

SYNTHESIS, CHARACTERIZATION, STRUCTURAL, THERMAL, POM STUDIES, ANTIMICROBIAL AND DNA CLEAVAGE ACTIVITY OF A NEW SCHIFF BASE-AZO LIGAND AND ITS COMPLEXATION WITH SELECTED METAL IONS

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ABSTRACT : Novel Co(II), Cu(II), Ni(II), Cd(II), Zn(II) complexes of Schiff Base- Azo Ligand namely [1,1'-(((1,1')-((3,3')-(ethane-1,2-diylbis(azaneylylidene))bis(1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl-3-ylidene))bis(diazene-2,1-diyl))bis(4-hydroxy-3,1-phenylene))bis(ethan-1-one)] are prepared from 4-amino antipyrine, 1-(4-hydroxyphenyl)ethan-1-one, and ethylene-1,2-diamine. The elemental composition and structural environment of the prepared metal complexes are confirmed by ¹H, ¹³C NMR, FT/IR, and CHN elemental analyzer. The ϵ_{\max} and molecular mass is measured with the support of mass and UV-visible spectrometer. All the compounds were screened for their antibacterial and antifungal activity by MIC method. Further, DNA cleavage activity by Agarose Gel Electrophoresis (AGE) method and antioxidant activity was performed by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The molar conductivity and magnetic susceptibility measurements signaled that the prepared complexes are designated with an octahedral arrangement. The DNA cleavage activity of the compounds on Calf-thymus DNA (Cat. No-105850) molecule showed moderate activity. Furthermore, the antimicrobial activity results revealed that the metal complexes were found to be more active than the free ligand. The newly synthesized ligand acts as N₄O₂ donor hexadentate chelate and coordinated through two atoms of azo nitrogen functions, two azomethine nitrogen and two oxygen atoms to the Cu, Co, Cd, Ni and Zn metal ions and form octahedral geometrical arrangements. All the complexes were found to be non-electrolytic in nature. Further, all the newly prepared compounds showed moderate biological activity.

Key words : Schiff Base-Azo Ligand, DNA cleavage activity, selected metal ions, thermal studies.

INTRODUCTION

One of the important and appealing sections of coordination chemistry is the chemistry of the transition metal complexes with azo ligands (Poole *et al*, 2000). Azo dyes are widely used with a tremendous number of industrial applications in the textile industry (Nejati *et al*, 2007), pharmaceuticals (Khanmohammadi *et al*, 2012), indicators (Gup *et al*, 2007) and synthetic organic dyes (Katz *et al*, 1987) are the largest and most versatile azo group that is characterized by a lone pair of orbital containing two electrons in the nitrogen atom (Abe *et al*, 1999), if linked to an aromatic ring carrying additional donor sites that is well suitable for chelation and its complexes containing both azo and azomethine groups (Maho *et al*, 2003). The azo group possesses excellent donor properties and is important in coordination chemistry (Rangnekar *et al*, 1999). Azo dyes derived from heterocyclic amines containing nitrogen in the aromatic rings and their metal complexes have been receiving the

attention of research groups due to their biological activities as antibacterial (Kondil *et al*, 1998), antitumor (Kurtođlu *et al*, 1999), antifungal (Peker *et al*, 2004). Also, Schiff bases and their metal complexes have gained interest owing to their structural diversity and a wide range of potential biological activities such as anticancer (Souza *et al*, 1985), antitumor (Qiao *et al*, 2011), antibacterial (Etaiw *et al*, 2011), antifungal (Panchal *et al*, 2006), anti-tubercular (El-Tabl *et al*, 2004), antioxidant (Nath *et al*, 2011), anti-inflammatory (Kosar *et al*, 2011) and corrosion inhibition (Robert *et al*, 2010). These observations encouraged me to synthesize a new azodye ligand from 4-aminoantipyrine and 1-(4-hydroxyphenyl)ethan-1-one, then synthesize Schiff base from (E)-4-((5-acetyl-2-hydroxyphenyl)diazonyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one and ethylene diamine their Co(II), Ni(II), Cu(II), Cd(II), Zn(II) complexes for evaluation of their biological activities and DNA cleavage activity.

Experimental Materials and Apparatus

All chemicals of highest purity were used in this work which supplied by Fluka and BDH. Spectrophotometric measurements were made with Shimadzu UV-Visible 1650 PC double beam. The FTIR measurements were made in Shimadzu 8400 Series Japan. ¹HNMR spectra were measured on BRUKER AV 400 Avance-III 400MHz instrument. ¹³CNMR spectra were measured on BRUKER AV 100 Avance-III 100MHz instrument. CE440 CHN Elemental Analyser was made by EAI. Differential Scanning Calorimeter DSC model STA PT-1000 Linseis. Electric molar conductivity measurements were made at room temperature using an Alpha digital conductivity model-UK 9300.

Synthesis of azo

The azo ligand under investigation was prepared by mixing (0.229g, 0.1 mmol) of 4-aminoantypyrine (0.203 g, 1mmol) in (10 ml) ethanol and (10 ml) distilled water. NaNO₂ (0.069 g, 1mmol) in an aqueous solution (8 ml) in drops added while preserving to solution the temperature between 0-5°C. Then by addition that the diazonium chloride respectively to (0.11 g, 01 mmol) 1-(4-hydroxyphenyl)ethan-1-one in cold ethanolic solution with constant stirring. After that added to the dark brown colored mixture, solution of 1M (1gm, 10 ml). The mixture was stirred at 0°C for 2h and acidified with 1 mL of conc. HCl in (pH = 2-4). The orange product was created after isolated by filtration washed several times with distilled water till the filtrate turns free from chloride ion by testing it with a few drops of silver nitrate solution, and recrystallized from aqua ethanol solution and dried.

Synthesis of [H₂AZDP] ligand

(15 ml) an ethanolic solution of azo ligand (0.3 g, 5 mmol) was added to a mixture including (0.3 g, 5 mmol) ethylene-1,2-diamine in (15 ml) an ethanolic solution and 3 drops of glacial acetic acid. The solution was heated in a water bath for 5 h at (70°C) in existence of K₂CO₃

after the addition of excess of Ethanol (50 ml). A yellow solid was formed, and then recrystallized from methanol. The product was dried over anhydrous CaCl₂ in vacuum. Yield is 71%, mp 187°C as shown in Scheme 2.

