Synthesis, Antioxidant, Molecular Docking And DNA Interaction Studies of Metal Based Imine Derivatives

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Research Article

Keywords: Antioxidants, DNA binding, free radicals, Schiff base metal complexes

Posted Date: August 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-778827/v1

Abstract

Background: Currently, numerous investigations are ongoing into the interaction of free radicals with biological systems such as lipids, DNA and protein.

Methods: In the present work, synthesis, characterization, antioxidant, DNA binding and molecular docking studies of Schiff base ligand and its Ni(II), Co(II), Cu(II) and Zn(II) were evaluated.

Results: The metal complexes shown significant dose-dependent antioxidant activities comparable to the classical antioxidants, ascorbic acid and ethylene diaminetetraacetic acid (EDTA). The DNA binding constants (*k*b) were found to be $3.487\times10^{-5}M^{-1}$, $1.858\times10^{-5}M^{-1}$, $3.090\times10^{-5}M^{-1}$, $1.367\times10^{-5}M^{-1}$ and $9.118\times10^{-5}M^{-1}$ for Ni(II), Co(II), Cu(II) and Zn(II) metal complexes, respectively. Binding constants (*Kb*) and free energy (ΔG) values calculated from molecular docking analysis were found to be in close agreement with experimental results.

Conclusion: The obtained results indicate the importance of synthesis complexes as a source of synthetic antioxidants and anticancer drugs.

Background

Free radicals are highly reactive species containing one or more unpaired electrons. They donate or take electrons from other molecules in an attempt to pair their elections and generate a more stable species [1, 2]. Free radicals are generated within the body during normal metabolic activities, stimulation of macrophages, leucocytes, aerobic respiration and other metabolic processes, on the other hand tobacco smoke, pollutants, ionizing radiations, organic solvents and pesticides are the major exogenous sources of free radicals production in biological systems. For instance, ROS/RNS are markedly involved in many signaling pathways that control development and maintain cellular homeostasis [3, 4]. However excess production of these free radicals either internally or transferred from environment have a great impact on human in the etiology of various diseases. Although, the body possesses defense mechanisms as antioxidant nutrients and enzymes which arrest the damaging properties of free radicals. Continuous exposure to chemicals and contaminants may increase the amount of free radicals in the body beyond its ability to control and cause irreversible oxidative damages [5].

Therefore, antioxidants with free radical scavenging potential may be relevant in the therapeutic and preventions of diseases where free radicals are implicated. In addition to natural antioxidants such as vitamin C, vitamin E, carotenoids flavonoids a number of synthetic antioxidants like butylatedhydroxyltoluene (BHT), butylatedhydroxyanisole (BHA), tertiary butylhydroquinone and Schiff base metal complexes have been prepared and their antioxidant capacity has been assessed for the prevention of various diseases [6, 7].In the present study, the synthesis, characterization, antioxidant, DNA binding activity, molecular docking studies of newly synthesized Schiff base ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were evaluated *in vitro*. The names of compounds along with their structures employed in the present work are given in Fig. 1.

Methods

Chemicals

Salicylaldehyde, aniline, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), ferrous sulphate, Ascorbic acid, ethylenediaminetetraacetic acid (EDTA), Tris HCl buffer, ferric chloride, O-phenenthroline, sulfuric acid, ammonium molybdate, Potassium phosphate (mono phosphate and diphosphate), Hydrogen peroxide, ethanol, Salmon fish DNA of analytical grade were purchased from Sigma Aldrich.

Synthesis of 2-[(*E*)-(phenylimino)methyl]phenol (H-Pimp)

The Schiff base ligand H-pimp was synthesized by reacting the methanolic solution of 5mmol of aniline with 5mmol salicyladehyde. Yellowish product was produced instantaneously upon mixing. Crude product was filtered and recrystallized

from concentrated metahanolic solution.

Yield: 63%, M.Pt: 48° C, Elemental analysis (C13H11NO): Calc. C(79.16%), H(5.62%), N(7.10%), Exp. C(80.63%), H(5.12%), N(7.01%), IR analysis (cm^{-1}): 3200 (w), 1569 (s), 1483(s), 1407(s), 1363(s), 1282(s), 1156(s), 1110(s), 987(s), 912(s), 857(s), 756(s), 688(s), ^{1}H NMR (300.13 MHz, CDCl3, 303k) δ = 6.8 (d, ^{3}JHH = 7.03 Hz, 1H, H13), 6.91 (d, ^{3}JHH = 7.03 Hz, 2H, H12 & H14), 6.94 (d, ^{3}JHH = 7.01 Hz, 2H, H4 & H5), 7.02 (d, ^{3}JHH = 7.2 Hz, 1H, H3), 7.11 (d, ^{3}JHH = 7.31 Hz, 2H, H11 and H15), 7.3 (d, ^{3}JHH = 6.9 Hz, 1H, H6), 8.53 (s, Ar HC N), 10.0 (s, OH), ^{13}C{^{1}H}-NMR (75.47 MHz, CDCl3, 303k), 110 (CH, C13), 112 (CH, C12 & C14), 112.1 (CH, C4 & C5), 113 (CH, C3), 126 (CH, C11 and C15), 133 (CH, C6), 133 (C, C2), 148 (C, C1), 153 (C, C10), 159 (CH, Ar HC-N).

