E-ISSN: 1116 - 3089 P-ISSN: 3026-9784
MPRI-JOMDR | INTERNATIONAL JOURNAL OF ORGANIC & MEDICINAL DEV. RESEARCH, VOL. 03 NO. 3 JANUARY, 2024

ENHANCEMENT OF THE ANTIBACTERIAL POTENCIES OF AMPICILLIN BY SCHIFF BASE FORMATION AND COMPLEXING WITH METAL (II)-IONS OF CHROMIUM, IRON AND ZINC

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Key words:
Ampicillin, Schiff Base, Synthesis, Antibacterial, Enhancement,

Abstract

The Schiff base of ampicillin with salicylaldehyde was synthesized according to standard established procedures. The synthesized Schiff base and its corresponding Metal(II)-ions of Chromium, Iron and Zinc were characterized using Fourier transformed infrared (FTIR), Ultra-Violet Spectroscopy, Molar conductivity, melting point and Solubility in: distilled water, methanol, ethanol, acetone, chloroform, Dimethylsulphoxide (DMSO) and Petroleum ether respectively. The FTIR result confirmed the formation of the Schiff base with an azomethine group (C=N) absorption band at 1633.71cm^{-1}. The synthesized Schiff base and its metal complexes were found to be soluble in DMSO and the molar conductivity result was found to be low in all the complexes which indicates their non-electrolytic nature. The in-vitro antibacterial activity test of Schiff base and its Metal complexes on gram-positive (*Staphylococcus aureus* and *Streptococcus pyrogen*) and gram-

negative (*Pseudomonas aeruginosa* and *Salmonella typhi*) showed an enhanced antibacterial activity than that of the control (Ampicillin) with a zone of inhibition ranging from 17-28mm at a concentration of 100mg/ml. The control (Ampicillin) showed inhibition zones ranges of 18-25mm on *S. aureus, S. pyrogen, P. aeruginosa* and *S. typhi*. This study therefore substantiates the use of Schiff base in improving the efficacy level of antibiotic drugs to which resistant has been developed.

INTRODUCTION

Ampicillin is a beta-lactam antibiotic that is widely used to treat bacterial infections clinically such as *Escherichia coli.* It belongs to the group of aminopenicillins having a molecular formular of $C_{16}H_{19}N_3O_4S$ with a molecular mass of 349.41g/mol. It's chemically regarded as 6([-2-amino-2-phenylacetyl]amino)-3-3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]neumoni-2-carboxylic acid. It has a strong broad spectrum action that was extended to both gram-positive and gram-negative bacteria by suppressing its cell wall synthesis (Katzung *et al,.* 2001).

Schiff bases are synthetic compounds obtained by condensation of primary amine and carbonyl compounds (aldehydes and ketones). They are important category of pharmacological active molecules that have a great concern by medicinal Chemists because they possess a variety of pharmacological properties. Large number of Schiff base derivatives have been reported to exhibit significant antibacterial, antifungal, antitubercular, antiturmor, antileishmanial, DNA-binding activities etc (Abdulsada *et al.,* 2018).

Schiff base inorganic complexes having transition metal ion possesses broad range of pharmacological activities like antiviral, anticancer, antibacterial, antifungal, anticonvulsant and anti-inflammatory activities that can be well explained by several commercially available drugs containing their functional group (Yousef *et al.,* 2017). Large number of drugs exhibits modified pharmacological and toxicological properties preferably Schiff base containing compound possessing wide range of biological activities and complexation of metal in the form of complexes exhibits

some degree of antibacterial, antifungal, antitumor and anti-inflammatory activities (Xavier and Srividhya 2014). The activities of organic drugs for which resistance has emerged can be restored through structure modification by incooperating a metal or a metal complexes in to the drug. It was reported in 1975 that substituting the aromatic groups in the antibiotics penicillin and Cephalosporine with ferrocenyl moieties produced compounds with altered antibacterial activity as compared to the original penicillin and Cephalosporine. The ferrocenyl penicillin showed comparable activity to modify the structure to contain a metal, benzyl-penicillin and also ù- lactamase, which is one of the enzymes responsible for bacterial resistance to penicillintype antibiotics against various strains of Staphyloccus aureus. Large number of synthetic drugs have been discovered over the years for the treatment of malarial disease like chloroquine, sulphadoxine and pyrimethamine being among the most effective, (Taghreed *et al*., 2016). In a long search of novel drugs that will act against antimicrobial resistance diseases, the use of inorganic complexes has received a considerable attention and these inorganic complexes having Schiff base ligands of hetero cyclic compounds are of great importance as structural models for biological system because of the presence of hetero atoms (nitrogen, oxygen and sulphur), (Mohammed *et al.,* 2016).

