Lafia Journal of Scientific & Industrial Research (LJSIR), Vol. 2(2), 2024
p-ISSN: 3026 – 9288 e-ISSN: 3027 – 1800 pages: 13 – 23
https://lafiascijournals.org.ng/index.php/ljsir/index ⇒ Published by the Faculty of Science,
Federal University of Lafia, Nasarawa State, Nigeria

Antibacterial and DNA Binding Effects of Cobalt(II) Complexes Containing Aminophenol Schiff Base Moiety

F. N. Ejiah[1,2]*, T. M. Fasina[1], N. Revaprasadu[2], F. T. Ogunsola[3] & O. B. Familoni[1]
[1]Department of Chemistry, University of Lagos, Nigeria
[2]Department of Chemistry, University of Zululand, South Africa
[3]Department of Medical Microbiology, University of Lagos, Nigeria

Abstract

Development of new compounds with potential effects against pathogenic organisms has become necessary due to the increase in microbial resistance reported for existing antibiotics. The present study was carried out to investigate the effect of substituent groups on the antibacterial activities of 2-aminophenol Schiff bases and their cobalt (II) complexes. In line with this, new cobalt (II) complexes with Schiff bases derived from 2-aminophenol and *p*-substituted benzaldehydes were synthesized. The compounds were characterized using elemental analysis, mass spectrometry, atomic absorption spectroscopy, electrospray ionization mass spectrometry, infrared spectroscopy, ^{1}H NMR and electronic absorption spectroscopy. Results indicate that all metal complexes had a 1:2 metal ligand ratio with magnetic moments characteristic of tetrahedral geometry around the metal ion. The Schiff bases and their metal complexes were screened for *in-vitro* antibacterial activities using disc diffusion and minimum inhibitory concentration methods against 6 human pathogenic bacteria usually found around the hospitals and homes; *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (ATCC10702), *Enterococcus faecalis* (ATCC 29212) and *Kribsella pneumonia* (ATCC 10031) with ampicillin used as the reference compound. DNA binding study using calf thymus DNA revealed intercalative mode of activity. The result showed that Schiff bases exhibited moderate inhibitory activity against the tested microorganisms while Schiff base metal complexes exhibited higher antibacterial activity when compared to ampicillin. Our results indicate that these complexes can be employed as active ingredients in development of broad-spectrum antibacterial agents.

Keywords: Aminophenol, Antibacterial, Cobalt (II) complexes, Schiff bases

Article History
Submitted
February 13, 2024
Revised
May 13, 2024
First Published Online
May 16, 2024

Corresponding author
F. N. Ejiah ✉
fejiah@unilag.edu.ng

doi.org/10.62050/ljsir2024.v2n2.314

Introduction

The tolerance of pathogenic microorganisms towards existing antibiotics is gradually increasing difficulties to prevent and treat bacterial infections [1]. Over the years, microbial infections have become a great burden to many low- and middle-income countries [2, 1]. Resistance of microbes can result from mutation of the genome of a bacterium or from foreign DNA. Clinically, mutations can occur and become fixed in the patient been treated, as such new antibiotics is paramount for such patient. Environmental emissions of both resistant faecal bacteria and residual antibiotics have also been associated with microbial resistance [3, 4]. Food, such as raw vegetables, is a possible exposure route as fresh produce often carries various resistant bacteria with diverse mobile elements [5-7].

Reports have shown that all approved antibiotic classes, whether natural, semi-synthetic or synthetic compounds, have experienced resistance in some of the pathogens they target [1]. Considering the global health challenge to predict evolutionary events that lead to the emergence of new resistance, there is therefore a need to synthesize and investigate new compounds on a range of microorganisms for use as antibacterial agents. Schiff bases have proved to be an important class in this row owing to a wide range of biological activities such as antibacterial, antifungal, anti-tuberculosis, herbicidal and analgesic [8-12].

The use of Schiff bases as ligands is based on the presence of other electronegative atoms such as nitrogen, oxygen which enhances the coordinating possibilities of these ligands to metal ions, hence Schiff base metal complexes have gained much attention due to diverse structural aspects and increased antibacterial activity. The coordination of Schiff base ligands with transition metal ions has resulted in formation of complexes with interesting biological properties. These biological properties usually depend on the type of metal ion employed in the coordination compound. Cobalt(II) metal ions can transport oxygen within the host system, which have led to its use as oxygen carriers [13-15]. Liu *et al.* in 2022 [16] reported aerobic oxidation reactivity of cobalt(II) and cobalt(III) aminophenol complexes. Abd-Elzaher evaluates the antimicrobial activity of cobalt(II) complex containing acetylferrocene with 2-aminophenol moiety [17]. Other cobalt complexes have also shown remarkable biological activity [18-21].

In our work, we have previously reported antibacterial activity of *ortho* substituted 2-aminophenol Schiff base cobalt(II) complexes [22]. Based on these reports, the aim of this work is to develop cobalt complexes from *para* substituted aminophenol Schiff base moiety to establish the effects of substituents on antimicrobial activity and DNA binding.

Materials and Methods

Reagents

All reagents and solvents were of analar/spectroscopic grades and used without further purification. Ethanol, chloroform, dimethyformamide, dimethyl sulfoxide, 2-aminophenol, 4-methoxybenzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, cobalt(II) chloride, were purchased from Aldrich-Sigma Company.

Physical measurements

Infrared (IR) spectra of the compounds were recorded on a Bruker FT-IR (ATR) tensor 27 spectrophotometer directly on small samples of the compounds in the range 400 to 4000 cm^{-1}. ^{1}H-NMR spectra in DMSO-d_6 solution of the ligands were recorded on a Bruker Avance III 400 *MHz*. Chemical shifts were reported in ppm relative to TMS as internal standard. Electronic absorption spectra of Schiff bases and metal complexes recorded from 200 to 1100 *nm* using freshly prepared dimethylformamide (DMF) solutions were measured on a Cary Model 50 spectrophotometer. Thermogravimetric analysis (TGA) was carried out at $15^oC\ min^{-1}$ heating rate using a Perkin Elmer Pyris 6 TGA from 50 to 1000^oC in a closed perforated aluminium pan. The differential scanning calorimetric (DSC) analysis was carried out at $20^oC\ min^{-1}$ heating rate from room temperature to 450^oC using a Perkin Elmer DSC 4000 series, calibrated with indium metal. Metal analysis was determined using Analyst 200 atomic absorption spectrophotometer (AAS) Perkin Elmer. Melting points were determined on a Reichert Thermovar melting-point apparatus and are uncorrected. Magnetic susceptibility measurements were performed using a Sherwood Scientific magnetic susceptibility balance on powdered samples. $Hg[Co(SCN)_4]$ was used as the calibrant and corrections for diamagnetism calculated from Pascal's constants. Microanalytical data were obtained on a Perkin Elmer model 2400 series II CHNS/O elemental analyzer. All at the University of Zululand, South Africa. Mass spectra were recorded on Agilent 7890 gas chromatograph mass spectrometer at the central research laboratory, University of Lagos. Electrospray Ionization (ESI) analyses were performed in positive ionization mode at the University of Birmingham on a Micro mass LCT Time of Flight Mass Spectrometer.