Synthesis of transition metal complexes with H-pimp Schiff base ligand

The transition metal complexes of the H-pimp Schiff base ligand were synthesized by following the same procedure as reported [8]. The metal salts were initially dehydrated by keeping the acetate salts of Co(II), Ni(II), Cu(II) and Zn(II) in an oven for 3-4 h at 110° C. Methanolic solution of H-pimp (5mmol) was added to the methanolic solution of metal acetate (2mmol) and stirred for 3h. The product was either instantly soon after the reaction or obtained through concentration using rotary evaporator.

Bis(2-[(*E*)-(phenylimino)methyl]phenolate)cobalt(II) (Co-pimp)

Yield; 43%, elemental analysis, C26H20CoN2O2, Calc. C(69.18%) H(4.47%) Co (13.06%) N(6.21%) Found C(69.88%) H(5.11%) Co(13.02%) N(6.12%) IR analysis: 1536(s), 1482(s), 1465(s), 1450(s), 1328(s), 1180(s), 1148(s), 1122(s), 1086(s), 1010(s), 976(s), 929(s), 858(s), 837(s), 758(s), 698(s) cm^{-1}, λmax = 860 nm (ε = 17.6 M^{-1} cm^{-1}, ^{2}A2g →^{2}B1g).

Bis(2-[(*E*)-(phenylimino)methyl]phenolate)nickel(II) (Ni-pimp)

Yield; 55%, elemental analysis, C26H20N2NiO2, Calc. C(69.22%) H(4.47%) N(6.21%) Ni(13.01%) Found. C(69.28%) H(4.90%) N(6.29%) Ni(12.11%), IR analysis: 1533(s), 1464(s), 1443(s),1416(s), 1343(s), 1260(s), 1224(s), 1181(s), 1147(s), 1123(s), 1033(w), 981(s), 946(s), 871(w), 821(s), 810(s), 761(s), 751(s), 728(s), 689(s), 671(s) cm^{-1}, λmax = 690 nm (ε = 28.3 M^{-1} cm^{-1}, ^{1}A1g →^{1}A2g).

Bis(2-[(*E*)-(phenylimino)methyl]phenolate)copper(II) (Cu-pimp)

Yield; 68%, elemental analysis, C26H20CuN2O2, Calc. C (68.48%) H (4.42%) Cu (13.94%) N (6.14%) Found. C (69.33%) H (4.92%) Cu (13.01%) N(7.44%) IR analysis: 1555(s), 1523(s), 1478(s), 1441(s), 1388(s), 1351(s), 1323(s), 1255(s), 1205(w), 1175(s), 1151(s), 1133(s), 1098(s), 1031(s), 1009(s), 987(s), 937(s), 834(s), 767(s), 699(s), 623(s) cm^{-1}, λmax = 660 nm (ε = 188.6 M^{-1} cm^{-1}, $dz^2 \rightarrow dx^2-y^2$).

Bis(2-[(*E*)-(phenylimino)methyl]phenolate)zinc(II) (Zn-pimp)

Yield; 55%, elemental analysis, C26H20N2O2Zn, Calc. C (68.20%) H (4.40%) N (6.12%) Zn (14.29%) Found. C (70.01%) H(4.78%) N (6.89%) Zn (14.95%), IR analysis: 1581(s), 1531(s), 1459(s), 1441(s), 1389(s), 1351(s), 1326(s), 1253(s), 1169(s), 1151(s), 1096(s), 1032(s), 1008(s), 927(s), 829(s), 789(s), 762(s), 688(s), 596(s) cm^{-1}.

Determination of *In-Vitro* antioxidant studies

DPPH radical scavenging assay

The antioxidant activity of the newly synthesized compounds were assessed using the stable DPPH free radical according to Ibrahim *et al.*2017 [7]. Various concentrations (50, 100, 200 and 400μM) of compounds were mixed with ethanolic solution containing 85μM DPPH radical. The decrease in absorbance was measured at 518 nm using a UV-Visible Spectrophotometer.

Ascorbic acid was used as positive control to determine the maximal decrease in DPPH absorbance. The values are expressed in percentage of inhibition of DPPH absorbance in relation to the control values without the compounds (ascorbic acid maximal inhibition was considered 100% of inhibition).

