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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION, BIOLOGICAL SCREENING AND POM ANALYSIS OF POTENTIALLY BIOACTIVE COPPER(II) CARBOXYLATE COMPLEXES

Muhammad Sirajuddin[1*], Changaiz Ahmad[1], Hizbuallah Khan[1], Imdad Ullahkhan[2], Muhammad Tariq[3] and Nazif Ullah[2]

[1]Department of Chemistry, University of Science and Technology Bannu, 28100, Pakistan
[2]Department of Biotechnology, Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan, Pakistan
[3]Instituteof Chemical Sciences, BahauddinZakariya University, Multan, Pakistan

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ABSTRACT. Here we are reporting the synthesis, characterization of four novel copper(II) complexes with carboxylate ligand and their evaluation for biological applications. The complexes have general formulas $C_{22}H_{18}CuN_4O_{12}$, $C_{32}H_{26}CuN_6O_{12}$, $C_{27}H_{23}CuN_5O_{12}$ and $C_{34}H_{26}CuN_6O_{12}$. The synthesized complexes were characterized by FT-IR and elemental analyses carried out through EDX. The deprotonation of the carboxylate ligand, 4-(2-methoxy-5-nitrophenylamino)-4-oxobut-2-enoic acid, was observed in FT-IR spectrum, indicating that complexation happens *via* the carboxylate moiety's oxygen. In complexes **1** and **3**, the value of Δν value is in between 120-180 cm^{-1} showing the bridging mode of the carboxylate moiety while in complexes **2** and **4**, its value is greater than 180 cm^{-1} indicating the monodentate binding mode. The findings of antimicrobial activity revealed that the complexes have adequate antibacterial and antifungal properties. The anti-promastigote analysis results showed that the complexes **1** and **4** have significant activity as compared to the standard drug. The binding mode of complexes with DNA was confirmed by UV-Visible spectroscopy and viscosity measurements and the results showed that complexes interact with DNA through intercalative mode. A new and efficient strategy was applied through POM (Petra, Osiris and Molinspiration) analyses for determining the toxicity assessment, bio-availability and drug score of the synthesized complexes. The results showed that the complexes have good bioavailability and drug scores.

KEY WORDS: Copper(II) carboxylate complex, DNA binding, Anti-promastigote analysis, Cytotoxicity, POM analysis, Docking study

INTRODUCTION

The Cu(II) carboxylate complexes have become a field of interest due to its significance to the nature of the active sites of various metal enzymes. Earlier research has also shown that copper(II) carboxylate complexes can effectively stimulate the binding of biopolymers like bovine serum albumin (BSA), deoxyribonucleic acid, etc. The existence of different metals in the coordination sphere of Cu(II) carboxylate complexes allow the regulation of the essential pharmacological factors and, hence, can alter the efficiency of the drug. These complexes have shown ferromagnetic interactions depending on the Cu-O-Cu connection angle and can be adjusted by making slight variations in their structural parameters [1-3]. They are extensively used in homogeneous catalysis, magneto-chemistry, bioinorganic chemistry and electrochemistry. The properties of the complex vary according to the metal ion and the ligands attributed to it. Furthermore, these properties can be modified by changing the substituents on the ligands. Carboxyl ligands are responsible for stabilizing the oxidation states of metal ions and the geometry of complexes. Such structures are common in complexes when the terminal ligand is O, N, or S donor or a halogen group such as Cl. A remarkable feature of copper carboxylate complexes is their adjustable redox chemistry and its biological application, as shown by its antitumor and enzymatic activity. The biological activity of metal complexes has been found to

*Corresponding author. E-mail: m.siraj09@gmail.com

be greater in dinuclear complexes than in mononuclear complexes [4, 5]. Cu(II) carboxylates with 1,10-phenanthroline unit show remarkable metal-centered paramagnetic, enzymatic, biological and deoxyribonucleic acid binding properties. Deoxyribonucleic acid binding study is a prerequisite for compounds aimed at anti-cancer treatment, as the anti-cancer drugs presently in use are eventually targeted at cellular DNA. These complexes are biologically more favorable than Pt based drugs because they are biologically important and non-toxic. The use of non-toxic ligands without the reported cytotoxicity helps the biocompatibility of copper based drugs [6-8]. The structure of the complexes becomes more stable if the nitrogen donor ligand is attached at axial position. One of these nitrogen donors, pyridine and its derivatives, helps form H-bonds and increase the stiffness of the crystal structure. Carboxylate groups show various binding modes like monodentate, chelate, monatomic crosslink, syn, syn-, syn-anti-, anti, anti-, "syn, anti, anti- and "syn, syn, anti-" [9-11].

Here copper(II) complexes with [O, N] donor ligands were successfully synthesized and evaluated for their antimicrobial, antileishmanial activities and carries lesser toxicity to human blood erythrocytes and DNA. The POM analyses results also revealed that they possess the drug-like characteristics.

