

SYNTHESIS, CHARACTERIZATION, POM ANALYSES AND BIOLOGICAL EVALUATION OF *N*-[(2-METHOXY-5-NITROPHENYL)]-4-OXO-4-[OXY] BUTENAMIDE BASED ZINC(II) CARBOXYLATE COMPLEXES

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ABSTRACT. The aim of medicinal chemistry is to link many scientific disciplines and allows the scientists in researching and developing new drugs with enhanced and targeted properties. In this article we are exploring the preparation of four new zinc(II) carboxylate complexes based on *N*-[(2-methoxy-5-nitrophenyl)]-4-oxo-4-[oxy]butenamide which were characterized through FT-IR and EDX studies. The DNA binding ability and binding type of complexes were assessed by spectroscopic (UV-Visible) and viscosity measurements, exhibiting an intercalative pattern of interaction. The synthesized compounds were also assessed to know theoretically about their nature by molecular docking studies resulting also in intercalation mode. Analysis of the complexes for biological applications such as anti-microbial, anti-leishmanial, cytotoxicity and DNA damage activities showed that these complexes carry good anti-microbial, anti-leishmanial activity with no toxicity to human blood thyrocytes and DNA. The bioavailability prediction and drug likeness score has also been evaluated through Insilco studies.

KEY WORDS: Zn(II) carboxylate complex, DNA binding, Anti-leishmanial activity, Cytotoxicity, Docking study

INTRODUCTION

Zinc has got importance because of its catalytic, enhanced biological activities of ligand along with highly inhibiting activities against fungal and bacterial growth [1-3]. The amide-based carboxylate ligands were designed for the current study because of the existence of peptide bonds in its structures which is highly important on the biological side because of its larger hydrophilic character as compared to the simple carboxylates [4, 5]. The carboxylate complexes of zinc(II) with ligands of nitrogen or oxygen donors have been proved highly significant in biochemical aspects, i.e. biological and chemical point of view in current era [6, 7].

In coordination chemistry, Zn is an attractive central metal ion and is an essential part of all enzyme classes. Zinc performs diverse physiological functions in many biological processes, for example, as a structural component, as a catalytic factor, or as a signaling mediator [8]. Curcumin based zinc(II) complexes having an aromatic spectator ligands displayed *in vitro* anticancer activity against various cancer cell lines [9]. Some Zn(II) carboxylate complexes have shown excellent anti-diabetic activity as Zn plays a vital role in insulin storage and secretion, as well as its reported insulin mimetic properties [10]. Zn(II) complexes have also displayed antitumor activity against human cancer cell lines because of the cytoprotective nature of Zn and suppresses apoptotic pathways [11]. Amide based Zn(II) carboxylate complexes have also been used for Alzheimer's disease treatment against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [12].

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New drugs with better cure rates and minimum side effects are required for the cancer treatment. Coordination chemistry presents lot of compounds having various geometries, different redox reactivity and different modes of attachment to DNA, e.g. *cis*-platin and other platinum coordination compounds are among the most widely used drugs for cancer treatment. Due to some severe side effect of Pt based drugs, coordination compounds of other than Pt metals with good antitumor activity got the attention of the researcher for the development of new anticancer agents [13]. Zinc(II) carboxylates possess various mode of interaction with DNA like intercalation, groove binding and electrostatic interaction [14-16].

The aim of this research work is to prepare *N*-[(2-methoxy-5-nitrophenyl)]-4-oxo-4-[oxy]butenamide based complexes of Zn with oxygen/nitrogen donor ligands capable of exploiting the antimicrobial, anti-leishmanial activities and carries lesser toxicity to human blood erythrocytes and DNA.

EXPERIMENTAL

Materials and methods

Solvents and other required chemicals of analytical grade purity were obtained from Sigma and Alfa Aesar and used as such as received. A digital Electro-thermal melting point instrument was used to determine the melting points of the prepared samples. FTIR spectra in the range of 4000–400 cm^{-1} of prepared compounds were obtained using Thermo fisher FTIR spectrophotometer. The DNA binding interaction was validated through UV-Visible spectrophotometer. EDX instrument was employed to determine the elemental compositions of the prepared compounds.

Synthesis of N-[(2-methoxy-5-nitrophenyl)]-4-oxo-4-[oxy]butenamide (HL) and NaL

The ligand HL was re-synthesized by dissolving in glacial acetic acid separately maleic anhydride and 2-methoxy-5-nitroaniline and then mixed them slowly. The mixture was stirred at 25 °C and precipitate formed after few min. The resulting precipitate was filtered and washed thoroughly using H_2O to get the desired product after air dry [17].