Ferrous ion-chelating assay

The ferrous ion chelating activity of newly synthesized compounds were analyzed by a standard method Puntel *et al.,* 2005[8]. Various concentrations (50μM, 100 μM, 200 μM, and

400 μM) of compounds were mixed with 0.2 ml of 3.6 mM ferrous sulphate, 0.3ml of 100 mM Tris-HCl (pH=7.4), 0.1 ml of 9 mM O-Phenanthroline and diluted up to 3.0 ml with ultra-pure distal water. The reaction mixture was shaken vigorously, incubated for 10 minutes and the decrease in absorbance was determined at 510 nm. EDTA (ethylenediaminetetraacetic acid) at the same concentrations utilized as a reference standard and without Schiff bases complexes .

Ferric Reducing / Antioxidant Power Assay

The ferric reducing power of the newly synthesized compounds were determined according to (Kumar *et al.,* 2012) [9]. Different concentrations (50, 100, 200, 100 and 200 μM) of compounds, 0.2 ml of 3.6 mM ferric chloride, 0.3ml of 100 mM tris buffer (pH=7.4), 0.1 ml of 9 mM O-phenanthroline and diluted up to 3.0 ml with ultra-pure distal water. It was shaken for 10 min vigorously and left to stand at room temperature. The increase in absorbance of the sample solution was measured at 510 nm using a UV-Visible Spectrophotometer. Ascorbic acid at the same concentrations was utilized as a reference standard and without compounds sample mixture as control.

Total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant capacity of newly synthesized compounds were evaluated by phosphomolybdenum assay (Sahaa *et al*., 2008)[10]. Reagent solution containing various concentrations (50, 100, 200 and 400 μM) of compounds aliquot in ethanol, 0.7 ml of 0.6 M sulphuric acid, 1.0 mM ammonium molybdate, 1.0 ml of 28 Mm pottasium pasphate and ultra pure distal water was incubated at 95°C for 90 min. After cooling, at room temperature the increase in absorbance of the mixture is measured at 695 nm using an V-730 UV-Visible/NIR Spectrophotometer. Ascorbic acid was utilized as reference standard and without compounds sample mixture as control.

Hydroxyl radical scavenging activity

The scavenging activity of all the synthesized complexes for hydroxyl radicals were measured with Fenton reaction by (Li et al., 2011)[11]. Reaction mixture of various concentrations (50, 100, 200 and 400 μM) of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes, 0.1mL of 7.5 mM O-phenanthroline, 0.5 ml of 0.2 M phosphate buffer (pH 6.6), 0.1 mL of 7.5 mM ferrous sulfate and 0.1 mL of H2O2 (0.1%) and diluted up to 3 mL with distilled water. The reaction mixture incubated at room temperature for 30 min and the absorbance was measured at 510 nm using a UV-Visible Spectrophotometer. The reaction mixture without Schiff base complexes has been used as control and without Schiff base complexes and H2O2 as a blank.

DNA Absorption spectroscopic studies

The interaction between metal complexes and DNA were studied using electronic absorption method. Solution of Salman fish DNA in the buffer 50 mM NaCl/ 5 mM Tris–HCl (pH 7.2) in water gave a ratio 1.9 of UV absorbance at 260 and 280 nm, indicating that the DNA was sufficiently free from protein [12]. The concentration of DNA was measured using its extinction coefficient at 260 nm ($6600M^{-1}$ cm^{-1}) after 1:100 dilution. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in ethanol and diluting suitably with the corresponding buffer to the required concentration for all of the experiments. The data were then fitted to the Equation 6 to obtain the *k*b values for interaction of the complexes with DNA.

[DNA] / (εa-εf) =DNA/ (εa-εf) + 1/ [*k*b (εb-εf)]

Where εa, εf, and εb are the apparent, free and bound metal complex extinction coefficients, respectively. A plot of [DNA]/ (εb-εf) versus [DNA], gave a slope of 1/ (εb-εf) and a Y-intercept equal to [*k*b/(εb-εf)]$^{-1}$; *k*b is the ratio of the slope to the Y-intercept.