Metal complexes

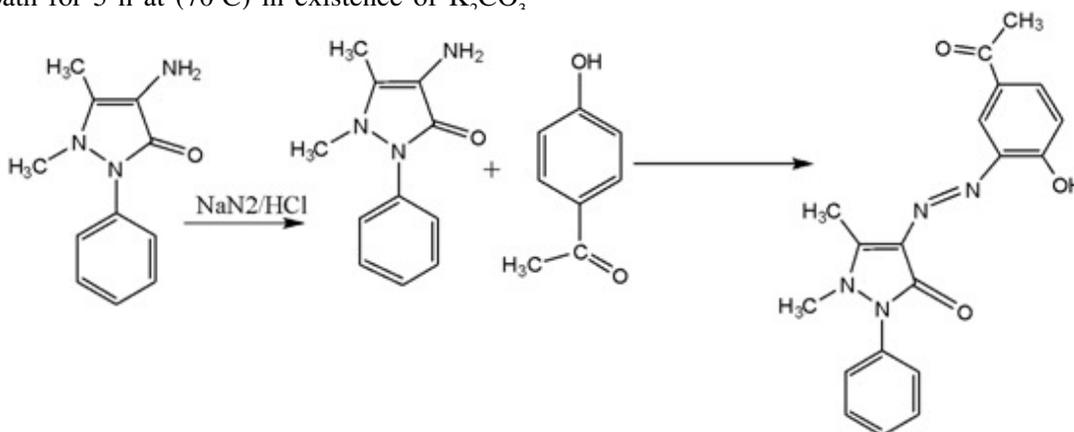
Following the effective synthesis of the ligand [H₂AZDP], attention was directed towards the chemical behaviour of the ligand [H₂AZDP] towards transition metal ions. The metal ions selected for this purpose were Co(II), Cu(II), Ni(II), Cd(II) and Zn(II). When a mixture of (0.01mole; 7.24g) of [H₂AZDP] ligand in (20ml) absolute ethanol was reacted with (0.01 mole) of the metal salts, to which 4 drops of KOH solution were added in (20ml) absolute ethanol to adjust the pH>9 for 2 hours a change in colour was observed by refluxing and the complex compounds precipitated.

The products were purified by filtering and washing several times with ethanol, and gave elemental analyses compatible with the suggested formulae given in Table 1. On the basis of elemental analysis data Table 1.

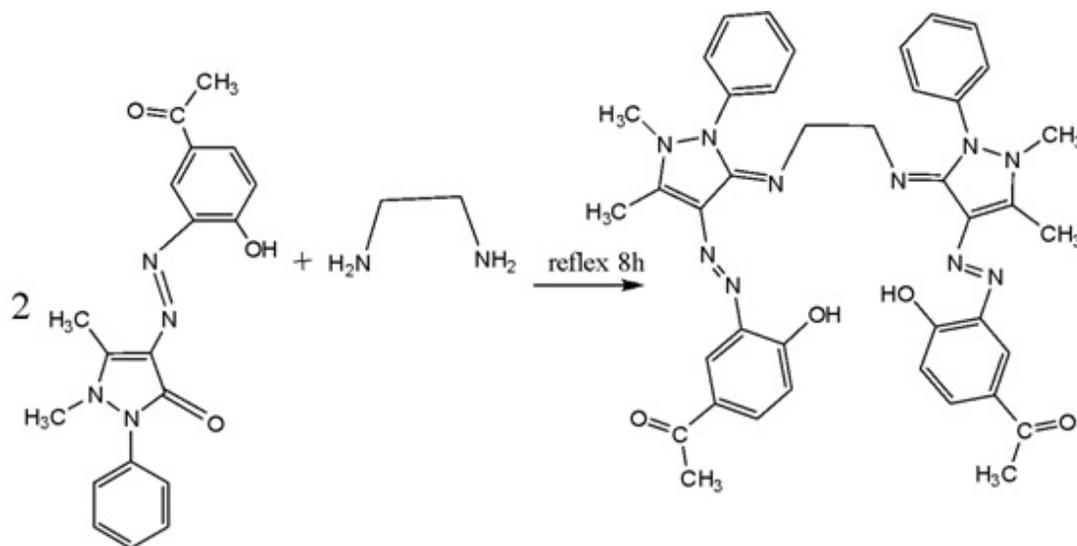
All the complexes have the general composition [M(AZDP)].nH₂O in which M = Co(II) and Cu(II), n=2; Ni(II), n=3; Cd(II), n=1; Zn(II),n=zero ions [H₂AZDP] is Schiff Base- Azoligand.

In vitro antibacterial activity testing using agar well diffusion assay NCCLS

Loop full evolutions from bacterial isolates were injected into alimentary broth incubated at 37°C for 18 hours. The bacterial suspensions were diluted normal saline. Detect themuddy and compare with standard tube (McFarland number 0.5) to yield a uniform suspension including 1.5 × 10⁸ CFU/ ml. A sterilized cotton swab was dipped and streak into adjustment suspension the entire Mueller Hinton agar (for all tested bacteria) surface of plates and the plates were left for one (5-15) minutes at room temperature to dry. Media were cut into six wells



Scheme 1 : Synthesis of the Azo ligand.



Scheme 2 : The structural of the $[H_2AZDP]$ ligand.

Table 1 : Some physical properties of prepared ligand (H_2AZDP) and its complexes.

Compounds	Formula	Molecular Weight	Colour	Yield %	M.P.	%Elemental Analysis Found % (Calculated)			
						C	H	N	M
H_2AZDP	$C_{40}H_{40}N_{10}O_4$	724.83	Yellow	71	187	66.11 (66.28)	5.24 (5.56)	19.23 (19.32)	-
$[Co(AZDP)]2H_2O$	$C_{40}H_{42}CoN_{10}O_6$	817.77	blue	69	240	57.87 (58.26)	5.03 (5.18)	16.58 (17.13)	7.11 (7.21)
$[Ni(AZDP)]3H_2O$	$C_{40}H_{44}Ni_{10}O_7$	835.55	Green	62	221	57.08 (57.50)	5.07 (5.31)	16.12 (16.67)	6.87 (7.02)
$[Cu(AZDP)]2H_2O$	$C_{40}H_{42}CuN_{10}O_6$	821.26	Pale-brown	73	235	57.89 (58.42)	4.89 (5.15)	16.87 (17.03)	7.26 (7.73)
$[Cd(AZDP)]H_2O$	$C_{40}H_{38}CdN_{10}O_4$	854.22	Pale-brown	68	264	56.02 (56.31)	4.88 (4.73)	16.13 (16.42)	12.87 (13.17)
$[Zn(AZDP)]$	$C_{40}H_{38}ZnN_{10}O_4$	786.19	Brown	65	239	66.32 (66.95)	4.17 (4.86)	17.24 (17.77)	7.96 (8.29)

Table 2 : 1H -NMR Chemical shifts for ligand (H_2AZDP) (ppm in DMSO).

$CH_3-C=$	CH_3-CO	CH_2	CH_3-N	$C-OH$	$CH-OH$	$CH_{(aromatic)}$
2.04	2.44	3.08	3.31	6.12	6.54	7.07-8.83

(5mm diameter) by cork borer and add (20 μ) of the test agent dilutions (The plates were performed in triplicates). All plates of the cultured plates were then allowed to incubate at 37°C for overnight. After (24 h) of incubation, the diameters of inhibition zones for all tested agent dilutions for each tested bacteria were measured by using a measuring scale in millimeter.

RESULTS AND DISCUSSION

The ligand $[H_2AZDP]$ is formed via the condensation of the azo dye Schiff base under study with p-phenylene diamine. It is characterized based on elemental analyses (Table 1). The results obtained are in good agreement with those calculated for the suggested formula (Scheme 2).

