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Synthesis, Spectral and Biological studies of transition metal complexes of schiff base derived from Ofloxacin

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Abstract

This research project is aimed to synthesis, characterization and biological evaluation of transition metal complexes of ofloxacin with nitrogen donor heterocyclic ligands. Schiff base ligands are synthesized by introducing an amino group to the ring of ofloxacin followed by the treatment with cinnamaldehyde. Schiff base ligands are complexed with the transition metals such as Ni(II), Mn(II), Cu(II), Zn(II) to form metal complexes. All the derivatives and metal complexes are analyzed by FTIR, TGA, DTA, elemental analysis, molar conductivity, UV-Spectral studies and atomic absorption spectroscopy. All the synthesized metal complexes are of ML_2 type. The ligands and metal complexes are also subjected to antimicrobial screening against bacteria i.e. *S. aureus* and *E. coli* as well as against fungi i.e. *A. flavus* and *A. niger*. The IR studies show that ligand is bi-dentate and has coordinate bonds to metal atom by N- azomethyine and phenolic –O. The conductivity test of Schiff base and metal complexes show non-electrolytic nature. The thermal analysis data shows that the water molecule has coordinate bond with transition metal atom or ion. All the synthesized ligands and metal complexes show better antibacterial activities than the ofloxacin.

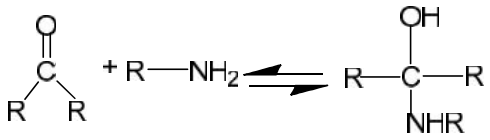
Keywords: Synthesis, Characterization, Schiff base, Ofloxacin, Complexes.

1. Introduction

Schiff bases are an important class of ligands in coordination chemistry. The Schiff base complexes exhibit important properties such as anti-inflammatory activity, antibiotic activity; antimicrobial activity and antitumour activity (Martina *et al.*, 2012). Some of the Schiff base complexes are used as model molecules for biological oxygen carrier systems. Schiff base is a nitrogen analogue of a ketone or an aldehyde.

Schiff bases have proved to be an important intermediate. This also includes the reaction of an amino or carbonyl group of the substrate with an enzyme. In this regard, the pyridoxal Schiff bases derived from the active form of vitamin-B6 (pyridoxal) and amino acids have been synthesized and screened from biological point of view (Lehlinger, 1975). The complexes of such kind of ligands with transition metals are important enzyme models. At pH 5 the degree of hydrolysis and solubility in water is highest for Schiff bases. Schiff bases have got a wide variety of applications in analytical chemistry, agrochemical, catalysis, food industry, fungicidal, dye industry and biological activities (Gaur 2003). Schiff bases have played a significant role in the progress and development of the coordination chemistry. They are usually synthesized by the condensation of a primary amine with an aldehyde or ketone in which C=N-R group replaces a C=O group.

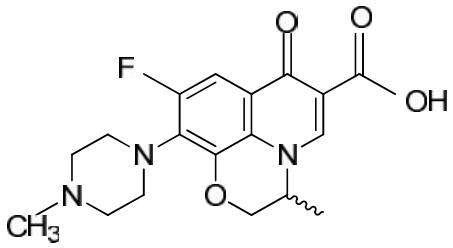
The following scheme illustrates the synthesis of Schiff base:



Here "R" can be aliphatic or aromatic group. Mostly they are bidentate, tridentate, tetradentate or polydentate ligands and they can form very stable complexes with transition metals (Munir *et al.,* 1985).

Ofloxacin is one of a new generation of fluorinated quinolones structurally related to nalidixic acid. It is an orally administered broad spectrum antibacterial drug. The pharmacokinetic profile of ofloxacin is superior to that of ciprofloxacin, with more rapid absorption and a peak serum concentration several times higher. Moreover, ofloxacin achieves high concentrations in most tissues and body fluids. Its IUPAC name of Ofloxacin, is(RS)-(oflo),(-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic x acid).

Fig 1 Structural formula of Ofloxacin



Fluoroquinolones (FQs) are broad-spectrum antibacterial agents widely used for treating bacterial infections. Their major mode of action is the inhibition of DNA replication in bacteria via interference of the normal function of DNA topoisomerase. The mechanism of bactericidal effect of Ofloxacin is based on the inhibition of the DNA gyraze of the bacteria, the enzyme that produces a negative supercoil in DNA and thus permits transcription and replication (Bukhari *et al* 2013).