Statistical analysis

Linear regression analysis was used to calculate IC50±SEM values from data and graphs by using Graph pad prism 6® (Motulsky and Neubig, 2001). Significant differences among the means of data were tested by the one-way ANOVA followed by the student´s t-test with significance level (P<0.05). All the tests were conducted in triplicate

Molecular docking methodology

The chemical structure of the Schiff base ligand and its complexes with Co(II), Ni(II), Cu(II) and Zn(II) were sketched and optimized on MOE2017 window using MOE molecular builder and were entered into MOE database. 3-D structure of DNA with PDB ID-1D66, resolution of 2.7 and sequence of CCGGAGGACTGTCCTCCGG was obtained from the RCSB protein Data Bank [13]. For the purpose of docking simulation PDB internal coordinates of DNA were optimized using molecular dynamic AMBER force field and semi-empirical PM3 approaches to attain minimum energy and stable conformation. Water molecules were removed from DNA structures by sequence editor of MOE to exclude effect of water on interaction of DNA with the ligand and its metal complexes. Structures was protonated with their standard geometry followed by their energy optimization tool using MOPAC 7.0. The resulting structures were subjected to systematic conformational search at default parameters with RMS gradient of 0.01 kcal/mol using Site Finder to find out active sites of DNA molecule. Finally a number of docking runs were carried out to get a final binding pose from scoring function. The best conformation was selected on the basis of energetic ground and the minimum Final Docking Energy (ΔG) [14-15].

Results And Discussion

The Schiff base ligand 2-[(*E*)-(phenylimino)methyl]phenol (H-pimp) was synthesized by the condensation reaction of salicylaldehyde with aniline. It was characterized through different spectroscopic and analytical techniques. The uncorrected melting temperature was observed around 48° C. The infrared spectral data of the H-pimp Schiff base ligand reveal a peak around 3200 cm^{-1} assigned to the NH stretch. The peak is weakly observed because of the possible intramolecular hydrogen bonding viz; given in scheme 1.

The broad peak for hydroxyl stretch is obscured by the NH band appearance. The azomethine is observed at the expected position. The formation of the ligand is further supported by the NMR spectra. The ^{1}H-NMR of the H-pimp Schiff base ligand reveal a singlet assigned each for azomethine moiety and the phenolic hydroxyl group. Rest of the spectrum show peaks for the aromatic protons. ^{13}C{^{1}H}-NMR show imine peak at 159 ppm, and the hydroxyl peak at 148 ppm. The Schiff base ligand was reacted further with metal ions in 2:1 molar ratio to produce metal derivatives of the anionic ligand with [M(pimp)2] compositions. The schematic representation of the metal derivatives is shown in scheme 2:

The IR spectra of the metal complexes share combine features of bonding irrespective of the metal center. The H-pimp ligand is behaving as monoanionic ligand offering coordination sites like O^{-} and HC = N. Both the stretching bands were seen displaced from the position observed in the spectrum of the free ligand. Rest of the spectra show differences due to the complexation. The elemental compositions in metal complexes also reveal closeness for the [M(pimp)2] compositions in all the metal complexes. Therefore, it has been observed unambiguously that the metal complexation occurs through bonding of two molecules of the ligand with metal centers. The metal complexes were also observed to be non-electrolyte in nature.

The UV-visible spectra of the metal complexes were recorded within the region 320–800 nm in methanolic solutions. The Co-pimp complex show a single and broad band at 860nm assigned to the distorted square planar ^{2}A2g $\rightarrow^{2}B_{1g}$ ^{1}A1g$\rightarrow^{1}$B1g

and $^{1}A1g \rightarrow Eg$ were considered enfolded within the broad $^{2}A2g \rightarrow {}^{2}B1g$ transition [2]. Similarly the visible spectrum of Ni-pimp metal complex also reveal a single transition $^{1}A1g \rightarrow {}^{1}A2g$ for the C2v symmetry. The Cu-pimp show a weak transition at 660 nm assigned to the $dz^2 \rightarrow dx^2 - y^2$ in distorted tetrahedral geometry. All the transitions in metal complexes were assigned charge transfer ligand to metal transitions where the imine group is involved in donating the electron density toward metal orbitals.

DPPH radical scavenging assay

The IC50 value for ligand was found to be 333.58±5.771μM highest among the tested compounds. The IC50 values for the synthesized complexes are ranging from 150 to 196μM. The IC50 value for ascorbic acid was found to be 121.07±15.94. These activities were dose reliant and maximum DPPH scavenging activity was observed at higher concentrations.

Ferrous ion-chelating assay

Figure 3 shows the Fe^{2+} chelating properties of ethanolic solution of Schiff base Ligand and its metal complexes and EDTA (ethylenediaminetetraacetic acid). The IC50 value for ligand was found to be 297.45 ± 5.771μM highest among the tested compounds. The IC50 values for the synthesized complexes are ranging from 138 to 180 μM. The IC50 values for EDTA was found to be 135.

Ferric Reducing / Antioxidant Power Assay

The compound reduction power may serve as a significant indicator of its potential antioxidant activity [6–7]. Table 3 and Fig. 4 shows the reductive competences of the Schiff base ligand and its metal complexes when compared to the standard, Ascorbic acid. The IC50 value for ligand was found to be 293.28 ± 6.538 μM highest among the tested compounds. The IC50 values for the synthesized complexes are ranging from 130 to 162 μM. The IC50 values for Ascorbic acid was found to be 99.