Biophysical experiment

Ultrapure water was used throughout all biophysical work. DNA samples were made up from Calf thymus DNA sodium salt (sigma Aldrich) by dissolving in milli-Q water and washed using a 10kda MWCO centrifuge tube. The solution was then quantified by UV-vis spectroscopy (Cary 5000 NIR spectrometer) by $\varepsilon 258 = 13200\ mol^{-1}\ dm^3\ cm^{-1}$ to give a concentration in DNA base pairs. This stock solution was kept in the fridge with fresh aliquot being taken out for each experiment. Fresh buffer was made up before each experiment. Cobalt complexes were dissolved in 1% DMSO with fresh solutions being used for each batch of experiments.

Circular dichroism (CD) spectra were recorded on a Chirascan+ spectrometer (Applied Photophysics limited). The samples were scanned in a 1 *cm* path length cuvette between 400 and 200 *nm* with 3 repeats at 1 *nm* step size and 0.5 *s* dwell time S37 per point. Titrations were carried out at a constant concentration of DNA, sodium chloride (40 *mM*) and Tris HCl buffer (200 *mM*, pH 7.4) by adding compensating solutions of 2x DNA/Buffer of equal volume to the titre of the complex solution. The concentration of complex in the cuvette was increased step wise by adding set volumes of a stock complex solution. The R value refers to the ratio of DNA base pairs to complex, i.e. R60 = 60*bp* for every 1 complex, R4 = 4*bp* per complex

Synthesis of Schiff bases (B1-B3)

Equimolar quantities (10 *mmol*.) of 2-aminophenol and *P*-substituted benzaldehydes were dissolved in ethanol (25 *ml*) and stirred under reflux at 70^oC for 6 *h*. The precipitate formed was separated by filtration, recrystallized from ethanol, dried and stored in a desiccator over silica gel.

Synthesis of Schiff base cobalt(II) complexes (B1 cobalt-B3 cobalt)

An ethanolic (40 *ml*) solution of Schiff base (4 *mmol*.) was mixed with Co(II) chloride (2 *mmol*.) in ethanol (20 *ml*) solution keeping ligand-metal ratio 2:1. The solution was made alkaline with triethlyamine and heated to reflux for 4 *h*. The solid product formed was collected by filtration, washed in ethanol, dried and stored in a desiccator over silica gel.

Antibacterial studies

Disc diffusion assay

The antibacterial sensitivity of ligands and metal complexes were individually tested against a panel of standard microorganisms namely, *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Bacillus cereus* (ATCC 10702), *Enterococcus faecalis* (ATCC 29212) *and Klebsiella pneumoniae* (ATCC 10031) using the paper disc diffusion method [23, 24]. The compounds were prepared in DMSO to obtain a final concentration of 10 *mg/ml*. Sterile Whatman No. 1 (6 *mm*) discs were separately impregnated with each sample to be tested and placed on the Mueller Hintoninoculated agar. The plates were incubated at 37^oC for 24 *h* and the diameterzones of inhibition measured at the end of the incubation period. Ampicillin was used as reference compound.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the 96-well micro-plate dilution method [25, 26]. Serial plate concentrations of 5.0, 2.5, 1.25, 0.625, 0.312, and 0.157, 0.078 and 0.039 *mg/mL* were prepared for each compound. Each was inoculated with $1.5x10^8$*CFU/ml* of 0.5 McFarland standard bacteria suspension and incubated for 24 *h* at 37^oC. As an indicator of bacterial growth, 20 μL of 0.2 mg/ml *p*-iodonitrotetrazolium solution (a colourless tetrazolium) was added to each well and incubated at 37^{o}C for 30 min. Growing bacteria metabolize this salt to give a red product (formazan). Inhibition prevents this conversion resulting in a clear well. MIC values were recorded as the lowest concentration of compound preventing bacterial growth.

Results and Discussion

Synthesis

The synthesis of Schiff base (**B1-B3**) and their cobalt (II) complexes (**B1 cobalt-B3 cobalt)** are presented in the Figs. 1-4. All the complexes are colored powders, stable in air, and insoluble in water and other common solvents; but soluble in polar coordinating solvents such as DMF and DMSO. The metal content analysis of the complexes revealed the percentage metal ion per mole of complex. All the complexes exhibited one mole of cobalt per mole of the complex. The physical characteristics, analytical data, and percentage metal content of the compounds are given in Table 1 and are in good agreement with the proposed formulation. The complexes exhibited the formula $[(B1)_2Co]$, $[(B2)_2Co]$ and $[(B3)_2Co]$ for B_1, B_2 and B_3 Schiff bases, respectively.

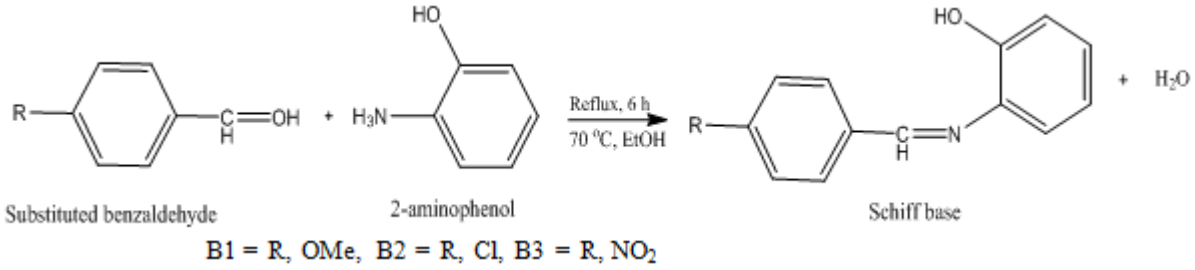

Figure 1: Synthesis of Schiff bases

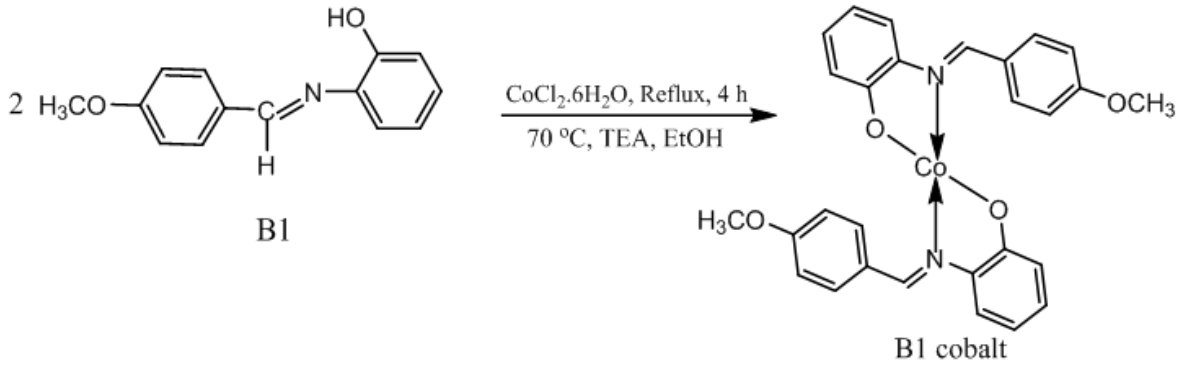

Figure 2: Synthesis of Schiff base cobalt(II) complexes (B1 cobalt)