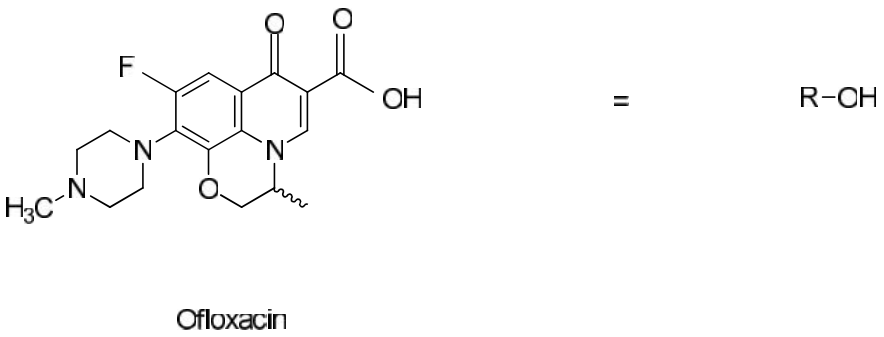
Ofloxacin also forms complexes with various metal ions or atoms and their characterization can be carried out by the electrophoresis. Ofloxacin is freely soluble in acetic acid, slightly soluble in water, methanol, ethanol or acetone.

2. Experimental

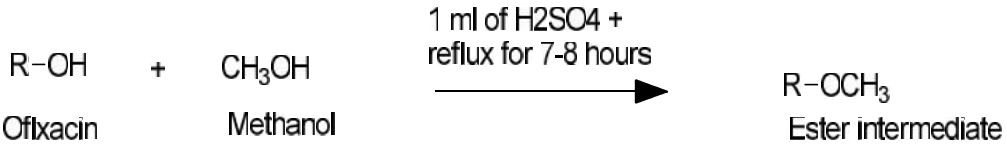
All the research work was carried out in Laboratory, Department of Chemistry in Government College University Faisalabad. The chemicals and reagents used were of analytical grade they were obtained from E.Merck/BDH and used without any further purification. Ofloxacin was obtained from pharmaceutical company and used without further purification. The IR spectras of the ligands and the complexes are recorded from the IR Prestge-21, SHIMADZU. UV spectra are obtained from UV-1700, SHIMADZU. Elemental analysis is done from CHNS-(England). Atomic absorption data obtained from AA-6300, SHIMADZU and TGA, DTA spectras are obtained from SDT Q 600, SHIMADZU. The antimicrobial activity was determined by the Disc Diffusion Method.

2.1 Synthesis of Ester from Ofloxacin

0.01 Moles of ofloxacin was dissolved in the 60 ml of methanol and poured it into the round bottom flask. 1 ml of conc. H_2SO_4 was added to the round bottom flask which served as a catalyst for esterification process. The mixture was then refluxed for about 7-8 hours till the complete consumption of ofloxacin (indicated by TLC). The light yellow colored crystalline product was filtered after cooling the hot solution and dried out under vacuum and reserved in a desiccator for further use.



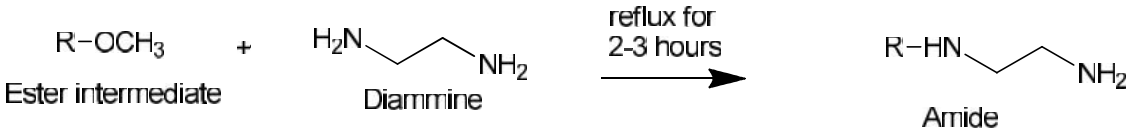
Ofloxacin



Oflxacin Methanol Ester intermediate

2.2. Synthesis of Amide from Ofloxacin

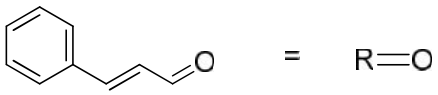
After that, 0.01 moles of ethylene diammine was added to the solution of ester intermediate and reaction mixture was again refluxed for 2-3 hours till the completion of reaction (indicated by TLC). The resulting complex in the form of creamy yellow colored crystals was isolated in crystallized form after reducing the volume of the solution by evaporation. The crystalline product was dried out and saved in desiccator for further use.



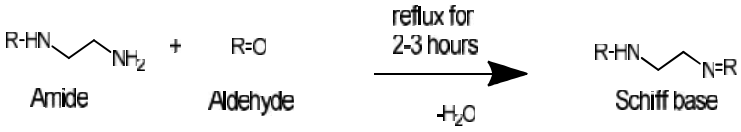
Ester intermediate Diammine Amide

2.3. Synthesis of Schiff Base of Ofloxacin

0.01 Moles of Cinnamaldehyde was then added to the amide reaction mixture and reflux was again carried out for 2-3 hours. The resulting complex in the form of crystals of light yellow color was isolated in crystallized form after volume reduction of the solution by evaporation. The crystalline product was dried out and saved in desiccator for further use.