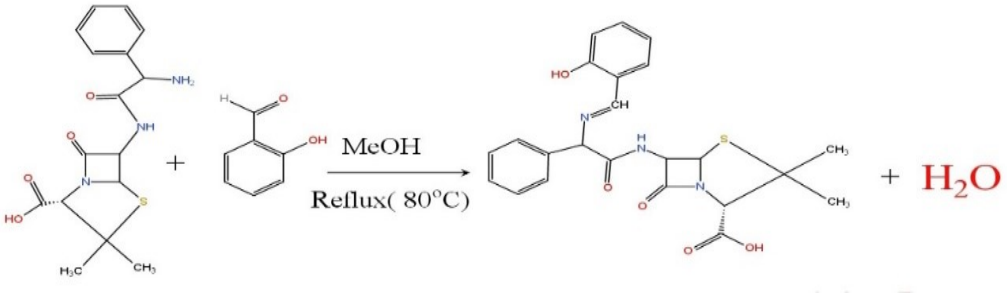
Material and Method:

All the chemicals used were of analytical grade from Sigma-Aldirich and BD and used without further purification. The metal salts used are in their chloride form. Melting point of the synthesized compounds were carried out by an open capillary method using electrothermal melting point apparatus. The fourier-Transform Infrared (FTIR) spectrometric was recorded on Perkin-Elmer in the wavelength range of 450 - 4000cm^{-1}and the UV-Visible spectrum of the complexes using DMSO as a solvent was also obtained.

Synthesis of Schiff Base ligand (HL$^{1)}$

The Schiff base was prepared by using a method as described by Aurora *et al.,*2015 in which 0.349g(1mmol) of ampicillin was dissolved in 25 mL methanol and mixed with 0.122g (1mmol) salicylaldehyde dissolved in 15mL of Methanol. In order to

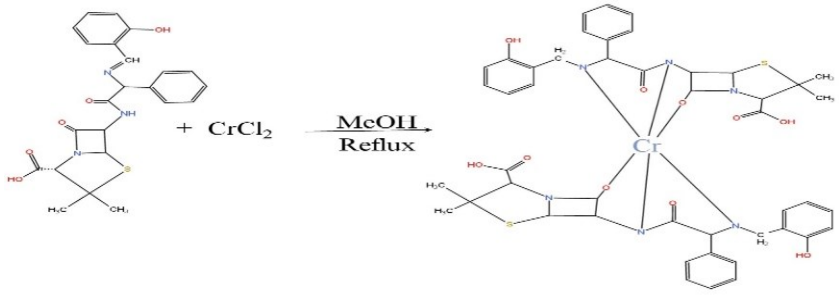
obtain the pH value between 7 to 8, then few drops of 1M NaOH solution was added and the mixture was refluxed for 2 hours at 80°C. The volume of the solution was reduced to one half by evaporation and an orange precipitate was formed. It was filtered, washed with methanol and dried in vacuum at room temperature under anhydrous $CaCl_2$ in desicator. Recrystallization from a mixture of ethanol – water (50:50) gave the Schiff base.