Sodium salt (NaL) was obtained by following the procedure described by Sirajuddin and co-worker [17] by the slow addition of $\text{NaHCO}_3(\text{aq})$ to a flask containing suspended solution of ligand, HL in distilled H_2O . After few min stirring at 25 °C, a clear solution was achieved which gives the required NaL product after rotary evaporation of H_2O (Scheme 1).

Synthesis of bis(N-(2-methoxy-5-nitrophenylamino)-4-oxo-4-[oxy]butenamide)zinc(II) (1)

Complex **1** was obtained by reacting an aqueous solutions of NaL (4 mmol, 1.16 g) and zinc sulfate (2 mmol, 0.574 g) in 2:1 stoichiometric ratio at room temperature [18] as presented in Scheme 1. Precipitate of the required complex **1** was appeared after stirring the resulting mixture at 25 °C. Precipitates of the required complex **1** was filtered, washed with distilled H_2O and dried. The desired complex was tried for crystallization in different solvents like methanol and DMSO but failed to get good quality crystals.

Synthesis of 2,2'-bipyridine bis(N-(2-methoxy-5-nitrophenylamino)-4-oxo-4-[oxy]butenamide)zinc(II) (2)

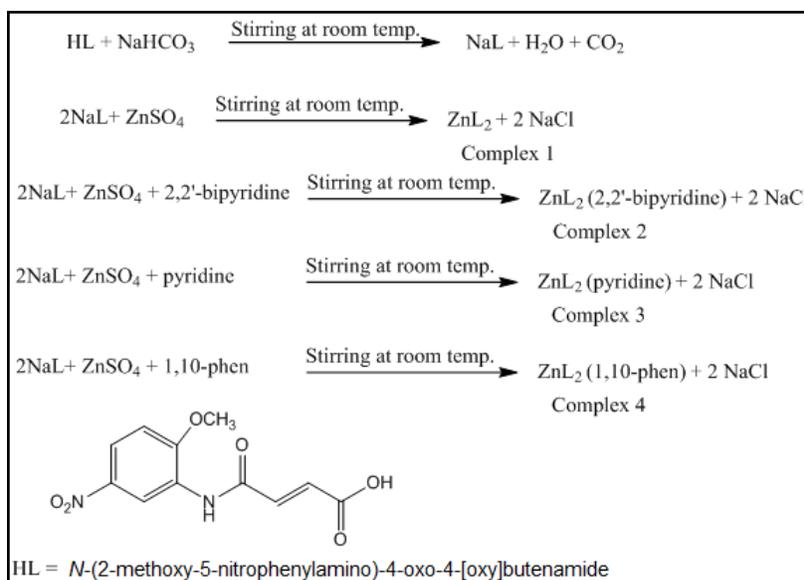
The same procedure as described for complex **1** was practiced for getting the complex **2** with additional step in which a 0.23 g (1.5 mmol) of 2,2'-bipyridine in 2-3 mL MeOH was slowly added to the mixture to get the desired product [18].

Synthesis of pyridine bis(N-(2-methoxy-5-nitrophenylamino)-4-oxo-4-[oxy]butenamide)zinc(II) (3)

The same procedure as described for complex **1** was practiced for getting the complex **3** with additional 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 [18].

Synthesis of 1,10-phenanthroline bis(N-(2-methoxy-5-nitrophenylamino)-4-oxo-4-[oxy]butenamide)zinc(II) (4)

The same procedure as described for complex **1** was practiced for getting the complex **4** with additional step in which a 0.29 g (1.5 mmol) of 1,10-phen. in 2-3 mL MeOH was slowly added to the mixture to get the desired product [18].



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

Complex-DNA binding study via UV-Visible spectroscopy

The deoxyribonucleic acid solution was obtained by the dissolution of small amount of sodium salt of SS-DNA in distilled water that was kept on stirring at room temperature for overnight to get the clear homogeneous solution. The ratio of n/p (nucleotide to protein) ratio for the prepared solution was obtained from the absorbance ratio of A_{260}/A_{280} and was about of 1.9 which indicates that DNA is free from protein. The concentration of DNA was calculated to be 1.97×10^{-4} M *via* using the molar absorptivity value $6600 \text{ M}^{-1} \text{ cm}^{-1}$ at 260 nm [19-21]. The DNA solution was stored at 4 °C. Complex solutions (20 μM) were prepared and screened for DNA-binding studies. The concentrations of complexes were kept constant while that of the SS-DNA was varied during the experiment. To both the complex and reference cell, an same quantities of DNA concentration were added to get variable absorbance [22, 23].

