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Electrochemical and Biochemical Investigations of Some New Coordination Compounds of Ni (II) with Azomethines Derived from Sulfa Drugs

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ABSTRACT

Some new coordination compounds of nickel (II) have been synthesized by the reaction of nickel (II) acetate with azomethines (Schiff bases) in 1:2 molar ratio using methanol as a reaction medium. Azomethines used in these studies have been prepared by the condensation of 1-acetyl-2-naphthol and 2-acetyl-1-naphthol with sulphadimidine, sulphaguinidine and sulphadiazene in ethanolic medium. An attempt has been made to probe their bonding and structures on the basis of elemental analyses, IR spectral evidences and Cyclic Voltametric studies. Ni (II) compounds have been found to be more active than their uncomplexed ligands as both of them were screened for antibacterial and antifungal studies. Anti-inflammatory activity studies showed the test compounds are comparable to the standard drug Diclofenac sodium.

Keywords: Azomethine, Nickel (II) complexes, Antibacterial and Anti-inflammatory activities, Spectral evidences.

INTRODUCTION

Active and well-designed Schiff base ligands are widely designed and prepared for its high yield and one-step procedure via condensation of amines and carbonyl compound [1]. They are very popular due to diverse chelating ability. The chemistry of Schiff bases has occupied a place of considerable importance because of their well-established biological [2,3], anticancer [4] and antimicrobial properties. The N, O and S containing ligands and their complexes have become important due to their wide biological activities [5]. Sulpha drugs are chemotherapeutic agents and possess SO_2NH moiety as an important toxophoric functional [6] Schiff bases and their transition metal complexes have attracted much attention for their importance in the field of coordination chemistry related to catalysis enzymatic reactions, magnetism and molecular architectures [7,8]. Derived sulpha drug ligands with nickel metal ions play an important role in biology, pharmacy and industry due to their coordination, catalytic and biological activities [9-14].

Therefore, we report the synthesis, characterization and biological studies of some new coordination compounds of Ni (II) with unsymmetrical Schiff-base ligands (derived from 1-acetyl-2-naphthol and 2-acetyl-1-naphthol with sulphadimidine, sulphaguinidine and sulphadiazene) The synthesized sulpha drug derived compounds (L^1H–L^6H) have been exposed to act as bidentate towards divalent metal atom solely

through the azomethine nitrogen and phenolic oxygen forming a stable six-membered chelate ring and increased activity when administered as metal chelates rather than as organic compounds. The structures of the ligand and its metal complexes are characterized and also discussed by elemental analysis, molar conductance, IR spectra, cyclic voltammograms and biological properties.

MATERIALS AND METHODS

Materials: All chemicals and solvents used for the synthesis were of reagent grade. 1-acetyl-2-naphthol, 2-acetyl-1-naphthol and sulpha drugs (Aldrich), nickel acetate tetra hydrate were obtained commercially and used as received.

Physical Techniques: All the reactions were carried out under strictly anhydrous condition. IR spectra were acquired using a model A-8400 S, Shimadzu Spectrophotometer by preparing in KBr medium. The cyclic voltametric data were recorded with a fully computer controlled Basic Electrochemistry System (model ECDA 001) in DMF solution at 300 K using a three electrode cell comprising reference Ag/AgCl auxiliary Pt and working glassy carbon electrodes. Elements were analyzed by Vario EL III Element Analyzer instrument. Nitrogen was estimated by the Kjeldal's method. Molecular weights were determined by the Rast's camphor method. Molar conductance measurements were made in anhydrous DMF at 34±1° using a Systronics Model 305 conductivity bridge. Melting points were measured by the melting point apparatus.

Synthesis of Ligands: The azomethines were synthesized by the condensation of 1-acetyl-2-naphthol and 2-acetyl-1-naphthol with sulpha drugs viz. sulphadimidine, sulphaguinidine and sulphadiazene in 1:1 molar ratio using ethanolic as a reaction medium. The solution was refluxed on a water bath from 4 to 6 h and then allowed to cool at room temperature. The dried crystalline solids were purified by washing with ethanol and recrystalized with the acetone. The physical properties and analytical data are recorded in table 1 and the structure of ligands (type A and B) proposed in the present work is given in figure 1.

