Electronic Supporting Materials (ESI†)

Palladium(II), platinum(II) and silver(I) complexes with 3-acetylcoumarin benzoylhydrazone Schiff base: Synthesis, Characterization, Biomolecules interaction, cytotoxic activity and computational studies.

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Supporting information

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Section1: Experimental

1.1. Materials and physical measurements

The precursors K_2PdCl_4, K_2PtCl_4, $AgNO_3$, 3-acetylcoumarin, and benzhydrazide were purchased from Alfa Aesar; Deoxyribonucleic acid calf thymus (CT DNA, Type XV), yeast ribonucleic acid (tRNA, Type IX). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and RPMI-1640 were procured from Sigma, while the bovine serum albumin (BSA, 98.5%) was obtained from Biomark. Fetal Bovine serum was obtained from GIBCO, UK. All solvents (HPLC grade) and materials were used as received. The cell lines, the normal human lung cells (WI38), breast cancer (MCF7), and human cervical carcinoma (HeLa) were obtained from ATCC (Cairo, Egypt).

Fourier-transform Infrared spectra (FTIR, KBr pellets, 4000 – 400 cm^{-1}) were recorded on a JASCO FT/IR-4100 spectrometer. The NMR spectra were measured on Bruker 400 in DMSO-d_6. Electronic spectra of the compounds (10^{-3} - 10^{-5} M in DMSO solvent) were measured in the range of 200-900 nm using a JASCO V-630 double-beam spectrophotometer. Fluorescence spectra measurements were carried out on a spectrofluorimeter (model 6285) in the spectral range of 200 – 700 nm. For BSA binding was measured using HASCO FP-8350 spectrofluorimeter.

Molar conductance values were measured using a 10^{-3} M solution of complexes in DMSO using CM-1K portable conduct meter. The magnetic susceptibility was measured using a Sherwood Scientific magnetic susceptibility balance (on powdered samples at room temperature). The thermometer melting point apparatus (RUMO 4000) was used to measure the melting points of the compounds.

1.2. Biological evaluations

1.2.1. ct DNA and tRNA interaction studies [1].

1.2.1.1. UV-Visible studies:

Electronic absorption titration studies were carried out using a fixed concentration of compounds in DMSO solution (50 μM) with increasing concentration of CT DNA or tRNA in 5 mM Tris HCl/50 mM NaCl buffer, pH 7.2 as early reported [2,3]. Purity of the CT DNA was verified by electronic absorption studies following the ratio of absorbance at 260 and 280 nm, gives 1.9 and suggested that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by examining the molar extinction coefficient of (ct DNA, ε = 6600 $M^{-1}cm^{-1}$ and tRNA, ε = 7700 $M^{-1}cm^{-1}$) at 260 nm. The electronic spectra were recorded at of 300 – 500 nm spectral range. An equal amount of ctDNA or tRNA was added to the reference cell as well as the sample cell to eliminate the absorbance of nucleic acid itself.

An analogous method was used for emission studies also. Ethidium bromide (EB) displacement experiments were performed by monitoring the changes in fluorescence intensity at excitation and emission wavelengths after addition of different concentration of compounds to the Tris-HCl buffer of EB bounded DNA (EB-DNA, 5 μM EB/ 50 μM ct DNA or tRNA).

2.5.2. Interaction with Bovine Serum Albumin (BSA):

In protein binding studies the excitation and emission wavelengths of BSA are around 280 and ~345 nm was monitored using BSA (50 μM) solution prepared in phosphate buffered saline (pH-7.2. the electronic spectral were performed after each addition of the test compound ((5 μM - 50 μM) in the 200 – 500 nm range.[4] The fluorescence quenching by was also monitored using the same concentrations and the decrease of the fluorescence intensity was recorded. The binding parameters of BSA complexes was calculated by Stern Volmer equations [5] and will be discussed in detail in the following section.

1.3. MTT assay

Cell viability was determined by the colorimetric MTT assay [6,7] which based on the conversion of the yellow tetrazolium salt MTT to purple formazan crystals after reacted with mitochondrial dehydrogenase of metabolically active cells.[7] Cells were treated with different concentrations of each compound (1.56, 3.125, 6.25, 12.5, 25, 50, and 100 μM), and cisplatin was used as a positive control after 24 and 48 h. The samples were prepared in DMSO and diluted with DMEM (The final concentration of DMSO did not exceed 0.5% v/v). The cells (2×10^4 cells per well) were plated in 96-well culture plates and 5 replica wells were used for controls as well as treated groups. After 24 h, culture medium was replaced by fresh medium containing various concentrations of HL and CuL and incubated for 24 h in a 5% CO2 humidified atmosphere. After 24 h of drug exposure, medium was removed and 100 μL medium and 10 μL of 5 mg/mL MTT in phosphate buffered saline (PBS, pH 7.4) was added to each well for an additional 2 h. Then the medium and MTT were removed and 100 μL of DMSO was added to dissolve the MTT formazan crystals. The absorbance of samples was measured at 570 nm with ELISA plate reader.

Cytotoxicity effect was revealed as the percentage of treated cells relative to untreated cells at A570 nm. Percent control was calculated using the following formula: % Control = [Mean O. D. of Drug treated well / Mean O. D. of control well] × 100

References

[1] S.A. Elsayed, S. Harrypersad, H.A. Sahyon, M.A. El-Magd, C.J. Walsby, *Molecules* 25, **2020**, 4284.