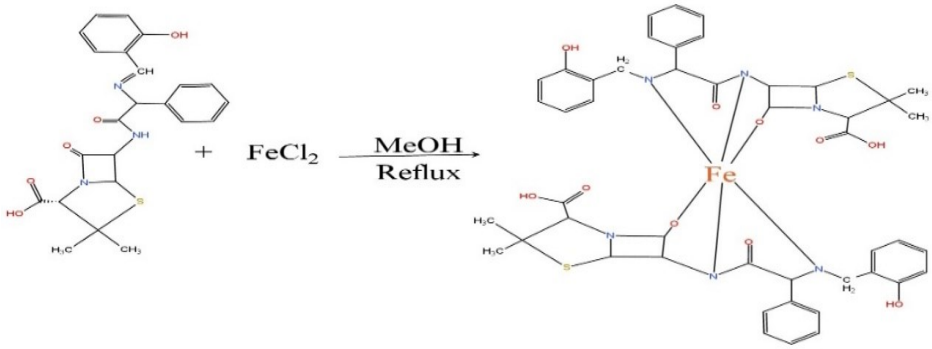
Scheme 1: Synthesis of HL^1

Synthesis of Schiff Base Metal Complex

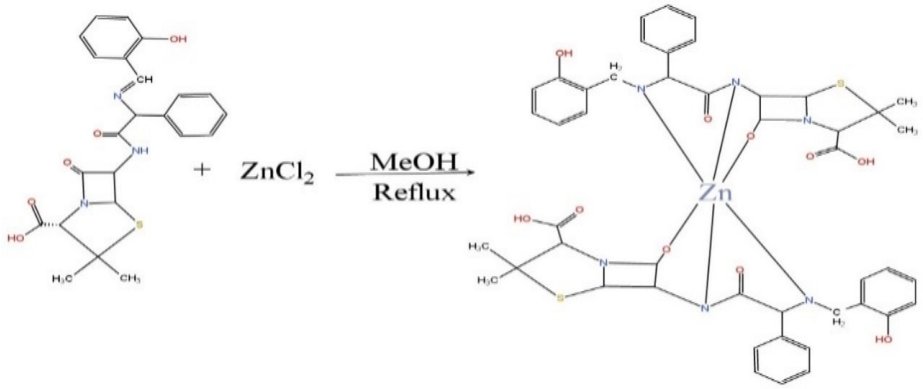
The $Cr(HL^1)_2$ complexes was synthesized by using a method as describe by Aurora *et al.,* (2015) in which 0.907g (2.0mmol) of the synthesized Schiff base dissolved in 25 mL methanol was mixed with 0.123g (1.0 mmol) of the Chromium(II)-Chloride($CrCl_2$). The mixtures pH value was adjusted between 7 to 8 by adding few drops of 1M NaOH solution and then the mixture was refluxed for 2 hours at 75°C. Finally, the volume of solutions was reduced to one half by evaporation, and an occurrence of colored precipitate was formed. It was filtered, washed with methanol and dried in desiccator. Recrystallization from hot methanol gave the metal complex. The same procedure was repeated with Iron(II)-Chloride and Zinc(II)-Chloride to obtain their complexes.



Scheme 2: Synthesis Of $Fe(HL^1)_2$ Complex



Scheme 3: Synthesis Of $Fe(HL^1)_2$ Complex



Scheme 4: Synthesis Of $Zn(HL^1)_2$ Complex

Result and Discussion

Physical Characterization

From Table the synthesized Schiff base ligands and their metal complexes show different colours ranging from brown, orange, yellow, grey, blue to green. The Schiff base HL^1 is orange in colour . The HL^1 complexes of Cr, Fe and Zn are green, brown and grey in colour respectively.

TABLE 1: Physical Characterization Results

COMPOUND	MOLECULAR FORMULAR	MOLECULAR WEIGHT	COLOUR	PERCENTAGE YIELD(%)	MELTING POINT (^{0}C)	MOLAR CONDUTIVITY (Scm2mol-1)
HL^1	$C_{23}H_{23}N_3O_5S$	453.53g/mol	Orange	68.95	238	57.8
$Cr(HL^1)_2$	$[Cr(C_{23}H_{23}N_3O_5S)_2]$	970.61g/mol	Green	51.25	252	39.3
$Fe(HL^1)_2$	$[Fe(C_{23}H_{23}N_3O_5S)_2]$	959.85g/mol	Brown	56.12	276	49.1

$Zn(HL^1)_2$	$[Zn(C_{23}H_{23}N_3O_5S)_2]$	969.41g/mol	Grey	60.32	265	38.6

The HL^1 metal complexes of Cr, Fe and Zn has a percentage yield of 51.25, 56.12 and 60.32% respectively. This is in agreement with the percentage yield values obtained by Abdulsada *et al.,* (2018). The low yield may be attributed to the incomplete reaction of the ligand with the metal ion.

The melting point show that the HL^1 and its metal complexes had a melting point range of 238-276°C and are in agreement with the values obtained from the Ndahi *et al*., (2018). The free HL^1 ligand has lower melting point than that of its correspondent complexes of Cr, Fe and Zn respectively.

The molar conductivities of HL^1 and its metal complexes in DMSO showed that Cr, Fe and Zn complexes had a conductivity values of 39.3, 49.1 and 38.6 respectively while the free ligand had a conductivity value of 57.8, the lower value of molar conductivity in the complex of Zn is an indicative of its non-electrolytic nature (Tawfiq 2011).