Complex-DNA binding study via viscometry

The viscosity of complex-DNA adduct was measured using the Ubbelohde Viscometer at room temperature to know the binding behavior of the complex-DNA interaction. In order to record mean flow time the process was done in triplicate. The data were plotted between the relative viscosity and the ratio of the complex-DNA concentration, i.e. $(\eta/\eta_0)^{1/3}$ vs. $([\text{Complex}]/[\text{DNA}])$. Here η is the viscosity in the presence of the complex and η_0 signifies the viscosity of the DNA in the absence of the complex [24].

Anti-bacterial activity assay

The anti-bacterial activity of the screened compounds against five bacterial strains (*Klebsilla pneumonia*, *Streptococcus auras* and *Escherichia coli*) were determined using well diffusion assay. All the experimental materials and nutrient agar media were sterilized while autoclaved for twenty min at 121 °C. Zinc(II) carboxylates complexes of 1 mg/mL to 6 mg/mL were prepared in DMSO. Azithromycin and DMSO were used +ve and -ve controls, respectively. A saline solution of 0.49 g NaCl in 50 mL distilled water was also prepared. A homogeneous solution of Stock nutrient Agar was obtained by dissolving 5.5 g of it taken using digital balance and was solubilized in 200 mL distilled H₂O [25-30].

Anti-fungal activity assay

Different dilutions of the screened samples were prepared having concentrations 1-6 mg/mL in DMSO. Respective solution of Turbinofine was also prepared in DMSO which was used as +ve control. Sabouraud dextrose agar media was prepared by dissolution of 4.5 g of SDA in 80 mL distilled water. After that all experimental materials and media (SDA) were sterilized while autoclaved for twenty min at 121 °C. The experimental activities were carried out in laminar flow which provide microbes free environment. The SDA was poured in test tubes and kept in slant position. After SDA solidification 60 μ L concentration from stock solutions of each complex were added in each corresponding labeled test tubes. The media along with samples were kept for some time to get solidified. After solidification fungal strains *Aspergillus niger* and *Aspergillus flavus* were applied on each test tube with the help of wire loop. All the test tubes were closed through cotton swabs and kept in incubator at 37 °C. The inhibition was measured once after 24 h and again after 72 h [31-33].

Anti-promastigote assay

The anti-promastigote activity of the prepared compounds was performed by following the procedure described by Mehwish and co-worker [34] using MTT assay [35]. The activity of the compounds at four different concentrations (prepared in serial dilution in the range of 500-62.5 μ g/mL) were accessed.

Cytotoxicity assay

The prepared compounds were tested to explore their cytotoxicity activity performed by following the procedure described earlier [34, 36]. Fresh human blood was collected from volunteers and RBC were obtained by centrifugation after washing three times with PBS. A 990 μ L of the remaining RBC (red blood cells) were treated with each sample (complexes 1-4) such that the final tested concentration reaches at 500, 250, 125 μ g/mL followed by incubation at 37 °C for 3 h and then the cells were centrifuged for five min at with 1000 rpm. The supernatants were collected and the released hemoglobin was confirmed at 576 nm using spectrophotometer.

Dimethyl sulfoxide and Triton X-100 (0.5%) were employed as -ve and +ve controls, respectively. With the help of Elisa plate reader, the OD was obtained at 576 nm and % hemolysis was determined by the formulashown in equation 1 [37].

$$\text{Hemolysis (\%)} = \frac{\text{OD 576 nm in sample solution} - \text{OD 576 nm in PBS}}{\text{OD 576 nm in 0.5\% Triton} - \text{OD 576 nm in PBS}} \times 100 \quad (1)$$

DNA damage analysis (DNA laddering assay)

The DNA damage was studied by DNA laddering assay as described earlier [38]. The DNA material was extracted from fresh human blood (obtained earlier for cytotoxicity assay) using phenol/chloroform (1:1) method. A 0.5 mg/mL proteinase K was added to harvest and re-suspend the cells in digestion buffer. In the presence of RNase A, the mixture was incubated at 37 °C for 3 h and precipitated by adding sodium acetate and ice-cold ethanol to the aqueous phase followed by overnight incubation at -20 °C and was treated with different concentrations (500 µg/mL) of selected compounds (complexes) for 3 h. The mixture was centrifuged, and pellets were collected, allowed to dry and re-suspended in *tris*-EDTA buffer. Using a *tris*-acetate-EDTA running buffer on 2% agarose gel, 20 µg DNA aliquots were then electrophoresed and photographed under ultraviolet light. [39-41].