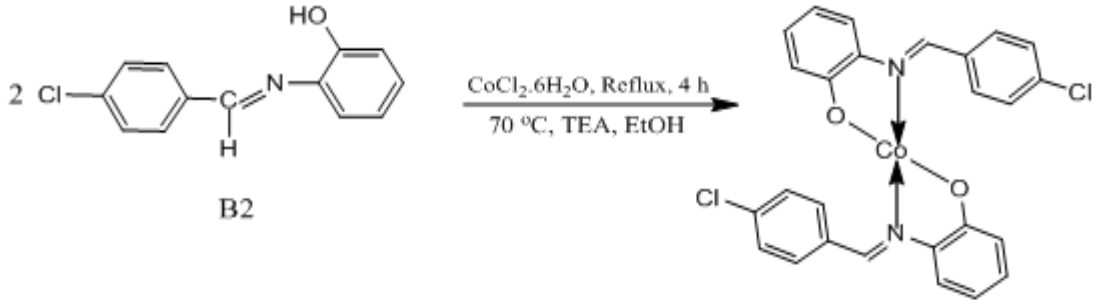

Figure 3: Synthesis of Schiff base cobalt(II) complexes (B2 cobalt)

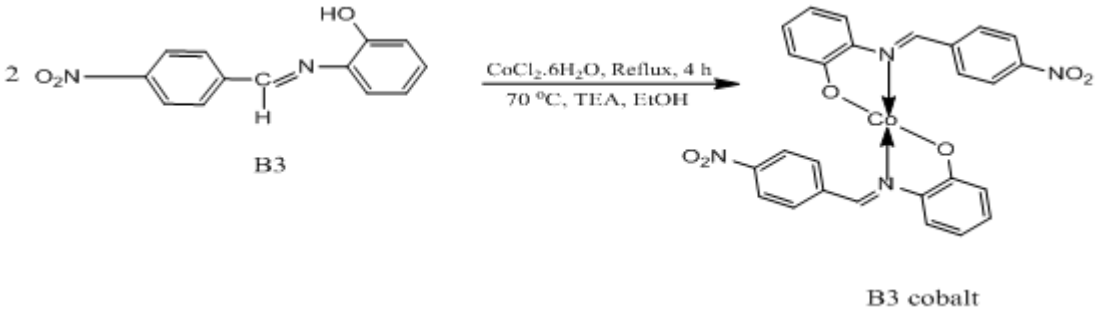

Figure 4: Synthesis of Schiff base cobalt(II) complexes (B3 cobalt)

Table 1: Physical and analytical data of substituted 2-aminophenol Schiff bases and cobalt complexes

Compound	R	Empirical Formula (*M.wt*)	Yield (%)	Color	M.pt (^oC)	% Found (cacld)			Metal (%)
						C	H	N	
B1	OMe	$C_{14}H_{13}NO_2$ (227)	69.10	Yellow	94-95	74.08 (73.99)	5.67 (5.77)	6.13 (6.16)	-
B1 cobalt	OMe	$C_{28}H_{24}CoN_2O_4$ (512)	56.57	Brown	>250	65.03 (65.76)	4.30 (4.73)	5.95 5.48)	10.13 (11.52)
B2	Cl	$C_{13}H_{10}NOCl$ (231)	62.83	Yellow	120-121	67.26 (67.39)	4.30 (4.35)	6.05 (6.05)	-
B2 cobalt	Cl	$C_{26}H_{18}Cl_2CoN_2O_2$ (519)	60.00	Brown	>250	60.08 (60.02)	3.27 (3.49)	5.60 (5.38)	11.34 (11.33)
B3	NO_2	$C_{13}H_{10}N_2O_3$ (242)	76.79	Yellow	163-165	64.82 (64.46)	4.07 (4.16)	11.64 (11.56)	-
B3 cobalt	NO_2	$C_{26}H_{18}CoN_4O_6$ (541)	87.34	Orange	>250	57.01 (57.68)	3.16 (3.35)	10.21 (10.35)	11.52 (10.89)

Table 2: Spectroscopic data of substituted 2-aminophenol Schiff bases and metal complexes

Compound	R	ν(O-H)	*ν*(C=N)	*ν*(C-O)	*ν*(C-Cl)	*ν*(M-O)	*ν*(M-N)	HC=N (s,1H) *ppm*	OH (s, 1H) *ppm*	OCH_3 (s, 3H) *ppm*
B1	OMe	3333	1618	1245	-	-	-	7.89	8.63	3.91
B1 cobalt	OMe	-	1593	1190	-	587	443	-	-	-
B2	Cl	3291	1601	1277	350	-	-	7.98	8.65	-
B2 cobalt	Cl	-	1577	1238	350	593	433	-	-	-
B3	NO_2	3304	1623	1299	-	-	-	8.37	8.77	-
B3 cobalt	NO_2	-	1596	1274	-	582	487		-	-

Infrared spectroscopy, ^{1}H NMR, Mass Spec and ESI-MS

The relevant FTIR data of the ligands and metal complexes are given in Table 2. The infrared for the ligands reveals bands in the region 1601-1623 cm^{-1}attributed to the imine bond. In order to determine the bonding mode of Schiff base to the metal ion, the IR spectra of the Schiff bases were compared with those of the corresponding metal complex. The heteroatoms, nitrogen and oxygen due to the presence of lone pairs of electrons are expected to be involved in complexation and therefore the position of bands for these functional groups will vary in metal and ligand. In the spectra of Schiff base and Schiff base metal complex for *p*-methoxy-substituted compound, a shift in IR band (1618-1593 cm^{-1}) was observed. Similar shifts were also observed for *p*-chloro-substituted compounds (1601-1577 cm^{-1}) and *p*-nitro-substituted compounds (1623-1596 cm^{-1}) which indicates coordination of azomethine nitrogen to metal ion. The presence of OMe and NO_2 groups increased IR stretching frequency for C=N bond while Cl group decreased the IR stretching frequency. This may be attributed to electronic effects, resonance effects associated with OMe and NO_2 compounds and inductive effects in Cl compounds. A shift to lower wavenumber due to coordination of metal ion to hydroxyl oxygen in the compounds reveal peaks at 1245-1190 cm^{-1}, 1277-1238 cm^{-1} and 1299-1274 cm^{-1} for Ome, Cl and NO_2 substituted compounds, respectively [27, 28]. The new bands in the spectra of metal complexes 433 – 487 cm^{-1} and 582 – 593 cm^{-1} are assigned to stretching frequencies of *ν*(M-N) and *ν*(M-O) bond, respectively.

The ^{1}H NMR of all Schiff bases exhibits a singlet in the region 7.98-8.37 *ppm* attributed to the imine proton. Schiff bases with electron donating groups appeared up-field due to increase in the electron density in the vicinity of the proton which causes shielding from the magnetic field while Schiff bases with electron withdrawing groups appear down field as a result of low electron density in the vicinity of the proton. These effect and trends were also exhibited in the phenolic proton. The three protons of OCH_3 group resonated in the region 3.91 *ppm* as expected.

The molecular ion peak was observed at *m/z* 227 and 231 for B1 and B2 as shown in Figs. 5 and 6 confirming their molecular weights, which corroborate with the calculated value. The presence of *nitro* group on the aromatic ring which induces thermal instability was observed in B3. This was evident in Fig. 7 at *m/z* 211 against *m/z* 242 corresponding to the loss of NO^+ (*m/z*= 31) in the ligand. For the cobalt complexes, ESI-MS confirmed the structures of the compounds as the found and calculated were in good agreement. Evident from Figs. 8, 9 and 10 are *ESI-MS* of B1 cobalt 512.12, *m/z*=512.11, B2 cobalt 519.10, *m/z*= 519.01 and B3 cobalt 542.06, *m/z*= 542.06. It was also observed that the cobalt ion in the nitro substituted complex stabilized the compound.

