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Synthesis, characterization and biological activity of a Schiff base derived from *o*-amino benzoic acid and its Co(II), Cd(II) and Ni(II) complexes

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ABSTRACT

Equimolar amounts of imidazoleacetophenone and 2-aminobenzoic acid were combined together and the Schiff base 4(1*H*-imidazole-yl)acetophenoneanthranilic acid was prepared as a new bidentate complexing agent. The synthesized ligand was reacted with cobalt(II), cadmium(II), and nickel(II) ions yielding air stable complexes. For quantification and characterization purposes, elemental analysis, infrared spectra, electronic spectra, proton nuclear magnetic resonance spectra and mass spectra studies were carried out on the obtained complexes and ligand. Thermogravimetric analysis and magnetic susceptibility measurements were also used for characterization. The ligand IR spectrum showed that the ligand acts as a bidentate coordinates to the metal ions through the nitrogen and oxygen atoms. Measurements of magnetic susceptibility for Ni(II) and Co(II) complexes were found to be 3.4 and 3.8 B.M., respectively, in the range normal for the octahedral geometry. The conductivity measurements revealed that the chelates are non-electrolytes. An *in vitro* antimicrobial investigation was also carried out for the free ligand and its metal complexes against a number of bacterial and fungal strains, to assess their antimicrobial properties by diffusion technique. Antimicrobial activity of the prepared complexes showed higher activity than the free ligand.

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1. Introduction

Schiff bases are nitrogen analogs of aldehydes or ketones in which the carbonyl group is replaced by carbodiimide (C=N-R) group, where R, may be an aliphatic (alkyl) or an aromatic (aryl) group. A Schiff base is usually formed by condensation of an aldehyde or ketone with a primary amine. Schiff bases that contain aryl substituent having effective conjugation are substantially more stable and more readily synthesized, while those which contain alkyl substituent are relatively unstable [1,2].

Survey of literature confirmed that *o*-amino benzoic acid derivatives with a number of Schiff bases having potential bioactive constituents act as antifungal [3], antibacterial [4-6] and antitumor activities [7,8]. These bases have been also used as complexing agents for complexation with metal ions [9,10]. In terms of industrial scale, they have wide range of application such as pigments and corrosion inhibitors [11]. Schiff bases are also applicable in modern technology as photo stabilizers and dyes for solar collectors and solar filters [12].

The objective of this work is to synthesize the ligand 4(1*H*-imidazole-yl)acetophenoneanthranilic acid, and its chelates with Co(II), Cd(II), and Ni(II) ions. The resulting compounds characterized by using different analytical techniques, such as elemental analysis, IR, UV-Vis, ^{1}H NMR, magnetic susceptibility measurements and thermal analysis. Biological activities of the ligand and complexes were also investigated.

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2. Experimental

2.1. Materials and measurements

Organic solvents (absolute ethyl alcohol (SDFCL, India), dimethyleformamide (DMF, 99%, GC-Lab Tech Chemical), dimetheylsulfoxide (DMSO, LabaChemie PVT. Ltd.) used without purification. $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CdCl_2.2H_2O$, 4(1*H*-imidazole-yl)acetophenone, antharanalic acid (Sigma, USA), ammonium hydroxide (SDFCL, India), glacial acetic acid (Scharlau, European Union) are of analytical grade and used as is. Double distilled water was used in all preparations.

2.2. Instrumentations

The magnetic susceptibility was measured on powdered samples using Gouy balance at Cairo University, Egypt. Elemental analyses were carried out in the Micro Analytical Unit, Cairo University, using chemical analyzer, Carlo-Erba model 1106. UV-vis spectra were carried out using Shimadzu model 3101 spectrophotometer. Infrared spectra of solid samples were recorded on Perkin-Elmer model 1650 and Shimadzu model 8400S spectrophotometer. ^1H NMR spectra were recorded in a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz using DMSO-d_6. Mass chromatograms were recorded on a Shimadzu GCMS-QP2010 Plus instrument. Thermal analysis measurements (TGA and DTA) were recorded on a Shimadzu TGA-50H and Shimadzu DTA-50. The electrical conductivity was measured using a conductivity meter (Jenway Ltd., Model 4010).

