

Synthesis, spectral characterization and in vitro biological studies of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases

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Abstract

A series of metal complexes of cobalt(II), nickel(II) and copper(II) have been synthesized with newly derived biologically active ligands. These ligands were synthesized by the condensation of 3-substituted-4-amino-5-hydrazino-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin. The probable structure of the complexes has been proposed on the basis of elemental analyses and spectral (IR, UV-Vis, magnetic, ESR, FAB-mass and thermal studies) data. Electro chemical study of the complexes is also reported. All these complexes are non-electrolytes in DMF and DMSO. All the ligands and their Co(II), Ni(II) and Cu(II) complexes were screened for their antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus pyogenes* and *Pseudomonas aeruginosa*) and antifungal (*Aspergillus niger*, *Aspergillus flavus* and *Cladosporium*) activities by MIC method. The brine shrimp bioassay was also carried out to study their in vitro cytotoxic properties.

Keywords: Synthesis, Biological activity, Electrochemical, 1, 2, 4-triazole, Coumarin, metal complexes

Introduction

Nowadays there is an increasing demand for the preparation of new antimicrobial agents due to the developing resistance towards conventional antibiotics [1–3]. Synthesis of 1,2,4-triazole derivatives has attracted widespread attention due to their diverse biological activities. Moreover, 1,2,4-triazoles are a new class of antimicrobial agents. For instance, fluconazole and itraconazole are used as antimicrobial drugs in medicine [4,5]. Beside these, some biheterocyclic compounds incorporating 1,2,4-triazole ring have been reported as antimicrobial agents [6–9]. Among these, the commonly known systems are generally triazoles fused with pyridines, pyridazines, pyrimidines, pyrazines, and triazines. Although, there is no attempt has been made to fuse the coumarin moiety to study their biological and structural importance. In addition to 1,2,4-triazole derivatives coumarin derivatives display an additional significant

biological [10–14] and pharmacological [15,16] properties. Many of these compounds possess antibacterial [15], antifungal [16] and insecticidal [13] activities and the hydroxycoumarins are the typical phenolic compounds and therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants [17]. Metal complexes of 1,2,4-triazole derivatives have been extensively investigated and reported from our laboratory [18–21].

Recently, a number of work has been made to obtain Co(II), Ni(II), Cu(II) and Zn(II) complexes with the Schiff bases derived from cinnamaldehyde and 4-amino-3-ethyl-5-mercapto-s-triazole and 4-amino-5-mercapto-3-n-propyl-s-triazole [22] and cobalt(II), Nickel(II) and copper(II) complexes with Schiff base derived from furfuraldehyde and 3-substituted-4-amino-5-mercapto-s-triazole have been isolated and characterized by elemental analyses,

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magnetic, spectral (IR, UV-Vs, EPR) and thermal studies [23].

In continuation of our efforts on 1,2,4-triazole derivatives we have synthesized a new series of Schiff bases derived from 3-substituted-4-amino-5-hydrazino-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin. The survey of the literature reveals that, no work has been carried out on the synthesis of metal complexes with these Schiff bases. These ligands have donor sites with the ONNO sequence and varied coordination abilities. Because of importance of triazole and coumarin compounds in biological and medicinal fields; it attracted our attention and aroused our interest in elucidating the structure of Co(II), Ni(II) and Cu(II) complexes with these bioactive Schiff bases (Figure 1). The Schiff bases are characterized by spectral, thermal and molar conductivity studies and these are evaluated for their antibacterial and antifungal properties against various pathogenic bacterial strains using the minimum inhibitory concentration (MIC) method.

Material and methods

All the chemicals used for preparing triazoles and their Schiff bases were of reagent grade. The 3-substituted-4-amino-5-mercapto-1,2,4-triazoles were prepared as reported [24,25]. 8-formyl-7-hydroxy-4-methylcoumarin was prepared according to the reported method [26]. The IR spectra of the ligands and their Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000 – 250 cm^{-1} region in KBr disk. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200–1100 nm. The ^1H NMR spectra of ligands and their metal complexes were recorded in DMSO- d_6 on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. Thermogravimetric analyses data were measured from room temperature to 1000°C at a heating rate of 10°C/min. The data were obtained by using a Perkin-Elmer Diamond TG/DTG instrument. The fluorescence study was carried out on model Hitachi F-7000. The FAB-mass spectra were recorded

on a Jeol SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 KV, 10 mA) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature. m-Nitrobenzyl alcohol was used as the matrix.

The mass spectrometer was operated in the positive ion mode. Molar conductivity measurements were recorded on a ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51. Electrochemical studies were carried out using CHI1110A-Electrochemical analyzer and magnetic moment was carried out by a faraday balance.

Chemistry

Synthesis of 3-substituted-4-amino-5-hydrazino-1,2,4-triazole. A mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazole and $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ in 1:1 molar proportions in EtOH was boiled under reflux for 4–5 h on a water bath. The reaction mixture was cooled at room temperature; within an hour the compound separated from the clear solution. It was filtered, washed and recrystallized from EtOH.

Synthesis of Schiff bases [I–IV]. The mixture of 3-substituted-4-amino-5-hydrazino-1,2,4-triazole (0.01 mol) and 8-formyl-7-hydroxy-4-methylcoumarin (0.02 mol) in ethanol containing a few drops of concentrated HCl was heated under reflux for about 3–4 h. The product separated on cooling was filtered and washed with ethanol and crystallized from ethanol.