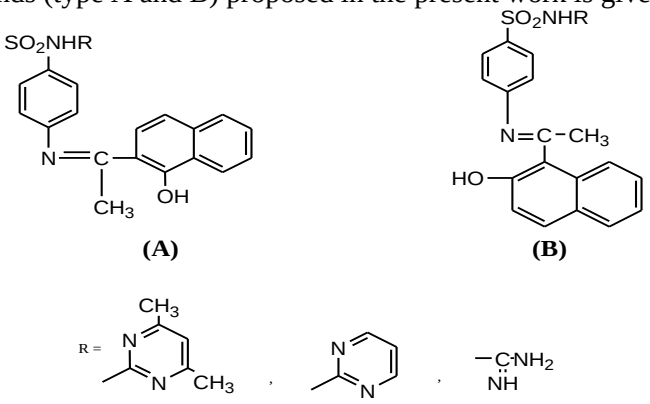

Figure 1
Structure of the Ligands

Synthesis of Complex: Azomethine of sulpha drugs was dissolved in methanol in a round bottom flask. At the same time, nickel (II) acetate was dissolved separately in dry methanol. Then, nickel (II) acetate solution was added drop wise into the flask containing the ligand solution. The contents were refluxed for about 4-5 hours. The solid compound obtained was filtered, washed repeatedly with ethanol and dried over anhydrous $CaCl_2$ in a desiccator. The purity of the compounds was checked by TLC using silica gel-G as an adsorbent. The physical properties and analysis of these complexes are listed in table 2.

Table 1. Analytical and physical data of the Ligands

Ligands	Colour & State	M.P. (^oC)	Analysis (%)				M.Wt Found (Calcd.)
			C Found (Calcd.)	H Found (Calcd.)	N Found (Calcd.)	S Found (Calcd.)	
$C_{24}H_{22}N_4O_3S$ (L^1H)	Yellowish Powder	186	64.56 (64.77)	4.97 (5.36)	12.55 (12.78)	7.18 (7.48)	446.52 (446.68)
$C_{24}H_{22}N_4O_3S$ (L^2H)	Shiny Yellow Solid	210	64.56 (64.85)	4.97 (5.07)	12.55 (12.95)	7.18 (7.45)	446.52 (446.94)
$C_{22}H_{18}N_4O_3S$ (L^3H)	White Solid	228	63.14 (63.34)	4.34 (4.52)	13.39 (13.87)	7.66 (7.89)	418.47 (418.87)
$C_{22}H_{18}N_4O_3S$ (L^4H)	Light Yellow Solid	214	63.14 (63.47)	4.34 (4.48)	13.39 (13.78)	7.66 (7.97)	418.47 (418.85)
$C_{19}H_{18}N_4O_3S$ (L^5H)	Cream Solid	238	59.67 (59.89)	4.74 (4.88)	14.65 (14.85)	8.38 (8.55)	382.44 (382.76)
$C_{19}H_{18}N_4O_3S$ (L^6H)	Light Green Solid	213	59.67 (59.96)	4.74 (4.94)	14.65 (14.70)	8.38 (8.65)	382.44 (382.89)