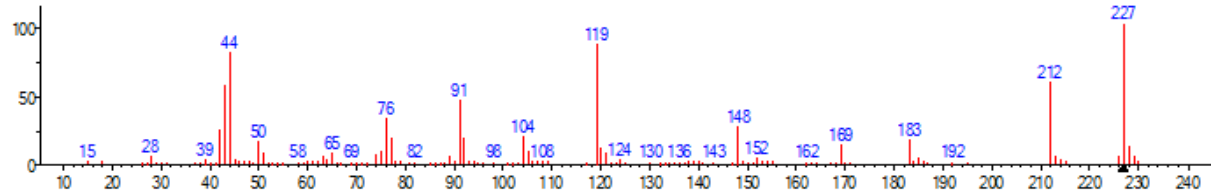

Figure 5: Mass spectrum of B1-methoxy substituted Schiff base (m/z 227)

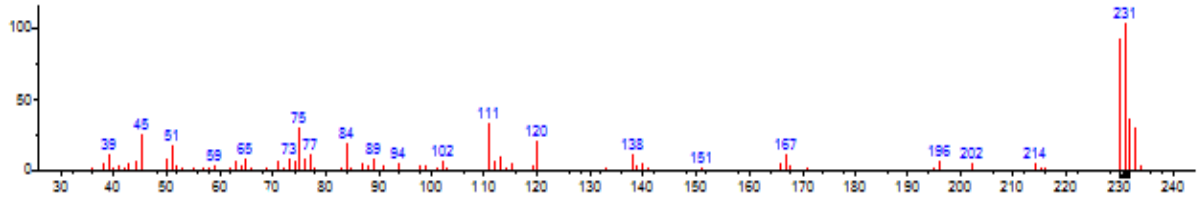

Figure 6: Mass spectrum of B2-chloro substituted Schiff base (m/z 231)

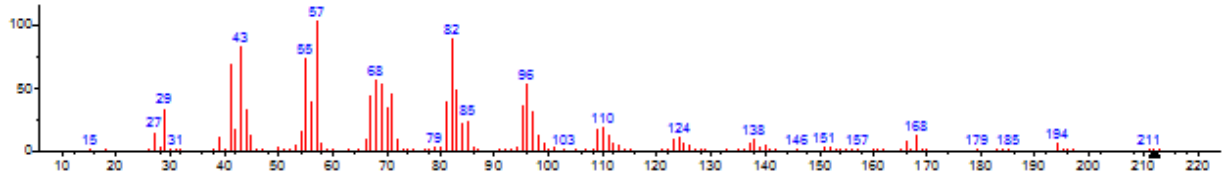

Figure 7: Mass spectrum of B3-nitro substituted Schiff base $[M\text{-}NO^+]^{-1}$ m/z 211

The presence of *nitro groups* on the aromatic ring frequently induces thermal *instability*, a characteristic feature of nitro derivatives.