EXPERIMENTAL

Materials and methods

Copper(II) acetate salt, 2-methoxy-5-nitro aniline, maleic anhydride, sodium bicarbonate, pyridine, 2,2'-bipyridine and *o*-phenanthroline were purchased from Sigma Aldrich (USA). Analytical grade solvents were obtained from Merck (Germany) and used as such received. Sodium salt of Salmon sperm-DNA obtained from Arcos Organics (USA).The M.P (melting points) of the synthesized compounds were determined in capillary tubes using a digital electro-thermal melting point device. Infrared spectra were recorded using a Thermo Nicolet-6700 FT-IR Spectrophotometer. The DNA interaction study was performed using UV-Vis spectrophotometer (Shamidzu-1800) at room temperature.

Synthesis of 4-(2-methoxy-5-nitrophenylamino)-4-oxobut-2-enoic acid (HL) and NaL

The ligand, 4-(2-methoxy-5-nitrophenylamino)-4-oxobut-2-enoic acid, was re-synthesized according to the reported method described by Sirajuddin and co-workers [12]. NaL, sodium 4-(2-methoxy-5-nitrophenylamino)-4-oxobut-2-enoate, was synthesized by the procedure described by Sirajuddin and co-workers [13] in which the ligand HL was suspended in distilled water and to which an aqueous solution of sodium bicarbonate was added. The mixture was stirred at 25 °C till the appearance of clear solution. After this the solution was rotary evaporated to acquire the desired NaL which was further used for complexation with Cu(II) acetate salt (Scheme 1).

*Synthesis of complex **1**: bis((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper*

For the synthesis of *bis*((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper, $Cu(CH_3COO)_2$ (2 mmol, 0.34 g) in distilled H_2O was added slowly to the clear aqueous solution of NaL (4 mmol, 1.16 g) [13] as presented in Scheme 1. With constant stirring at 25 °C we get green color precipitate. After filtration the precipitate was washed with distilled H_2O to remove unreacted species and then was air dried. For crystal formation the resultant complex was dissolved in solvents like DMSO and methanol but failed to get good quality crystals.

*Synthesis of complex **2**: 2,2'-Bipyridine bis((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper*

For the synthesis of 2,2'-bipyridine *bis*((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper same procedure as described for complex **1** was adept for getting the complex

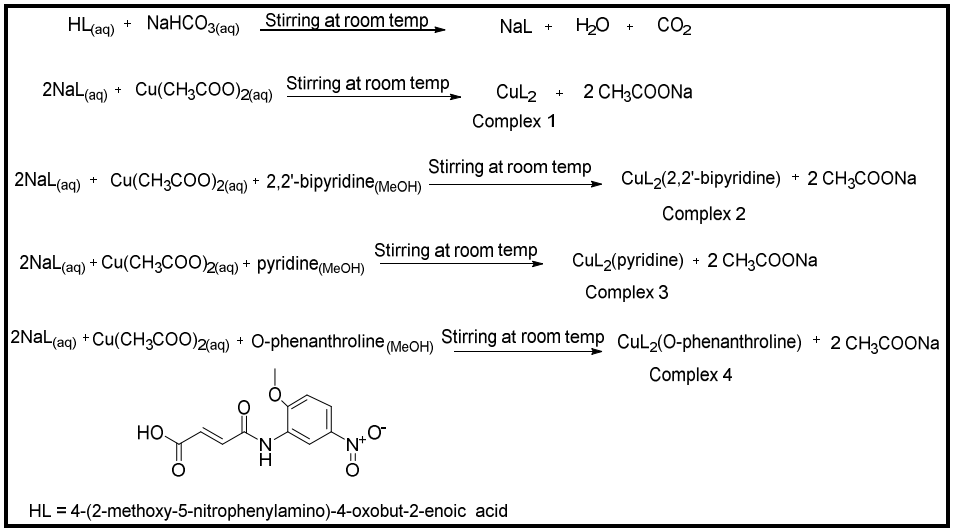
2 with supplementary step in which a 0.23 g (1.5 mmol) of 2,2´-bipyridine in 2-3 mL MeOH was slowly added to the mixture to get the desired product [13].

*Synthesis of complex **3**: Pyridine bis((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper*

For the synthesis of pyridine *bis*((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper, same procedure as described for complex **1** was adept for getting the complex **3** with supplementary step in which a 0.12 mL (1.5 mmol) of pyridine in 2-3 mL MeOH was slowly added to the mixture to get the desired product [13].

*Synthesis of complex **4**: O-phenanthrolinebis((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper*

For the synthesis of *o*-phenanthroline *bis*((4-((2-methoxy-5-nitrophenyl)amino)-4-oxobut-2-enoyl)oxy)copper same procedure as described for complex **1** was adept for getting the complex **4** with supplementary step in which a 0.29 g (1.5 mmol) of 1,10-phenanthroline in 2-3 mL MeOH was slowly added to the mixture to get the desired product [13].



Scheme 1. Synthetic way for the preparation of complexes **1-4**.

Complex-DNA binding study via UV-visible spectroscopy

A solution of sodium salt of salmon sperm DNA (SS-DNA) was prepared by dissolving its small amount (2 mg) in distilled water and was kept on stirring for overnight at room temperature. The value of absorbance ratio for DNA solution at 260 and 280 nm (A_{260}/A_{280}) was found to 1.8, which shows that DNA is almost free of protein. The concentration of DNA solution was determined using the value of ε (6,600 M^{-1} cm^{-1}) and was found 2.94 $\times 10^{-3}$ M. The DMSO and water in 1:1 ratio (v/v) was used to dissolve the complexes at a concentration of 1 mM. By adding varying concentration of SS-DNA to the fixed concentration of compound, absorption titrations study was performed. To exclude the absorbance of DNA itself in cuvettes, equivalent amount of SS-DNA was added to the complex and reference solutions. The solutions were first incubated for about 10

min at room temperature and then measurements of the UV data for complex-DNA were taken in cuvettes having path length of 1 cm [14-18].