Table 2
Free energy (ΔG), binding constant (Kb) of ligand and its metal complexes calculated from molecular docking data.

Complex	Free Energy (-ΔG)/kJ/mol	Binding Constants (K_b)/M^{-1}
H-pimp	31.56	3.14×10^5
$Ni(pimp)_2$	30.29	1.88×10^5
$Co(pimp)_2$	31.22	2.73×10^5
$Cu(pimp)_2$	29.87	1.59×10^5
$Zn(pimp)_2$	32.79	5.04×10^5

Table 3
Data set of selected electronic descriptors calculated from molecular docking data.

Complex	E_{Total}/kcalmol	E_{HOMO}/kcal/mol	E_{LUMO}/kcal/mol	$E_{electro}$/kcal/mol	E_{vandr}/kcal/mol	E_{IP}/kcal/mol
H-pimp	-449908.307	-8.8801	3.9700	-296836.46	0.00000	8.8801
$Ni(pimp)_2$	-	-	-	-	0.00000	-
$Co(pimp)_2$	-	-	-	-	0.00000	-
$Cu(pimp)_2$	-	-	-	-	0.00000	-
$Zn(pimp)_2$	-99731.257	-1.18563	-0.4894	-875361.812	0.00000	-1.18563

Total antioxidant activity (Phosphomolybdenum assay)

Total antioxidant capacity of Schiff base ligand and its metal complexes have been evaluated by phosphomolybdate method with ascorbic acid as a standard. The Mo(VI) is reduced to Mo(V), in the presence of drugs which shows maximum absorbance at 695 nm. The IC50 value for ligand was found to be 264.41 ± 7.532μM highest among the tested compounds. The IC50 values for the synthesized complexes are ranging from 120 to 155 µM. The IC50 values for Ascorbic acid was found to be 86.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the Schiff base ligand and its metal complexes was investigated by O-phenanthroline method. The IC50 value for ligand was found to be 49.63 ± 8.391μM highest among the tested compounds. The IC50 values for the synthesized complexes are ranging from 36 to 43 µM. The IC50 values for Ascorbic acid was found to be 29.

Ligand (Z)-2-((phenyl-imino)methyl)phenol containing hydroxyl group at ortho position to benzene ring and nitrogen atom are interconnection between two rings. Oxygen atom of hydroxyl group having high electron donating power and acts as ring activating group.

Hence, the overall ligand acts as a rich π electrons species and their antioxidant power have further enhanced by complexation with transition metals.

All the metal complexes containing the same two bidentate Ligands (Z)-2-((phenyl-imino)methyl)phenol attached at the same position and the antioxidant potential depend only on central metal atom. Zn(II) and Cu(II) Complexes shows almost same antioxidant as compared to Ni(II), Co(II) metal complexes.

DNA Binding activity

The electronic absorption spectroscopy is the most common way to investigate the interactions of various Schiff base metal complexes with DNA. In general, complex bound to DNA through intercalation usually results in hypochromism and red shift (bathochromism), due to the strong stacking interaction between aromatic chromophore of the complex aromatic π rings and the base pairs of DNA. The H-pimp, Ni-pimp, Co-pimp, Cu-pimp, and Zn-pimp complexes showed absorption bands at 331, 325, 427.5, 387 and 321 nm with increasing concentration of DNA. All the complexes showed hypochromicity and a red-shifted charge transfer peak maxima in the absorption spectra. The absorption spectra of the H-pimp, Ni-pimp, Co-pimp, Cu-pimp, and Zn-pimp complexes in the absence and presence of SH DNA are given in Figs. 5, 6, 7, 8 and 9, respectively. With the addition of DNA, the absorption intensities are gradually decreased. A total of 10% (for H-pimp), 15% (for Ni-pimp), 17% (for Co-pimp), 14% (for Cu-pimp) and 16% (for Zn-pimp) of hypochromicity with 2.0, 1.0, 1.5, 2.5 and 1.0 of red shift were obtained. The intrinsic binding constants for H-pimp, Cu-pimp, Ni-pimp, Co-pimp,] and Zn-pimp complexes are found to be

$3.487\times10^{-5}M^{-1}$, $1.858\times10^{-5}M^{-1}$, $3.090\times10^{-5}M^{-1}$, $1.367\times10^{-5}M^{-1}$ and $9.118\times10^{-5}M^{-1}$, respectively (illustrated in Table 1) indicating a moderate intercalation between the complexes and Salmon fish DNA. These *k*b values are much smaller than the typical classical intercalators. In order to compare the binding strength of the complexes with Salmon fish DNA the *k*b were obtained by monitoring the changes in the absorbance for the complexes with increasing concentration of DNA. The *k*b was obtained from the ratio of slope to the intercept from the plot of [DNA]/(□a-□f) versus [DNA].