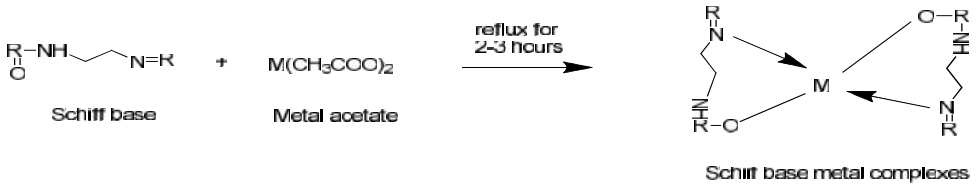
cinnamaldehyde



Amide Aldehyde Schiff base

2.4. Synthesis of Schiff Base Metal Complexes

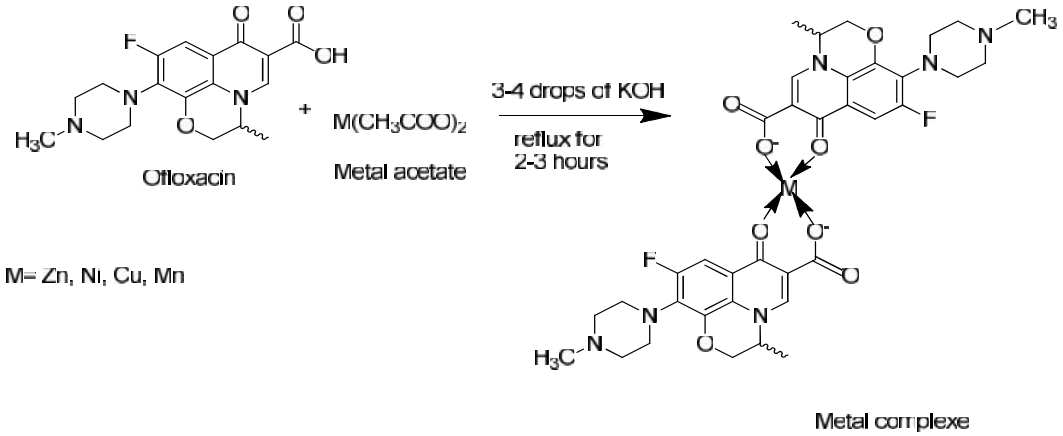
This Schiff base complex (≈0.2g) was then mixed with 0.lg of metal acetate solution prepared in methanol (where M= Mn, Zn, Ni and Cu) and reflux was again carried out for 2-3 hours. Then Schiff base transition metal complexes were dried out under vacuum and reserved in a desiccators.



Schiff base Metal acetate Schiff base metal complexes

2.5. Synthesis of Transition Metal Complexes of Ofloxacin

0.2g of ofloxacin was dissolved in 10 ml of methanol and added to the solution of transition metal (M=Cu, Mn, Zn, Ni), which was prepared by dissolving 0.1g of metal acetate in 10 ml of methanol. 3-4 drops of 0.1 M KOH solution was also added to this solution to adjust the pH between7-8. This mixture was poured into the round bottom flask and refluxed for 2-3 hours until a colored solution was obtained. Those transition metal complexes of ofloxacin were isolated after volume reduction by evaporation in crystallized form. Those crystalline complexes were dried and reserved in desiccators.



2.6 Antimicrobial Study

For antibacterial study of both ligand and complexes, the disc diffusion method 2 is adopted using peptone, beef extract, sodium chloride and agar-agar as the agar medium. The discs which are used in this method have 5 mm diameters. 0.01 ml of DMF (as a solvent) is used for equally amount of ligand and complexes each (about 30 µg). After that the filter paper discs were dipped in the above mentioned solutions, and then these discs were dried and placed in the Petri dishes cultured with the investigation organisms. The plates were incubated for 24 hrs at 37°C. After 24 hrs the inhibition zone was evaluated in the each case.