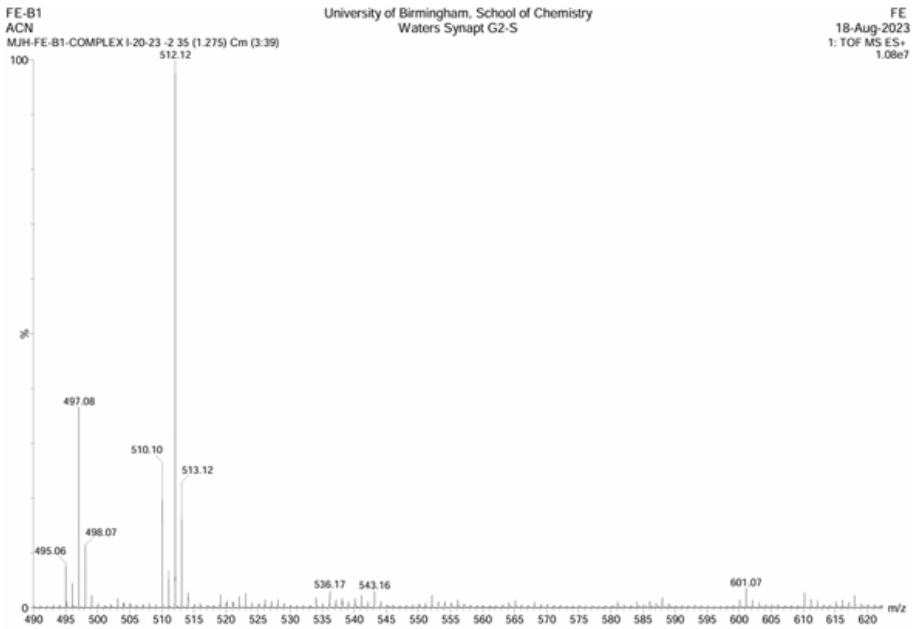

Figure 8: ESI-MS spectra of B1 cobalt 512.12, m/z=512.11

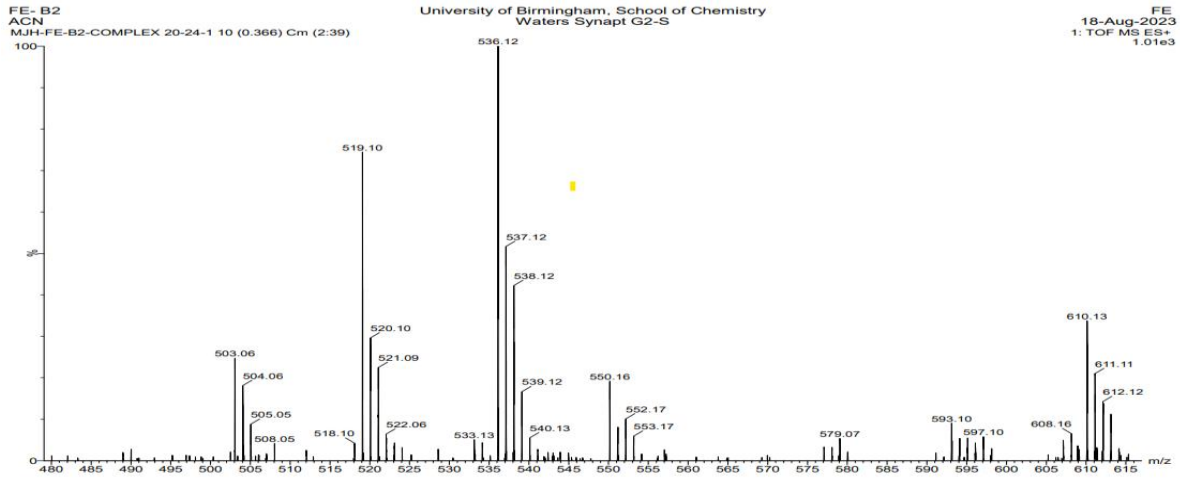

Figure 9: ESI-MS spectra of B2 cobalt 519.10, m/z= 519.01

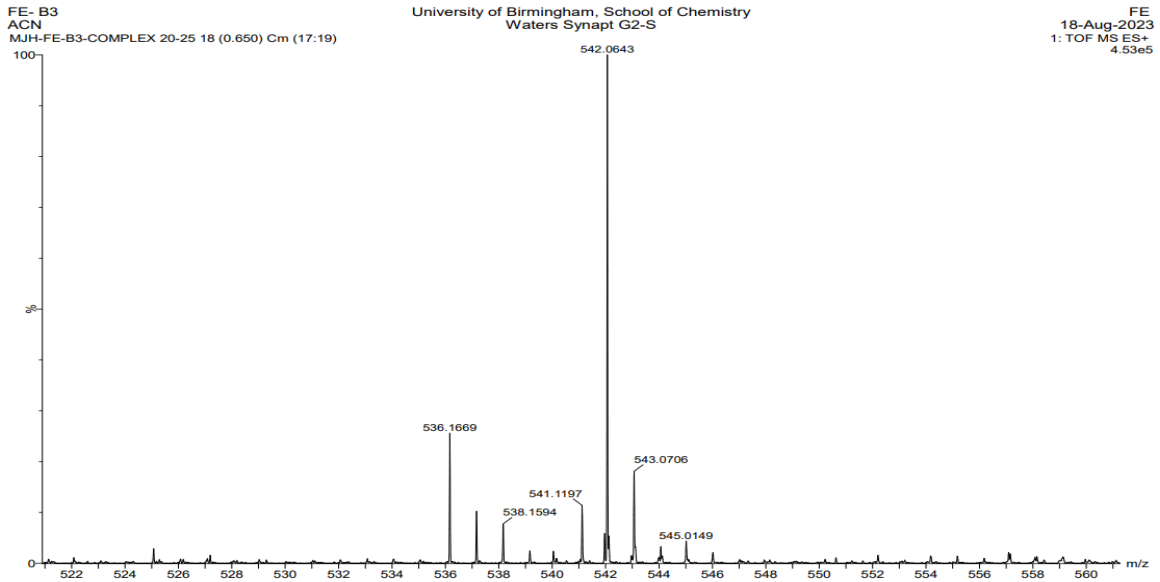

Figure 10: ESI-MS spectra of B3 cobalt 542.06, m/z= 542.06

Electronic absorption data and magnetic susceptibility measurements

The electronic absorption spectra of Schiff bases and their metal complexes were recorded at room temperature using DMF as solvent. The Schiff bases exhibited two absorption bands; a high energy band 260-276 *nm* attributed to π→π* transitions of the aromatic ring and a lower energy band 350-380 *nm* due to n→π* transition of the non-bonding electrons present on the nitrogen of the azomethine group [29].

Schiff bases with methoxy and chloro substituents (Figs. 11 and 12) shift the imine bands to shorter wavelength (350, 355 *nm*), the opposite is true for nitro substituted Schiff base (380 *nm*) as shown in Fig. 13. After the complex formation, the n→π* of the imine group shifted to lower wavelength 321, 321 and 326 *nm* for methoxy, chloro and nitro substituted ligands [30]. The bands due to ligand to metal charge transfer transitions confirming the coordination of Schiff base to metal ion was observed at longer wavelength at 427, 436 and 489 *nm* for methoxy, chloro and nitro substituted complexes, respectively [31, 32].

The range of moments for tetrahedral cobalt (II) complexes is 4.26-5.00 *BM* [33] values of 4.92, 4.65 and 4.28 *BM* presented for the magnetic moments of **B1, B2** and **B3** cobalt complexes respectively are within the expected range for mononuclear tetrahedral complexes [34-37].

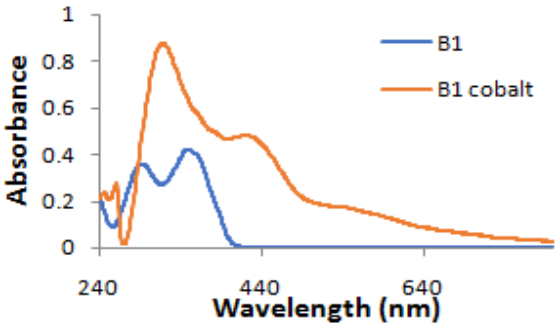

Figure 11: UV-Vis spectrum of B1 and B1 cobalt(II) complex

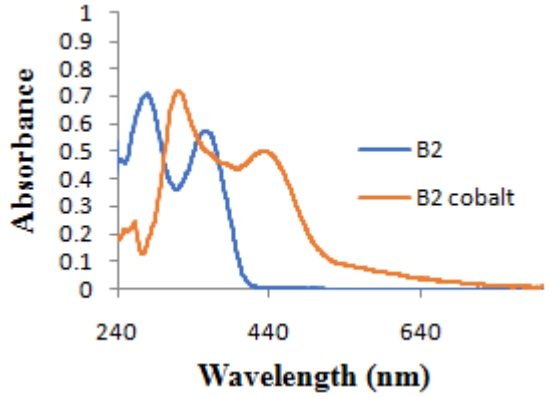

Figure 12: UV-Vis spectrum of B2 and B2 cobalt(II) complex

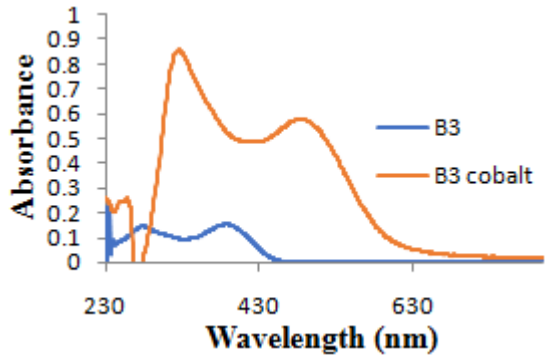

Figure 13: UV-Vis spectrum of B3 and B3 cobalt(II) complex

Antibacterial activity

The antibacterial activity of synthesized Schiff bases and their metal complexes are related to the nature and position of substituent groups. Structure activity-relationship of Schiff bases of 2-aminophenol derivatives were studied in relation to nature and position of substituent groups. The Schiff bases **B1-B3** and their cobalt(II) complexes **B1 cobalt-B3 cobalt** were tested against six human pathogenic bacteria; *Escherichia coli (ATCC 8739), Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 19582), Bacillus cereus (ATCC 10702), Enterococcus faecalis (ATCC 29212)* and *Klebsiella pneumoniae (ATCC 10031) with* Ampicillin used as a reference compound. The diameter zones of inhibition are shown in Table 3. The *in-vitro* antibacterial activity of synthesized Schiff bases presented in Table 4 show that **B**1 exhibited moderate activity against the tested organisms when compared to the low activity observed for **B2** and **B3.** The activity may have arisen from the electron donating ability of the methoxy substituents at *p*-position which play an important role in antibacterial activity [38-40]. A comparative study of minimum inhibitory concentration of Schiff bases and its metal complexes indicates that metal complexes exhibited higher antibacterial activity than the free ligands. This is probably due to the greater lipophilic nature of the complexes. Such increased activity of the metal complexes can be explained on the basis of overtone's concept and Tweedy's chelation [32, 41]. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms. This result corroborates Al-Sha'alan, 2007 report that lipophilicity of Schiff base complexes leads to inhibition of metabolic pathway by deactivation of various cellular enzymes [42]. In addition, there was an increased activity of Schiff base metal complexes with methoxy and chloro substituted compounds. These complexes exhibited very low MIC values in the range 0.31-0.62 *mg/ml* for OMe substituent and 0.62-1.25 *mg/ml* for Cl substituent. Bacterial strain is considered to display tolerance when an antibiotic does not contribute to 99.9% reduction in the number of bacteria used in the test, usually with MBL / MIC > 32 *mg/L* [43].

A minimum inhibitory concentration value of 0.28-1.27 *mg/ml* has been attributed with extremely strong activity while MIC values of 1.81-8.85 *mg/ml* are attributed with weak activities [44]. The low MIC values can be attributed to the substituents at *para* position which have the ability to intercalate between bases of DNA. These results are in agreement with Xia *et al*. which reports that methoxy and chloro groups at *p*-position significantly increase activity [45]. In addition, electron-donating substituents such as *p*-methyl and *p*-methoxy exhibiting better antimicrobial activity in comparison to halogen substituted (*p*-fluoro and p-chloro substitution) analogues, where the activity was considerably lost against all the bacterial strain tested have also been reported [46, 47].