The 1H NMR spectrum of the $[H_2AZDP]$ ligand was recorded utilizing in solvent (d_6 -DMSO) (Souza *et al*, 1985) (Table 2, Fig. 2). This spectrum evidenced its composition by the presence of CH_3-N protons signals

at $\delta = 2.04$ ppm. Also, the 1H NMR of the ligand exhibits signals at δ (ppm) = 2.42 (s, 6H, $2CH_3, CH_3-C$), 3.01 (s, 6H, $2CH_3, CH_3-CO$), 3.31 (s, 4H, $2CH_2$), 7.34–8.83 (m, 18H, 16Ar H), 6.12 (s, 2H, $2CH, 2CH-OH$), and 6.54 (brs, 2H, 2OH).

The ^{13}C NMR spectral data of the $[H_2AZDP]$ ligand, is based on ^{13}C shifts in similar Schiff base ligand (Qiao *et al*, 2011). The chemical shifts for carbons of azo (=N-C and azothine (N=C) groups) was observed between 95.56 and 152.21 ppm. The signal observed at 162.24 ppm is assigned to (C=O) phenolic carbon. Also, the spectrum showed peaks at 118–135 ppm corresponding to carbons of the phenyl ring (Etaiw *et al*, 2011). And another data for the $[H_2AZDP]$ ligand given in the Table 3, Fig. 3.

The electron impact mass spectrum of the $[H_2AZDP]$ ligand, confirms the proposed formula by showing a peak at 724 u corresponding to the ligand moiety [$(C_{40}H_{40}N_{10}O_4)$] atomic mass [724.83 u]. The series of peaks in the range, *i.e.* 45, 55, 76, 77, 79, 91, 107, 111, 135, 163, 168, 186, 273, 334, 349, 375 and 724 u, attributable to different fragments of the ligand. These values the condensation of amino group with keto group

Table 3 : ^{13}C -NMR Chemical shifts for ligand (H_2AZDP) (ppm in DMSO).

$\text{CH}_3\text{-C=}$	$\text{CH}_3\text{-CO}$	$\text{CH}_3\text{-N=}$	$\text{CH}_2\text{-N}$	C-N=	C-N-	CH_2 (aromatic)	C=N	=C-CH_3	C-OH
7.9	26.11	35.76	60.43	95.56	101.22	118.11-135.42	152.21	153.76	162.24

Table 4 : Electronic spectral data of prepared compounds.

Compound	$\Lambda_{\text{mohm.cm}^2 \text{ mole}^{-1}}$	μ_{eff}	λ_{nm}	ν' wave number cm^{-1}	Assignments	B complex	β	$10\text{Dq} (\nu_1)$ theoretical cm^{-1}
H_2AZDP	-	-	247	40485	$\pi \rightarrow \pi^*$	-	-	-
			332	30120	$n \rightarrow \pi^*$			
$[\text{Co}(\text{AZDP})]2\text{H}_2\text{O}$	17	1.25	340	29411	C.T	878.7	0.81	8432
			439	22774	C.T			
			498	20080	${}^4\text{T}_{1\text{g}(\text{F})} \rightarrow {}^4\text{T}_{1\text{g}(\text{P})} (\nu_3)$			
			645	15503	${}^4\text{T}_{1\text{g}(\text{F})} \rightarrow {}^4\text{A}_{2\text{g}(\text{F})} (\nu_2)$			
$[\text{Ni}(\text{AZDP})].3\text{H}_2\text{O}$	10	1.54	346	28901	C.T	651.6	0.71	11456
			421	23752	${}^3\text{A}_{2\text{g}(\text{F})} \rightarrow {}^3\text{T}_{1\text{g}(\text{F})} (\nu_3)$			
			691	15105	${}^3\text{A}_{2\text{g}(\text{F})} \rightarrow {}^3\text{E}_{1\text{g}(\text{F})} (\nu_2)$			
			789	12674	${}^3\text{A}_{2\text{g}(\text{F})} \rightarrow {}^3\text{T}_{2\text{g}(\text{F})} (\nu_1)$			
$\text{Cu}(\text{AZDP})]2\text{H}_2\text{O}$	15	1.76	368	36900	C.T	-	-	-
			434	23041	C.T			
			534	14577	$\text{Eg}_{(\text{F})} \rightarrow \text{T}2\text{g}_{(\text{F})}$			
$\text{Cd}(\text{AZDP}).\text{H}_2\text{O}$	12	-	346	37174	C.T	-	-	-
			450	29761	C.T			
$[\text{Zn}(\text{AZDP})]$	13	-	342	38461	C.T	-	-	-
			467	28409	C.T			

Table 5 : The main frequencies of the ligand [H_2AZDP] and its complexes.

Compounds	$\nu(\text{OH})$	$\nu(\text{C=N})$	$\nu(\text{N=N})$	$\nu(\text{C-O})$	$\nu(\text{M-O})$ $\nu(\text{M-N})$
H_2AZDP	3432	1633	1487	1262	-
$[\text{Co}(\text{AZDP}).]2\text{H}_2\text{O}$	-	1607s.	1451 s.	1316 sh.	565 w.458m.
$[\text{Ni}(\text{AZDP}).]3\text{H}_2\text{O}$	-	1616 s.	1459 s.	1354 s.	567 w.418m.
$[\text{Cu}(\text{AZDP}).]2\text{H}_2\text{O}$	-	1609 s.	1457 sh.	1340s.	497w. 441m.
$[\text{Cd}(\text{AZDP}).]\text{H}_2\text{O}$	-	1611sh.	1454 sh.	1326 sh.	512w. 476m.
$[\text{Zn}(\text{AZDP})]$	-	1610 s.	1460 sh.	1331s.	555 w.461m.

s= strong, br=broad, w = weak, sh = sharp, m = medium

(Panchal *et al*, 2006). The molecular ion peak (724 u) is in good agreement with the suggested molecular formula indicated from elemental analyses. The mass spectrum of the ligand [H_2AZDP] shows the fragmentation pattern in Fig. 4.

