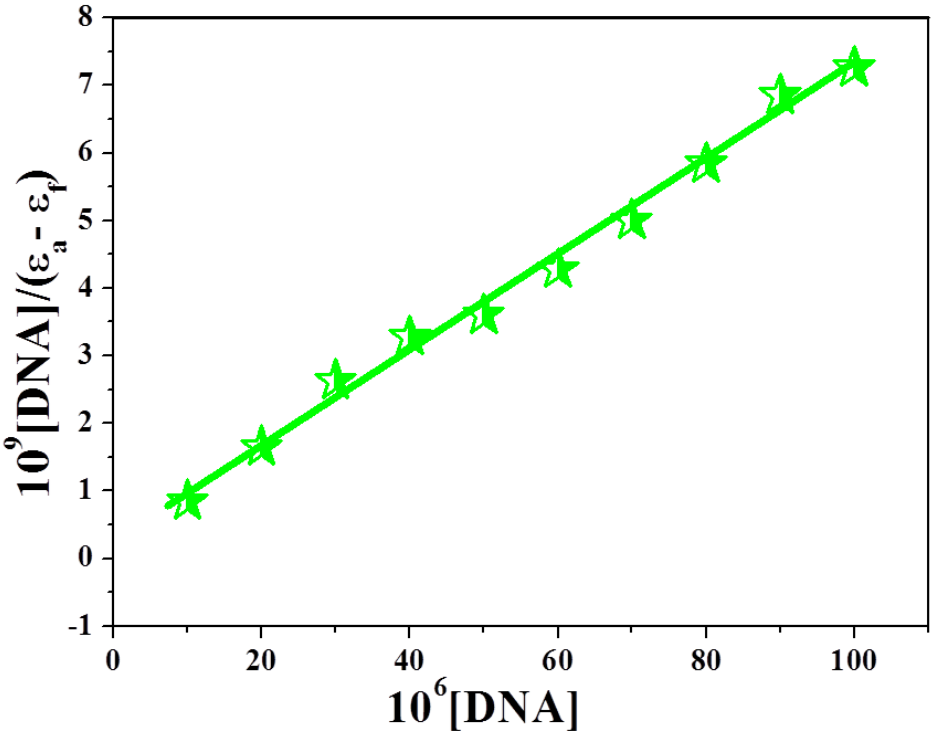

Figure S13: plott [DNA] / (ε_a–ε_b) versus [DNA] for binding DNA with CuL_2 complex.

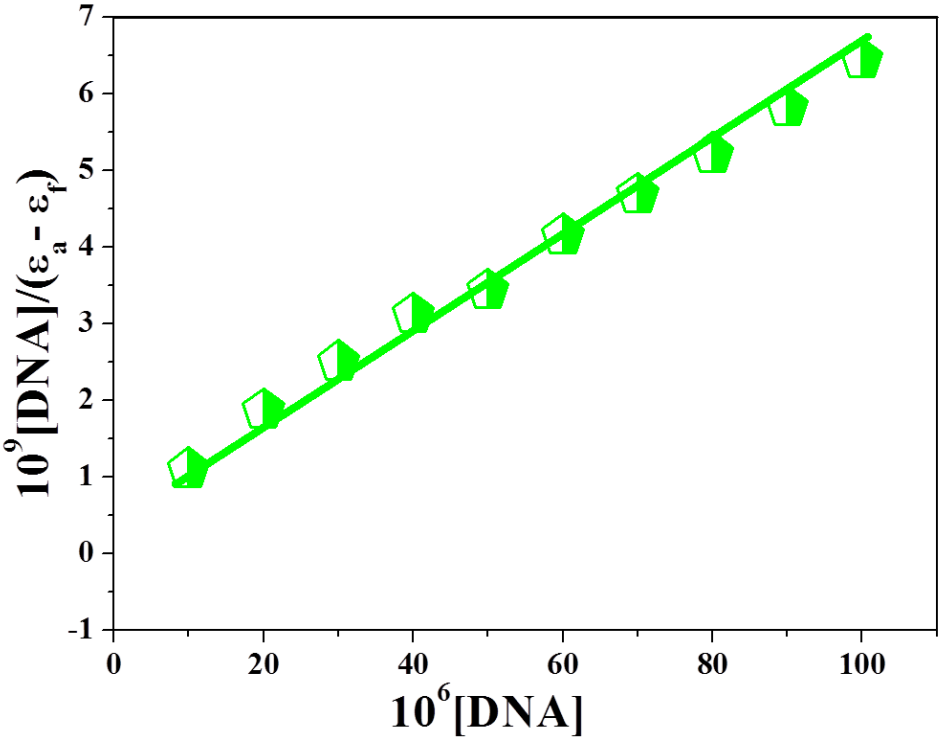

Figure S14: plott [DNA] / (ε_a–ε_b) versus [DNA] for binding DNA with PdL complex.