Theoretical study by molecular docking

Molecular docking study was performed to get the information about the interaction between the drug target and compound [42]. For this MOE-Dock program (www.chemcomp.com) was used. MOE (molecular operating environment) software was employed to draw the 3D structure of the metal-complex analogs [43]. A 3D protonated structure of all compounds was drawn which were then minimized by using the default parameters of the MOE. From the protein databank (PDB), the 3D crystal structure of the DNA (1 bna) was obtained and the tested compounds were docked into the active site of the target receptor in MOE by the default parameters as described in earlier [4] and by using MOE software the best poses were obtained after docking.

RESULTS AND DISCUSSION

The physical data including the molecular formula, mass, physical state, solubility, color and melting points of the prepared Zn(II) carboxylate complexes is given in Table 1. The complexes were freely soluble in DMSO and some also in methanol. The sharp melting points of the synthesized compounds show their purity.

Characterization of synthesized compounds by FTIR

Table 1 presents the selected FTIR peaks observed in the synthesized compounds spectra. The deprotonation of the ligand HL was confirmed by the absence of OH peak at 3250 cm⁻¹. The symmetric vibration peaks in complexes are present in the range of 1337-1344 cm⁻¹ while the asymmetric vibration peaks appears in the range of 1522-1530 cm⁻¹. A decrease in the value of symmetric vibration and increase in the value of asymmetric vibration was observed after complexation compared to that of the free ligand because of the attachment of oxygen of the carboxylate moiety with Zn atom [44]. The difference between asymmetric and symmetric vibrations ($\Delta\nu$) for complexes **1-4** are 183, 192, 188 and 182 cm⁻¹, receptively, indicating bidentate mode of coordination of carboxylate moiety to Zn atom as $\Delta\nu$ for complexes $\ll \Delta\nu$ for NaL[5]. The complex formation was also confirmed by the appearance of Zn-O absorption bands at 428, 430, 424 and 420 cm⁻¹, respectively in complexes **1-4** [5, 45]. The attachment of 2,2'-bipyridine, pyridine and 1,10-phenanthroline group in complexes was observed at 516, 470 and 479 cm⁻¹ because of Zn-N peak [5].

Table 1. Physical and FTIR data (cm⁻¹) of HL NaL and corresponding zinc(II) carboxylate complexes **1-4**.

Physical data						
Comp. No.	Mol. formula	Mol. mass	Nature	Color	Solubility	M. P. (°C)
1	C ₂₀ H ₁₈ N ₄ O ₁₂ Zn	571.77	Powder	Light yellow	DMSO	150-152
2	C ₃₀ H ₂₆ N ₆ O ₁₂ Zn	727.95	Powder	Dark yellow	DMSO, MeOH	118-120
3	C ₂₅ H ₂₃ N ₅ O ₁₂ Zn	650.87	Powder	Off white	DMSO, MeOH	147-149
4	C ₃₂ H ₂₆ N ₆ O ₁₂ Zn	751.97	Powder	Yellow	DMSO	98-100
FTIR data (cm ⁻¹)						
IR Peak	HL	NaL	1	2	3	4
v(OH)	3120	Absent	Absent	Absent	Absent	Absent
v(COO) _{asy}	1540	1552	1527	1530	1525	1522
v(COO) _{sym}	1276	1301	1344	1338	1337	1340
Δv	264	251	183	192	188	182
v(C=C)	1594	1664	1633	1681	1648	1645
v(Zn-O)	Absent	Absent	428	430	424	420
v(Zn-N)	Absent	Absent	Absent	516	470	479
v(N-H)	3347	3364	3505	3550	3499	3367
v(C=O)	1716	1714	1700	1702	1705	1704

EDX analysis

The prepared complexes were also characterized by EDX to study the presence of Zn. Since EDX is used mainly for the detection of heavy metals and is not suitable for the lighter elements like carbon or oxygen atoms. The presence of Zn metal in the composition confirms the complex formation as shown in Table 2. The variation in the percentage composition is due to the fact that EDX is only good for heavy metal detection.