The UV-Vis spectra were recorded in ethanol solution with a concentration (1.0×10^{-5} M) for the Schiff base and its complexes in the wavelength range from 200 to 1000 nm (Table 4). The Schiff base-azo [H_2AZDP] showed a higher energy peak at 247 nm and showed a peak at 332 nm. These nm, the ligand [H_2AZDP] bands assigned due to $\delta \rightarrow \delta^*$ and $n \rightarrow \delta^*$ transitions encompassing molecular orbitals particularly centered on

the azomethine chromophore and benzene ring (El-Tabl *et al*, 2004). The spectra of the complexes, exhibited that the azomethine chromophore $n \rightarrow \delta^*$ transitions shifted to 340 nm for $[\text{Co}(\text{AZDP})]2\text{H}_2\text{O}$, 346 nm for $[\text{Ni}(\text{AZDP})]3\text{H}_2\text{O}$, 368 nm for $[\text{Cu}(\text{AZDP})]2\text{H}_2\text{O}$, 346 nm for $[\text{Cd}(\text{AZDP})]\text{H}_2\text{O}$ and 342 nm for $[\text{Zn}(\text{AZDP})]$ complexes that splitted to peak at indicating that the imino nitrogen is involved in coordination to the metal ion (Nath *et al*, 2011). All the complexes exhibited a broad band with maximum in range (434-467) nm due to metal to ligand charge transfer. The $\text{Cu}(\text{AZDP})]2\text{H}_2\text{O}$ complex shows a band at 434 nm with a shoulder at 534 nm could be assigned due to MLCT transition's (Cu (II)-phenolate

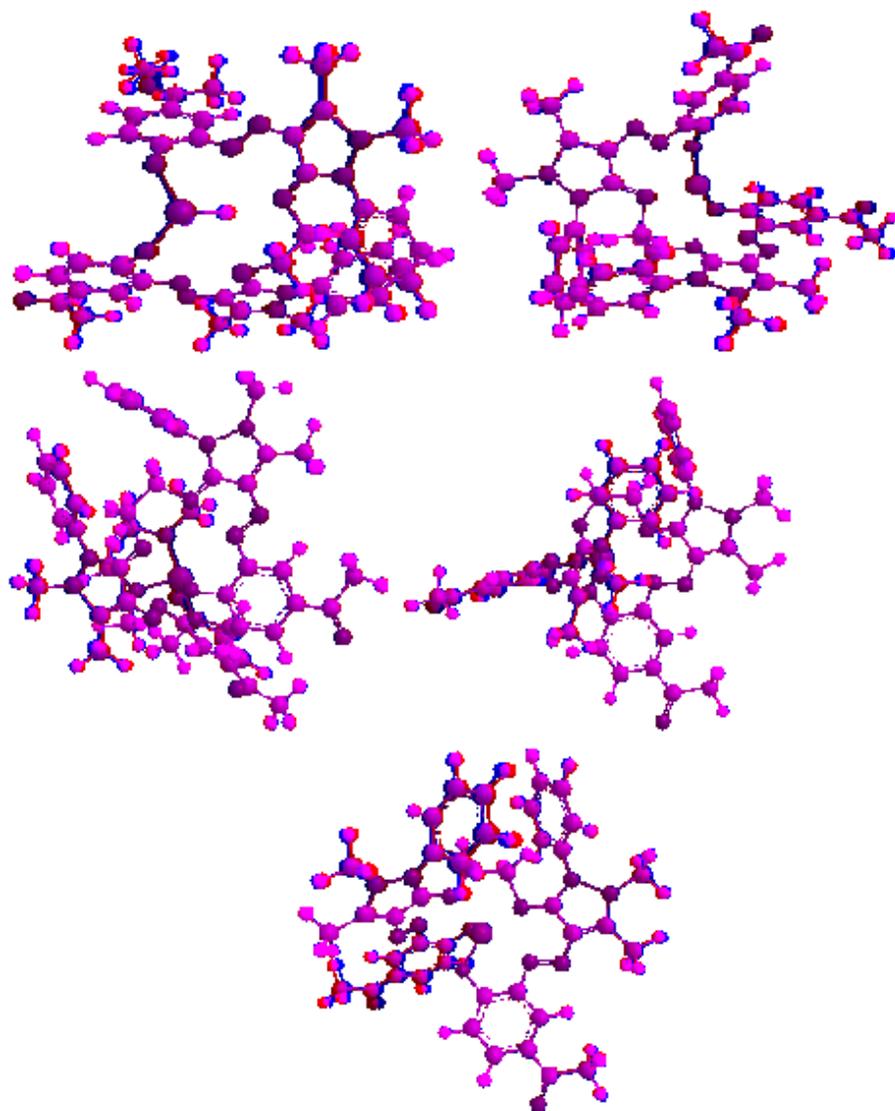


Fig. 1 : Structures of Co(II), Cu(II), Ni(II), Cd(II), Zn(II) complexes.

Table 6 : Antibacterial activity of AZO compounds on Bacterial isolates -Inhibition Zone in (mm) at concentration of (1×10^{-5}).

Bacteria	H2AZDP	Co complex	Ni complex	Cu complex	Cd complex	Zn complex
<i>S. aureus</i>	12	16	22	19	25	27
<i>Streptococcus spp.</i>	15	15	17	19	20	20
<i>E. coli</i>	6	18	22	27	24	30
<i>K. pneumonia</i>	9	9	11	17	17	19
<i>Proteus spp.</i>	12	13	14	17	20	20
<i>S. typhi</i>	15	18	20	20	21	26
<i>Acinetobacter spp.</i>	7	8	8	11	11	12
<i>V. cholera</i>	20	20	25	25	25	26

(δ^*) transition; CT band from the filled d ($3d_{xz}$, $3d_{yz}$) orbitals of copper (II) to the antibonding orbitals of the phenolic residue) $E_{g(F)} \rightarrow {}^2T_{2g(F)}$ and d- δ^* transitions, respectively. The UV-Vis spectrum of the $[Co(AZDP)]2H_2O$ complex exhibits three d-d bands observed at 421, 498 and 645 nm assignable to ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}(\delta_3)$ and ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}(\delta_2)$ transitions,

which are characteristic of an octahedral structure. The spectrum of $[Ni(AZDP)].3H_2O$ complex showed absorption bands at 691 and 789 nm which, could be attributed to the ${}^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}(\delta_3)$, ${}^3A_{2g(F)} \rightarrow {}^1E_{1g(F)}(\delta_2)$ and ${}^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}(\delta_1)$ transitions corresponding to the octahedral geometry (Kosar *et al*, 2011). In the Cd(II) and Zn (II) complex, an octahedral structure was proposed

and the diamagnetic behavior were confirmed. The fourth band is a CT band and the electronic parameters such as Dq , B , \hat{a} , δ_2/δ_1 are calculated by using following equations given in the Table 3.

The Racah parameter (B) of the Ni(II) and Co(II) complexes are set up to be less than the free ion data and the parameter (\hat{a}) for both the complexes are less than one. All these surveillances indicate which, nature of bonds are covalent in the metal ligand complexes.

The complexes of Co(II), Cu(II) and Ni(II) are paramagnetic while Cd(II) and Zn(II) are diamagnetic. The magnetic susceptibility data of Co(II), Cu(II) and Ni(II) complexes are 1.25, 1.54 and 1.76 BM for ligand $[H_2AZDP]$, respectively, that was regular with existence of a single unpaired electron (Robert *et al*, 2010).