Table 2. Analytical and physical data of the Ni (II) complexes

Complex Molecular Formula	Molar Ratio C/L	Yield %	Colour & State	M.P (^oC)	Mol.wt. Found (Calcd.)	% Analysis Found (Calcd.)				
						Ni Found (Calcd.)	C Found (Calcd.)	H Found (Calcd.)	N Found (Calcd.)	S Found (Calcd.)
$NiC_{48}H_{46}N_8O_8S_2$	1:2	77	Light Brown Solid	218	985.75 (986.05)	5.95 (6.14)	58.48 (58.67)	4.70 (5.00)	11.37 (11.56)	6.51 (6.68)
$NiC_{48}H_{46}N_8O_8S_2$	1:2	73	Yellow Brown Solid	227	985.75 (985.99)	5.95 (6.17)	58.48 (58.59)	4.70 (4.96)	11.37 (11.49)	6.51 (6.72)
$NiC_{44}H_{38}N_8O_8S_2$	1:2	71	Cream Solid	239	929.64 (929.94)	6.31 (6.65)	56.85 (62.97)	4.12 (4.26)	12.05 (12.25)	6.90 (7.12)
$NiC_{44}H_{38}N_8O_8S_2$	1:2	76	Shiny Green Solid	245	929.64 (929.88)	6.31 (6.48)	56.85 (56.12)	4.12 (4.34)	12.05 (12.28)	6.90 (7.10)
$NiC_{38}H_{38}N_8O_8S_2$	1:2	79	Brown Solid	249	857.58 (857.76)	6.84 (6.98)	53.22 (53.53)	4.47 (4.65)	13.07 (13.32)	7.48 (7.68)
$NiC_{38}H_{38}N_8O_8S_2$	1:2	81	Reddish Brown Solid	234	857.58 (857.98)	6.84 (7.08)	53.22 (53.48)	4.47 (4.58)	13.07 (13.26)	7.48 (7.58)

Antibacterial activity: The pathogenic microbial were used to test the antimicrobial potential of the Schiff base Ni(II) complexes against bacteria viz., *Staphylococcus aureus* which was representative type of gram positive bacterial group and *Escherichia coli* which was representative type of gram negative bacterial group and evaluated by the disc diffusion method. Standard nutrient agar is used as medium for testing the activity of microorganisms as antibacterial agents and a lawn of microorganisms was prepared by pipetting and evenly spreading inoculums on to agar set in Petri dishes. The compounds under investigation were dissolved in DMSO to get final concentration of 500 and 1000ppm. The paper disc (Whatman no.1) having diameter of 5 mm were soaked in these solutions and placed in appropriate medium previously seeded with tested organism in Petri dishes. The plate incubated for 48 h at 37°C. During this period, the test solution was diffused and affected the growth of the inoculated bacteria. The zone of inhibition developed on the plate was measured.

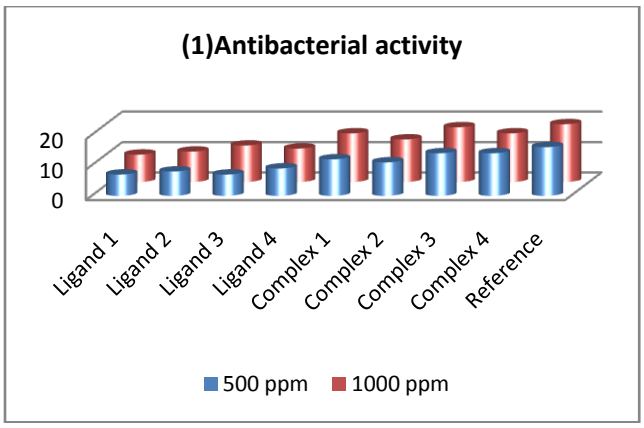

Figure 2. Ligand 1 & 2 = $C_{24}H_{22}N_4O_3S$, Ligand 3& 4 = $C_{22}H_{18}N_4O_3S$
Complex 1&2 = $NiC_{48}H_{46}N_8O_8S_2$, Complex 3&4 = $NiC_{44}H_{38}N_8O_8S_2$, Reference = Streptomycin

(1) Effect of different concentrations of ligand and Ni(II) complexes with *Escherichia coli(-)*
(2) Effect of concentration of ligand and Ni (II) complexes with *Staphylococcus aureus(+)*

Antifungal test: An antifungal activity of the ligands and their corresponding complexes was found in vitro against *Aspergillus niger* and *Rhizopus phaseoli* by the agar plate technique [15]. In this method, the medium used is potato dextrose agar medium(composition : potato slices – 200g, dextrose – 20g, Agar – Agar – 15g and distilled water 1000 mL). The solutions of the compounds in different concentration (100 and 200ppm) in DMF were then mixed with the medium. The linear growth of the fungus was recorded by measuring the diameter of colony and the percent inhibition was calculated. Micostatin was used as reference compound for antifungal activities (Table 3).