3. Results and Discussion

The physical, microanalytical data, IR, electronic absorption thermal data and antimicrobial studies of ligand and complexes is given in the tables 1-5. It summarizes the elemental percentage of the carbon, hydrogen and nitrogen of ligand and complexes. The results obtained from the elemental analysis show that the ligand is formed in the reaction of the antibiotic with Salicylaldehyde. The results gained from elemental analysis and atomic absorption also show that there is 1:2 molar ratio in all the complexes formed from the reaction of the metal salts with the ligand having ML2 stoichiometry. The solubility of ligand and complexes is checked in water and many organic solvents. DMF, DMSO, chloroform, methanol, ethanol and distilled water were used to check solubilities. Complexes showed little solubility in chloroform but most of complexes were found to be soluble in DMF and Distilled water. Some complexes were also soluble in methanol and ethanol.

Table 1: Physical Properties of Intermediates, Ligands and Complexes

Complex No.	Complex Code	Color	Physical state	Melting/Decomposition points °C
Drug	OF	Yellow	Crystalline solid	272
1	OE	Light Yellow	Crystalline solid	260
2	OA	Creamy Yellow	Crystalline solid	240
3	OS	Light Yellow	Crystalline solid	268
3a	OS_1 (Cu)	Brown	Crystalline solid	272
3b	OS_2 (Mn)	Dark Yellow	Crystalline solid	288
3c	OS_3 (Zn)	Yellowish White	Crystalline solid	244
3d	OS_4 (Ni)	Greenish Yellow	Crystalline solid	240
4a	O_1 (Cu)	Light Blue	Crystalline solid	228
4b	O_2 (Zn)	Off White	Crystalline solid	206
4c	O_3 (Mn)	Yellow	Crystalline solid	280
4d	O_4 (Ni)	Green	Crystalline solid	255

Table 2: Microanalytical data of intermediates, ligands and its metal complexes

Sr. No	Sample Code	Elemental Analysis data % Found (Experimental/calculated) C	H	N	O
1	OF	58.75 (58.81)	9.21 (9.25)	3.58 (3.62)	12.30 (12.33)
2	OE	58.40 (58.43)	9.09 (9.12)	3.57 (3.60)	12.26 (12.30)
3	OA	58.42 (58.45)	9.16 (9.20)	3.56 (3.61)	12.28 (12.31)
4	OS	58.73 (58.71)	9.22 (9.20)	3.57 (3.61)	12.39 (12.35)
5	OS_1 (Cu)	62.17 (62.21)	8.96 (8.99)	4.94 (4.90)	23.91 (23.96)
6	OS_2 (Mn)	61.05 (61.01)	7.99 (7.96)	5.04 (5.01)	23.36 (23.42)
7	OS_3 (Zn)	60.40 (60.44)	7.85 (7.91)	4.65 (4.71)	23.27 (23.31)
8	OS_4 (Ni)	60.69 (60.67)	7.90 (7.94)	4.69 (4.73)	23.39 (23.42)
9	O_1 (Cu)	60.42 (60.50)	7.88 (7.92)	4.66 (4.71)	23.27 (23.31)
10	O_2 (Zn)	60.21 (62.45)	7.09 (7.15)	4.15 (4.10)	22.23 (22.25)
11	O_3 (Mn)	61.04 (61.12)	7.56 (7.60)	4.65 (4.68)	23.78 (23.85)
12	O_4 (Ni)	60.40 (60.46)	7.45 (7.49)	4.90 (4.94)	23.33 (24.38)

Table 3: I.R Spectra of Ligands and Their Complexes

Complex No.	Complex code	D(-NH_2)	D(-O-H)	U (-N-H)	U (-N=C)	U (-M-O)	U (-M-N)	U (-C=O)
Drug	Of	-	3520	-	-	-	-	1710
1	OE	-	-	-	-	-	-	1710
2	OA	3410	-	3165	-	-	-	1706
3	OS	-	-	3160	1621	-	-	-
3a	OS1 (Cu)	-	-	3145	1612	370	470	-
3b	OS2 (Mn)	-	-	3142	1608	380	466	-
3c	OS3 (Zn)	-	-	3140	1604	382	463	-
3d	OS4 (Ni)	-	-	3146	1602	378	469	-
4a	O1 (Cu)	-	3420	-	-	505	-	1645
4b	O2 (Zn)	-	3420	-	-	509	-	1640
4c	O3 (Mn)	-	3400	-	-	506	-	1630
4d	O4 (Ni)		3410			502		1600