Table 1
The IC50(μM) ± SEM values of Ligand (H-pimp) and its metal complexes M(pimp)2a for different assays.

Compound	DPPH assay	FRAP assay	TAA assay	•OH assay
H-pimp	333.58 ± 5.771	293.28 ± 6.538	264.41 ± 7.532	49.63 ± 8.391
$Ni(pimp)_2$	196.70 ± 11.30	145.90 ± 11.00	134.78 ± 11.29	39.41 ± 14.32
$Co(pimp)_2$	159.44 ± 9.066	153.66 ± 11.65	134.42 ± 11.55	43.19 ± 13.62
$Cu(pimp)_2$	150.51 ± 10.95	130.47 ± 13.31	120.93 ± 11.76	36.04 ± 12.84
$Zn(pimp)_2$	170.34 ± 11.85	162.90 ± 10.61	155.77 ± 11.76	36.91 ± 15.01
Ascorbic acid(AA)	121.07 ± 15.94	99.612 ± 16.91	86.729 ± 17.21	29.25 ± 17.21
[a]Standard used is Ascorbic acid (AA)				

Table 4
Data set of selected steric descriptors calculated from molecular docking data.

Complex	SlogP	HF	M_R	Dipole moment	V_{surf}
H-pimp	3.1428	28.3960	10.7179	3.4950	0.00000
$Ni(pimp)_2$	5.5234	———	7.9865	0.0000	0.00000
$Co(pimp)_2$	4.5117	———	9.4579	0.0000	0.00000
$Cu(pimp)_2$	6.1117	———	6.1803	0.0000	0.00000
$Zn(pimp)_2$	2.3745	96.1966	11.7197	16.9428	0.00000

Structural analysis and Molecular docking

Structural analysis of (Z)-2-((phenylimino) methyl)phenol ligand and its metal complexes were carried out using semiepirical PM3 method Fig. 10(a-e) for their charge distribution and molecular docking with doubly stranded DNA (PDB:1D66). Molecular docking studies determined all possible configuration of computationally anticipated Schiff base ligand H-pimp, Ni-pimp, Co-pimp, Cu-pimp, and Zn-pimp complexes with DNA to understand the molecular mechanism and physical mode of interaction. DNA is the primary pharmacological target of number of anticancer compounds, and henceforth, the interaction between DNA and metal complexes are of vital prominence in understanding the mechanism. Transition metal complexes interact with DNA via both covalent and / or noncovalent interactions. Non-covalent DNA interactions include intercalative, electrostatic and groove binding of metal complexes with a DNA helix (Zhang *et al*., 2001). Pose view analysis of (Z)-2-((phenylimino) methyl)phenol and its metal complexes was also performed to substantiate the mode of DNA binding and have been shown in Fig. 11(A). Least energy confirmation pose of (Z)-2-((phenylimino) methyl)phenol revealed that planar aromatic part of ligand intercalates between stacked base pairs of DNA by π-stacking interactions. 2D lig plot of the ligand

showed that H of H-O group develops H-bonding with adenine DA (A8) of DNA whereas O of H-O group builds H-bonding with H atom of Guanine DG(A7) as shown in Fig. 7B(i). H-bonding characteristics of ligand rendered significantly high binding affinity with DNA (Table-2). Ni(II) complex of ligand exhibited mixed mode of intercalation and groove binding. It can be seen that Ph- ring of Zn(II) complex intercalates between DNA base pairs and rest of bulky structure stucks into minor groove of DNA establishing Vander Waal's interactions with groove as visible in Fig. 11. B(ii).It was observed that Co(II) and Cu(II) complexes of (Z)-2-((phenylimino) methyl) phenol fit well into minor groove of DNA via hydrophobic and Vander Waal's interactions (Fig. 11.B (iii-iv))Interactions of Zn(II) complex with DNA are attributed to the intercalation of aromatic planar ring with flanking base pairs more importantly with DC(A14), DG(B26) and DT(A15) of DNA Fig. 11B(v). An substantial observation was that Zn(II) complex showed enhanced interactions and binding affinity in terms of highest *Kb* value as compared to free ligand and other complexes (Table-2). Negative value of free energy depicted that physical interaction mechanism of compounds with DNA has been proved to be spontaneous.

For the comprehension of microscopic interactions between DNA and (Z)-2-((phenylimino) methyl)phenol ligand as well as its complexes a number of physico chemical descriptors were calculated listed as electronic descriptors (Table − 3) and steric descriptors (Table − 4).