2.3. Synthesis of the ligand (HL)

Antharanalic acid (2.74 g, 0.02 mol) was placed in 100 mL round bottomed flask. Absolute ethanol (25 mL) was added and the mixture was heated until the dissolution was completed. Imdazoleacetophenone (3.72 g, 0.02 mol) dissolved in 25 mL hot absolute ethanol and added to solution, followed by addition of few drops of glacial acetic acid. The mixture was then refluxed on hot plate with constant stirring for two hours. On cooling, yellow powder was precipitated. The precipitate was filtered, washed with ethanol and left to dry in air. The product was recrystallized from ethanol [13,14].

2-((1-(4-(1H-imidazol-1-yl) phenyl)ethylidene)amino) benzoic acid (4(1H-Imidazole-yl)acetophenoneanthranilic acid) (HL): Color: Yellow. Yield: 84%. M.p.: 80-82 °C. FT-IR (KBr, ν, cm^{-1}): 3455 (OH) (sh, acid), 1674 (C=O) (acid), 1627 (C=N) (imine). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.606 (s, 3H, CH_3), 6.492 (t, 1H, Ar-H), 6.731 (d, 1H, Ar-H), 7.170 (d, 1H, Ar-H), 7.213 (t, 1H, Ar-H), 7.680 (d, 1H, Ar-H), 7.81-7.87 (m, 3H, Ar-H), 8.080 (d, 2H, Ar-H), 8.418 (s, 1H, Ar-H), 8.523 (sb, 1H, OH). MS (EI, *m/z* (%)): 305 (M^+, 100), 259, 228, 210, 196, 166, 150, 120, 90, 79. Anal. calcd. for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 67.00; H, 3.50; N, 12.90%. Λ_m (S.m^2.mol^{-1}): 46.

2.4. Synthesis of complexes

2.4.1. Synthesis of [Ni(L)$_2$(H$_2$O)$_2$]

$NiCl_2.6H_2O$ (0.238 g, 0.001 mol) placed in 100 mL beaker dissolved in 20 mL of warm absolute ethanol was added drop wise, with stirring, to a solution of 4(1*H*-Imidazole-yl)acetophenoneanthranilic acid (0.61 g, 0.002 mol) in 20 mL ethanol. The mixture was refluxed for two hours. On partial evaporation of the solvent, the green precipitate separated out was filtered, washed with ethanol and left to dry in air [13]. Color: Green. Yield: 82.5%. M.p.: 178-179 °C. FT-IR (KBr, ν, cm^{-1}): 1680 (C=O) (Ketone), 1610 (C=N) (imine). Anal. calcd. for $C_{36}H_{32}NiN_6O_6$: C, 61.47; H, 4.59; N, 11.95. Found: C, 61.40; H, 4.12; N, 11.50%. Λ_m (S.m^2.mol^{-1}): 30.

2.4.2. Synthesis of [Co(L)$_2$(H$_2$O)$_2$].H$_2$O

$CoCl_2.6H_2O$ (0.238 g, 0. 001 mol) placed in 100 mL beaker dissolved in 20 mL of warm absolute ethanol was slowly added, with stirring, to a solution of ligand (0.61 g, 0.002 mol) in 20 mL of absolute ethanol. The mixture was refluxed on hot plate with constant stirring for two hours, followed by addition of few drops of ethanolic solution of ammonia. The pink precipitate was collected by filtration, washed with ethanol and left to dry in air [13]. Color: Pink. Yield: 81%. M.p.: 180-182 °C. FT-IR (KBr, ν, cm^{-1}): 1672 (C=O) (Ketone), 1595 (C=N) (imine). Anal. calcd. for $C_{36}H_{34}CoN_6O_7$: C, 59.92; H, 4.75; N, 11.65. Found: C, 60.50; H, 4.63; N, 12.40%. Λ_m (S.m^2.mol^{-1}): 41.