Molar conductivities of metal complexes

Molar conductance ($\ddot{E}M$) measurements of the complexes (Table 1), carried out using DMSO as the solvent at the concentration of 10M, indicate non-electrolyte behaviour of the complexes and conductivity values were found in the range $10\text{--}17 \text{ } \ddot{U}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ (Nath *et al*, 2011), (Table 1) (Chaur *et al*, 2011). Thus the complexes may be formulated as $[M(AZDP)]_n \cdot nH_2O$ in which $M = \text{Co(II); Cu(II), } n = 2; \text{Ni(II), } n = 3; \text{Cd(II), } n = 1; \text{Zn(II), } n = \text{zero ions}$. Further insight concerning the structure of the ligand is obtained from IR, UV-vis. The IR and UV-vis measurements, of $[H_2AZDP]$ ligand will be discussed with its metal complexes.

IR spectra and mode of bonding

The infrared spectra assignment of the proposed structures of the Schiff base of azo-dye complexes was made through consideration of their infrared spectra. The coordinated stretching vibration bands of the isolated products were suggested by using a comparison technique of infrared spectra comparing the spectra of the free ligand and its metal complexes (Refat *et al*, 2006). The IR spectra of the free ligand and metal complexes were completed in the range $4000\text{--}400 \text{ cm}^{-1}$ (Table 5). The IR spectra of the complexes appear as a sharp band in the range $1607\text{--}1616 \text{ cm}^{-1}$, referred to $\dot{i}(C=N)$, that is moved to higher frequency on going from the free ligand at 1633 cm^{-1} to the complexes. This is indicative of the coordination of the imine nitrogen to the metal (Tuđba Eren *et al*, 2014). In the far IR spectra of all the complexes, the non ligand bands observed at $476\text{--}418 \text{ cm}^{-1}$ region assigned to $\dot{i}(M-N)$ stretch. The disappearance of the free ligand $\dot{i}(OH)$ band around 3432 cm^{-1} in the spectra of all complexes indicating deprotonation of organic ligand prior to coordination (Rezaeian *et al*, 2014). On the other hand, the $\dot{i}(C-O)$,

which occurs at 1262 cm^{-1} for the ligand, was moved to higher frequencies, $1345\text{--}1316 \text{ cm}^{-1}$ after complexation, this shift confirms the participation of phenolic oxygen of the ligand in $C-O-M$ bond formation. Conclusive evidence regarding the bonding of oxygen to the metal ions is provided by the occurrence of bands at $567\text{--}521 \text{ cm}^{-1}$ region due to $\dot{i}(M-O)$. In the free ligand, the sharp band spotted at 1487 cm^{-1} is a result from $\dot{i}(N=N)$ stretching frequency of azo dye that is moved to a range of $1460\text{--}1451 \text{ cm}^{-1}$ in metal complexes that suggests bonding of azo nitrogen with the metal ions (Sanmartín *et al*, 2000). The spectra of complexes reveal the presence of band in range $3354\text{--}3391 \text{ cm}^{-1}$ result to the vibration frequency of O-H of lattices coordinated carry water. The thermal values asserts the nature of these water molecule (Jaworska *et al*, 1994).

Thermal analysis

TG/HDSC analysis is a very helpful process for assaying the thermal decomposition of solid essences encompassing straightforward metal salts in addition to complex compounds. The thermo gram directs the minimized in example weight with the linear rise in heat remediation temperature 10°C up to $400^\circ\text{C min}^{-1}$. The decomposition happens at lower three main discoverable steps, each step does not refer generally to a single process, but rather it reflects on two or three overlapping processes. The target of the thermal analysis is to get acquaintance with reference to the thermal constancy, of the investigated complexes as shown in Fig. 5, to resolve whether water molecules are outside or inside the coordination sphere (Kakanejadifard *et al*, 2009). The thermal conductance of metal completely shows which, the hydrated complexes forfeit molecules of hydration first: followed by decomposition of ligand molecules inches subsequent Steps.

For Co(II) complex ($C_{40}H_{42}CoN_{10}O_6$), the values received backup the suggested frameworks and indicate that Co(II) complex underwent three step degradation reaction. The first step shows weight between $50\text{--}140^\circ\text{C}$ and occurs at maximum peak lying in 131.2°C , corresponding to the loss of 6.6% the weight loss associated with this step agrees quite well with the loss of three lattice water molecules. The second step shows weight between $150\text{--}210^\circ\text{C}$ occurs at $T_{\text{max}} 195.1^\circ\text{C}$, corresponding to the loss of 55.18% and it referred to loss the $[C_{11}H_{13}N_3]$ moiety in 4-aminoantipyrine and ethylene diamine. The third decomposition step occurs at $T_{\text{max}} (429.7^\circ\text{C})$ corresponding to the loss of 44.81% audits suggests the formation of the $[C_8H_9NO]$ moiety in 1-(4-hydroxyphenyl)ethan-1-one and an ultimate pyrolysis product metal oxide. It's reflective of two or

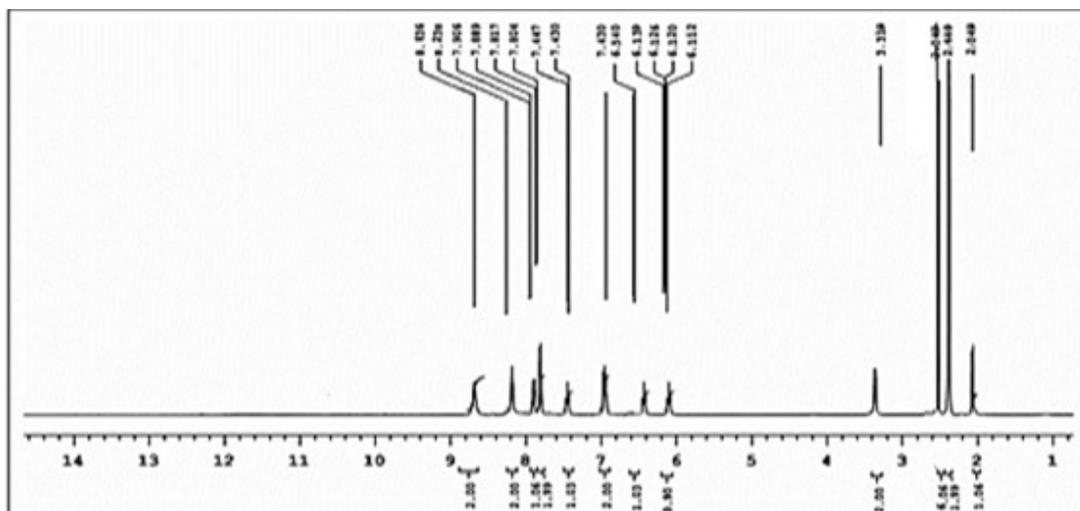


Fig. 2 : ^1H -NMR Chemical shifts for ligand (H_2AZDP).