Table 3: Zones of inhibitionof substituted 2-aminophenol Schiff bases and metal complexes

Compound	Diameter (*mm)* zones of inhibition (10 *mg/ml*)					
	S. aureus **(ATCC 6538)**	***E. faecalis*** **(ATCC 29212)**	***B. cereus*** **(ATCC 10702)**	***E. coli*** **(ATCC 8739)**	***P. aeruginosa*** **(ATCC 19582)**	***K. pneumonia*** **(ATCC 10031)**
B1	10.0±0.0	7.0±0.0	9.0±0.0	8.0±0.0	10.0±0.0	8.0±0.0
B1 cobalt	11.0±0.0	12.0±0.0	12.0±0.0	13.0±0.0	12.0±0.0	10.0±0.0
B2	10.5±0.7	9.5±0.7	10.0±0.0	10.0±0.0	0.0±0.0	11.0±0.0
B2 cobalt	11.0±0.0	13.0±0.0	17.0±0.0	11.5±0.7	12.0±0.0	11.0±0.0
B3	11.0±0.0	11.0±0.0	0.0±0.0	9.5±1.4	9.5±0.7	0.0±0.0
B3 cobalt	11.0±0.0	12.0±0.0	12.0±1.4	12.0±0.0	10.5±0.7	11.0±0.0
Ampicillin	13.0±0.0	11.0±0.2	14.0±0.2	10.0±0.0	9.0±0.1	11.0±0.1

Table 4: Minimum inhibitory concentration (MIC) of substituted 2-aminophenol Schiff bases and metal complexes

Compound	MIC (*mg/ml*)					
	S. aureus **(ATCC 6538)**	***E. faecalis*** **(ATCC 29212)**	***B. cereus*** **(ATCC 10702)**	***E. coli*** **(ATCC 8739)**	***P. aeruginosa*** **(ATCC 19582)**	***K. pneumonia*** **(ATCC 10031)**
B1	5.00	2.50	2.50	1.25	2.50	1.25
B1 cobalt	0.31	0.62	0.31	0.62	0.62	0.31
B2	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
B2 cobalt	0.62	1.25	0.62	1.25	1.25	0.62
B3	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
B3 cobalt	0.62	2.50	1.25	1.25	1.25	0.62
Ampicillin	2.50	5.00	5.00	1.25	5.00	2.50

DNA binding study

To further investigate the biological activity spectroscopically and to corroborate the effects of substituents on DNA binding study, circular dichroism was employed. We used circular dichroism (CD) to probe the interaction of cobalt complexes with calf thymus-DNA. The DNA alone shows a strong positive signal at 275 *nm* and negative signal at 245 *nm* due to the DNA bases. The circular dichroism spectroscopy shows the DNA structure retained for nitro substituted cobalt complex (Fig. 16). This was observed at low and high loading of complex:DNA ratio. A significant and interesting result was observed for methoxy substituted cobalt complex (Fig. 14). An increase in the magnitude of the 275 *nm* signal induces an ordered expanded structure as the complex binds to the DNA, leading to a loss in orientation and increase in magnitude in the 275 *nm* signal [48]. This is due to binding into the groove. Also, metal complexes get inserted between DNA base pairs, leading to the separation of base pairs with an increase in the DNA shape [49]. An increase in molar ellipticity was observed in the positive and negative peaks. We observed minor binding into the groove for chloro substituted cobalt complex (Fig. 15).

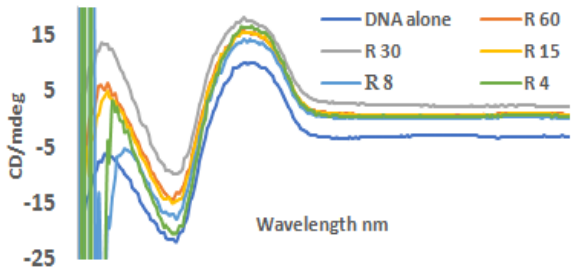

Figure 14: Circular dichroism spectroscopic studies of B1 cobalt complex with CT-DNA. CT-DNA (100 *μM* in base pairs), B1 cobalt complex (200 *Mm*),DMSO (1%), sodium chloride (40 *mM*), and tris buffer (200 *mM*, pH 7.4). The R-value is the ratio of DNA base pairs to complex

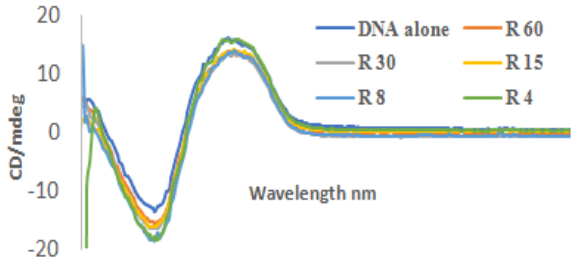

Figure 15: Circular dichroism spectroscopic studies of B2 cobalt complex with CT-DNA. CT-DNA (100 *μM* in base pairs), B2 cobalt complex (200 *Mm*), DMSO (1%), sodium chloride (40 *mM*), and tris buffer (200 *mM*, pH 7.4). The R-value is the ratio of DNA base pairs to complex

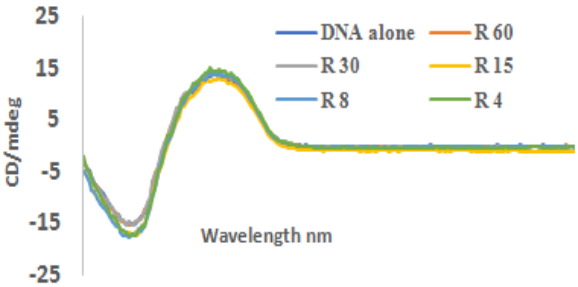

Figure 16: Circular dichroism spectroscopic studies of B3 cobalt complex with CT-DNA. CT-DNA (100 *μM* in base pairs), B3 cobalt complex (200 *Mm*), DMSO (1%), sodium chloride (40 *mM*), and tris buffer (200 *mM*, pH 7.4). The R-value is the ratio of DNA base pairs to complex

Generally, we observed a similar trend in the antimicrobial activity with methoxy, chloro and nitro substituent, with methoxy substituted cobalt complex exhibiting the lowest MIC followed by chloro. The nitro substituted cobalt complex showed the least activity.

Conclusion

The synthesis and characterization of Schiff base cobalt complexes have been realized with physicochemical and spectroscopic methods. The ligands are bidentate to the metal ion through imine nitrogen and hydroxyl oxygen. The complexes adopt tetrahedral geometry. The antibacterial study reveals that the complexes have higher activity than the free ligands. Antibacterial activities were compared with the activity of ampicillin as a reference compound and all the cobalt complexes were found to be more active than ampicillin.

The effect of Schiff base ligands with metal ions on microorganisms was found to be synergistic. This study sheds further light on the effect of substituent groups on the chemical and the biological behavior of the ligands in the presence of cobalt metal ion which may help to understand this class of ligands. The results obtained validate the hypothesis that Schiff bases having methoxy and chloro substituent groups at the phenyl ring are required for antibacterial activity while nitro group in the aromatic ring have varying antibacterial activity. Biological studies of cobalt(II) Schiff base complexes has shown potentials as an active ingredients in the formulation of antibacterial agents. We are also studyiny the in-silico perspectives to establish the most favorable conformations within the protein active site. This would be published hereafter.

Conflicts of interest: There are no conflicts of interest to declare.