Complex-DNA binding study via viscometry

Viscosity measurements were made at room temperature with the help of Ubbelodhe viscometer. Digital stopwatch was used to record the flow time of the samples. Experiments were repeated in triplicate to measure the average flow time. The data was platted as $(\eta/\eta_o)^{1/3}$*vs*. r(r = [Complex]/[DNA]). The equations 1 and 2 was used for the determination of viscosity of SS-DNA in the presence and absence of the compound [19-23]:

$$\eta_o = t - t_o \quad (1)$$

$$\eta = \frac{t - t_o}{t_o} \quad (2)$$

where η_o = viscosity of DNA in the absence of compound, η = viscosity of DNA in the presence of compound, t_o = time flow of DNA in the absence of compound, t = time flow of DNA in the presence of compound.

Petra/osiris/molinspiration (POM) analyses

POM analysis was used for the identification and indication of the type of pharmacophores site that affects biological activity with a change in the chemical substitution. Petra /osiris/molinspiration identify the biological activities of the molecules. POM analysis presents the relationships between biological activity and electrostatic property which is in the form of pharmacophores site. This provides essential characteristics for the receptor topology and also for ligand-receptor interaction [24].

Antibacterial assay

Three bacterial strains, two Gram-negative (*Klebsiella pneumonia*, *Escherichia coli*) and one gram-positive (*Staphlococcus aureus*) were taken and the synthesized complexes were checked for their antibacterial activity against these bacterial strains. Agar well diffusion method was used to determine the antibacterial activity. Agar medium (75 mL) used act as nutrient at 45 °C. Sterile petri plate having a diameter of 14 cm was taken and agar media was poured gently. Then the petri plate containing mixture was allowed to solidify. After that all petri plates were applied by bacterial strains with the help of cotton buds. The cotton buds were disposed after single use. After this 7 mm wells were dug in the medium with the help of sterile metallic borer. The wells were labeled. The sample solutions were made in DMSO. 100 µL sample solution (at 1 mg/mL) was added to the respective labeled wells. Azithromycin (standard drug) at a concentration of (1 mg/mL) was used as positive control while the DMSO functioned as negative control. All plates were incubated (aerobic) at 37 °C for twenty four hours. The preparation of plates for this activity was made in triplicate. Around each well the diameter of the zone of inhibition was measured. The measured diameter of zone of inhibition was used to determine the antibacterial activity of the compounds [24].

Antifungal activity

Agar tube dilution method was used for the determination of the antifungal activity using two fungal strains (*Aspergillus flavus* and *Aspergillus niger*). The media was prepared by dissolving 4.5 g of sabourd dextrose agar (SDA) in 80 mL distilled water. Screw capped test tubes was loaded with 4.5 mL SDA. Test tubes were autoclaved at120 °C for 15 min. The test tubes were left for cooling at 40 °C. Sample solution in DMSO was made at a concentration of 12.0 mg/mL. The

sample solution (66.0 µL) was added to the non-solidified SDA to make 200.0 µg/mL final concentrations. The screw capped test tubes were kept in slanting position to solidify at 25 °C. Fungal strains were applied through the wire loop when the tubes get solidify. The tubes were containing terbinafine at concentration of (200.0 µg/mL) act as a positive control while DMSO acts as a negative control. The media were incubated at 37.0 °C. Growth was measured (in mm) after 24 and 72 hours. The growth inhibition was measured shown by in equation 3 [24]:

$$\%\ \text{growth inhibition} = 100 - \frac{\text{Linear growth in test sample (mm)}}{\text{Linear growth in control (mm)}} \times 100 \quad (3)$$

Anti-promastigote assay

The anti-promastigoate activity of the prepared compounds was performed by following the procedure described by Nadhman and co-worker [25] using MTT assay. The activities of the compounds at four different concentrations (prepared in serial dilution in the range of 500-31.25 µg/mL) were tested [24].

Cytotoxicity assay

The prepared compounds were texted to explore their cytotoxicity activity performed by following the procedure described earlier [24, 26]. Fresh human blood was collected from volunteers (requires approval of the Ethics Committee of the Faculty of Biotechnology, Abdul Wali Khan University Mardan). The red blood cells were then isolated by centrifugation and washed 3 times with PBS. The remaining 990 µL of red blood cells were treated with complexes **1-3** and the final concentrations analyzed were reached to 500, 250 and 125 µg/mL. Then incubate at 37 °C for 3 hours. Subsequently, the cell suspension was centrifuged at 1000 rpm for 5 min. The supernatant was collected after centrifugation and the free hemoglobin was confirmed at 576 nm using a spectrophotometer. DMSO was applied as a negative control and Triton X-100 (0.5%) as a positive control. Optical density (OD) was taken at 576 nm using an Elisa plate reader (Biotek Elx-800). The hemolysis rate was calculated using the formula in equation 4 [24]:

$$\text{Hemolysis}\ (\%) = \frac{\text{OD at 576 nm in the sample solution} - \text{OD 576 nm in PBS}}{\text{OD at 576 nm in positive control} - \text{OD at 576 nm in PBS}} \times 100 \quad (4)$$