[2] S. Zehra, T. Roisnel, F. Arjmand, *ACS Omega* 4, **2019**, 7691-7705.

[3] K.A. Meadows, F. Liu, J. Sou, B.P. Hudson, D.R. McMillin, *Inorg. Chem.* 32, **1993**, 2919-2923.

[4] A. Ray, B.K. Seth, U. Pal, S. Basu, *Spectrochim. Acta - A: Mol. Biomol.* 92, **2012**, 164-174.

[5] M.E.K. Wahba, N. El-Enany, F. Belal, *Anal. Methods* 7, **2015**, 10445-10451.

[6] T. Mosmann, *J Immunol Methods* 65, **1983**, 55-63.

[7] F. Denizot, R. Lang, *J. Immunol. Methods* 89, **1986**, 271-277.

Section 2: Figures

2.1. FTIR

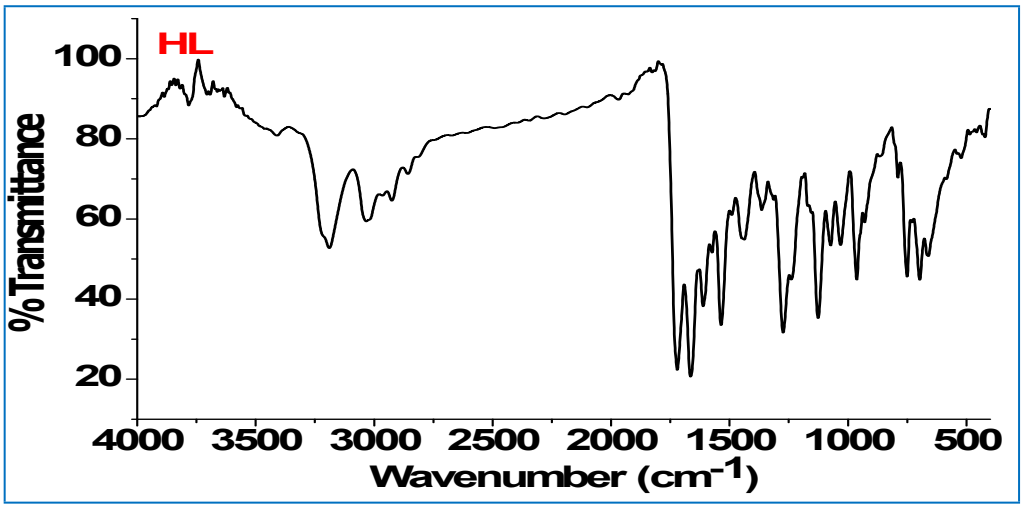


Fig. S1a. FTIR spectrum of 3-acetylcoumarin benzoylhydrazone Schiff base (HL).

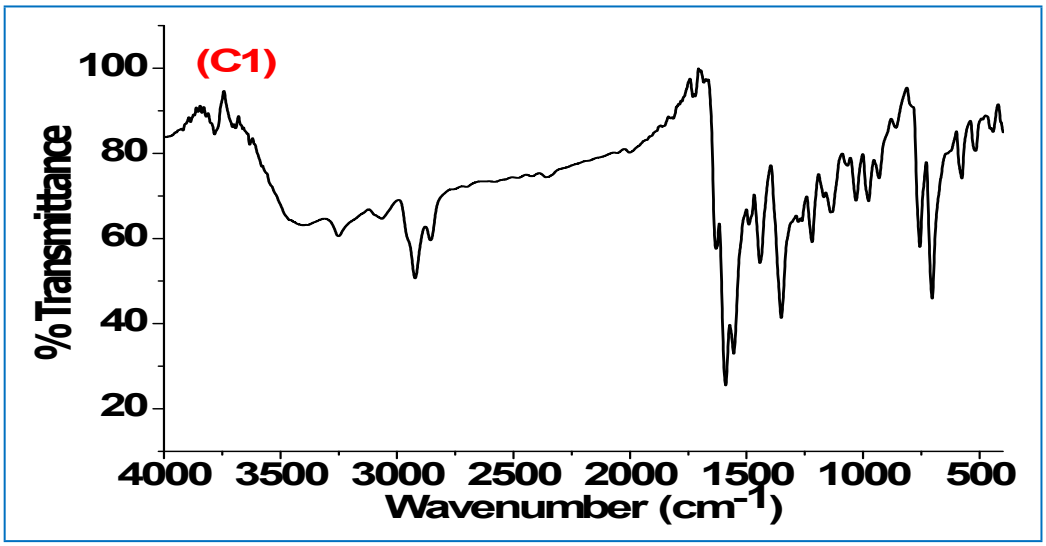


Fig. S1b. FTIR spectrum of [Pd(L)Cl] (**C1**) complex.