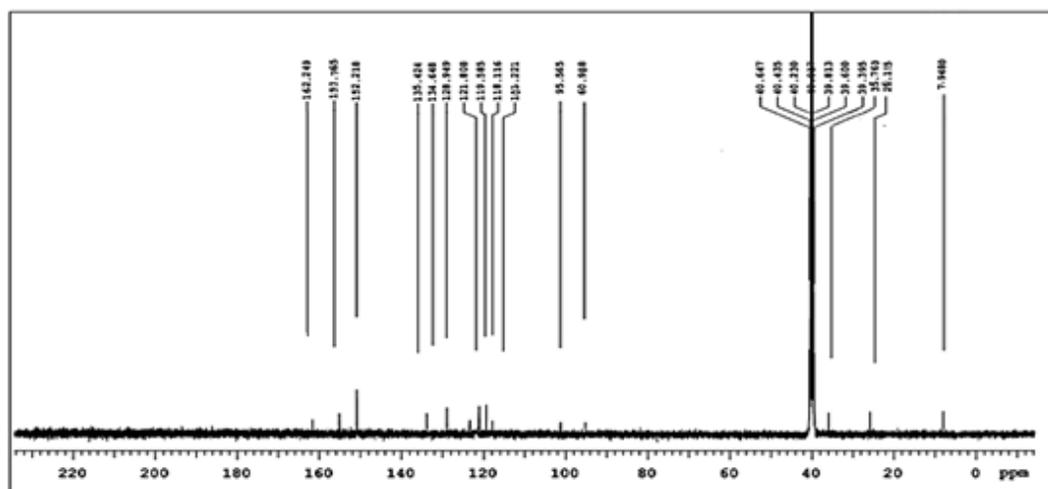


Fig. 3 : ^{13}C -NMR Chemical shifts for ligand (H_2AZDP).

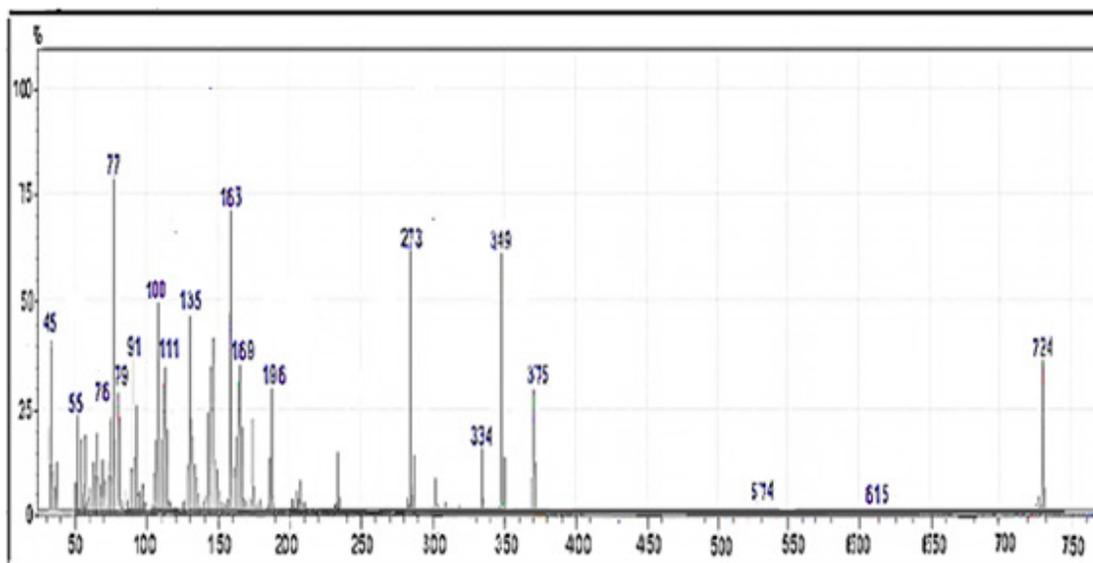


Fig. 4 : Mass for ligand (H_2AZDP).

three overlapping processes and attributed to loss of the 4-aminoantipyrine and another moieties (Kakanejadifard *et al*, 2008).

Ni(II) complex ($C_{40}H_{44}N_{10}NiO_7$), the TG of this complex reveals a mass loss between 25-110°C occurs at the maximum temperature of 82.5°C corresponding to the loss of two lattice water molecules and with a mass loss of 2.66%. The next and last decomposition step occurs in the temperature of 275.3°C and 357.7°C with a mass loss of 25.33% and 32%, respectively referred to a single process, but it's reflective of two or three overlapping processes and attributed to the loss of the 4-aminoantipyrine and ethylene diamine moieties, product metal oxide and another remind moieties (Shakir *et al*, 2007).

For Cd complex ($C_{40}H_{38}CdN_{10}O_4$), a mass loss between 15-120°C occurred and within the temperature 91.6°C corresponding to the loss of 1% for one molecule of lattice water and terminal methyl groups in 4-aminoantipyrine moiety. The temperature 179.7°C at a loss of 5.33%, referred to a single process, but it's reflective of two or three overlapping processes and attributed to the loss of referred to a single process, but it's reflective of two or three overlapping processes corresponding to a loss of one molecule [N_2CH_4] and remaining organic moieties at higher temperature 286.1°C (Ilhan *et al*, 2007). The residual is in agreement with cadmium oxide at the end of the thermogram at higher temperature 366.2°C.

For Cu complex ($C_{40}H_{42}CuN_{10}O_6$), the data obtained support the proposed structure and indicate that Cu(II) complex underwent three step degradation reaction. The first step occurs at maximum peak lying in 85.6°C, corresponding to the loss of 4.3% the weight loss associated with this step agrees quite well with the loss two lattice water molecules and terminal methyl groups in 4-aminoantipyrine moiety. The second step occurs at T_{max} 150.7°C, corresponding to a mass loss of 3% for two OCH_3 in 1-(4-hydroxyphenyl)ethan-1-on. The third decomposition step occurs at T_{max} 354.6°C corresponding to the loss of 25% to a loss of N_2CH_4 molecule the remaining organic moiety (Rezvani *et al*, 2006). The residual is in agreement with Cu oxide.

Zinc complex ($C_{40}H_{38}ZnN_{10}O_4$), this complex is anhydrous component that decompose in two steps and is thermally stable up to 178°C the first step occurs in the temperature of 150.8°C with a mass for 4-aminoantipyrine of 25.6% and the last decomposition step occurs in the temperature of 353.1°C with a mass loss of 54.0% referred to the loss of zinc oxide and other moieties (Yilmaz

et al, 2008).

POM Analyses of ligand

From the values evaluated in Table 1, all structures are given to be non-mutagenic when run through the mutagenicity assessment of chelated arrangement and, as far as irritating and reproductive effects are concerned, all the compounds are at low risk comparable with standard dyes used. The hydrophilicity character of ligand has been expressed in terms of the cLogP value. It has been established that the absorption or permeation is greatly affected by the hydrophilicity (data of cLogP) (Kakanejadifard *et al*, 2014). Accordingly, when cLogP is higher than 5, the absorption or permeation decreases. On this basis, ligand has cLogP values within the acceptable criteria and are active at different IC_{50} because another crucial parameter should be taken in consideration.