DNA damage analysis (DNA laddering assay)

The Promastigotes (1 x 10^6 cells/mL) was cultured for 72 hours in the presence and absence of different concentrations (500, 250 and 125 µg/mL) of the compounds selected. Cells were reaped and re-suspended in digestive buffer with 0.5 mg/mL proteinase K added. The mixture was incubated in the presence of RNase A at 37 °C for 3 hours. The DNA material was extracted by processing using phenol/chloroform (1:1), sodium acetate and ice-cold ethanol were added to the aqueous phase for precipitation, followed by overnight incubation at -20 °C. The mixture was centrifuged; pellets were collected, dried and resuspended in *Tris*-EDTA buffer. In addition, DNA aliquots (20 µg) were electrophoresed on a 2% agarose gel using *Tris*-acetate-EDTA running buffer and photographed with UV light [24, 27].

Molecular docking

Docking is an important tool for finding interactions between ligands and drug target. Molecular docking was performed using the MOE-Dock program (www.chemcomp.com) to study the binding interactions of synthesized complexes at the active sites of DNA. The three-dimensional (3D) structure of the metal-complex derivatives was designed by the MOE (molecular operating environment) software. Each has been minimized by using 3D protonated compound and the

MOE predefined parameters. The 3D crystal structure of the DNA (1bna) was obtained from protein databank (PDB). The synthesized compounds were docked to the active site of the target receptor of MOE by a predetermined parameter. That is, Positioning: Triangle Matcher, Rescoring 1: London dG, Purification: Force field, Rescoring 2: London dG. Ten conformations were created for each ligand and the highest score conformation was selected based on the docking score for further molecular docking studies. After molecular docking, MOE software was used to analyze the best stances with polar, arene-arene, H-pi, and pi-H interactions [28, 29].

RESULTS AND DISCUSSION

Table 1 presents the physical data including the molecular formula, mass, physical state, solubility, color and melting points of the prepared Co(II) carboxylate complexes. The complexes were freely soluble in DMSO and also were air stable. The sharp melting points of the synthesized compounds mentioned in Table 1, show their purity.

Characterization of synthesized compounds by FT-IR

Thermo Nicolet-6700 FT-IR Spectrophotometer was used to record the infrared spectra of HL, NaL and Cu(II) carboxylate complexes and is shown in Table 1. Synthesis of NaL was confirmed by disappearance of OH peak of carboxylic acid, showing the attachment of sodium (Na) with carboxylic acid. NaL was then used for synthesis of other complexes. The presence of new peaks in the range of 410-435 cm^{-1} for ν (Cu-O) confirms the synthesis of the new complexes, because these peaks were absent in ligand spectrum. In complexes **2-4** having the 2,2'-bipyridine, pyridine and *o*-phenanthroline groups, an extra peak in the range of 500-510 cm^{-1} of ν(Cu-N) was also detected. The presence of this new peak confirms the coordination of the nitrogen atom of these groups to the copper atom [1].

According to the literatures, the $\Delta\nu = [\nu(COO)_{asym} - \nu(COO)_{sym}]$ value below 200 cm^{-1} means to the Cu-carboxyl coordination is considered to act as bidentate ligand, while a value above 200 cm^{-1} indicates monodentate coordination [30, 31]. The Δν for complexes **1** is 168 cm^{-1} while for complexes **2-4** are 219, 209 and 204 cm^{-1}, respectively, indicate the carboxylate moiety as bidentate ligand in complex **1** and as monodentate in complexes **2-4**.

Table 1. Physical and FTIR data (cm^{-1}) of HL, NaL and corresponding copper(II) complexes **1-4**.

Physical data						
Comp. #	Mol. formula	Mol. mass	Nature	Color	Solubility	M. P. (°C)
1	$C_{22}H_{18}CuN_4O_{12}$	593.02	Powder	Green	DMSO	211-213
2	$C_{32}H_{26}CuN_6O_{12}$	750.14	Powder	Light blue	DMSO	147-149
3	$C_{27}H_{23}CuN_5O_{12}$	673.05	Powder	Light green	DMSO	198-200
4	$C_{34}H_{26}CuN_6O_{12}$	774.16	Powder	Green	DMSO	188-190

FTIR data (cm^{-1})						
IR Peak	**HL**	**NaL**	**1**	**2**	**3**	**4**
ν(OH)	3119	Absent	Absent	Absent	Absent	Absent
$\nu(COO)_{asy}$	1540	1538	1533	1578	1576	1541
$\nu(COO)_{sym}$	1327	1260	1365	1359	1367	1337
Δν	213	278	168	219	209	204
ν(C=C)	1594	1664	1682	1618	1650	1645
ν(Cu-O)	Absent	Absent	420	412	414	432
ν(Cu-N)	Absent	Absent	Absent	507	510	503
$\nu(C\text{-}H)_{str\ Al}$	2997	2732	2952	3061	2943	3081
$\nu(C\text{-}H)_{strAr}$	3091	3113	3149	3108	3125	3143
ν(N-H)	3446	3566	3352	3389	3479	3358