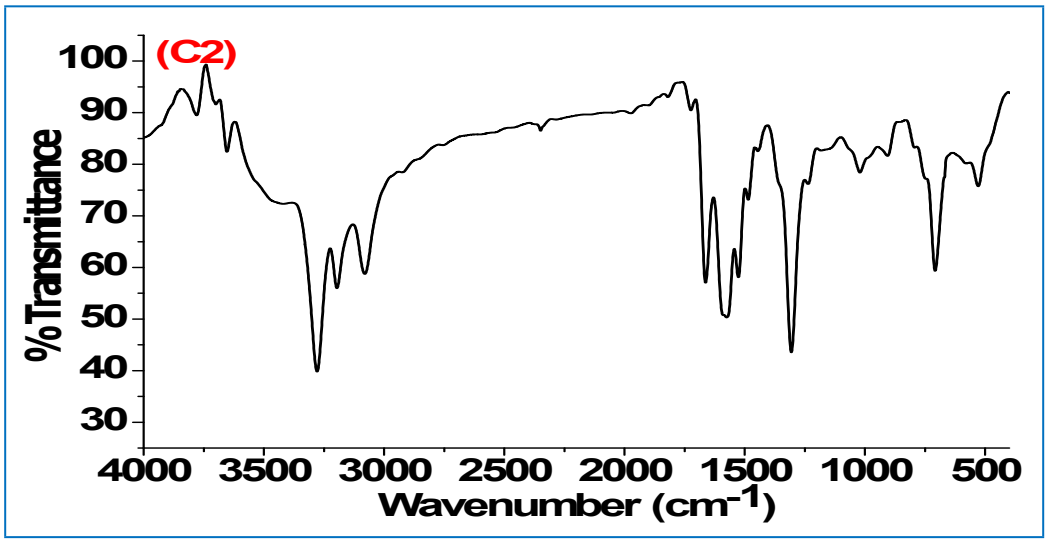


Fig. S1c. FTIR spectrum of [Pt(HL)Cl]Cl (**C2**) complex.

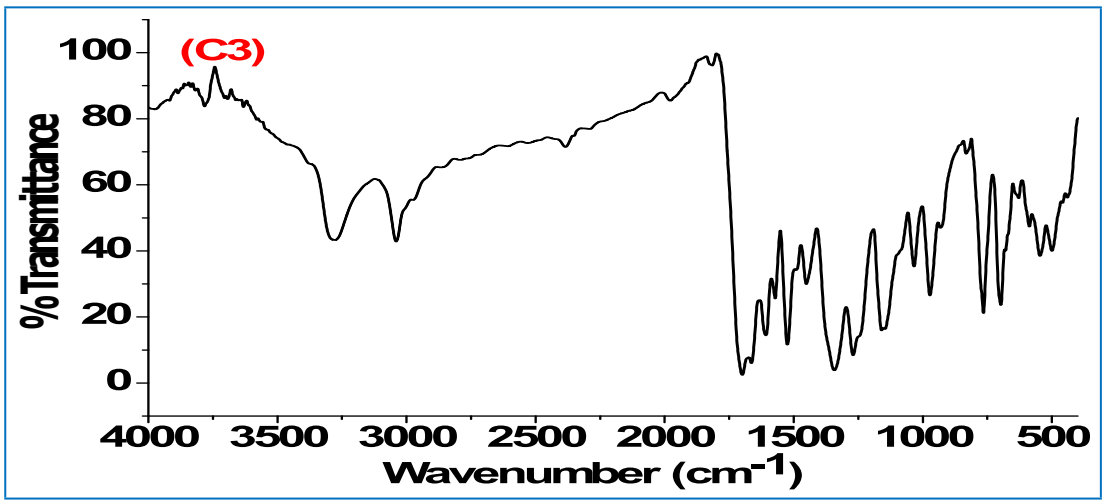


Fig. S1d. FTIR spectrum of $[Ag(HL)_2]NO_3$ (**C3**) complex.