Table 3. Antifungal activity of the ligands and Ni (II) complexes

Micostatin	L^1H		$NiC_{48}H_{46}N_8O_8S_2$		L^2H		$NiC_{48}H_{46}N_8O_8S_2$	
	100ppm	200ppm	100ppm	200ppm	100ppm	200ppm	100ppm	200ppm
IZ	13	15	18	19	14	16	14	18
A. niger (Al)	(1.70)	(1.65)	(1.35)	(1.35)	(1.72)	(1.67)	(1.00)	(1.25)
IZ	12	16	23	22	15	17	17	18

R. phaseoli (Al)	(1.90)	(1.50)	(1.52)	(1.54)	(0.98)	(1.84)	(1.16)	(1.23)

IZ = Inhibition zone (diameter in ppm); AI = Activity index (inhibition zone of tested compounds) L^1H and L^2H = $C_{24}H_{22}N_4O_3S$

Anti-inflammatory activity: A novel series of Schiff bases and Ni (II) complexes were screened for their anti-inflammatory activity. The anti-inflammatory activity was determined in male Wistar albino rats-with a body weight between 175 to 225 g using carrageenan induced rat paw edema method (Winter et al., 1962; K Srinivasa1 et al., 2013) [16,17]. The animals were divided into seven groups containing six in each and labeled I-VII. Group I served as control (0.2 mL normal saline). In Group II rats were given carrageenan (0.1 mL of 1% mg kg^{-1}, bw, p.o.) and group III was treated with carrageenan (0.1 mL of 1% mg kg^{-1}, bw, p.o.) single dose plus drug Diclofenac (12.5 mg kg^{-1} bw, p.o.) The other groups IV, V, VI and VII were treated (i.p.) with the synthesized compound (200 mg kg^{-1}) respectively. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.1 ml of 1% solution of carrageenan into the plantar side of the right hand paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured by using plethysmograph immediately after injection and procedure was repeated at **1,** 2, 3 and 4 h after carrageenan injection. The mean paw volume at different time intervals was calculated and compare with control and the percentage inhibition was calculated by using following formula-

$$\text{Percent edema inhibition} = 100(1 - Vt/Vc)$$

Where, Vc = Volume of the edema in the control group, Vt = Volume of the edema in the treated group, respectively [24].

RESULTS AND DISCUSSION

The bimolar reactions that led to formation of the complexes by Ni(II) acetate with aforementioned ligands of sulpha drugs in a 1:1 molar ratio in methanol as shown below-

$$Ni(OAc)_2.4H_2O + 2LH \xrightarrow[\text{reflux}]{\text{methanol}} NiL_2 + 2AcOH + 2H_2O$$

Where LH is Schiff base of sulpha drug

The acetic acid liberated during these reactions remains soluble in the reaction mixture and the complexes could be separated by filtering through sintered funnel. The newly synthesized complexes are solid, colored with good yield and soluble in DMF and DMSO. The molecular weight determined by the Rast camphor method them reveals to be monomer and Molar conductance of 10^{-3} M solution of the complexes ranges 8-12 Ω^{-1} cm^2 mol^{-1} shows their non electrolytic behavior. This in turn suggests that the water molecules are coordinated with the metal ions and present inside the coordination sphere in all the complexes. The probable structure of complexes (type C and D) proposed in the present work is given in Figure 3.