DNA binding study by Ultraviolet-visible spectroscopy

The interaction between the complex and SS-DNA was studied using electronic absorption spectroscopy and the results are shown in Figure 1. Complex **1** has one strong absorption peak at 254 nm with absorbance of 1.441, complex **2** at 300 nm having absorbance 1.517, complex **3** at 288 nm with absorbance 1.117 and complex **4** at 273 with absorbance 1.937. These strong absorption peaks are due to the π-π* transition of the aromatic ring. This strong absorption peak in complexes**1-4** decreased steadily as the concentration of DNA increases and the wavelengths show slight red shift. Due to presence of nitro and carbonyl groups some compounds show second peak that's because of the *n*-π* transition. During the interaction of the complexes **1-4** with DNA, shifting of peak towards the longer wavelength side was observed that could be used for understanding of binding mode of the compounds with DNA. Due to the existence of strong stacking (π-π*) between the complexes and the base pairs of DNA, hypochromic and bathochromic effects were observed which is the indication of intercalative binding mode [23]. The association constant (binding constant) of the complex with DNA was determined by using the Benesi-Hildebrand's equation [32]:

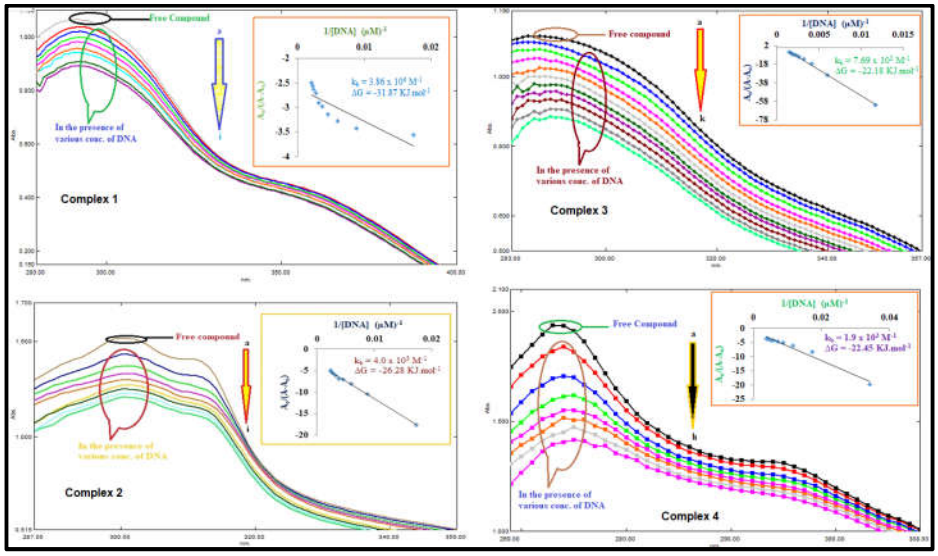


Figure 1. Absorption spectra of complexes **1-4** in the absence and presence of various conc. of SS-DNA. The inset graph is used for calculation of binding constant and Gibb's free energy.

Viscosity measurement

The binding mode of DNA-complexes was further confirmed by viscosity measurement. The hydrodynamic measurements which are length dependent are considered to be less ambiguous test for confirmation of binding mode. In intercalation binding mode, elongation of the DNA helix occur as complex bind to DNA resulting in separation the base pairs, hence increases the viscosity. As complex concentration increases, the viscosity of the compounds-DNA mixture was also increased steadily. Figure 2 presents the plot of relative viscosity *vs*. concentration ratio (r) of compound and DNA. The steady increase in the relative viscosity of the DNA upon the addition of various concentration of complexes**1-4** indicate the intercalative mode of binding [22].