Optimization of structure (Fig. I)

Total charge of heteroatoms (Fig. II)

DNA cleavage activity

The ligand and its Co(II), Cu(II), Ni(II), Cd(II), Zn(II) complexes were screened for their DNA cleavage activity with Calf-thymus DNA (Cat. No-105850) as a goal molecule by agarose gel electrophoresis process and the gel picture exhibiting cleavage is depicted in Fig. 6. Treatment of DNA on the ligand and complexes revealed that all the complexes have acted on DNA as there was a molecular weight difference between the treated DNA samples and the control. The difference was observed in bands of lanes compared to the control Calf-thymus DNA. The results indicate the important role of nitrogen and oxygen atoms to the metal ions in these isolated DNA cleavage reactions. On the basis of the cleavage of DNA observed in case of ligand and its Cu, Co, Ni and Zn complexes (Kose *et al*, 2013), it can be concluded that all the compounds under present study inhibited the growth of pathogenic organism by DNA cleavage as has been observed on the DNA cleavage of Calfthymus DNA (Dasa *et al*, 2014).

Antimicrobial sensitivity results

All of screened compounds showed salient antibacterial sensitivity with screened bacteria. A comparative search of the antibacterial effectiveness data of the ligand and their complexes indicate that the metal complexes exhibited higher antibacterial activity compared to the free ligand. There is very few research attached to the investigation of the biological sensitivity of Azo compounds on pathogenic bacteria. The antimicrobial researches of the ligand [H_2AZDP] and its

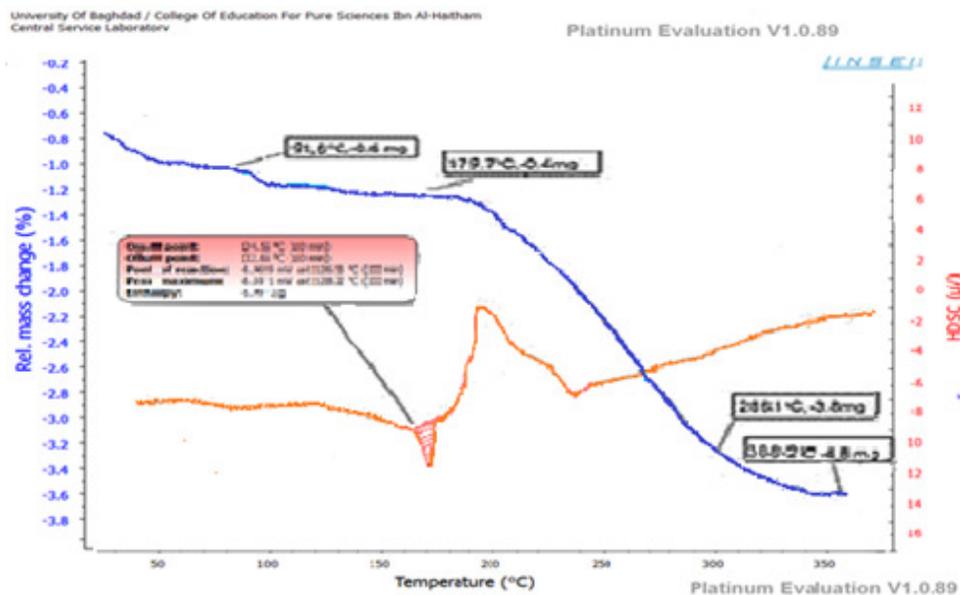


Fig. 5 : TG/HDSC Thermal for Cd complex.

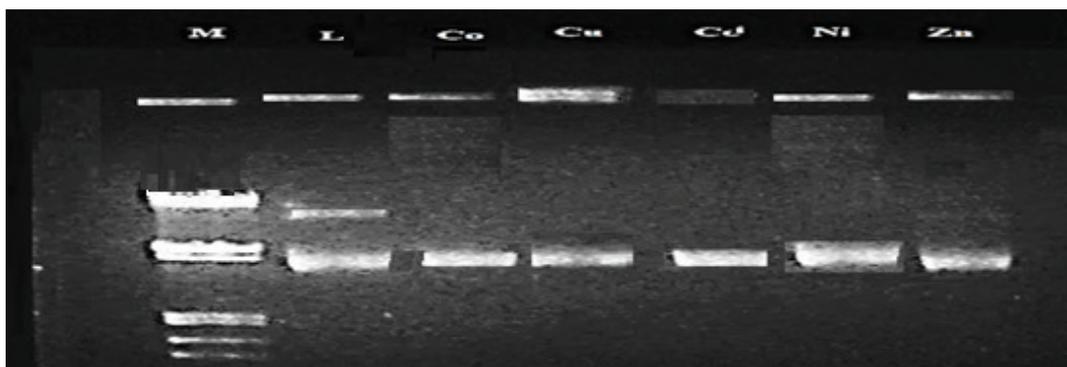


Fig. 6 : Agarose gel diagram showing cleavage studies of ligand L and complexes Co, Cu, Cd, Ni & Zn with CT-DNA at RT.

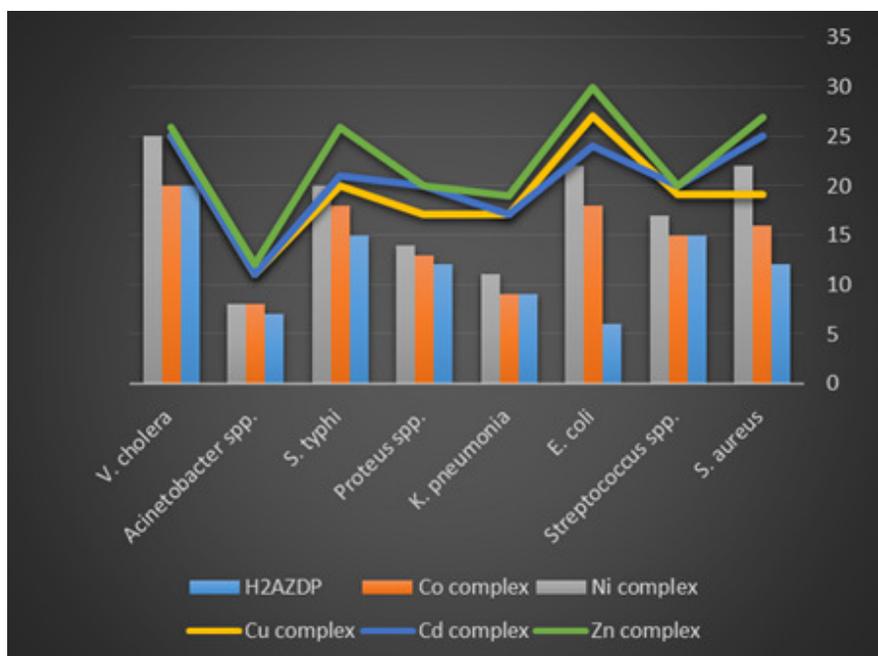


Fig. 7 : Antimicrobial studies of ligand L and complexes Co, Cu, Cd, Ni & Zn.