Proposed structure of the complexes:

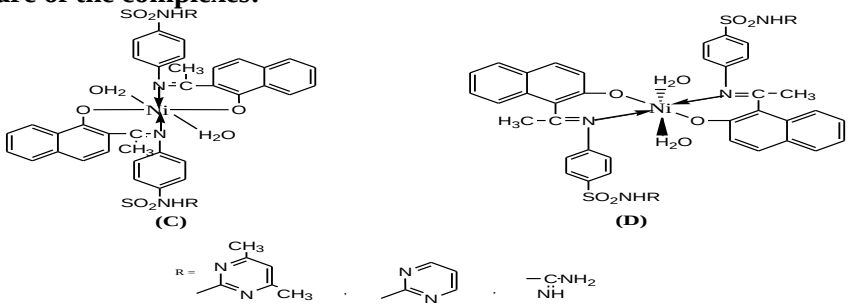

Figure 3
Structure of the Complex

Spectroscopic Characterization

IR spectra: In the azomethines, two broad bands in the region 3450-3150 cm^{-1} are observed which can be assigned to υ(NH) and υ(OH) phenolic vibrations. No significant change in υ(NH) band in the spectra of complexes indicated non-involvement of NH group in coordination, however band due to –OH group disappears in the complexes showing the coordination of metal through oxygen after deprotonation. A strong band at ~ 1215 cm^{-1} in the ligands may be due to the phenolic C-O stretching vibrations. In the resulting complexes, a shift of this band to higher frequency (1285 cm^{-1}) indicates the bonding of the ligand through the phenolic oxygen. All the ligands display a strong and sharp band in the region 1615-1626 cm^{-1} which is due to the υ(>C=N-) stretching frequency in the free ligands. It shifts to lower (~10 cm^{-1}) in the spectra of complexes. The lowering may be attributed to a decreases in the >C=N- bond order as a result of Ni←N bond formation. New bonds in the region360-365 cm^{-1} and 412-424 cm^{-1} in the nickel complexes may be attributed to υ (Ni←N) and υ(Ni-O) vibration respectively.

Cyclic Voltammetry: The Ni (II) complexes were studied by cyclic voltammetry (CV) at room temperature using a three electrode combination system. The three electrodes were connected to the electrochemical cell. The cyclic voltammogram of Ni(II) complex (1×10^{-2}M) in DMF solution in the absence of molecular oxygen at room temperature in 1.0 to –1.0 V potential range at scan rate 100-500 mVs indicating irreversible one-electron process [18]. A noteworthy feature has been observed in the cyclic voltammogram of Ni (II) complex. During the forward scan it shows one cathodic reduction peak, at -0.469 V which are attributed to reduction of Ni(II) →Ni(I) shown in fig . Cyclic voltammetric parameters are reported in table -4.