2.4.3. Synthesis of [Cd(L)$_2$(H$_2$O)$_2$]

$CdCl_2.2H_2O$ (0.228 g, 0.001 mol) placed in 100 mL beaker dissolved in 20 mL warm absolute ethanol was added slowly, with stirring to a solution containing 0.61 g (0.002 mol) of imidazole acetophenoneanthranalic acid dissolved in 20 mL of absolute ethanol. The mixture was refluxed on hot plate with constant stirring for two hours, followed by addition of few drops of ethanolic solution of ammonia. The white precipitate was collected by filtration, washed with ethanol and left to dry in air [13]. Color: White. Yield: 86%. M.p.: 168-170 °C. FT-IR (KBr, ν, cm^{-1}): 1673 (C=O) (Ketone), 1598 (C=N) (imine). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.609 (s, 6H, CH_3), 6.481 (t, 2H, Ar-H), 6.702 (d, 2H, Ar-H), 7.146-7.173 (d, 4H, Ar-H), 7.711 (d, 2H, Ar-H), 7.852-7.823 (d, 4H, Ar-H), 7.899 (s, 2H, Ar-H), 8.081 (d, 4H, Ar-H), 8.440 (s, 2H, Ar-H). Anal. calcd. for $C_{36}H_{32}CdN_6O_6$: C, 57.11; H, 4.26; N, 11.10. Found: C, 60.50; H, 4.63; N, 12.40%. Λ_m (S.m^2.mol^{-1}): 32.

2.5. Biological activity

The Schiff base ligand and its metal complexes were tested for their antimicrobial activities at the Medical Mycology Laboratory, University of Alazhar, Cairo, Egypt.

2.5.1. Antibacterial screening

The antibacterial activity of the ligand and its metal complexes were tested by using diffusion method, against *Streptococcus pneumoniae* and *Bacilli's subtilis*, as Gram positive and *Pseudomonas aeruginosa* and *Escherichia coli* as Gram negative bacteria [15]. The test compounds were dissolved in DMSO. Twenty five milliliters nutrient agar was placed in petri plates. After solidification, the test bacteria were spread over the medium using springer. The discs of Whatman No. 1 filter paper saturated with the test compounds were placed at four equidistant places from the center in the inculcated petri plates. Filter paper disc treated with DMSO severed as control and ampicillin and gentamicin were used as standard drugs. The Petri dishes were kept in refrigerator for 24 hours for pre-diffusion and then incubated for 72 hours at 38 °C and the inhibition zone around each disk was measured. The zone of inhibition was carefully calculated in millimeters [9].

2.5.2. Antifungal screening

The antifungal activity of the synthesized ligand and its metal complexes were tested against *Aspergillus fumigates* and *Candida albicans* by serial dilution method.

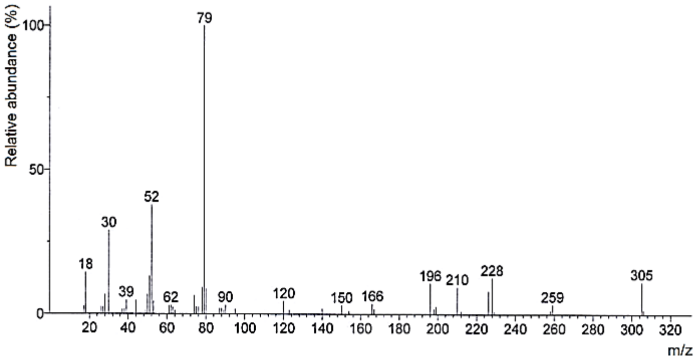

Figure 1. Mass spectrum of the ligand (HL).

Potato-dextrose agar (PDA) was used to evaluate the effect of the compound under investigation on the mycelia linear growth of the two tested fungi. Fifty milliliter of the medium was poured into 100 mL conical flask and autoclaved at 121 °C for 20 minutes. Three drops of lactic acid were added to prevent bacterial contamination. Dilutions for each of the tested compounds were done by dissolving appropriate amounts of each compound in 10 mL. Equal volumes of DMSO containing diluted compounds were added to PDA to get series of different concentrations for each compound [16,17].

Zero concentration treatment was prepared for each fungus which contains equivalent volume of DMSO only and used as control. Compounds amended PDA was dispensed aseptically into 9 cm diameter petri dish, plugs of mycelium were cut from the margins of actively growing culture of the fungi and placed in the center of compound amended and unamended PDA plates with four replicate plates for each fungus. All plates were incubated at 25 °C. Colony diameters (in mm) was measured after three days [13].

The growth inhibition percentage diameter was calculated on the basis of the average diameter of the fungal colony, percentage inhibition is equal to 100(C-T)/C, where C is the diameter of the fungus colony in the control plate after three days and T is the diameter of the fungus colony in the tested plates after the same period of time [18,19].