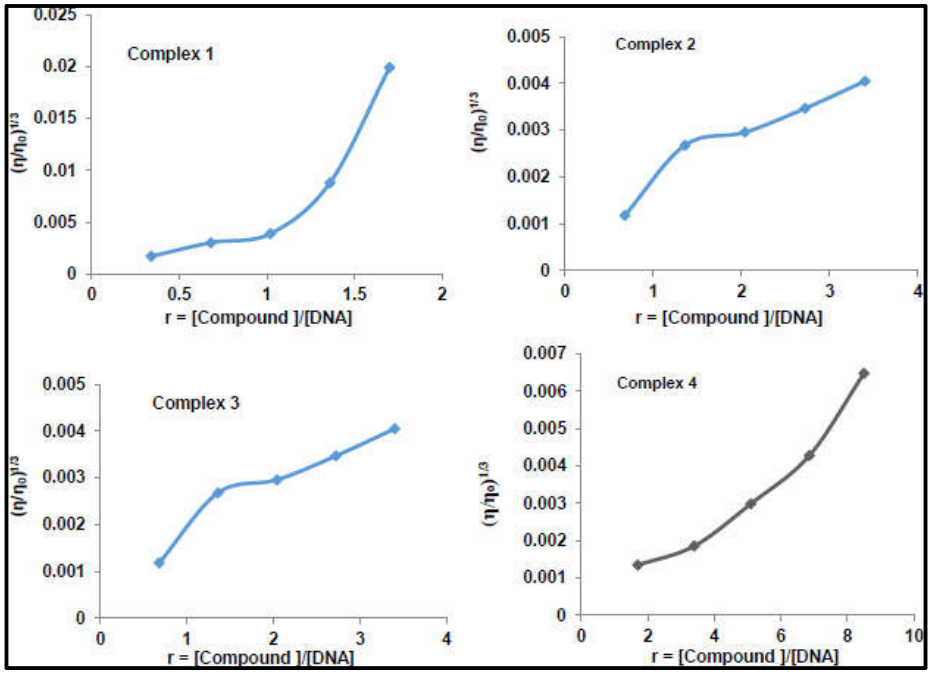


Figure 2. Effect of increasing concentration of complexes **1-4** on relative viscosity of DNA.

EDX analysis

The chemical compositions of the representative complex **1** was determined with the help of energy dispersive X-ray (EDX). Major elements and their percentages detected by EDX are listed in Table 2. The variation in the percentage composition is due to the fact that EDX is only good for heavy metal detection. The presence of Cu metal in the composition confirms the complex formation.

Table 2. Elemental analysis of complex **1** by EDX.

Element	Line type	Apparent conc.	Wt.%	Factory standard
C	K series	39.84	53.08	Yes
N	K series	0	0	Yes
O	K series	43.92	39.14	Yes
Cu	L series	4.97	7.78	Yes
Total			100	

Antibacterial activity results

In vitro antibacterial activity (Table 3) was conducted for the synthesized complexes against three bacterial strains; *Klebsiella pneumonia*, *Escherichia coli* (Gram-negative) and *Staphlococcus aureus* (Gram-positive). Agar well diffusion method was used for this activity. Each attempt was made in triplicate. Azithromycin was used as standard medicine. By comparing Cu(II) carboxylate complexes and standard biologically activity drugs, it was observed that the Cu(II) carboxylate derivatives are active against the tested bacterial strains. The minimum activity was shown by **1** mg/mL while maximum activity was shown by complex **4** at concentration of 6 mg/mL [24].

Antifungal activity results

The complexes were evaluated after incubation period for their antifungal activity using Terbinafine (shows maximum inhibition) and DMSO (shows zero inhibition) as +ve and -ve controls, respectively (Table 3). The maximum antifungal activity was shown by complex **4** at concentration of 6 mg/mL while comparing with standard antifungal drug and negative control.

Table 3. Antimicrobial activity results of the screened compounds at 6 mg/mL.

Comp. No.	Zone of inhibition (mm) for antibacterial activity results		
	K. pneumonia	*S. auras*	*E. coli*
1	16± 0.15	17± 0.14	18± 0.17
2	15± 0.14	16± 0.15	17± 0.15
3	16± 0.15	18± 0.12	17± 0.14
4	17± 0.10	18± 0.11	19± 0.09
Azithromycin	23± 0.14	22± 0.14	24± 0.07

Antifungal activity results				
Comp. No.	Zone of inhibition (mm)		% Inhibition	
	A. niger	*A. flavus*	*A, niger*	*A. Flavus*
1	20± 0.20	28± 0.14	40± 0.14	52± 0.15
2	19± 0.10	25± 0.18	39± 0.11	45± 0.12
3	22± 0.15	18± 0.15	43± 0.15	38± 0.14
4	23± 0.14	32± 0.11	47± 0.18	52± 0.17
DMSO	0	0	0	0
Terbinafine	50± 0.15	50± 0.10	100± 0.09	100 ± 0.11

Anti-Promastigote analysis results

The results were checked and analyzed in comparison with standardand negative control. The complexes **1-4** show good to better activities against *Promastigote L. tropica* when treated after incubation period as the percent inhibition goes from high to higher as shown in Table 4. Due to the better results of complexes as anti-promastigote, these complexes can be developed as drug.

Table 4. % inhibition of promastigote *L. tropica* treated with various conc. of complexes 1-4 after 72 h of incubation and % hemolysis of complexes **1-4** after 3 h of incubation at various conc.

Comp. No.	Anti-promastigote activity			
	Concentration in (µg/mL)			
	500	250	125	62.5
1	90.5	78.0	70.8	74.3
2	99.2	99.9	99.8	98.3
3	51.6	30.5	28.3	19.1
4	98.7	99.0	91.2	92.5
Amphotericin B (0.33-0.004 µg/mL)	100			

Cytotoxicity analysis			
Comp. No.	Concentration in (µg/mL)		
	500	250	125
1	1.19	1.44	1.35
2	1.00	1.11	1.22
3	5.71	1.82	1.41
4	1.25	1.75	1.34
Triton X 100 (0.5%)	100		
PBS	0.004		

Cytotoxicity analysis results

The cytotoxic effects of complexes were analyzed after being incubated for a given time. The cytotoxic effects were considered as lower at low values and *vice versa* because complexes were analyzed at different concentrations and they have variable effects as shown in Table 4. It was concluded that all the complexes behave as less cytotoxic to human blood erythrocytes which illustrate that the complexes can be design as a novel drug against leishmanial disease.