Acknowledgements: The authors acknowledge support from National Research Foundation (NRF), University of Zululand, South Africa; Department of Medical Microbiology, College of Medicine, University of Lagos and University of Birmingham.

References

[1] Larsson, D. G. J. & Flach, C-F. (2022). Antibiotic resistance in the environment. *Nat. Rev. Microbiol.*, 20(5), 257-269. https://doi.org/10.1038/s41579-021-00649-x

[2] Laxminarayan, R., Boeckel, T. V., Frost, I., Kariuki, S., Khan, E. A., Limmathurotsakul, D. & et al. (2020). Commission on antimicrobial resistance: 6 years later. *Lancet Infect. Dis.*, 20(4), e51-e60. https://doi.org/10.1016/S1473-3099(20)30003-7

[3] Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J. & Jo Handelsman, J., (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.*, 8(4), 251-259. https://doi.org/10.1038/nrmicro2312

[4] Ahammad, Z. S., Sreekrishnan, T. R., Hands, C. L., Knapp, C. W., & Graham, D. W. (2014). Increased water borne blaNDM-1resistance gene abundances associated with seasonal human pilgrimages to the upper ganges river. Environ. Sci. Technol., 48(5), 3014-3020. https://doi.org/10.1021/es405348h

[5] Kookana, R. S., Williams, M., Boxall, A. B. A., Larsson, D. G. J., Gaw, S., K. Choi, K., & et al. (2014). Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high-income countries. Philos. Trans. R. Soc. Lond. B Biol. Sci., 369(1656), 20130586. https://doi.org/10.1098/rstb.2013.0586

[6] Uyttendaele, M., Jaykus, L-A., Amoah, P., Chiodini, A., Cunliffe, D., Jacxsens, L. & et al. (2015). Microbial hazards in irrigation water: Standards, norms, and testing to manage use of water in fresh produce primary production. *Compr. Rev. Food Sci. Food Saf.*, 14(4), 336-356. https://doi.org/10.1111/1541-4337.12133

[7] Reid, C. J., Blau, K., Jechalke, S., Smalla, K. & Djordjevic, S. P. (2020). Whole Genome sequencing of *Escherichia coli* from store-bought produce. *Front. Microbiol.*, 10, 3050. https://doi.org/10.3389/fmicb.2019.03050

[8] Blau, K., Bettermann, A., Jechalke, S., Fornefeld, E., Vanrobaeys, Y., Stalder, T. & et al. (2018). The transferable resistome of produce. *mBio.*, 9(6), e01300-e01318. https://doi.org/10.1128/mBio.01300-18

[9] Khan, S. A., Siddiqui, A. A. & Bhatt, S., (2002). Analgesic Activity of Isatin Derivatives. *Asian J. Chem.*, 14(2), 1117-1118.

[10] Wang, Y-E., Yang, D., Huo, J., Chen, L., Kang, Z., Mao, J. & Zhang, J. (2021). Design, synthesis, and herbicidal activity of thioether containing 1,2,4-Triazole Schiff bases as transketolase inhibitors. *J. Agric. Food Chem.*, 69(40), 11773-11780. https://doi.org/10.1021/acs.jafc.1c01804

[11] Sivakumar, K. K., & Rajasekaran, A. (2013). Role of provisional restorations in endodontic therapy. *J. Pharm. Bioallied Sci.*, 5(1), S120-S124. https://doi.org/10.4103/0975-7406.113311

[12] Ejiah, F. N., Fasina, T. M., Familoni, O. B. & Revaprasadu, N. (2017). Synthesis and biological activity of cobalt complexes of aminophenol Schiff bases. *J. Chem. Soc. Nigeria.*, 42(1), 93-97.

[13] Raczuk, E., Dmochowska, B., Samaszko-Fiertek, J. & Madaj, J. (2022). Different Schiff bases-structure, importance and classification. *Molecules.*, 27(3), 787. https://doi.org/10.3390/molecules27030787

[14] Tine, M. R. (2012). Cobalt complexes in aqueous solutions as dioxygen carriers. *Coord. Chem. Rev.*, 256(1-2), 316-327. https://doi.org/10.1016/j.ccr.2011.10.009

[15] Emara, A. A. A., Ali, A. M., El-Asmy, A. F. & Ragab, E-S. (2014). Investigation of the oxygen affinity of manganese(II), cobalt(II) and nickel(II) complexes with some tetradentate Schiff bases. *J. Saudi Chem. Soc.*, 18(6), 762-773. https://doi.org/10.1016/j.jscs.2011.08.002

[16] Liu, J., Lorraine, S. C., Dolinar, B. S. & Hoover, J. M. (2022). Aerobic oxidation reactivity of well-defined cobalt(II) and cobalt(III) aminophenol complexes. *Inorg. Chem.*, 61(16), 6008-6016. https://doi.org/10.1021/acs.inorgchem.1c03686

[17] Abd-Elzaher, M. M. (2004). Synthesis, characterization, and antimicrobial activity of cobalt(II), nickel(II), copper(II) and zinc(II) complexes with ferrocenyl Schiff bases containing a phenol moiety. *Appl. Organomet.*, 18(4), 149-155. https://doi.org/10.1002/aoc.608

[18] Patil, S. A., Unk, S. N., Kulkarni, A. D., Naik, V. H., Kamble, U. & Badami, P. S. (2011). Spectroscopic, in vitro antibacterial, and antifungal studies of Co (II), Ni (II), and Cu (II) complexes with 4-chloro-3-coumarinaldehyde Schiff bases. *J. Coord. Chem*., 64(2), 323-336. https://doi.org/10.1080/00958972.2010.541240

[19] Ali, S., Singh, V., Preeti, J. & Tripathi, V. (2019). Synthesis, antibacterial, anticancer and molecular docking studies of macrocyclic metal complexes of dihydrazide and diketone. *J. Saudi Chem. Soc*., 23(1), 52-60. https://doi.org/10.1016/j.jscs.2018.04.005

[20] Renfrew, A. K., O'Neill, E. S., Hambley, T. W. & New, E. J. (2018). Harnessing the properties of cobalt coordination complexes for biological application. *Coord. Chem. Rev*., 375, 221-233. https://doi.org/10.1016/j.ccr.2017.11.027

[21] Fernandes, L. P., Silva, J. M. B., Martins, D. O. S., Santiago, M. B., Martins, C. H. G., Jardim, A. C. G. & et al. (2020). Fragmentation study, dual anti-bactericidal and anti-viral effects and molecular docking of cobalt(III) complexes. *Int. J. Mol. Sci*., 21(21), 8355. https://doi.org/10.3390/ijms21218355

[22] Fasina, T. M., Ejiah, F. N., Oloba-Whenu, O. A., Revaprasadu, N. & Familoni, O. B. (2017). Synthesis, characterization and structure activity relationship of Schiff bases derived from 2-aminophenol and substituted benzaldehydes. *FUW Trends in Science & Technology Journal*, 2(1), 252-256.