Spectra of the ligands and metal complexes were taken in the range of 4000-400 cm^{-1} for confirmation of the complexation. The absence of band in the range of 3520-3580 cm^{-1} suggests deprotonation of carboxylic group. It is absent in complexes 4a-4d but present in the spectra of ofloxacin. A weak band in the range of 3420-3410^{-1} cm may represent the lattice water (Jamil *et al.*, 2009). The band of (C=O) appeared at 1710 cm^{-1}in the spectrum of drug. The metal complexes of drug 4a-4d showed this spectrum in the range of 1630-1645 cm^{-1}suggesting that carbonyl oxygen is involved in the complexation with metal atoms. For complexes 3, 3a, 3b, 3c, and 3d this wavelength disappeared due to C=N formation. For complex 2 it appeared at 1708-1710 cm-like ofloxacin (Sultana *et al.*, 2013). A wavelength at 3410 cm^{-1}suggests -NH2 is present in complex 2 which disappeared in complexes 3, 3a-3d upon Schiff base formation (Chauhan & Shaik, 2005). A new band in the range of 1590-1630 cm^{-1} appeared in complexes 3, 3a-3d indicating the formation of C=N. It appeared at 1621 cm^{-1} for complex 5. The band shifted to lower frequency in complexes 3a-3d due to metal complex formation (Imran *et al.*, 2007). The metal complexes 4a-4d exhibit a band in the range of 500-515 cm^{-1} suggesting M-O bond formation which also confirms the involvement of ring oxygen in bonding with metals because C=O bond frequency shifted to lower frequencies at the same time. M-N bond frequencies appeared in the range of 450-550 $cm^{-1}$for the complexes 3a, 3b, 3c and 3d confirming the nitrogen association with the metals (Patel *et al*., 2011).

The thermal decomposition of the complexes has been studied in the nitrogen atmosphere by using the TGA and DTA techniques.

The sharp decrease from 200 to 500 °C showed the loss of ligand from Schiff based transition metal complexes. The complexes begins to lose of weight around 80 °C showing the presence of the moisture and around 120 °C suggesting the presence of the coordinated water, it is the dehydration stage at which the water molecules are eliminated. This indicates that the complexes are hygroscopic as well as hydrated. A sharp decrease from 200 to 500 °C in the weight indicate lose of the Schiff base ligand from the complexes.

Four different strains were used for antimicrobial screening of the complexes. Among these two are bacterial strains and two strains are fungi. Bacterial strains are *S. aureus* and *E. coli.* While fungal strains are *A. flavus* and *A. niger.*

Table 4: Thermal data

Complex	Temp. range ºC	Stage	TG Wt. Lose in %	DTA Temp. Peak ºC	Evolved moiety Formula	mass calculated %
OS1 (Cu)	100-200 210- 600	8.1 20.65	1 2	541.57 (exo) 580.65 (endo)	Water ligand	11.62 60.78
OS2 (Mn)	100-200 210- 600	1 2	12.2 58.85	499.76 (exo) 508.05 (endo)	Water ligand	11.12 60.05
OS3 (Zn)	100-200 210- 600	1 2	12.62 57.35	338.24 (exo) 366.34 (endo)	Water ligand	3.85 60.5
OS4 (Ni)	100-200 210- 400	1 2	14.11 60.22	264.33 (exo) 395.20 (endo)	Water ligand	12.85 60.7
O1 (Cu)	100-200 210- 600	1 2	11.55 60.03	544.65 (exo) 579.00 (endo)	Water ligand	11.76 60.10
O2 (Zn)	100-200 210- 600	1 2	3.75 60.5	335.129(exo) 365.29 (endo)	Water ligand	3.98 60.9
O3 (Mn)	100-200 210- 600	1 2	11.2 59.85	495.77 (exo) 505.10 (endo)	Water ligand	11.28 60.06
O4 (Ni)	100-200 210- 600	1 2	13.25 60.09	265.55 (exo) 394.19 (endo)	Water ligand	12.80 60.9

TGA = Thermogravimetric analysis and DTA= Differential thermal analysis

Table 5: Antimicrobial studies by Zone of Inhibition (mm)

Complex Code	*S. aureus*	*E. coli*	*A. flavus*	*A. niger*
Of	22	20	00	00
OS	24	22	10	8
OS_1 (Cu)	23	24	9	7
OS_2 (Mn)	25	21	10	8
OS_3 (Zn)	23	20	9	6
OS_4 (Ni)	21	19.5	8.5	6.5
O_1 (Cu)	22.5	21.5	9	6
O_2 (Zn)	23	19	7	5
O_3 (Mn)	21	22	9	6.5
O_4 (Ni)	20	18	8	5

Standard Drug, Ofloxacin (positive control) Concentration l0 mg/mL in DMSO

The ligands and their respective metal complexes were divided into different series. Values of antimicrobial activities are given in Table 5, while the comparative explanation is given below.