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

Element	Line type (series)	Apparent concentration	Weight %	Factory standard
C	K	40.32	53.10	Yes
N	K	0.15	0.88	Yes
O	K	43.36	37.01	Yes
Zn	L	6.82	9.03	Yes
Total			100	

DNA binding studies by UV-Visible spectroscopy

While studying absorption spectra for complexes one prominent peak was observed having variable wavelength and absorbance in the absence of DNA. The resultant peak appears as a result of π - π^* transitions along with aromatic chromosphere in complex. After the addition of DNA solution into the complex solution, changes take place in absorbance and wavelength, which shows the interaction between complex and DNA [46]. A hypochromic with slight red shift recorded in the spectra of the screened complexes upon the addition of DNA solution which is an indication of the intercalation type of interaction between the DNA and the compound. The π^* orbital of intercalated complex molecule interact with the π orbital of the DNA bases resulting in the reduction of π - π^* transition probability giving the bathochromic effect and supports the intercalation binding mode. The DNA binding constant K_b (M⁻¹) was obtained from the intercept to slope ratio of a plot of $A_{\nu}/(A-A_{\nu})$ vs. $1/[DNA]$ [47]. The DNA-complex interaction was stable and the binding was spontaneous which was confirmed from the -ve ΔG value. The effects for complexes **1**, **2**, **3** and **4** are shown in Figure 1.

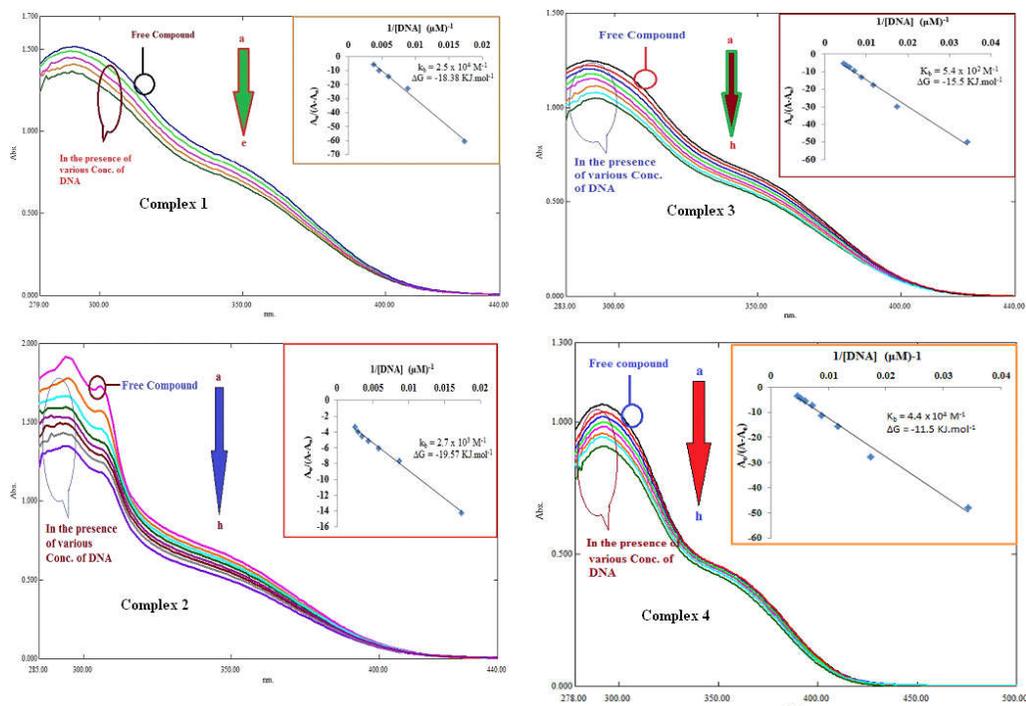


Figure 1. UV-Visible spectrum of 1 mM complexes (1-4)-DNA adduct with and without the presence of various concentrations of DNA: a (free comp.) and b-g (10-70 μM DNA with 10 μM DNA increment).

DNA binding studies by viscometry

The complex binding mode to deoxy ribonucleic acid (DNA) was also confirmed using viscometer. The time flow for various concentrations was recorded. The graph for viscosity was plotted as $(\eta/\eta_0)^{1/3}$ vs. $r = [\text{Compound}]/[\text{DNA}]$. The intercalated binding mode of complex to DNA was confirmed from the increase in viscosity. The relative viscosity of the free DNA and in the presence of various concentration of the complexes were determined from time flow using equations 2 and 3 [48]:

$$\eta_0 = t - t_0 \quad (2)$$

$$\eta = \frac{t-t_0}{t_0} \quad (3)$$

where t_0 and t are the time flow in the absence and presence of DNA, respectively.

The representative plot of complex 2 with DNA shows the intercalative mode of interaction in Figure 2A.