DNA damage analysis (DNA laddering assay) results

The UV light photograph in Figure 3 of the DNA damage analysis shows that the complexes express no effect on human DNA intactness. From the photograph the comparison between complexes **1-4** and control compound, i.e. H_2O_2 were carried out. The complexes have no contribution to DNA damage and the DNA can be targeted by the complexes as a drug with low replication probability. Cytotoxic Cu(II) complexes often show DNA cleavage activity [33] but the screened complexes are generally not-toxic as evidenced from their POM analysis.

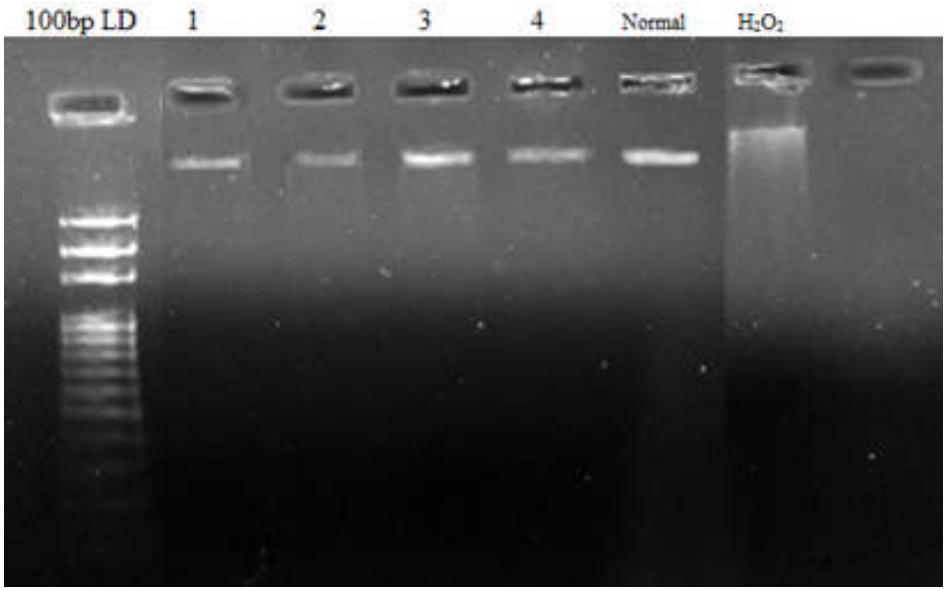


Figure 3. DNA laddering assay showing different compounds, samples bears no such effect on human DNA intactness at 500 µg/mL with control on right side.

POM analyses results

Modern drug discovery relies largely on high performance of small molecules that target macromolecular disease sites provided that the establish method have environment friendly compounds (drug-like) or not friendly (Pb-like compounds). For drug like properties, the synthesized compounds were compared with standard reference drugs.

The Osiris calculations are presented in Table 5. The Osiris methodology was used to calculate the mutagenicity, tumorogenicity, irritation and reproduction (toxicity risks) and to calculate Log*P*, solubility, drug-like and drug-score like properties (physiochemical properties). Toxicity risk within a molecule is predicted by toxicity risk predictor. The harmfulness of the compounds

depends that in which category of toxicity risk it comes. Tabulated data shows that the complexes **1-4** may be non-tumorigenic, non-irritant and have no reproductive effects when compared to reference drugs. But these compounds are mutagenic. The high cLog*P* values shows that the compound is poorly absorbed or permeated. For compounds to be able to absorb well, their cLog*P* value must not be higher than 5. Table 5 shows that all the compounds have cLog*P* values in the acceptable range. The complexes which have low solubility, they have low absorption and low distribution properties. Table 5 also shows the drug likeness of the synthesized complexes [34-36].

Table 5. Toxicity assessment, bio-availability and drug score of the synthesized compounds.

Comp. #	Toxicity risks			
	Mutagenic	Tumorigenic	Irritant	Reproductive effect
HL	not toxic	not toxic	not toxic	not toxic
NaL	not toxic	not toxic	not toxic	not toxic
1	Toxic	not toxic	not toxic	not toxic
2	Toxic	not toxic	not toxic	not toxic
3	Toxic	not toxic	not toxic	not toxic
4	Toxic	not toxic	not toxic	not toxic

Comp. #	Bio-availability and drug score				
	cLogP	S	TPSA	Drug likeness	Drug score
HL	-0.25	-2.22	121.4	-4.3	0.48
NaL	-1.26	-2.08	116.4	-5.8	0.29
1	-1.26	-2.08	116.4	-5.8	0.29
2	-1.26	-2.08	116.4	-5.8	0.29
3	-1.26	-2.08	116.4	-5.8	0.29
4	-1.26	-2.08	161.4	-5.8	0.29

Table 6. Molinspiration calculations of the synthesized compounds and standard drugs used.