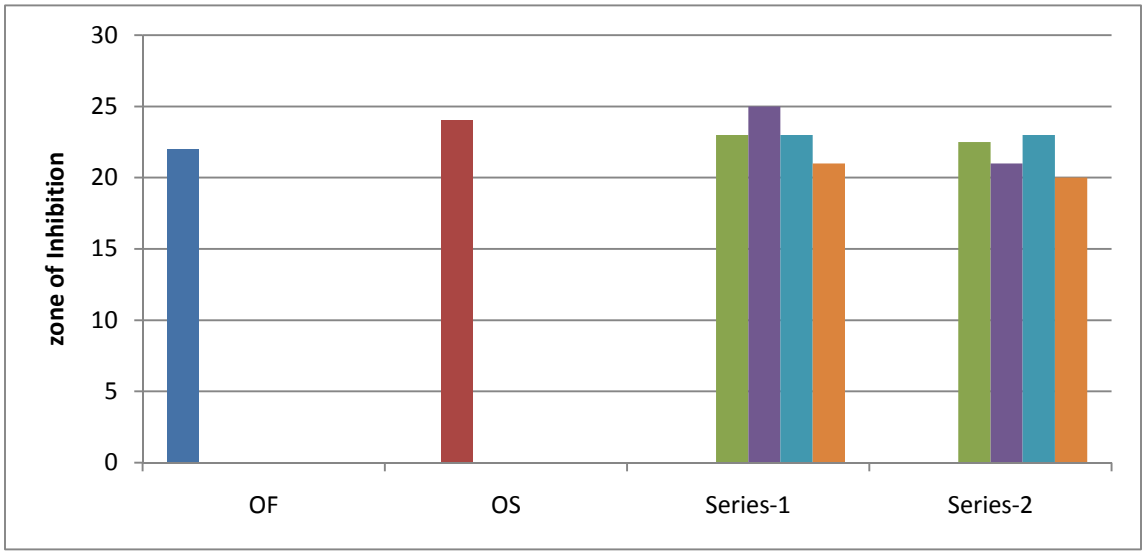


Fig. 3: Comparison of biological activity of ligands and their metal complexes against *S.aureus*

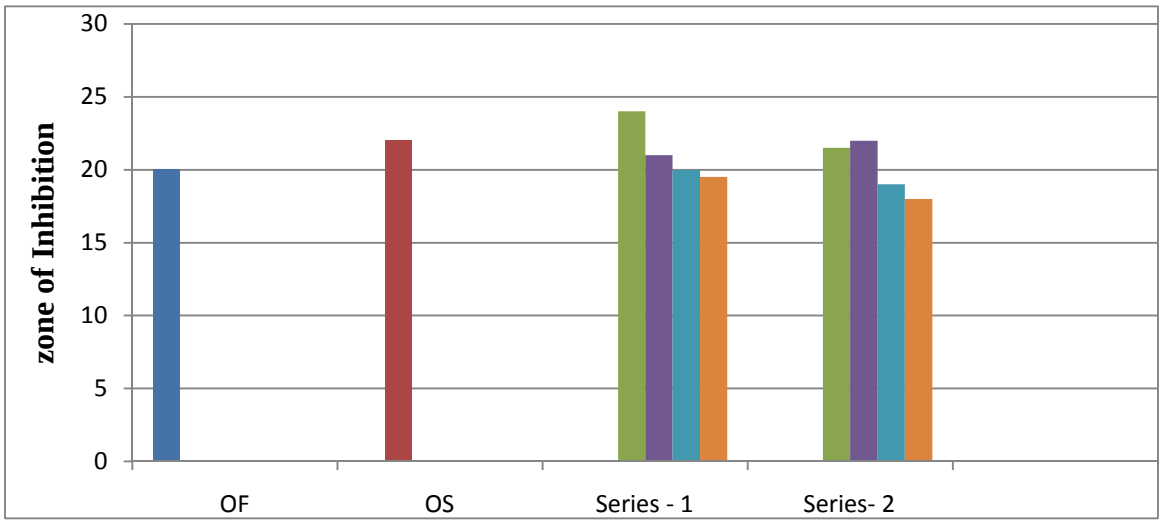


Fig. 4: Comparison of biological activity of ligand and their metal complexes against *E.coli*.