Fourier transformed infrared spectroscopy

The FTIR spectra of the synthesized Schiff bases and their metal complexes were recorded on Perkin Elmer spectrum version 10.03.09 in the wave number range of 450 - 4000cm^{-1} and the result obtained was recorded on the Table 2

TABLE 2: FTIR Result of the ligands and their metal complexes

COMPOUND	v(O-H)	vC=C	vC=N	vM-N	vM-O
HL^1	3194.82	1525.16	1633.71		
$Cr(HL^1)_2$	3435.00	1531.27	1614.56	697.55	527.78
$Fe(HL^1)_2$	3486.76	1457.22	1650.97	526.00	453.97
$Zn(HL^1)_2$	3479.30	1525.16	1645.00	526.00	466.62

From Table 2 the absorption band at 1633.71cm^{-1} is assigned to v(C=N) (azomethine) group of HL^1. This data confirmed the formation of HL^1 Schiff base through the -C=N- bond and this is in line with the result obtained by Aurora *et al.,* 2015. In HL^1 metal complexes the v(C=N) was moved to higher values of 1650.97cm^{-1} for Fe and 1645.00

cm^{-1} for Zn respectively, while Cr moved down to a lower wave number of 1614.56cm^{-1} and this shift in absorption of the free ligand with that of the metal complexes is an indicative of the coordination of the Schiff base ligands with the metals ions. This is in line with the work of Abdulsada *et al.,* (2018) who find out that upon complexation of the free ligand with the metal ion the absorption band of azomethine shift to lower or higher wavenumber. In the free ampicillin the ν(C=N) absorption band is lacking because the ampicillin does not have the azomethine group.

The presence of strong absorption band at 3194.82 cm^{-1} in the spectrum of HL^1 was assigned to *ν*(O-H) vibration. The band was shifted to 3435.00 cm^{-1}, 3486.76 cm^{-1} and 3479.30 cm^{-1} upon complexation with Cr, Fe and Zn respectively which is indicative of coordination with metal ions. This is in line the reported literature of Nasiru *et al.,* (2018), who found out that on complexation of ligand with metal ions the absorption band shifts to longer frequencies. Furthertmore, in free ampicillin the ν(OH) appeared at 3445.43 cm^{-1} due to the presence of OH group attached to the carboxylic group of the ampicillin. In the complexes there is an existence of weak bands at 527.00 cm^{-1}, 526.00 cm^{-1}, 547.87 cm^{-1}, 500.40 cm^{-1}, 561.79 cm^{-1}, 526.47 cm^{-1} and 578.79cm^{-1} which are assigned to *ν*(M-N) while the absorption bands at 453.97 cm^{-1}, 466.62 cm^{-1}, 518.27 cm^{-1}, 467.01 cm^{-1}, 485.94 cm^{-1}, 467.39 cm^{-1} and 464.24cm^{-1} were assigned to *ν*(M-O) and this also correspond to that of Nasiru *et al*., (2018).

Solubility test

The solubility test result of the synthesized Schiff base and their metal complexes were determined in ditilled water, acetone, methanol, ethanol, chloroform, dimethyl sulphoxide (DMSO) and petroleum ether as given in Table 3.

TABLE 3: Solubility Test Results of the Schiff bases and their complexes

COMPOUND	Water		MeOH		EtOH		ACTN		$CHCl_3$		DMSO		P.ETH	
	RT	ET	RT	ET	RT	ET	RT	ET	RT	ET	RT	ET	RT	ET
HL^1	IS	IS	PS	SS	PS	PS	PS	SS	IS	IS	SS	SS	IS	IS
$Cr(HL^1)_2$	IS	IS	PS	SS	PS	SS	IS	IS	IS	IS	SS	SS	IS	IS
$Fe(HL^1)_2$	IS	IS	SS	SS	PS	SS	SS	SS	IS	IS	SS	SS	PS	PS

$Zn(HL^1)_2$	PS	PS	SS	SS	IS	IS	IS	IS	IS	IS	SS	SS	IS	IS

IS= Insolube, PS = Partially soluble, SS = Soluble, RT = Room temperature(25°C), ET= Elevated temperature(75°C), P. Eth= Petroleum ether, DMSO = Dimethylsulphoxide

ACTN = Acetone, $CHCl_3$ = Chloroform

From Table 3 the Schiff base and its metal complexes were insoluble or partially soluble in water and petroleum ether and this is in line with a work reported by Nasiru *et al.,* (2018) were he find out that some of his complexes are insoluble or slightly soluble in water and hexane. The Schiff bases HL^1 together with its metal complexes exhibit varying degree of solubility in methanol, ethanol, chloroform and acetone ranging from partially soluble to strongly soluble but all are strongly soluble in DMSO while the free ampicillin is also strongly soluble in DMSO. This is in line with the result obtained by Nnamani et al., 2020 in which all his complexes and the Schiff base are strongly soluble in DMSO.