3. Results and discussion

3.1. Spectral studies

The analytical data of the ligand and its metal complexes was given in experimental section, coincide with empirical formula. The electronic impact mass spectrum of the ligand, Figure 1, shows molecular ion (M$^+$) peak at *m/z* 305, corresponding to the species [$C_{18}H_{15}O_2N_3$]$^+$, confirming the empirical formulae of the ligand. The spectrum also shows a series of peaks corresponding to the various fragments of the compound. The intensities of the peaks give an idea of the relative stability of the fragments [20-22]. The IR spectral data of the ligand shows strong band at ν 3455 cm^{-1} which is assigned to stretching mode of carboxylic acid group (COOH). The band at ν 1674 cm^{-1} is assigned to stretching mode of carbonyl group (C=O). On condensation, the ν(C=O) in the parent ketone frequency at ν 1710 cm^{-1} disappeared and replaced by a band at ν 1627 cm^{-1} which corresponds to ν(C=N). The strong absorption band at ν 1356 cm^{-1} is attributed to ν(C-O) [13]. The ^{1}H NMR spectrum of the ligand exhibited proton signals at δ 8.523 ppm which is assigned to COOH, the signal at δ 2.606 ppm assigned to CH_3 proton and the signals between δ 6.492 and 8.418 ppm assigned to aromatic protons. The ^{1}H NMR spectrum of the cadmium complex exhibited the broad signal at δ 3.34 ppm assigned to 2H (H_2O), the signal at δ 2.609 ppm assigned to CH_3 proton and the other signal between δ 6.481 and 8.081 ppm assigned to aromatic hydrogen atoms [13,23].

The band at ν 1627 cm^{-1} assigned to ν(C=N) in the free ligand which is shifted to lower frequency, namely to ν 1610 cm^{-1} in the Cd complex which is indicating the coordination of the azomethine nitrogen to the Cd(II) ion. The band at ν 3455 cm^{-1} in the free ligand assigned to OH of the carboxylic acid is disappeared in the complex spectrum indicating that the involvement of COO$^-$ in the bond formation process [24]. In the Cd(II) complex, the peak at δ 8.523 ppm in the ^{1}H NMR spectrum is disappeared due to the carboxylate group is involved in the coordination to the Cd(II) ion, indicating deprotonation of the ligand. The electronic absorption spectral data of the Cd(II) complex in DMF show one broad band at 21008 cm^{-1} (476 nm) due to charge transfer from ligand to metal atom [26]. On the basis of electrical, conductance and spectral data, octahedral geometry is assigned to cadmium complex (Figure 2).

In the Co complex, the ν(C=N) absorption band at ν 1627 cm^{-1} in the spectrum of the free ligand is shifted to 1595 cm^{-1} in the spectrum of Co(II) complex, indicating the coordination of the azomethine nitrogen to the cobalt ion. The band at 1356 cm^{-1} due to ν(C-O) in the free ligand is shifted to 1306 cm^{-1}. This indicates the coordination of the carboxylate group. The presence of the H_2O molecule is confirmed by the thermal analysis. The electronic absorption spectral data of the Co complex in DMF shows bands at 20161 cm^{-1} (496 nm) and 31645 cm^{-1} (316 nm). These bands may be due to the ligand to metal charge transfer (LMCT). The observed magnetic moment for the Co complex is 3.85 B.M. suggesting octahedral geometry (Figure 2) [25-27].

The band at ν 1627 cm^{-1} assigned to ν(C=N) in the free ligand is shifted to ν 1598 cm^{-1} in the Ni complex indicating the coordination of the azomethene nitrogen to the Ni(II) ion. The band at ν 3455 cm^{-1} assigned to ν(OH) of carboxylate group in the free ligand is absent in the Ni complex spectra. This indicates that the deprotonation of the ligand. The strong band at ν 1356 cm^{-1} assigned to ν(C-O) group in the free ligand is shifted to ν 1336 cm^{-1} in the complex spectrum suggesting coordination through the carboxylate group in complexation [28]. The electronic absorption spectral data of the Ni complex show two absorption bands at 26882 cm^{-1} (372 nm) and 31250 cm^{-1} (320 nm) corresponding to the ligand to metal charge transfer. The observed magnetic moment was found to be 3.4 B.M.. This confirms the presence of octahedral geometry (Figure 2) [1].