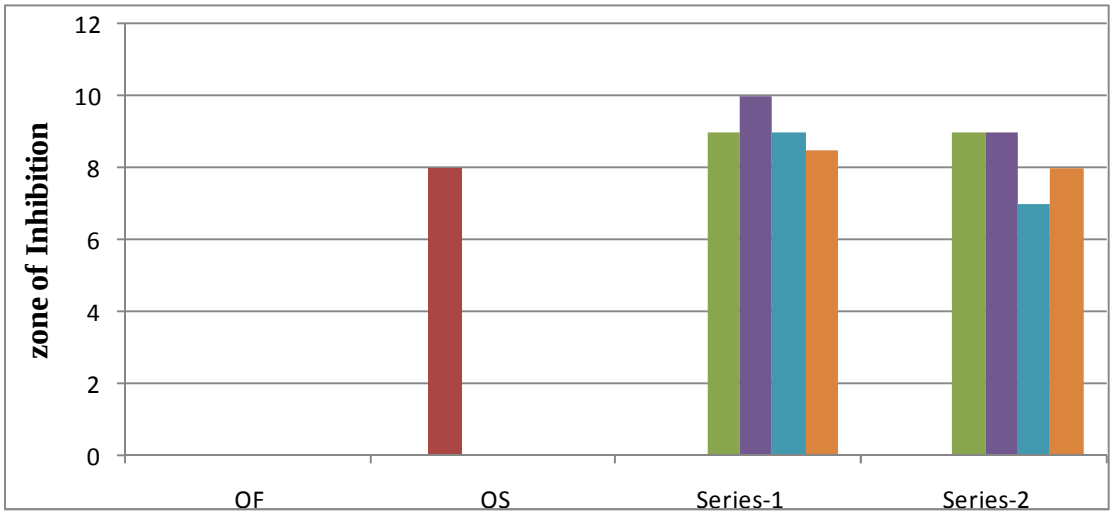


Fig. 5: Comparison of biological activity of ligands and their metal complexes against *A.flavus*

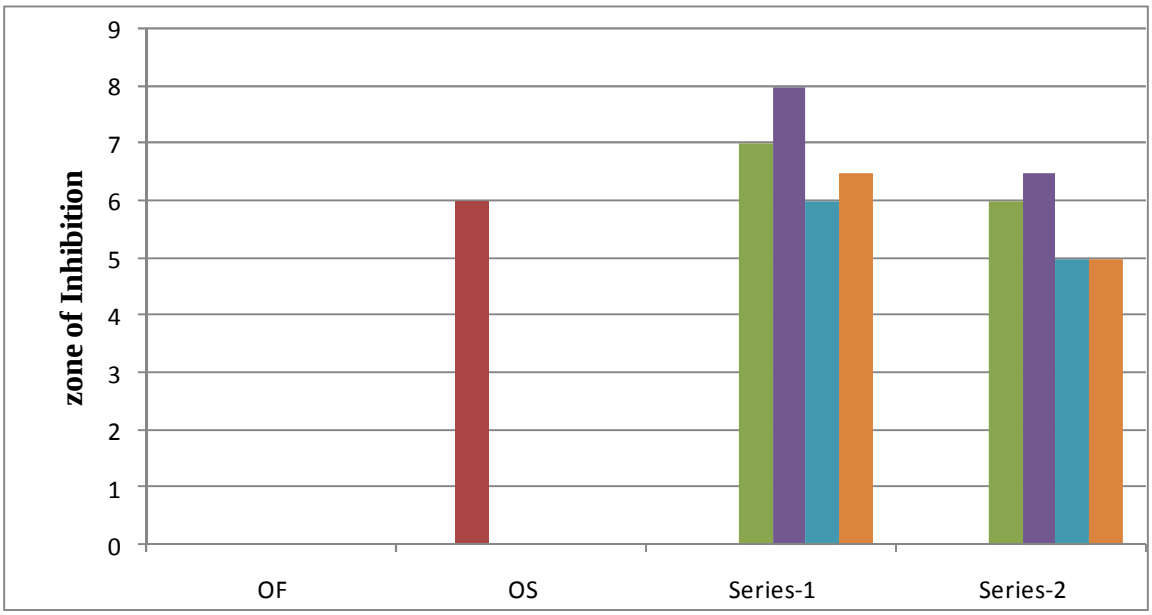


Fig. 6: Comparison of biological activity of ligands and their metal complexes against *A.niger*

Fig. 7: Comparison of antibacterial activity of ligands and their metal complexes