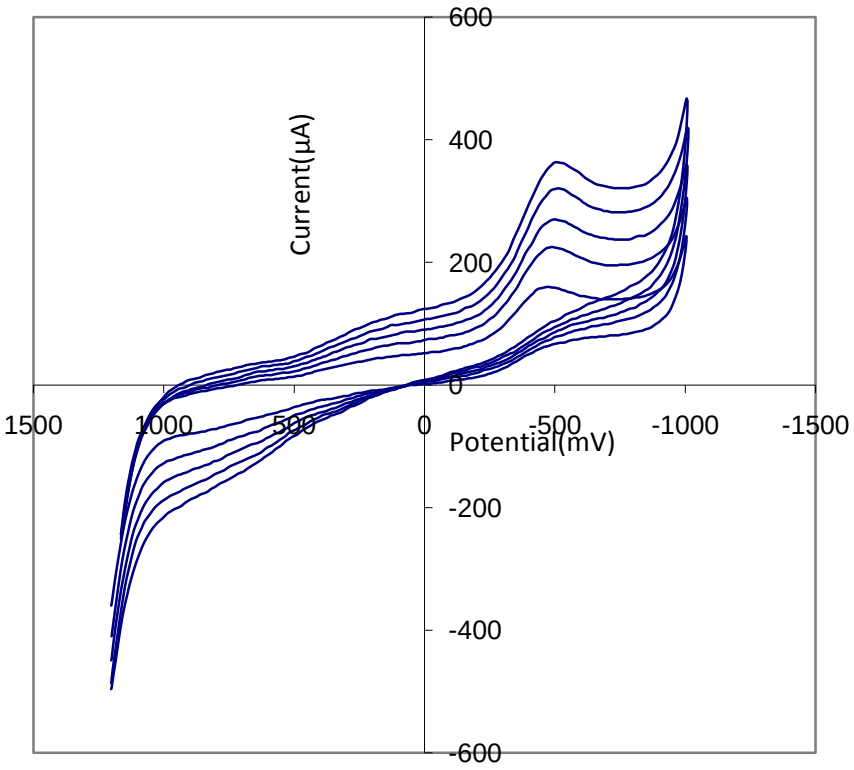

Fig 4: Cyclic Voltammograms of complex- $NiC_{48}H_{46}N_8O_8S_2$

Table 4. Cyclic Voltammetry parameters for 1 mM solution of the Ni (II) complex in DMF containing $NaClO_4$ as supporting electrolytes

Scan rate(v) mV s^{-1}	E_{pc} mV	I_{pc} µA	$E_{p/2}$ mV	$I_{pc}/v^{1/2}$
100	-426	154	-236	15.4
200	-448	216	-252	15.27
300	-458	261	-254	15.06
400	-461	306	-260	15.3
500	-469	351	-261	15.69

APPLICATIONS

Antimicrobial results*:* The results of the antimicrobial screening of the Schiff bases and their complexes against Gram negative (*Escherichia coli*) & Gram positive (*Staphylococcus aureus*) bacteria and some selected fungi (*Aspergillus niger* and *Rhizopus phaseoli)* have been found. The inhibition zones were measured in mm and results have been recorded in tables 3 and 5 (Figure 5). The experimental data clearly indicate that the metal complexes have more potent activity in inhibiting the growth of microorganisms than the ligands. The results further conclude that antimicrobial activity of the complexes increases due to metallation of its ligands [17].

Table- 5: Effect of Standard drug and tested compounds on Carrageenan induced paw edema in Rats (Volume of paw edema)

Group	Initial Reading	0 h	1 h	2 h	3 h	4 h
I	3.40 ± 0.053	3.42 ± 0.063	3.44 ± 0.072	3.41 ± 0.081	3.40 ± 0.033	3.40 ± 0.031
II	3.32 ± 0.079	3.89 ± 0.067	6.10 ± 0.043	6.82 ± 0.017	6.77 ± 0.066	5.82 ± 0.074
III	3.36 ± 0.051	3.91 ± 0.031	4.76 ± 0.081	4.78 ± 0.081	3.89 ± 0.047	3.37 ± 0.052
IV	3.37 ± 0.062	3.88 ± 0.076	5.42 ± 0.058	5.17 ± 0.093	4.76 ± 0.063	3.52 ± 0.042
V	3.35 ± 0.024	3.91 ± 0.060	5.46 ± 0.051	5.21 ± 0.067	4.68 ± 0.023	3.67 ± 0.033
VI	3.28 ± 0.076	3.85 ± 0.081	5.42 ± 0.086	5.83 ± 0.076	4.72 ± 0.083	3.65 ± 0.071
VII	3.37 ± 0.052	3.91 ± 0.031	5.58 ± 0.023	5.89 ± 0.033	4.68 ± 0.041	3.62 ± 0.042

Group
I Control
II Only carrageen treated 0.1 ml of 1%
III Carrageen + Diclofenac sodium 12.5 mg kg^{-1}
IV Carrageen + Drug extract-I 200mg kg^{-1}
V Carrageen + Drug extract-II 200mg kg^{-1}
VI Carrageen + Drug extract-III 200mg kg^{-1}
VII Carrageen + Drug extract-IV 200mg kg^{-1}

Anti-inflammatory results: The anti-inflammatory activity of the azomethine complexes were evaluated by carrageenan-induced rat paw edema method and the results are shown in table 5 and 6 and showed good anti-inflammatory activity. The percentage inhibition was 95.54%, 90.44%, 88.71% and 92.58% for nickel complexes at 200 mg kg^{-1}, respectively. However, these percentage inhibitions were significantly lower as compared to the reference drug Diclofenac (99.70%) at all dose levels. So the anti inflammatory activity may be due to the presence of the respective functional groups as evidence in literature [16].