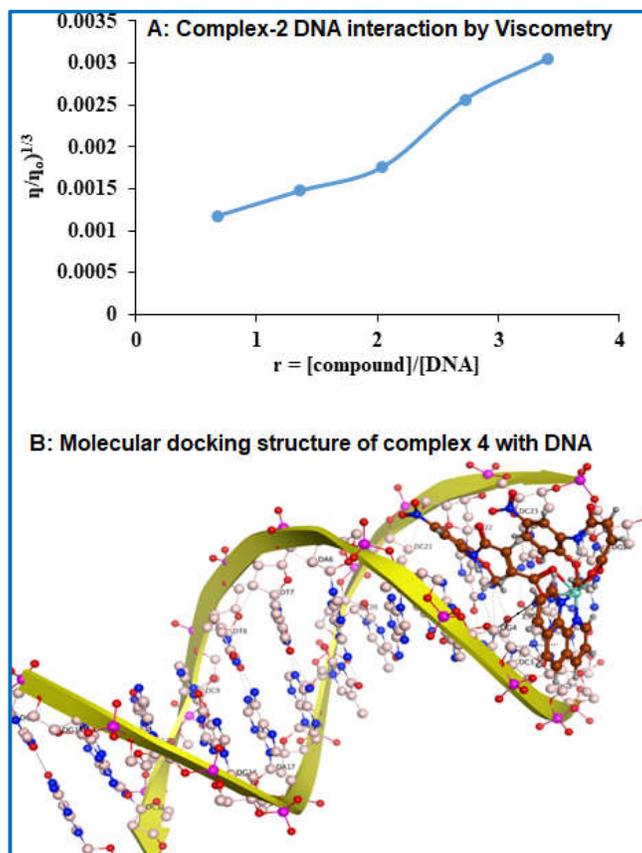


Figure 2. (A) Plot of relative viscosity vs. $r = [\text{compound}]/[\text{DNA}]$ in which the conc. of complex 2 is varied DNA conc. is constant and (B): molecular docking structure of complex 4 with DNA.

Analysis of anti-microbial activity

The synthesized complexes were screened for their anti-bacterial potential and their activity was compared with standard antibiotic, Azithromycin. The maximum activity of 23, 22 and 24 mm were shown by Azithromycin against *Klebsiella pneumonia*, *Streptococcus aureus* and *Escherichia coli*, respectively. It was observed that the antibacterial activities were increased with increasing complex concentration (Table 3). The minimum activity was shown by 1 mg/mL while maximum activity was shown by complex having concentration of 6 mg/mL.

The complexes were evaluated after incubation period for their anti-fungal activity using Terbinafine (shows maximum inhibition) and DMSO (shows zero inhibition) as +ve and -ve controls, respectively (Table 3). It was summarized that the minimum anti-fungal activities were shown at concentration of 1 mg/mL while maximum activity shown at 6 mg/mL. The recorded data was compared with standard anti-fungal drug and negative control. It was summarized that with the increase in complex concentration increase the anti-bacterial activity as shown by various concentration results.

Table 3. Antimicrobial activity results of the screened compounds.

Complex No.	Zone of Inhibition (mm) for anti-bacterial activity results at 6 mg/mL		
	<i>K. Pneumonia</i>	<i>S. Auras</i>	<i>E. Coli</i>
1	12	14	15
2	15	15	17
3	13	18	17
4	15	16	16
Azithromycin	23	22	24
Anti-fungal activity results at 6 mg/mL			
Complex No.	Zone of Inhibition (mm)		% Inhibition
	<i>A. niger</i>	<i>A. flavus</i>	<i>A. niger/A. flavus</i>
1	18	24	36/ 48
2	18	21	36/ 42
3	19	18	38/ 36
4	25	30	50/60
DMSO	0	0	0
Terbinafine	50	50	100

Anti-leishmanial activity of zinc(II) carboxylate complexes

Anti-promastigote assay of the tested compounds was performed by MTT assay using Amphotericin B as a standard and their data displayed that against *L. tropica* promastigote these compounds are highly active at all concentrations. Percentage inhibition of promastigote *L. tropica* treated with various concentrations of complex after 72 h of incubation is given in Table 4. The highest activity was shown by complexes **4** > **3** > **1** > **2**. All the complexes showed over 90% inhibition at 500 µg/mL tested against promastigote. The highest activity of complex **4** may be due to greater lipophilic character due to the presence of 1,10-phenanthroline moiety. The data clearly indicate that dose and time dependent activity was observed for the screened compounds.

Cytotoxicity analysis

The quality of chemical and other substances being lethal to cells is called cytotoxicity for example certain immune cells and venoms [49]. The interaction of molecules with the cells (RBC's) results in the formation of membrane pores which ultimately results in the hemolysis of cell membrane by colloid osmotic mechanism to release hemoglobin. The hemolytic activity of the screened compounds was then determined spectrophotometrically by measuring the released hemoglobin. Previously this assay has been used as a reliable model for screening pharmaceutical agents designed for intracellular delivery of biologic drugs. Keeping in view the importance of the test compounds it is worth testing the possible cytotoxic effect toward human red blood cells. The percent hemolytic activity of the screened compounds explored that the screened compounds were non hemolytic to human blood erythrocytes at all tested concentrations (Table 4).