Data	**HL**	**1**	**2**	**3**	**4**	StD-1	StD-2
LogP	0.82	-1.59	-1.59	-1.59	-1.59	2.73	5.72
TPSA	121	116	116	116	116	180	3.24
natoms	19	18	18	18	18	52	22
MW	266	253	253	253	253	749	291
nON	8	8	8	8	8	14	1
nOHNH	2	1	1	1	1	5	0
Nviolations	0	0	0	0	0	2	1
Nrotb	5	6	6	6	6	7	4
Volume	219	206	206	206	206	736	307
GPCR ligand	-0.41	-0.39	-0.39	-0.39	-0.39	-0.60	0.29
ICM	-0.38	-0.29	-0.29	-0.29	-0.29	-1.50	0.37
KI	-0.47	-0.44	-0.44	-0.44	-0.44	-1.35	0.10
NRL	-0.34	-0.54	-0.54	-0.54	-0.54	-1.40	0.44
PI	-0.48	-0.47	-0.47	-0.47	-0.47	-0.28	0.19
EI	-0.25	-0.17	-0.17	-0.17	-0.17	-0.82	0.68

NRL: Nuclear receptor ligand; ICM: ion channel modulator; KI: kinase inhibitor; PI: protease inhibitor; EI: enzyme inhibitor; StD-1: azithromycin; StD-2: turbinofine.

Table 6 described the molinspiration data of the tested compounds. The values of lipophilicity (cLog*P*) and total polar surface area (TPSA) are two important characteristics for prediction of per oral bioavailability of drugs. The lipophilicity and polar surface area values for complexes were compared with the standard drugs. The measured lipophilicity value for all complexes was -1.59. According to Lipinski's rule those drugs having cLog*P* are less than 5 are able to penetrate

through bio-membranes, hence these compounds penetrate through bio-membranes, and show good bioavailability. The low value of the cLog*P* is the prediction of good water solubility. Those complexes which have a total polar surface area values nearly around 160 or greater are expected to show poor intestinal absorption. For the prediction of oral bioavailability of a drug cLog*P* and polar surface area are the two key factors, but not enough criteria [34-36].

Molecular docking analysis of DNA

Molecular docking studies were carried out in order to find out the binding mode of the complexes with DNA. It was also confirmed by Molecular docking studies that the binding mode of complex to DNA is intercalative. In intercalation due to the insertion of the intercalator into the DNA base pairs, the DNA double helix arrangements may be stabilize, stiffen, lengthen and unwind. The complexes **1-4** were docked with DNA and the results showed that the most potent inhibitor was complex **2**. It was observed that this complex made three pi-H interactions with active site residue DT8, DT19 and DT20 of DNA, respectively [23] as shown in Figure 4.

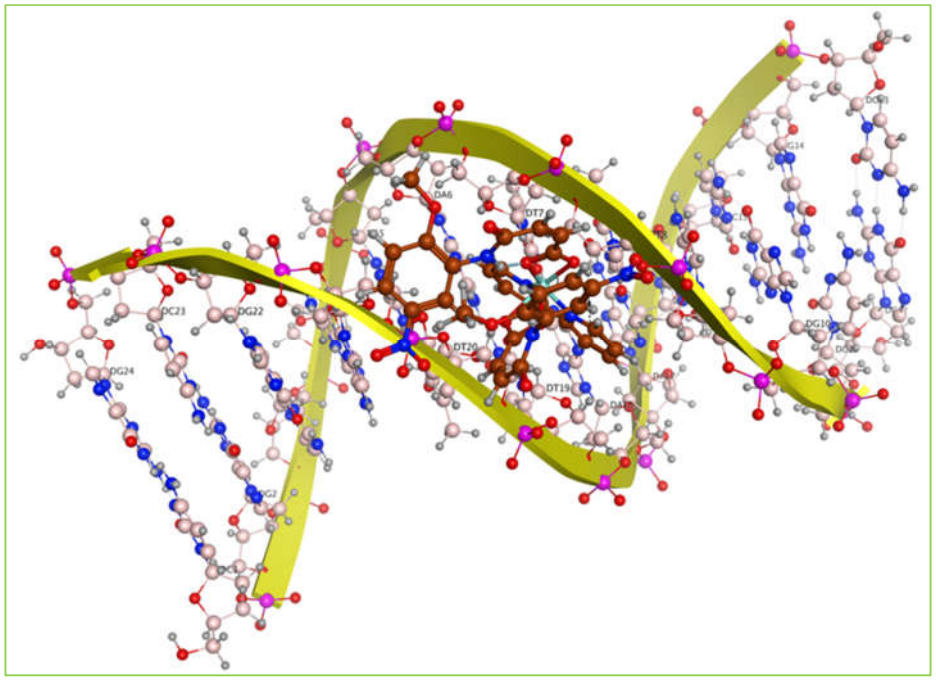


Figure 4. Binding mode of complex **2** inside the active site of DNA.

CONCLUSION

All the complexes were synthesized in a good yield. The expected functional groups were confirmed by FT-IR while the expected compositions were confirmed by EDX analysis. It was shown that the synthesized compounds interact with DNA through intercalative binding mode which was checked using UV-Visible spectroscopy. The intercalative mode of binding was futher confirmed through viscometry and molecular docking studies. The synthesized complexes were proved to have biological applications and exhibit significant antimicrobial, cytotoxic and

antileishmanial activities. The POM analyses were also carried out which demonstrate that the synthesized complexes may be an innovative contribution in drug development. The synthesized complexes lack the DNA laddering ability which indicates that there is no risk of DNA replication.

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