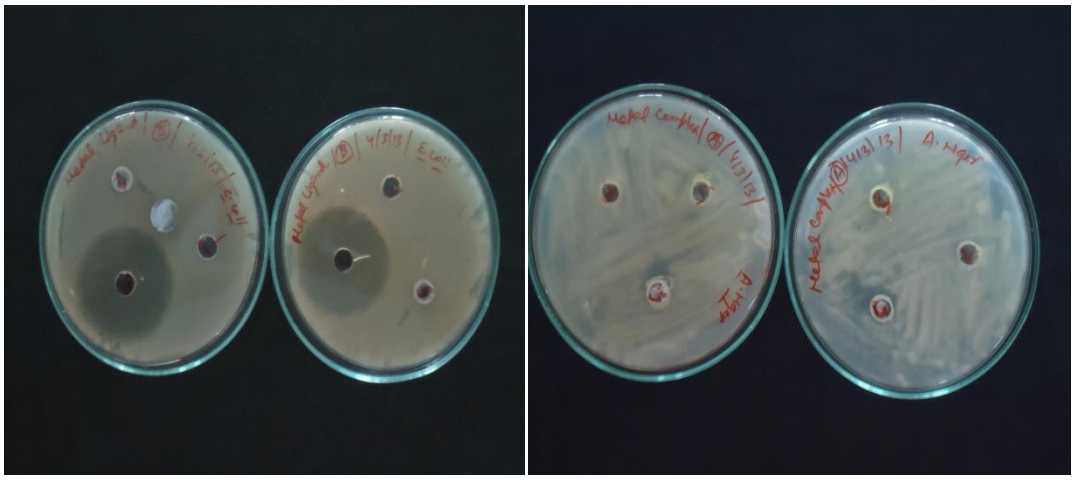

Figure 5. Antibacterial and Antifungal Activity of $NiC_{48}H_{46}N_8O_8S_2$

Table- 6: Effect of Standard drug and tested compounds on Carrageenan induced paw edema in Rats (Percentage of Inhibition)

Group	Initial Reading	2 Hrs.	Percentage of Inhibition after 2 Hrs. of treatment	4 Hrs.	Percentage of Inhibition after 4 Hrs. of treatment
I	3.40 ± 0.053	0.01	-	0.00	-
II	3.32 ± 0.079	3.5 ± 0.02	-5.14 %	2.5 ± 0.01	24.69 %
III	3.36 ± 0.051	1.4 ±0.03	58.33%	0.01 ±0.02	99.70%
IV	3.37 ± 0.062	1.8 ± 0.02	46.58%	0.15 ± 0.03	95.54%
V	3.35 ± 0.024	1.86 ± 0.06	44.47%	0.32 ± 0.07	90.44%
VI	3.28 ± 0.076	2.55 ± 0.09	22.25%	0.37 ± 0.05	88.71%
VII	3.37 ± 0.052	2.52 ± 0.02	25.22%	0.25 ± 0.02	92.58%

Group

I Control
II Only carrageen treated 0.1 ml of 1%
III Carrageen + Diclofenac sodium 12.5 mg kg^{-1}
IV Carrageen + Drug extract-I 200mg kg^{-1}
V Carrageen + Drug extract-II 200mg kg^{-1}
VI Carrageen + Drug extract-III 200mg kg^{-1}
VII Carrageen + Drug extract-IV 200mg kg^{-1}

CONCLUSIONS

In our present studies we have synthesized biologically active azomethines of sulpha drug ligands and their complexes. On the basis of above evidences octahedral geometry may be concluded for resulting complexes. The antibacterial and antifungal activity against (*Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Rhizopus phaseoli)* tested microorganisms of the studied complexes (fig.2&4) revealed that the activity increases upon complexation as compared to its ligand. The compounds showed toxicity against all species of fungi and inhibition zone growth of fungi depends on the concentration of the compounds and our results confirm that compounds exhibit a marked anti-inflammatory property at all dose levels.

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