2.2. ^{1}H NMR spectrum

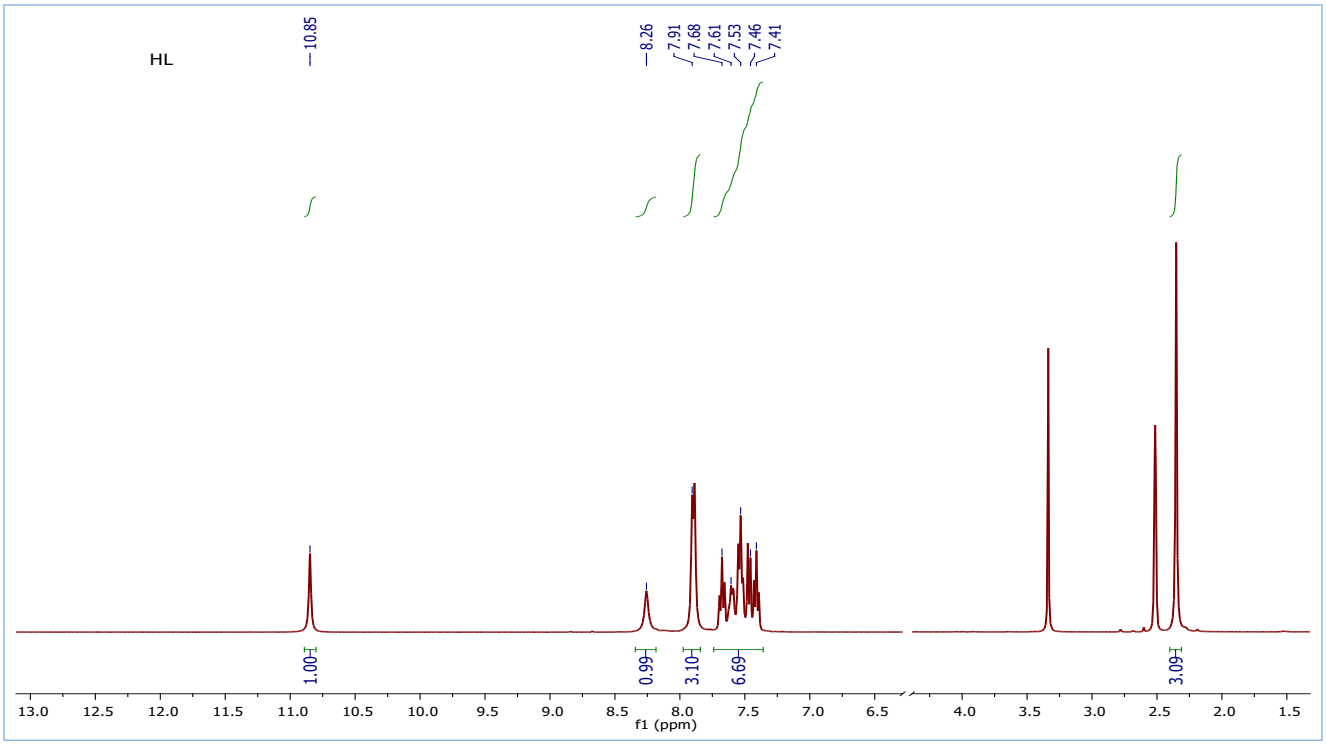
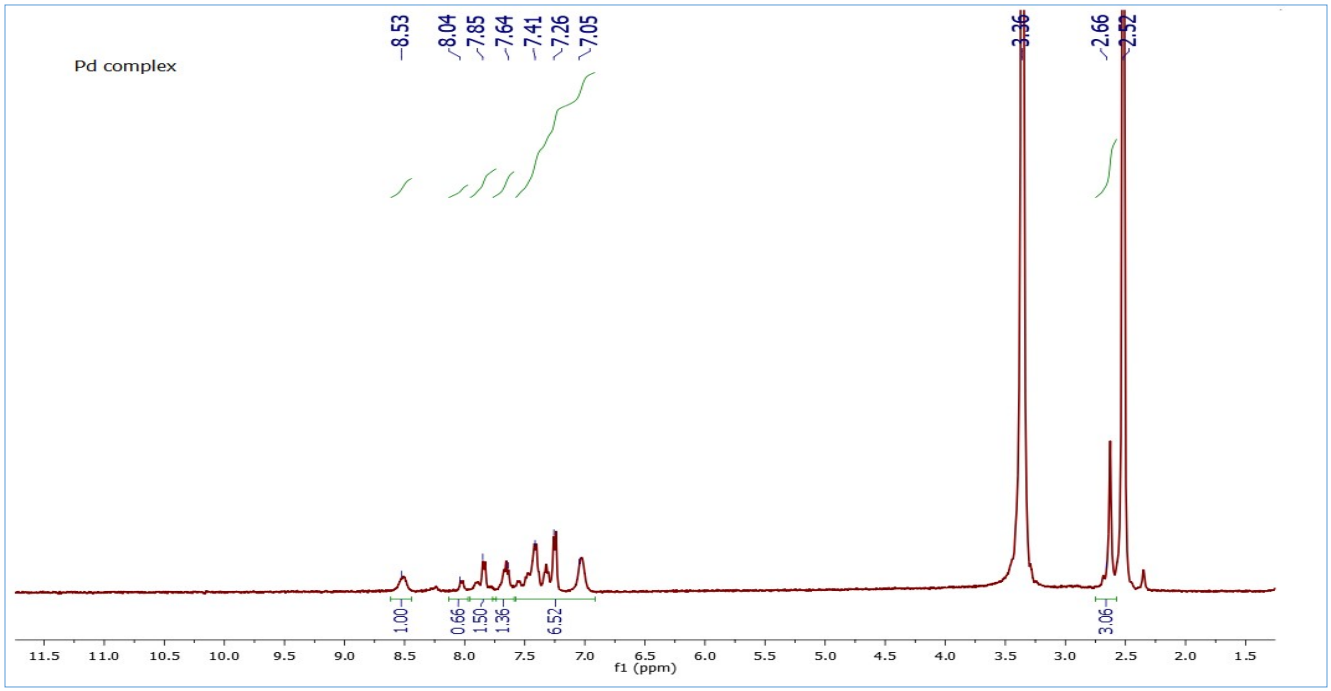


Fig. S2a. ^{1}H NMR spectrum of 3-acetylcoumarin benzoylhydrazone Schiff base (HL). **Fig.**



S2b.^{1}H NMR spectrum of [Pd(L)Cl] (**C1**) complex.