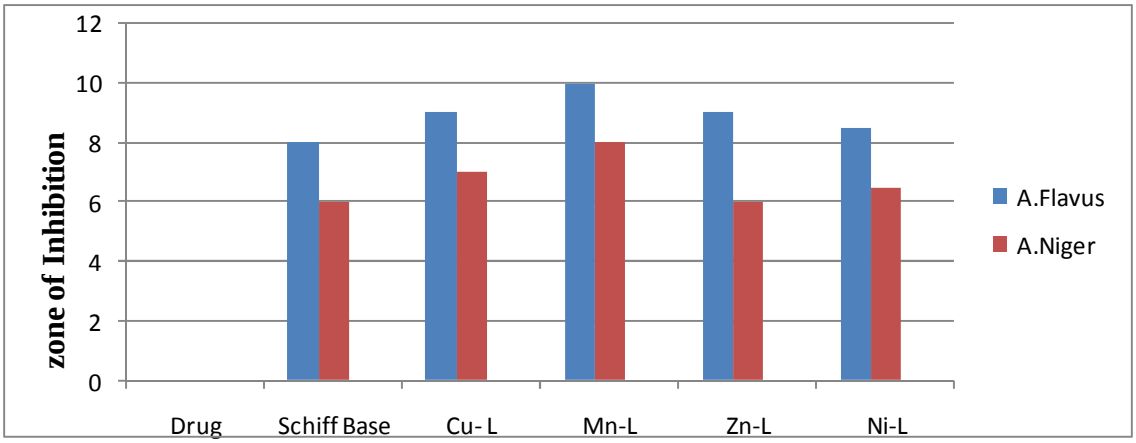


Fig. 8: Comparison of antifungal activity of ligands and their metal complexes

Series 1 contains Schiff base as a ligand and its four metal complexes. From the graph it is obvious that the metal complexes of ofloxacin showed more activity against *S. aureus*. All metal complexes except Ni complex showed enhanced activity with the Mn complex being more active. Similarly against *E.coli*, metal complexes showed somewhat comparable or enhanced activity than the parent drug except Ni complex which has just lower activity than the drug. Series 2 contains antibiotic and its metal complexes. It can be seen that the ligand showed better activity than the parent drug. The metal complexes of the ligand showed even higher activities than the ligand and the drug against both the bacterial strains. However ester intermediate of ofloxacin exhibited lower activity than the parent drug. Metal complexes of this derivative showed comparable or enhanced activity than the drug and the ligand as well. However Ni complex remained slightly lower in activity against *E.coli*. The Schiff base ligand exhibited better activities against both bacterial strains while its metal complexes showed even better activities than drug and the Schiff base ligand as well. Antibacterial activities of these derivatives are comparable with the drug; however metal complexes of these derivatives showed slightly higher activity than the drug and the ligand. The order of antibacterial activity is as follows; Metal Complexes of ligands > Ligands > Parent Drug.

It reveals that on complexation with transition metals, antibacterial activity increases (Singh & Varshney, 2006). Ofloxacin is an antibacterial drug so it does not show any activity against fungi. In case of antifungal activities, we know that some other quinolones show very low activities against any fungi. Besides very poor antifungal records of quinolones, we managed to measure the antifungal properties of our prepared complexes in order to know any effect on certain fungi. The prepared complexes were used on two fungal strains i.e *A. flavus* and *A. niger*. The prepared ligands showed just enhanced activities than the parent drug. However these activities of ligands are not better enough to be declared as good antifungal agents. Metal complexes of these ligands also exhibited very slight increase in antifungal activities.

4. Conclusion

It was found that metal complexation of the drug and its derivatives posed a positive effect on the antimicrobial efficacy of ofloxacin. The trend of antibacterial and antifungal activity was; Transition metal complexes > Ligands > Parent drug. Ofloxacin is known to be an antibacterial drug but its metal complexes also exhibited somewhat enhanced but comparatively low antifungal activities than the antibacterial potency.

The physio analytical data showed that all the complexes are formed from the reaction of the ligand and the metal salts. The 1:2 (ML2) of the Schiff base metal complexes is suggested by the elemental analysis, here “M” represents tranistion metal and “L” represents the ligand, showing that with the central metal ion two ligand are coordinated via "N" and "O" atoms, this is confirmed by the FT-IR spectral analysis. The thermal analysis shows that there is also coordination of water molecules with the metal ions. The minimum inhibition zone data suggested that the Schiff base transition metal complexes have more anti-bacterial activity than the ligand and the parental drug. So these complexes have better antibacterial yield.

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