UV-Visible Spectroscopy

The UV-Visible spectrum of the synthesized Schiff base and their corresponding metal complexes using DMSO as a solvent and the result obtained was recorded on the Table 4

TABLE 4: UV-Visible results of the Schiff base and their metal complexes

COMPOUND	Absorption maxima(nm)	Assigned Transition
HL^1	250 350	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
$Cr(HL^1)_2$	300 450	$n \rightarrow \pi^*$ $^6A_{1g} \rightarrow {}^4T_{2g}$
$Fe(HL^1)_2$	300 235 400	$n \rightarrow \pi^*$ LMCT $^6A_{1g} \rightarrow {}^4T_{2g}$

$Zn(HL^1)_2$	300 400	n →π* $^6A_{1g}$→$^4T_{2g}$

LMCT= Ligand to metal charge transfer

From table 4 it shows that HL^1 ligand has two absorption maxima at 250nm and 350nm which are assigned to π→ π* and n→π* electronic transitions respectively. Upon complexation with Cr, Fe and Zn there is appearance of new band of maximum absorption within the range of 250nm to 450nm in all the three complexes and this is assigned to $^6A_{1g}$→$^4T_{2g}$ electronic transition. This result is also in agreement with the one obtained by Ndahi *et al.*, (2018). The UV result indicates that the metal complexes of HL^1 possess an octahedral geometry because all the electronic transitions confers with that of the octahedral complexes.

Antibacterial activity

The antibacterial activities of the Synthesized Schiff base and its corresponding metal complexes was conducted according to standard procedures and the result obtained were presented on figure below

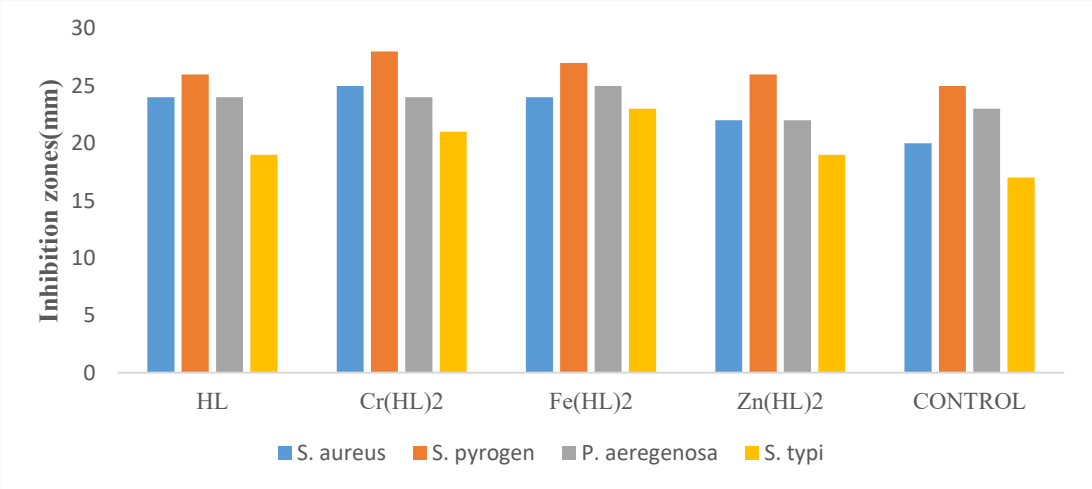


Figure 1: antibacterial activities of the Synthesized Schiff base and its metal complexes

From figure 1 the measured zone of inhibition of HL^1 and its metal complexes of Cr, Fe and Zn against *Staphylococus aureus, Sreptococcus pyrogen* (gram positive), *Pseudomonas aregenosa and Salmolena typhi* (gram negative). The activity of the

ligand and the metal complexes were found to be slightly higher than that of the control drug on *Staphylococus aureus* (gram positive), *Pseudomonas aregenosa and Salmolena typhi* (gram negative) and *Sreptococcus pyrogen* in all the metal complexes. The result is in agreement with the one obtained by Abdulsada *et al.,* (2018) who find out that metal complexes of copper with terephthalaldehydeneampicillin and terephthalaldehydenecephalexin inhibits the bacterial activity of *E.coli, S.aureus and P.aeruginosa* than the free ligands (Abdulsada *et al,.* 2018).