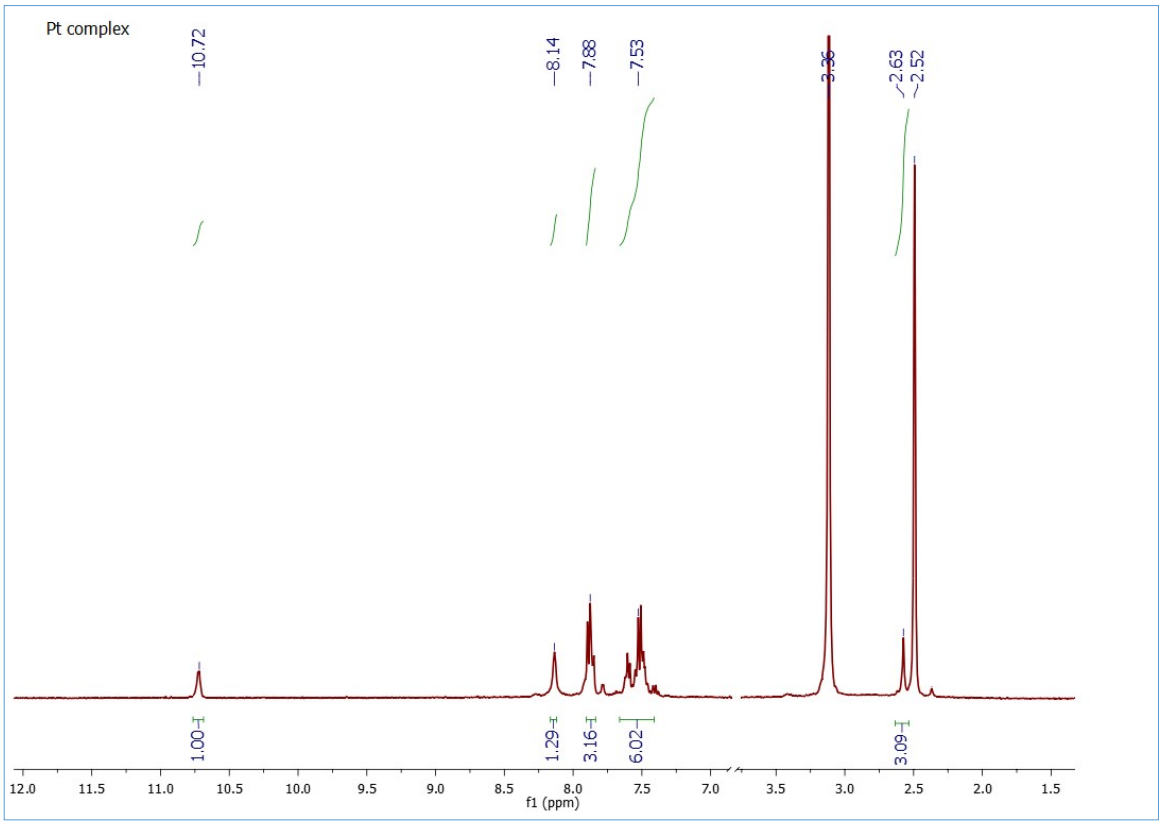


Fig. S2c. ^{1}H NMR spectrum of [Pt(HL)Cl]Cl (**C2**) complex.

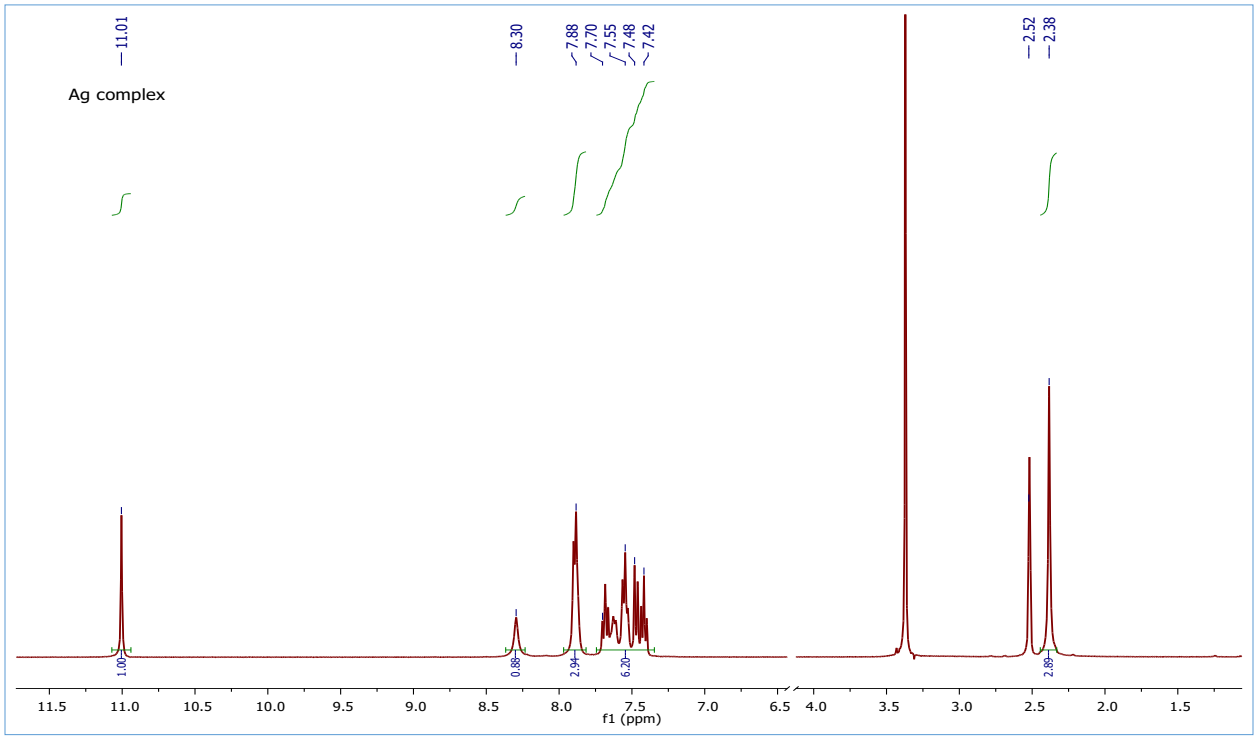


Fig. S2d. ^{1}H NMR spectrum of $[Ag(HL)_2]NO_3$ (**C3**) complex.