[23] Bauer, A. W., Kirby, W. N. N., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., 45(4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

[24] Jorgensen, J. H. & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin. Infect. Dis., 49(11), 1749-1755. https://doi.org/10.1086/647952

[25] O'Shaughnessy, M., Hurley, J., Dillon, S. C., Herra, H. C., Pauraic, M., Malachy, M., & et al. (2023). Antibacterial activity of metal-phenanthroline complexes against multidrug-resistant Irish clinical isolates: a whole genome sequencing approach. *J. Biol. Inorg. Chem*., 28(2), 153-171. https://doi.org/10.1007/s00775-022-01979-8

[26] Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*., 64(8), 711-713. https://doi.org/10.1055/s-2006-957563

[27] Rodríguez-Tudela, J. L., Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., Denning, D., & et al. (2003). Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. Clin. Microbiol. Infect., 9(8), 1-8. https://doi.org/10.1046/j.1469-0691.2003.00789.x

[28] Nishat, N., Khan S. A. & Rasool, P. S. (2010). Antimicrobial agents: synthesis, spectral, thermal, and biological aspects of a polymeric Schiff base and its polymer metal(II) complexes. *J. Coord. Chem*., 63(22), 3944-3955. https://doi.org/10.1080/00958972.2010.526207

[29] El-Sonbati, A. Z., El-Mogazy, M. A., Nozha, S. G., Diab, M. A., Abou-Dobara, M. I., Eldesoky, A. M. & et al. (2022). Mixed ligand transition metal(II) complexes: characterization, spectral, electrochemical studies, molecular docking and bacteriological application. *J. Mol. Struct*., 1248, 131498. https://doi.org/10.1016/j.molstruc.2021.131498

[30] Jarząbek, B., Kaczmarczyk, B. & Sęk, D. (2009). Characteristic and spectroscopic properties of the Schiff-base model compounds. *Spectrochim. Acta A Mol. Biomol. Spectrosc*., 74(4), 949-954. https://doi.org/10.1016/j.saa.2009.08.045

[31] Çetin, Z. & Dede, B. (2023). A novel Schiff base ligand and its metal complexes: Synthesis, characterization, theoretical calculations, catalase-like and catecholase-like enzymatic activities. *J. Mol. Liq*., 380, 121636. https://doi.org/10.1016/j.molliq.2023.121636

[32] El-Wahab, Z. H. (2007). Mononuclear metal complexes of organic carboxylic acid derivatives: Synthesis, spectroscopic characterization, thermal investigation and antimicrobial activity. *Spectrochim Acta A Mol Biomol. Spectrosc*., 67(1), 25-38. https://doi.org/10.1016/j.saa.2006.05.038

[33] Mounika, K., Anupama, B., Pragathi, J. & Gyanakumari, C. (2010). Synthesis, characterization and biological activity of a Schiff base derived from 3-ethoxy salicylaldehyde and 2- aminobenzoic acid and its transition metal complexes. *J. Sci. Res*., 2(3), 513-524. https://doi.org/10.3329/jsr.v2i3.4899

[34] West, B. O. (1962). The magnetic moments and structures of some *N*-substituted salicylideneimine complexes of cobalt(II). *J. Chem. Soc*., 260, 1374-1378. https://doi.org/10.1039/JR9620001374

[35] Abualy, M. M. & Khalil, M. M. H. (2001). Synthesis and spectroscopic study of Cu (II), Ni (II), and Co (II), complexes of the ligand salicylaldehyde-2-amino thiophenol. *Spectrosc. Lett*., 34(4), 495-504. https://doi.org/10.1081/SL-100105095

[36] Tuna, S., Canpolat, E. & Kaya, M. (2006). Synthesis and characterization of a new 4-methoxysalicyliden-p-aminoacetophenoneoxime and its complexes with Co(II), Cu(II) and Zn(II). *Pol. J. Chem*., 80(10), 1651-1656.

[37] Kashar, T. I. & Aal, S. A. (2021). Spectral, DFT-TDDFT computational investigation and biological studies of transition metal complexes of dehydroacetic acid Schiff base. *J. Iran. Chem. Soc*., 18, 1625-1640. https://doi.org/10.1007/s13738-020-02134-3

[38] Sari, N., Arslan, S., Logoglu, E. & Sakiyan, I. (2003). Antibacterial activities of some amino acid Schiff bases. *Gazi Univ. J. Sci*., 16(2), 283-288.

[39] Liew, S. K., Malagobadan, S., Arshad, N. M. & Nagoor, N. H. (2020). A review of the structure-activity relationship of natural and synthetic antimetastatic compounds. *Biomolecules*, 10(1), 138. https://doi.org/10.3390/biom10010138

[40] Mohamed, G. G. (2006). Synthesis, characterization and biological activity of bis(phenylimine) Schiff base ligands and their metal complexes. *Spectrochim Acta A Mol Biomol. Spectrosc*., 64(1), 188-195. https://doi.org/10.1016/j.saa.2005.05.044

[41] Saritha, T. J. & Metilda, P. (2021). Synthesis, spectroscopic characterization and biological applications of some novel Schiff base transition metal (II) complexes derived from curcumin moiety. *J. Saudi Chem. Soc*., 25(6), 101245. https://doi.org/10.1016/j.jscs.2021.101245

[42] Al-Sha'alan, N. H. (2007). Antimicrobial activity and spectral, magnetic and thermal studies of some transition metal complexes of a Schiff base hydrazone containing a quinoline moiety. *Molecules*, 12(5), 1080-1091. https://doi.org/10.3390/12051080

[43] Kowalska-Krochmal, B. & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens*, 10(2), 165. https://doi.org/10.3390/pathogens10020165

[44] Aligiannis, N., Kalpoutzakis, E., Mitaku, S. & Chinou, I. B. (2001). Composition and antimicrobial activity of the essential oils of two Origanum species. *J. Sci. Food Agric*., 49(9), 4168-4170. https://doi.org/10.1021/jf001494m

[45] Xiao, Z. P., Xue, J. Y., Tan, S. H., Li, H. Q. & Zhu, H. L. (2007). Synthesis, structure, and structure-activity relationship analysis of enamines as potential antibacterials. *Bioorg. Med. Chem*., 15(12), 4212- 4219. https://doi.org/10.1016/j.bmc.2007.03.060

[46] Aneja, B., Azam, M., Alam, S., Ahmad, P., Ronan, M., Umesh, Y. & et al. (2018). Natural product-based1,2,3-triazole/sulfonate analogues as potential chemotherapeutic agents for bacterial infections. *ACS Omega*., 3(6), 6912-6930. https://doi.org/10.1021/acsomega.8b00582

[47] Guoa, H-Y., Chenb, Z-A., Shenaand, Q-K. & Quan, Z-S. (2021). Application of triazoles in the structural modification of natural products. *J. Enzyme Inhib. Med. Chem*., 36(1), 1115-1144. https://doi.org/10.1080/14756366.2021.1890066

[48] Craig, J. S., Melidis, L., Williams, H. D., Dettmer, S. J., Heidecker, A. A., Altmann, P. J. & et al. (2023). *Organometallic pillarplexes* that bind DNA 4-way Holliday Junctions and forks. *J. Am. Chem. Soc.*, 145(25), 13570-13580. https://doi.org/10.1021/jacs.3c00118

[49] Maiti, S. K., Kalita, M., Singh, A., Deka, J. & P. Barman, P. (2020). Investigation of DNA binding and bioactivities of thioether containing Schiff base copper(II), cobalt(II) and palladium(II) complexes: Synthesis, characterization, spectrochemical study, viscosity measurement. *Polyhedron*., 184, 114559. https://doi.org/10.1016/j.poly.2020.114559

Citing this Article

Ejiah, F. N., Fasina, T. M., Revaprasadu, N., Ogunsola, F. T. & Familoni O. B. (2024). Antibacterial and DNA binding effects of cobalt(II) complexes containing aminophenol Schiff base moiety. *Lafia Journal of Scientific and Industrial Research, 2*(2), 13 – 23. https://doi.org/10.62050/ljsir2024.v2n2.314