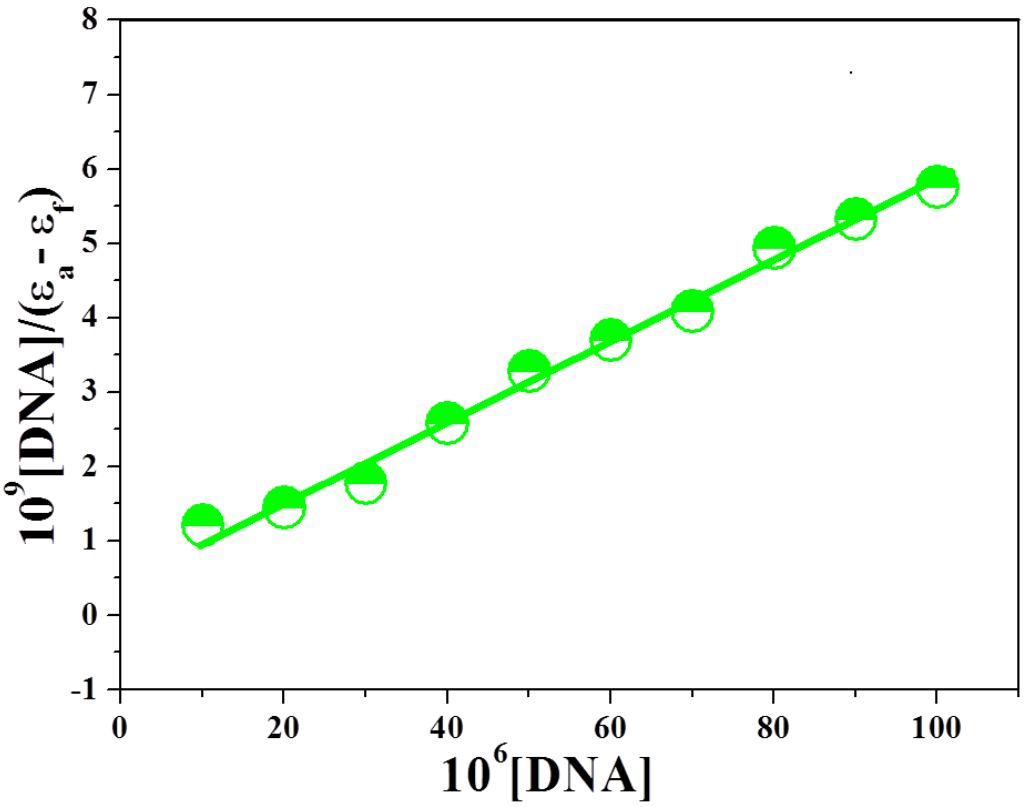

Figure S15: plott [DNA] / (ε_a–ε_b) versus [DNA] for binding DNA with AgL complex.

Table S1: Ground state geometrical parameters of the studied complexes using B3LYP/GENECP

Bond length Aº Bond angels º	**CuL_2**	**PdL**	**AgL**
M - O12		**1.866**	**2.217**
M - O16	**1.749**		
M - O18	**1.937**		
M - O26		**1.954**	
M - O27			**2.336**
M - O28			**2.347**
M - O29		**1.954**	
M - O48	**2.011**		
M - O49	**2.057**		
M - N7	**1.952**	**1.939**	**2.157**
M - N21	**1.904**		
N7 - C6			**1.394**
C6 - C5			**1.410**
C5 - O12			**1.337**
< O28MO27			**108.7**
< O27MN7			**124.4**
< O29MN7		**119.2**	
<MN7O16	**89.3**		
< MN21O18	**84.1**		
< O27MN7			**124.4**
< MN7C6			**114.5**

< O26MO12		**117.5**	
< O28MO12			**112.5**
< O29MO26		**107.3**	
< MO12C5			**109.6**
< O18MN7	**97.01**		
< O48MO16	**88.6**		
< O49MN21	**86.1**		
< O27MN7C6			**85.1**
< O29MN7C6		**113.2**	
< O28MO12C5			**36.8**
< O26MO12C5		**77.1**	
<O49MO18C19	**112.6**		
<O48MN7C1	**89.7**		

Table S2: Total energy, energy of the HOMO, LUMO, energy gap (E_g), chemical hardness (η), electronegativity (X), chemical potential (V), electron affinity (A), ionization potential (I) and chemical softness(S)of the studied complexes using B3LYP/GENECP

Parameters	**CuL_2**	**PdL**	**AgL**
E_T a.u.	-1640.009	-1037.465	-944.181
E_{HOMO} a. u.	-0.1771	-0.1989	-0.1694
E_{LUMO} a. u.	-0.0998	0.1472	-0.0747
E_g, eV	2.102	1.402	2.575
I, eV	4.817	5.411	4.607
A, eV	2.714	4.004	2.032
X, eV	0.13845	0.02585	0.122
ŋ, eV	0.03865	0.17305	0.047
S, eV^{-1}	12.9366	2.8893	10.638
V, eV	-0.13845	-0.02585	-0.122

Table S3: Cytotoxic activity (IC50) of the studied compounds against Hep-G2 cell line (hepatocellular carcinoma), MCF-7 cell line (breast carcinoma) and HCT-116 cell line (colon carcinoma)

Compounds	**HCT-116 cell line**	**Hep-G2 cell line**	**MCF-7 cell line**
HL	233	150.80	121.30
CuL_2	33.4	20.5	12.8
PdL	64.8	52.2	45
AgL	71.2	59.9	51
Vinblastine standard	13.56	7.89	4.44