2.3. ^{13}C NMR spectrum of the ligand

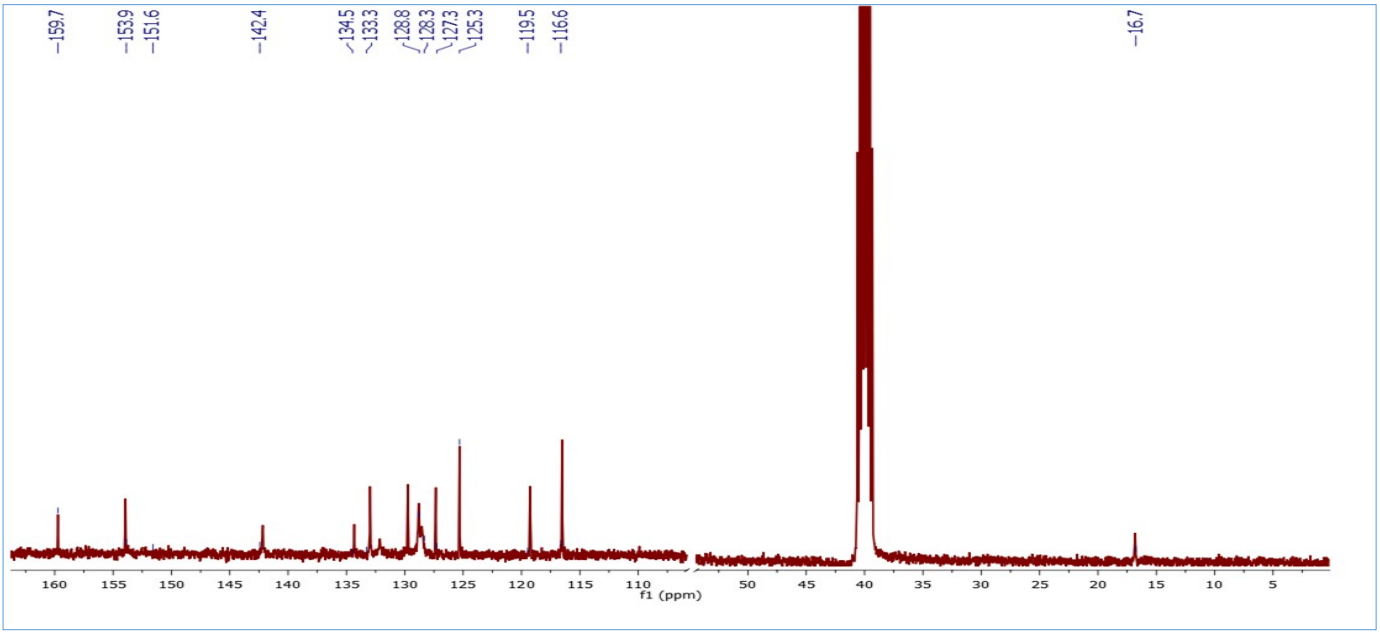


Fig. S3a. ^{13}C NMR spectrum of 3-acetylcoumarin benzoylhydrazone Schiff base (HL).