Conclusion

The synthesized Schiff Base of Ampicillin and its metal(ii) complexes of Cr, Fe and Zn has enhanced invitro Antibacterial potentials than the normal Ampicillin. Therefore, this study substantiates the possible use of Schiff base in improving the efficacy level of antibiotic drugs to which resistant has been developed.

Acknowledgement

The authors of this research wish to acknowledge the effort of Tertiary Education Trust Fund (TetFund) for the Funding of this Research under the Institutional Research Grant; indeed, we are very grateful for that.

References

Abdulsada H.A, Noor H.N, Ahmed K.H (2018). Design Synthesis And Preliminary Antibacterial Evaluation of Schiff Base Metal Complexes of Ampicillin and Cephalexin. *Acta Chimica and Pharmaceutica Indica.* Research 8(1) 1-9.

Aurora R. Adriana S. Geogeta C. Irina D. (2015). Synthesis, Spectral Characterization and Thermal Behaviour of new Metal(II)-Complexes with Schiff Base derived from Amoxicillin, *J. Chil. Chem. Soc.,* 60(3) 3074-3079.

Katzung , B.G (2001). Basic and Clinical Pharmacology. 8th Edition International Edition, Longman Medical Books, McGraw-Hill, New York.

Mohammed, S. Sumayya H, Mohammed S, Owais M, MohammedA, Saud I.A (2016). Pharmacophore hybrid Approach of New Modulated Bis-diimine Cu^{II}/Zn^{II} Complexes Based on 5-Chloro Isatin Schiff Base Derivatives: Synthesis, Spectral Studies and Comparative Biological Assessment. *Journal of Photochemistry and Photobiology* 157, 39-56.

Nasiru Y.P, Ndahi N.P, Bako L. Madugu M.L.,Zulqiflu A., and Mamman Y. (2018). Synthesis and Partial Characterization of Two Schiff Base Ligands with (2 and 4-Nitroaniline) and their Transition Metal(II) (Co and Cu) Complexes. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)* 4: 2635-3490.

Ndahi N.P, Garba H., Waziri I, Osunlaja A.A., Putaya Habila A.N (2018). Complexes of Mn(II) And Fe(II) with Schiff Bases derived from Trimethroprim with Salicylaldehyde and Benzaldehyde as Potential Antimicrobial Agents. *Nigerian Journal of Pharmaceutical and Biomedical Research,* vol 3(1)

Nnamani J. O, Nasiru P. Y., Wilson. L. D, Mohammed U. (2020): Synthesis, Characterization and Evaluation of Antimicrobial Potency of Fe(II), Mn(II), Cu(II), Co(II) and Zn(II) ion complexes with Schiff Base, Derived from Salicylaldehyde and p.toluidine. World Journal of Innovative Research (WJIR) Volume-8, Issue-2, Pages 16-21, ISSN: 2454-8236.

Taghreed H.A, Raheem T.M And Ahmed H.I (2016). Mixed Ligands (Schiff bases, Antibiotics Drugs) Metal complexes. LAP Lambert Academic Publishing pp 3-16.

Tawfiq A.A. (2011).Synthesis and Characterization of Some Divalent Transition Metals Complexes of Schiff Bases Derived from Salicylaldehyde Diamine Derivatives. *Al-Mustansiriya J. sci* 21(1):101-108

Xavier A. And Srividhya N. (2014). "Synthesis and Study of Schiff base Ligands. *IOSR Journal of Applied Chemistry (IOSR-JAC)* 7(11) Ver. I. pp 06-15 www.iosrjournals.org.

Yousef E, Barbara M, Maryam M, Moj K (2017). A Novel Cationic Cobalt(III) Schiff Base complexes: Preparation, Crystal Structure, Hirshfeld Surface Analysis, Antimicrobial Activities and Molecular docking. *Journal of Microbial Pathogenesis.* 113, 160-167 .