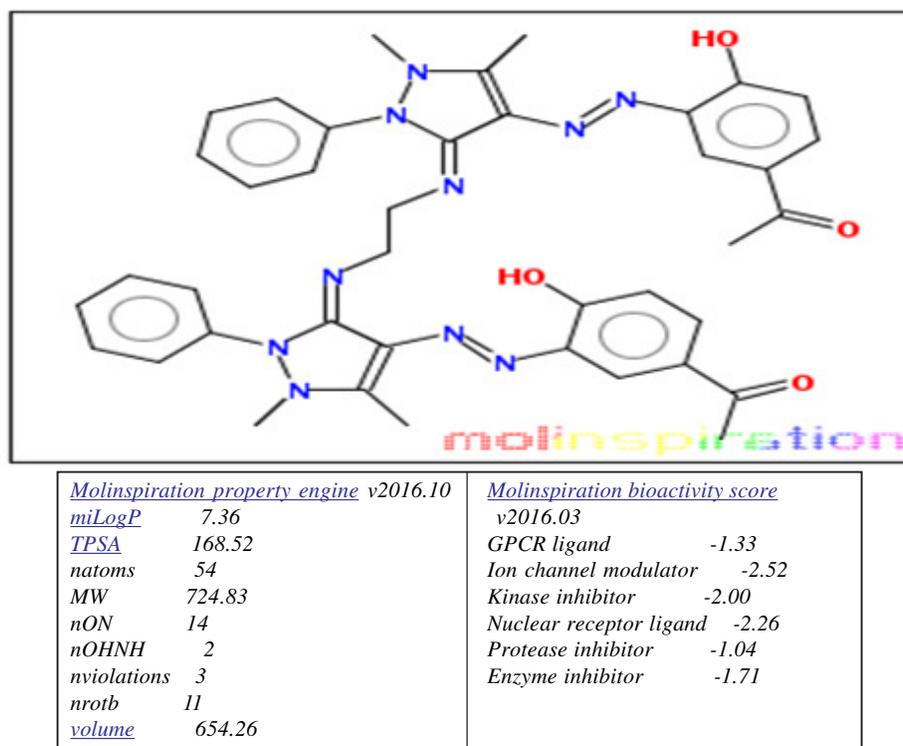


Fig. I : Optimization of structure.

^[a]TPSA: Total molecular polar surface area; NONH: number of OH—N or O—NH interaction, NV: number of violation of five Lipinsky rules; VOL: volume[33]. ^[b]GPCRL: GPCR ligand; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor.

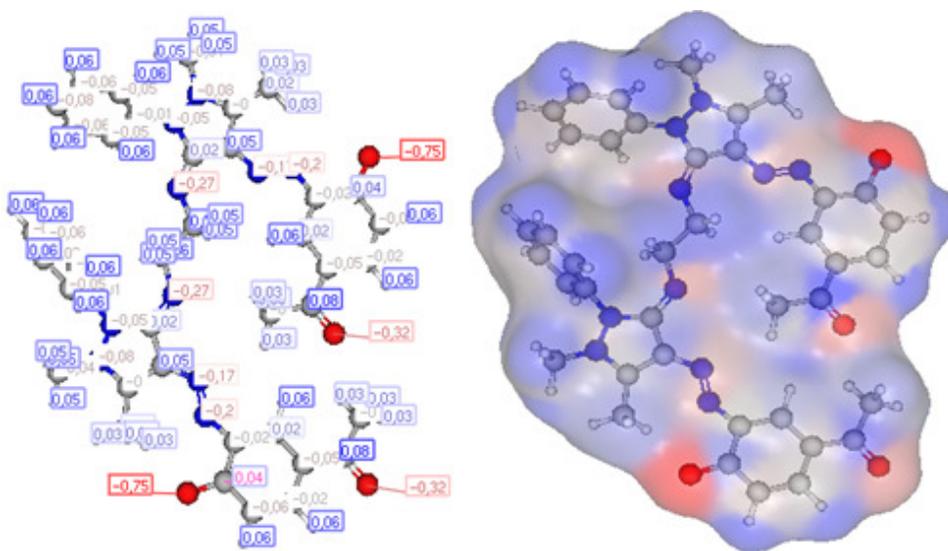


Fig. II : Total charge of heteroatoms.

metal complexes suggest which of the metal complexes appeared greater antimicrobial effectiveness than the free ligand against microorganisms such as (*E. coli*, *Streptococcus* spp., *Klebsiella pneumonia*, *Streptococcus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *V. cholera*) by disc diffusion way (Table 6). Consider activity was spotted as anti-bacterial effectiveness against Gram negative bacteria (*Klebsiella pneumonia*, *Vibrio cholera*,

Salmonella typhimurium, *Pseudomonas aeruginosa* and *Escherichia coli*) Gram-positive (*Staphylococcus aureus* and *Streptococcus* spp.) when azo-Schiff base compound screened using disc diffusion way. They found that (Azo-Schiff base) gave lowest activity against *E.coli* and *P.aeruginosa* had highest sensitivity, while cobalt, copper nickel, zinc and cadmium(II) complexes of azo ligand (Fig. 7) tested to disclose the antimicrobial sensitivity of Azo complexes on some influenced of all

types bacteria (Kulkarni *et al.*, 2012). The tested Cu and Zn(II) complexes have good antibacterial sensitivity against *S. aureus* and *V. cholera*. While the tested Co and Ni(II) complexes have good antibacterial sensitivity against *S. typhi* and *K. pneumonia*. The results that may be various result to different factors like:-the eventuality of utilizing different concentrations of the ligand and its complexes in testing the biological sensitivity or variances in chemical synthesis techniques of Azo compounds and utilizing diverse metals as ligands may affect the results or the biological activity of these compounds has been referred to its scavenging activity against reactive nitrogen and oxygen species (ROS and RNS), as well as to scavenger of the ROS hydroxyl radical (HO and there may be δ -electron delocalization over the whole coordinating system, as a consequence, there will be an growing in the lipophilic property of the metal complexes which favored its breakthrough through the lipid layer (Parmar *et al.*, 2010).

CONCLUSION

The spectral data revealed that the coordinating ability of the ligand has been proved in complexation reaction with Co(II), Cu(II), Ni(II), Cd(II) and Zn(II) ions. The newly synthesized ligand acts as ONO donor tridentate chelate. Metal ions are coordinated through a nitrogen atom of azo function, azomethine nitrogen and phenolic oxygen atom to the Cu, Co, Ni and Zn metal ions and form octahedral geometrical arrangements. Also, the reflectance spectra along with magnetic measurements confirm the octahedral geometry for all synthesized metal complexes. The process of chelation dominantly affects the biological sensitivity of the complexes that are active with pathogens. In general azo ligand and its complexes had emphatic leverage with *E. coli*, *Streptococcus* spp., *Klebsiella pneumonia*, *Streptococcus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *V. cholera*. The metal complexes appeared higher antibacterial sensitivity than free ligand. The electrophoretic studies indicated that Cu (II) and Co(II) complexes have good efficiency towards DNA cleavage. Based on the analytical data and spectral studies, the proposed structure of all the complexes is depicted in Fig. 5.

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