The molar conductivity values of Cd(II), Co(II) and Ni(II) chelates of the ligand are in the range of 30-41 Ohm^{-1}cm^2 mol^{-1} indicate that these complexes are non-electrolytes [29].

Table 1. Thermoanalytical results of the complexes of HL.

Compound	TG range (°C)	Number of steps	Found (calculated)		Assignments	Metallic residue
			Mass loss %	Total mass loss %		
$[Co(C_{18}H_{15}N_3O_2)_2(H_2O)_2].H_2O$	46-553	4	2.05 (2.49)	85.47 (87.53)	Loss H_2O	CoO
			21.12 (22.33)		Loss $H_2O+C_9H_7N_2$	
			48.08 (48.27)		Loss $C_{22}H_{10}N_3O_2$	
			14.22 (14.44)		Loss part of ligand	
$[Ni(C_{18}H_{15}N_3O_2)_2(H_2O)_2]$	21-559	5	5.90 (4.80)	89.26 (89.92)	Loss of H_2O	NiO
			49.90 (50.20)		Loss of $C_{21}H_{11}N_4O+2H_2O$	
			17.80 (17.92)		Loss of $C_8H_7N_2$	
			15.66 (17.00)		Loss of $C_7H_{10}O_2$	
$[Cd(C_{18}H_{15}N_3O_2)_2(H_2O)_2]$	47-701	2	8.50 (8.50)	83.50 (83.10)	Loss H_2O+CO	CdO
			75.00 (74.60)		Loss $C_{35}H_{28}N_6O_2$	

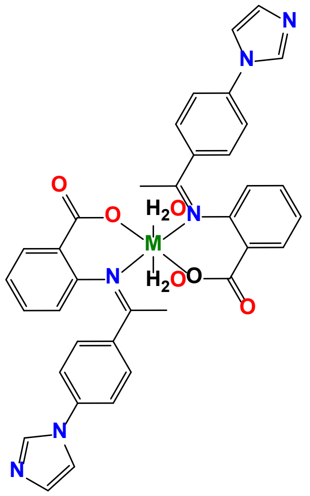

Figure 2. The probable structure of the complexes (M: Co(II), Cd(II) or Ni(II)).

3.2. Thermogravimetric analysis of the complexes

The thermogravimetric analyses of the complexes along with the % weight loss at different temperature range are shown in Table 1.

The curves show two decomposition steps for Cd(II), four decomposition steps for Co(II), and five decomposition steps for the Ni(II) complexes and the total % weight loss of the complexes corresponds to the loss of ligand after considering the transfer of one oxygen atom to the metal ion [24].

The thermogram of Co(II) complex shows four decomposition steps within the temperature range 46-553 °C. The first step of the decomposition within the temperature range 46-139 °C corresponds to mass loss of 2.05% (calculated 2.49%). Because of the low temperature can be regarded as lattice water. The second step within the temperature range 140-290 °C corresponds to loss of coordinated water molecule and $[C_9H_7N_2]$ with an estimated mass loss of 21.80% (calculated 22.33%) at 225 °C. The third decomposition step with an observed mass loss of 48.08% (calculated 48.27%) within the temperature range 290-514 °C corresponds to the removal of $[C_{22}H_{10}N_3O_2]$ at 459 °C. The fourth step within the temperature range 514-553 °C corresponds to removal of the remaining part of the ligand molecule as gases loss in the last stage is 14.22% (calculated 14.44%). The overall weight loss amount to 85.47% (calculate 87.53 %) [24].

The TGA curve for the Ni(II) complex shows five stages of decomposition within the temperature range of 21-559 °C. The mass loss equal to 5.90 % (calculated 4.80%) is equivalent to the loss of two water molecules in the temperature range 21-124 °C. The mass loss at 82 °C is due to the removal of lattice water molecules [30]. The second step of the decomposition within the temperature range 125-290 °C corresponds to loss of two coordinated water molecules and $[C_{21}H_{11}N_4O]$ at 219 °C, the mass loss is 49.90% (calculated 50.20%). The third and fourth decomposition within the temperature range 291-506 °C correspond to the removal of $[C_8H_7N_2]$ with an estimated mass loss 17.80% (calculated 17.92%). The fifth stage is in the temperature range 506-539 °C. It involves removal of the remaining part of the ligand, $[C_7H_{10}O_2]$, with estimated mass loss 15.66% (calculated 17.00%) at 526 °C, leaving NiO as residue [31,32]. The overall mass loss is 89.62% (calculated 89.92%).