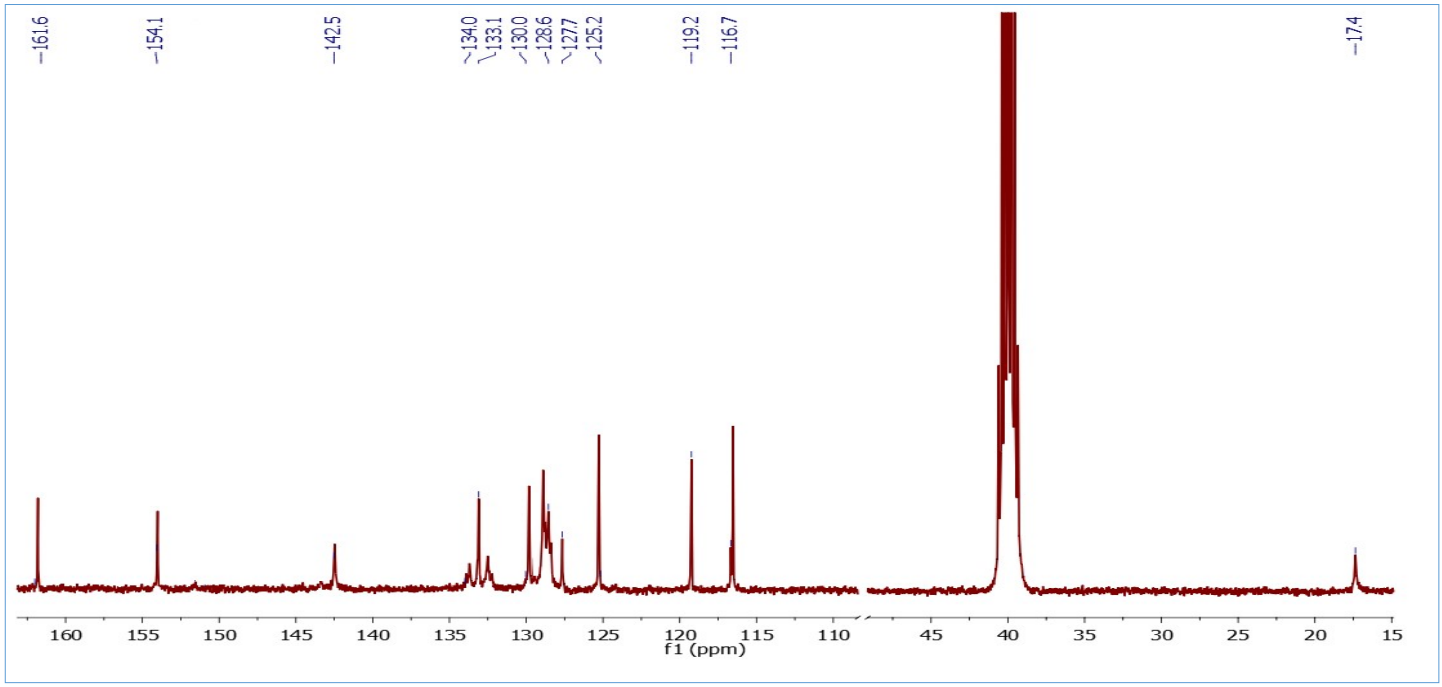


Fig. S3b. ^{13}C NMR spectrum of $[Ag(HL)_2]NO_3$ (**C3**) complex.

2.4. Solution stability of the ligand and its complexes in PBS.

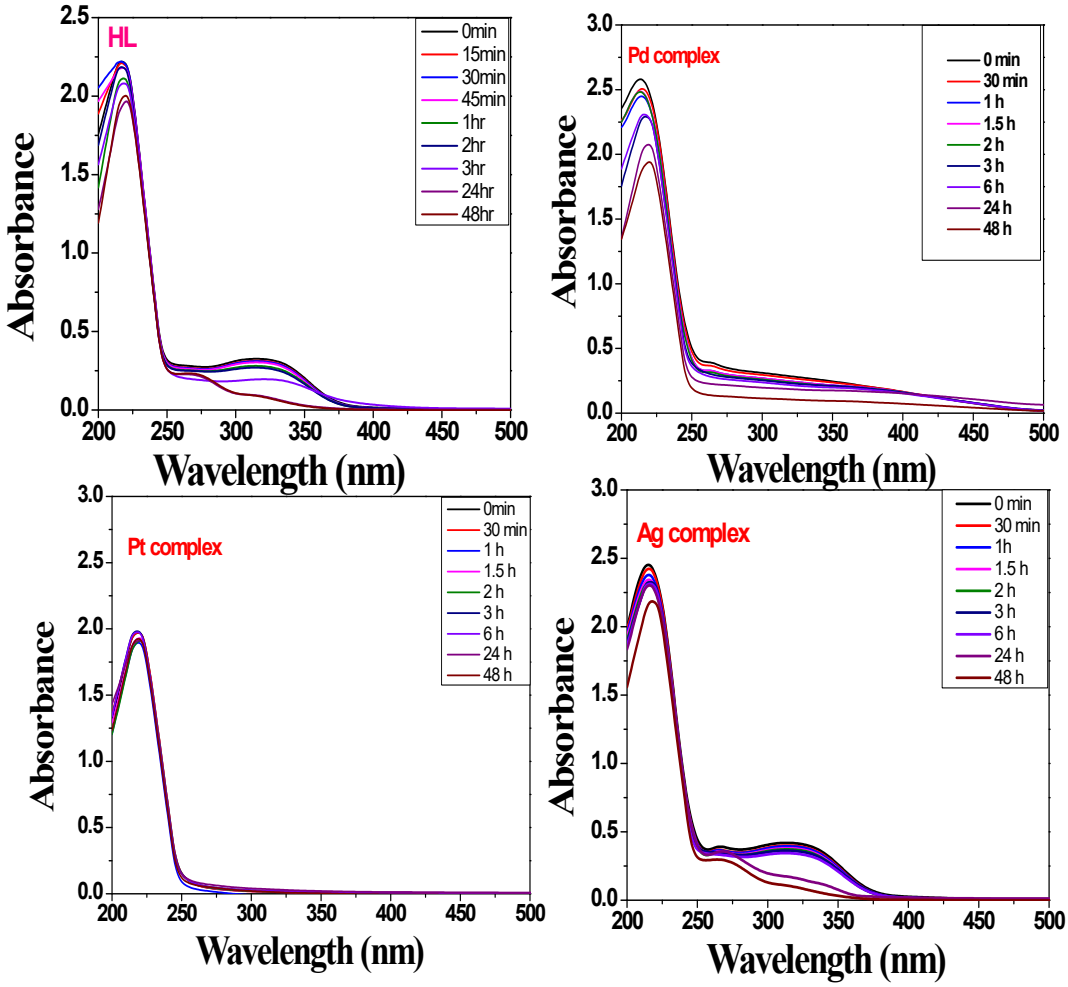


Fig. S4. Time-dependent electronic spectra of 10^{-5} M of the ligand and its complexes in % PBS, 1.6% DMSO.