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

Complex No.	Anti-leishmanial activity			
	Concentration in ppm			
	500	250	125	62.5
1	95.31154	89.82110	83.09685	69.33991
2	92.16533	88.21715	41.70265	38.92659
3	95.1851	91.48674	90.00617	45.71252
4	99.81210	99.10140	89.71452	88.31020
Amphotericin B (0.33-0.004 µg/mL)	100			
Complex No.	Cytotoxicity analysis			
	Concentration in ppm			
	500	500	500	500
1	1.1	1.0	1.0	1.0
2	1.0	1.1	0.9	0.9
3	1.0	1.01	1.0	1.0
4	1.4	1.1	1.3	1.3
Triton X 100 (0.5%)	100			
PBS	0.004			

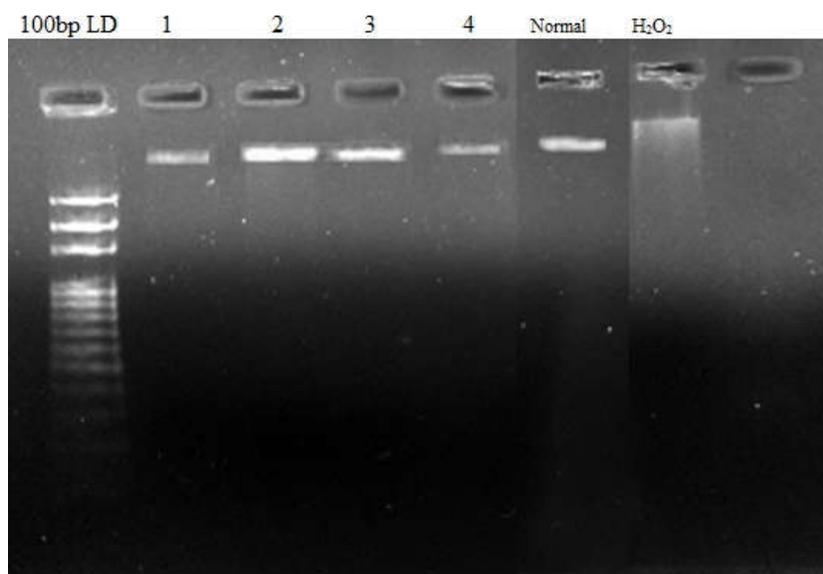


Figure 3. DNA laddering assay showing no prominent DNA degradation effects of different compounds **1, 2, 3, 4** at 500 µg/mL, with control on the rightmost and ladder on the leftmost side.

DNA damage analysis (DNA laddering assay)

DNA damage may be referred to as the ability of chemical agent that damages the genetic material inside the cell causing unexpected changes such as mutations, inappropriate event

activation and other structural changes which may cause serious illness such as cancer [50]. This assay has the importance that it not only confirms degradation or damage occurring to DNA but it also gives an idea about apoptosis. Our findings suggested that these complexes have no obvious DNA laddering/smears/tailing profile when compared with the ladder and positive control H₂O₂ (Figure 3). Lower DNA damage activity and nontoxic nature further increments the significance of these complexes in the field of drug development.

POM analysis

POM physico-chemical analysis or ADME/T is important to qualify drugs and their efficacy as leading candidates against various diseases. The POM physicochemical calculations included a partition coefficient (cLogP), aqueous solubility, donor hydrogen bond and drug likeness, which are evaluated in terms of Lipinski's rule-of-five. To qualify oral bioavailability, the topological polar surface (TPSA) should be < 140 Å² [33]. In our compounds the value of TPSA is < 140 except only for complex 1. The presence of two different pharmacophore sites: NH-C-C-N=O (potential antibacterial pharmacophore site) and O=C-C-C-C=O (potential antifungal/antiviral pharmacophore) make the synthesized complexes quit interesting from biological point of view [47].

From molinspiration data (Table 5) it was concluded that the synthesized complexes obey the rule of Lipinski behave as a drug and to have kinase and enzyme inhibition properties. As our synthesized complex has Molecular weight less than 450, so it may be highly absorbed because most of the traded drugs, i.e. approximately 80% has Molecular weights in this range. The complex with molecular weight 595.79 also shows drug likeness as 20% traded drug has molecular weight more than 450. The logP value of the synthesized complexes fall in the standard range, i.e., less than 5 so these compounds may be highly hydrophilic and thus meet the criteria of market drugs.