The thermogram of Cd(II) complex shows two decomposition steps within the temperature range 47-701 °C. The first step in the temperature range 47-347 °C corresponds to removal of two coordinated water molecules and CO, with an estimated mass loss of 8.50% (calculated 8.50%) at 255 °C. The second step within the temperature range 347-701 °C corresponds to the removal of the remaining organic part of the ligand $[C_{35}H_{28}N_6O_2]$ at 658 °C with estimated mass loss 75.00% (calculated 74.60%), leaving CdO as residue [33]. The overall weight loss amount to 83.50% (calculated 83.10%).

3.3. Antifungal activity of the ligand and its complexes

The results of antifungal activities are shown in Table 2. The ligand is not active against all the tested fungi, while all complexes are active under identical experimental conditions.

3.4. Antibacterial activity of the ligand and its complexes

The results of the *in vitro* antibacterial activities are shown in Table 3. The tests of the ligand and its metal complexes were carried out using the culture of *Staphylococcus pneumoniae* and *Bacillus subtilis* as Gram positive bacteria and *Pseudomonas aeruginosa* and *Escherichia coli* as Gram negative bacteria [34]. The experiments were carried out using the agar diffusion test.

Table 2. Antifungal activity of 4(1*H*-Imidazole-yl)acetophenoneanthranilic acid ligand, its complexes and negative control (DMSO) measured by agar diffusion test (Unit, mm).

Sample	**Zone of inhibition**	
	Aspergillus flavus	***Candida albicans***
Ligand (HL)	Not active	Not active
$[Ni(C_{18}H_{15}N_3O_2)_2(H_2O)_2]$	10	12.1
$[Cd(C_{18}H_{15}N_3O_2)_2(H_2O)_2]$	16.2	12.5
$[Co(C_{18}H_{15}N_3O_2)_2(H_2O)_2].H_2O$	20	15.2
Amphotericin B (Antifungal agent)	23.7	25.4

Table 3. Antibacterial activity of ligand, its metal complexes, positive control (Ampicillin and Gentamicin) and negative control (DMSO) measured by agar diffusion test (Unit, mm).

Sample	**Zone of inhibition**			
	Gram positive bacteria		**Gram negative bacteria**	
	S. pneumoniae	***B. subtilis***	***P. aeruginosa***	***E. coli***
Ligand HL	10.4	11.9	Not active	Not active
$[Ni(C_{18}H_{15}N_3O_2)_2(H_2O)_2]$	11.7	13.2	10.1	11.9
$[Cd(C_{18}H_{15}N_3O_2)_2(H_2O)_2]$	14.6	14.3	11.7	10.8
$[Co(C_{18}H_{15}N_3O_2)_2(H_2O)_2].H_2O$	13.1	10.8	13.4	12
Ampicillin	23	32	-	-
Gentamicin	-	-	17.3	19.9

Ampicillin and gentamicin (20 mg/mL) were used as standard drugs and DMSO was used as negative control. The susceptibility of the bacteria to the test samples was determined by formation of zone of inhibition (mm) produced using (5 mg/mL) concentration of the tested samples. Table 3 shows inhibition zone (mm) of each compound. The experimental results show that the metal complexes are less active than the standards. The ligand is not active against *Pseudomonas aeruginosa and Escherichia coli* [33-35].

4. Conclusion

In conclusion, this study reports a new bidentate ligand from the reaction between imidazoleacetophenone and 2-amino benzoic acid and the Schiff base 2-((1-(4-(1*H*-imidazol-1-yl)phenyl)ethylidene)amino)benzoic acid. Three stable colored metal ion complexes were synthesized from the reaction between the prepared complexing agent and three metal ions namely Co(II), Cd(II) and Ni(II). The ligand and its metal complexes were characterized using different spectroscopic analytical techniques such as MS, IR and ^{1}H NMR spectra. *In vitro* antimicrobial potential of the ligand and complexes were also investigated. Higher antifungal and antibacterial activities were observed from the metal ion complexes compared to that of the free ligand bioactivity. The complexes may be developed to drugs in the future for treatment of diseases caused by the tested pathogenic fungal and bacterial strains.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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