2.5. tRNA interaction

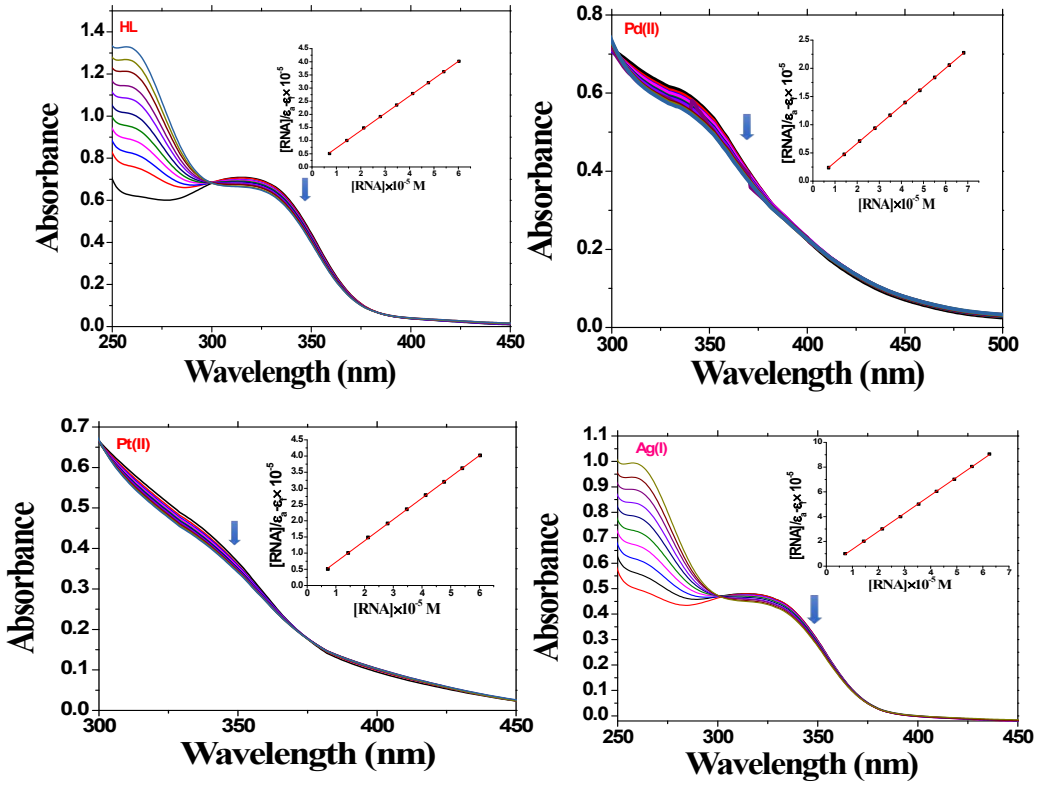


Fig. S5. Absorption spectra of HL ligand and its complexes (**C1** – **C3**) in absence and presence of increasing amounts of tRNA concentration (5 -50 μM) in Tris-HCl buffer (pH =7.2).

Section3: Tables

Table S1. FTIR spectral data (ν, cm^{-1}) of 3-acetylcoumarin benzoylhydrazone Schiff base (HL) and its complexes

No.	Compound	ν (NH)	ν(C=O) Lactone	ν(C=O) hydrazone	ν(C-O) New	ν(C=N)	$\nu(C=N)_{New}$	ν(N-N)	ν(M-O)	ν (M-N)
HL	$C_{18}H_{14}N_2O_3$	3192	1721	1664	--	1611	--	963	--	--
(**C1**)	[Pd(L)Cl]	--	1620	--	1221	1589	1554	975	575	442
(**C2**)	[Pt(HL)Cl]Cl	3278	1724	1663	--	1575	--	1021	529	435
(**C3**)	$[Ag(HL)_2]NO_3$	3277	1698	1664	--	1608	--	971	545	439

Table S2 ^{1}H NMR spectral data (δ, ppm) of 3-acetylcoumarin benzoylhyrazone (HL) ligand and its complexes.

	Compd.	δ (NH) (s, 1H)	δ H(4) (s, 1H)	δ H(6,9) (d, 2H)	δ H(7, 8) (t, 2H)	δ H(16,18) (t, 2H)	δ H(15, 19) (d, 2H)	δ (H17) (t, 2H)	δ (H12) (s, 3H)
HL	$C_{18}H_{14}N_2O_3$	10.85	8.26	7.91	7.68	7.53	7.46	7.41	2.35
(**C1**)	[Pd(L)Cl]	--	8.53	8.04 7.85	7.65	7.32	7.42 7.24	7.03	2.66
(**C2**)	[Pt(HL)Cl]Cl	10.72	8.84	8.13	7.89	7.60	7.77	7.50	2.63
(**C3**)	$[Ag(HL)_2]NO_3$	11.01	8.30	7.88	7.70	7.55	7.48	7.42	2.38

Table S3. ^{13}C NMR (δ, ppm) spectra of spectra data of the ligand and silver complex (**C3**)

No.	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)	C(9)	C(10)	C(11)	C(13)	C(14)	C (15,19)	C (16,18)	C(17)	C(CH3)
HL	159.7	127.3	142.4	119.5	116.5	125.3	133.5	128.7	151.6	153.9	128.3	134.5	130.0	129.7	128.8	16.7
(**C3**)	161.6	127.7	142.5	119.2	116.7	125.2	133.3	128.6	151.6	154.1	128.7	134.0	129.7	129.5	128.6	17.4

TableS4: Electronic spectral data of HL and its complexes (**C1**) – (**C3**).

Compound	Electronic spectra, λ_{max} (nm), (ε, $M^{-1}cm^{-1}$)	μ_{eff} (BM)
HL	232 (32000), 336 (36000)	--
Pd(II)	276 (27600), 330 (21200)	Dia
Pt(II)	276 (10000), 327 (2400)	Dia
Ag(I)	226 (96000), 332 (28000)	Dia