Electronic parameters of the ligand and complexes were calculated, however semi empirical calculations of transition metal compounds were complicated due to partially filled d-orbitals of the metal ions that are responsible for the multifarious structures with large variety of possible coordination number and geometries [16–17].Hence electronic parameters of only ligand and Zn complex (completely filled d-orbital) were determined. EHOMO and ELUMO values gave an estimate of the electron-donating or electron-accepting character of a given compound, consequently, a compound is considered more electron-donating as its EHOMO value increases and more electron accepting as its ELUMO value decreases. Table 3 depicts that Zn (II) complex is more electron donating as compared to ligand due to higher value of its EHOMO.

An excellent correlation of two steric parameters i.e., partition coefficient, (log P) and Molar refractivity (MR) with binding constant (*Kb*) has been perceived, Partition coefficient (log P) is illustrative of hydrophobicity of the molecule. In this work *slogP* revealed a reasonable inverse correlation with the *Kb* of ligand and its metal complexes (R^2 = 0.885), indicating that the compounds with a lower *slogP* are anticipated to constitute stronger complexes (Fig. 12A).

Another imperative steric descriptor calculated in present work was Molar refractivity (*MR*), a measure of the total polarizability of a mole of a substance and is dependance on the temperature, the index of refraction, and the pressure. A direct correlation of the MR (R^2 = 0.8281) with *Kb* was indicative of the fact that compounds with higher MR have more binding affinity with DNA (Fig. 12B)

Conclusions

In the present study we performed the antioxidant and DNA binding study of a Schiff base ligand and its metal complexes. This preliminarily screening of these synthetic compounds reveals interesting antioxidant and DNA binding activities, however, these results cannot be extended directly to *in vivo* systems which are differ and more complex from *in vitro* studies. Additionally, the obtained result showed that it is interesting to note that metal complexes presented higher DPPH radical and OH radicals activities, Fe^{+2} chelation, reducing Fe(III) to Fe(II) and Mo(VI) to Mo(V) higher than free ligand. The findings of this study clearly indicated that the antioxidant effects of Schiff base ligand and its metal complexes, presented DPPH radical-scavenging activity, demonstrating the mechanism by which these drug displayed antioxidant activities. The ability of Schiff base Ligand and its metal complexes to show significant reducing power and to hunt DPPH radicals suggests that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions. In conclusion the results presented in the present studies give information about the nature of antioxidants and DNA Binding intercalators found in Schiff base metal complexes. Structural analysis, Molecular docking and QSAR investigations presented highest binding affinity Zn(II) complex and lowest affinity for Cu(II) complex against DNA. Moreover computational calculated *Kb* values complemented experimentally determined *Kb* for UV-Vis spectroscopic results

Abbreviations

EDTA:ethylene diaminetetraacetic acid; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; BHT: butylatedhydroxyltoluene; BHA: butylatedhydroxyanisole

Declarations

Acknowledgements

This Research grant was supported by King Saude University ,Deanship of Scientific Research, College of Sciences Research Centre.

The authors gratefully acknowledge Abdul Wali Khan University Mardan (AWKUM) and King Saud University,

Funding

The authors are thankfull for the financial support of King Saude University ,Deanship of Scientific Research, College of Sciences Research Centre.

Availability of data and materials

All data generated or analyzed during this study are included in this published article .

Authors' contributions

Each author participated sufficiently in taking public responsibility for appropriate portions of the content. Study conception and design: MoI, FP, NM, and MuI, conceived the idea and designed experiments and wrote manuscript. HUN,AA and FP performed the experiments;, MI, JPK analyzed the data; JBR revised the manuscript. All authors reviewed and approved the final version.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

References

1. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine, fourth ed. Oxford University Press, Oxford. 2006
2. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006; 160:1–40.
3. Perez-De La Cruz V, Gonzalez-Cortes C, Pedraza-Chaverri J, Maldonado PD, Andres-Martinez L, Santamaria A Protective effect of S-allylcysteine on 3-nitropropionic acid-induced lipid peroxidation and mitochondrial dysfunction in rat brain synaptosomes. Brain Res Bull. 2006;68:379–383.
4. Perez-Severiano F, Rodriguez-Perez M, Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Ortiz-Plata A, Sanchez-Garcia A, Villeda-Hernandez J, Galvan-Arzate S, Aguilera P, Santamaria A S-allylcysteine, a garlicderived antioxidant,

ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. Neurochem Int. 2004; 45:1175–1183.
5. Castill J, Rama R, Davalos A Nitric oxide-related brain damage in acute ischemic stroke. Stroke 2000; 31. 852–857.
6. Ibrahim M, Hassan W, Meinerz DF, Dos Santos MV, Klimaczewski CM, Deobald A, Costa MS, Nogueira CW, Barbosa NB, Rocha JB. *In Vitro* Antioxidant evaluation and DNA binding ability of Ni(II), Co(II), Cu(II) and Zn(II) metal complexes containing bidentate Schiff base. *IOSR J Appl Chem*. 2012;*10*:06–14.
7. Ganji N, Chityala VK, Marri PK, Aveli R, Narendrula V, Daravath S, Shivaraj, DNA incision evaluation, binding investigation and biocidal screening of Cu(II), Ni(II) and Co(II) complexes with isoxazole Schiff bases. J Photochem Photobio Bio 2017; 175:132–140.
8. Puntel RL. Nogueira CW, Rocha JB. Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in rat brain in vitro. *Neurochem Res.* 2005;*30*:225–235.
9. Kumar, RS, Raj KB, Perumal P. Antioxidant activities of Indigo feracassioides Rottl.Ex. DC. Using various in vitro assay models. *Asian Pacif J Tro Dis* 2012; *2*:256–261.
10. Sahaa MR, Hasana SMR, Aktera R, Hossaina MM, Alamb MS, Alam MA, Mazumder MEH In vitro free radical scavenging activity of methanol extract of the leaves of Mimusopselengi Linn. *Bangl J Vetery Medic* 2008;6: 197–202.
11. Li P, Huo L, Su W, Lu R, Deng C, Liu L, Deng Y, Guo N, Lu C, He C Free radical-scavenging capacity, antioxidant activity and phenolic content of Pouzolziazeylanica. *J. Serbian Chem Soc* 2011:*76;* 709–717.
12. Parnham MJ, Graf E Pharmacology of synthetic organic selenium compounds. *Prog Drug Res.* 1991; 36:9–47. https://www.rcsb.org/structure/1D66
13. Abdel-Hamid MK, Abdel-Hafez AA, El-Koussi NA, Mahfouz NM, Innocenti A, Supuran CT.Design, synthesis, and docking studies of new 1,3,4-thiadiazole-2-thione derivatives with carbonic anhydrase inhibitory activity. Bioorg Med Chem 2007;15:6975–6984.
14. Zhang QL, Liu JG. Chao H, Xue GQ, Ji LN DNA-binding and photocleavage studies of cobalt(III) polypyridyl complexes. J Inorg Biochem. 2001; 83:49–55.
15. Peter Comba, Trevor. W. Hambley and Bodo Martin.K.M. Honorio, A.B.F. Da Silva. Molecular Modeling of Inorganic compounds-Peter, Int. J. Quant. Chem. 2003;95: 126.
16. Patrick G Medicinal Chemistry by, Department of Chemistry and Chemical Engineering,fourth ed., Paisely University Paisley, Scotland. 2009

Figures

Figure 1

Structure of Schiff base ligand and their metal (Ni(II), Co(II), Cu(II), Zn(II)) complexes.

Figure 2

A: DPPH radical scavenging activity of Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and Ascorbic acid). Mean ± SEM was used to expressed the values Significantly a $p < 0.0006$, b $p < 0.0017$, c $p < 0.0027$, d $p < 0.0086$ B Ferrous ion-chelating activity of Ligand and their metal complexes. (Ligand, Ni(II) Complex, Co(II) Complex, Cu(II) Complex, Zn(II) Complex and EDTA) (Ethylenediaminetetraacetic acid). Values are expressed as mean ± SEM (triplicate tests were conducted for each sample) Significantly a $p < 0.0010$, b $p < 0.0454$, c $p < 0.0086$, d $p < 0.0062$, e $p < 0.0095$, f $p < 0.0033$ C Ferric ion reducing activity of Ligand and their metal complexes. Values are expressed as mean ± SEM (triplicate tests were conducted for each sample). D Phosphomolybdenum assay of Ligand and their metal

complexes. Values are expressed as mean ± SEM (triplicate tests were conducted for each sample). E Hydroxyl scavenging assay of Ligand and their metal complexes. Values are expressed as mean ± SEM (triplicate tests were conducted for each sample).

Figure 3

A: Absorption spectra of H-pimp in buffer pH 7.2 at 25 ºC in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (□a-□f) × 10-9M2cm versus [DNA] × 10-5 M for titration of DNA with H-pimp. A: Absorption spectra of H-pimp in buffer pH 7.2 at 25 ºC in the presence of increasing amount of DNA. Arrows indicate the changes in absorbance upon increasing the DNA concentration. Inset: plot of [DNA] / (□a-□f) × 10-9M2cm versus [DNA] × 10-5 M for titration of DNA with H-pimp.

Figure 4

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Figure 5

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Figure 10

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Figure 11

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Figure 12

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Supplementary Files

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