Table 5. Molinspiration calculations of the complexes 1-4 and standard drugs.

Data	1	2	3	4	Azithromycin	Terbinafine
LogP	1.98	-1.59	-1.59	-1.59	2.73	5.72
TPSA	220.92	116.45	116.45	116.45	180.09	3.24
Natoms	39	18	18	18	52	22
MW	595.79	253.19	253.19	253.19	749.00	291.44
nON	16	8	8	8	14	1
nOHNH	2	1	1	1	5	0
Nviolations	2	0	0	0	2	1
Nrotb	14	6	6	6	7	4
volume	460.31	205.99	205.99	205.99	736.45	306.73
GPCR ligand	-0.18	-0.39	-0.39	-0.39	-0.60	0.29
ICM	-0.36	-0.29	-0.29	-0.29	-1.50	0.37
KI	-0.20	-0.44	-0.44	-0.44	-1.35	0.10
NRL	-0.22	-0.54	-0.54	-0.54	-1.40	0.44
PI	-0.17	-0.47	-0.47	-0.47	-0.28	0.19
EI	-0.16	-0.17	-0.17	-0.17	-0.82	0.68

ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease Inhibitor; EI: Enzyme Inhibitor.

Osiris calculations

The Osiris calculations are given in Table 6. Toxicity risks and physico-chemical properties of complexes were evaluated and analyzed by the methodology developed by Osiris. It is necessary for a good drug to have high hydrophilicity and low cLogP value (cLogP value must not be

greater than 5.0). It may lead to poor absorption and permeation if a drug lacks such contents. Compounds having $c\text{LogP} > 5$ have a good absorption ability. Upon such recommendations all the complexes exhibit $c\text{LogP}$ values in the satisfactory range. Here 'S' shows the aqueous solubility capability of a compound. The screened compounds may be recommended to be introduced as an innovative drug because they have the 'S' value range (Table 7) which meets about 80 % of Drug market criterion (S value greater than -4). The toxicity effects of the synthesized complex were also determined through Osiris analysis which helps in determining risky fragment. All the synthesized complexes have less toxicity risk as a whole and fulfill the requirements of the drug market up to some extent.

Table 6. Toxicity assessment of synthesized compounds.

Compounds	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

Table 7. Bio-availability and DS* of the synthesized compounds.

Compounds	Bio-availability and DS				
	$c\text{LogP}$	S	TPSA	Drug likeness	DS
HL	-0.25	-2.22	121.4	-4.28	0.48
NaL	-0.4	-2.21	119.4	-4.24	0.43
1	-1.28	-2.11	115.4	-5.4	0.26
2	-1.268	-2.11	115.4	-5.4	0.26
3	-1.28	-2.11	115.4	-5.4	0.26
4	-1.28	-2.11	115.4	-5.4	0.26

*DS: Drug score; S: solubility.

Molecular docking studies

Molecular docking studies were carried out in order to find out the binding mode of the metal complexes and to information about the proper orientation of DNA bounded complex yielding a stable adduct. It was also confirmed by Molecular docking studies that the binding mode of complex to DNA is intercalative. In intercalation due to the introduction of an intercalator into DNA base pairs the DNA double helix arrangements may be stabilize, stiffen, lengthen and unwind. The Zn carboxylate complex analogs were docked with DNA and the results showed that the most potent inhibitor was complex 4. It was observed that this complex made one H-acceptor and two pi-H interactions with DC3 and DG4 residues of DNA respectively as shown in Figure 2B.

CONCLUSION

All the complexes were synthesized in good yield. The expected composition was confirmed by EDX analysis. It was shown that the complex interact with DNA through intercalative binding mode which was checked using UV-Visible Spectroscopy. The intercalative mode of binding was also confirmed through viscometry and Molecular docking studies. The result of the Molecular docking study revealed that complex interacts with one H-acceptor and two pi-Hs with DC3 and DG4 residues of DNA, respectively. The results of DNA damage activity show

that the synthesized complexes compared to H₂O₂ (control) have no obvious DNA laddering profile. Further the lower DNA damage activity of these complexes enhances their importance in drug development field. The hemolytic activity results exhibited that the screened complexes showed concentration dependent hemolysis. The POM analysis were also carried out which demonstrated that the synthesized complexes might be an innovative addition in drug development. The lack of DNA degradation ability and cytotoxicity to human blood erythrocytes increment the significance of these complexes in the field of drug development.

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