Synthesis of Co(II), Ni(II) and Cu(II) complexes [1–12]. An alcoholic solution (25 mL) of Schiff base (I–IV) (1 m mol) was refluxed with 1 m mol of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 25 mL ethanol on a steam bath for 2 h. Then, to the reaction mixture 2 mmol of sodium acetate was added and refluxation was continued for 3 h. The separated complex was filtered, washed thoroughly with water, Ethanol, Ether and finally dried in vacuum over fused CaCl_2 .

Biology

In vitro antibacterial and anti fungal assay. All the synthesized Schiff bases (I–IV) and their corresponding Co(II), Ni(II) and Cu(II) (1–12) complexes were screened *in vitro* for their biological activity by using four bacteria, namely *E. coli*, *S. aureus*, *S. pyogenes* and *P. aeruginosa*, and three fungi namely *A. niger*, *A. flavus* and *cladosporium* by the reported method [27 & 28]. The stock solution (1 mg mL) of the test chemical was prepared by dissolving 10 mg of the test compound in 10 mL of *N,N*-dimethyl formamide (DMF) solvent. The stock solution was suitably diluted with sterilized

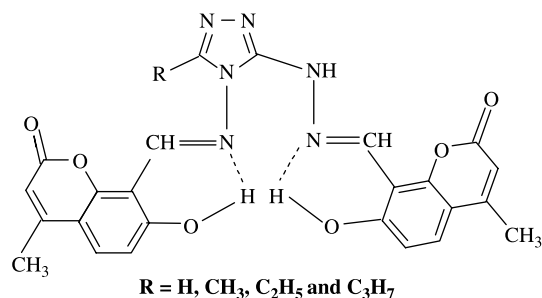


Figure 1. Structure of Schiff bases.

distilled water to a dilution of 100, 50 and 25 $\mu\text{g mL}^{-1}$. Control for each dilution was prepared by diluting 10 mL of solvent instead of stock solution with sterilized distilled water.

The bacteria were subcultured in agar medium. The petridishes were incubated for 24 h at 37°C. Standard antibacterial drug (gentamycine) was also screened under similar conditions for comparison. The fungi were subcultured in potato dextrose agar medium. Standard antifungal drug (fluconazole) was used for comparison. The petridishes were incubated for 48 h at 37°C. The wells were dug in the agar media using a sterile metallic borer. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm). Growth inhibition was compared with the standard drugs. In order to clarify any effect of DMF on the biological screening, separate studies were carried out with solutions alone of DMF and they showed no activity against any microbial strains.

Minimum inhibitory concentration (MIC). Compounds showing promising antibacterial/antifungal activity were selected for minimum inhibitory concentration studies.

In vitro cytotoxicity. The synthesized Schiff bases and their Co(II), Ni(II) and Cu(II) complexes were screened for their cytotoxicity (brine shrimp bioassay) using protocol of Meyar et al [29]. Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm) filled with artificial sea water, which was prepared with a commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg

of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was open to ordinary light.

After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 100, 50 and 10 $\mu\text{g/mL}$ were transferred to 9 vials (three for each dilutions were used for each test sample and LD₅₀ is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD₅₀ values [30].

Results and discussion

Chemistry

All the Co(II), Ni(II) and Cu(II) complexes were stable in room temperature, non-hygroscopic, slightly soluble in many common organic solvents but they are soluble in DMF and DMSO. The elemental analyses shown in Table I are agree well with the formation of 1:1 stoichiometry of the type ML₂.2H₂O. The molar conductivity measurements at the concentration of 10⁻³ M in DMF indicating that, these complexes are non electrolytes. The metal contents were estimated gravimetrically [31]. Nitrogen was estimated by Duma's method. The carbon and hydrogen were determined by CHN analyzer and the data is well agreed with calculated values.

Table I. Elemental analysis of Co(II), Ni(II) and Cu(II) complexes and their magnetic and molar conductance data.

Comp. No.	Empirical formula	M%		C%		H%		N%		Molar conductance Ohm-1 cm-2 mole ⁻¹	Mag. Moments (μ_{eff} BM)
		Obsd	Calcd	Obsd.	Calcd	Obsd.	Calcd	Obsd.	Calcd		
I	C24H18N6O6	–	–	59.17	59.25	3.67	3.70	17.22	17.28	–	–
II	C25H20N6O6	–	–	59.91	60.00	3.94	4.00	16.77	16.80	–	–
III	C26H22N6O6	–	–	60.62	60.70	4.21	4.28	16.31	16.34	–	–
IV	C27H24N6O6	–	–	61.31	61.36	4.49	4.54	15.88	15.90	–	–
1	Co(C24H16N6O6).2H2O	10.16	10.18	49.72	49.74	2.73	2.76	14.48	14.51	22	4.62
2	Co(C25H18N6O6).2H2O	9.91	9.94	50.57	50.59	3.02	3.04	14.15	14.17	24	4.90
3	Co(C26H20N6O6).2H2O	9.69	9.71	51.38	51.41	3.27	3.29	13.81	13.84	20	4.52
4	Co(C27H22N6O6).2H2O	9.45	9.49	52.15	52.18	3.53	3.54	13.51	13.53	27	4.63
5	Ni(C24H16N6O6).2H2O	10.11	10.14	49.75	49.76	2.75	2.76	14.50	14.52	23	3.35
6	Ni(C25H18N6O6).2H2O	9.88	9.90	50.61	50.62	3.01	3.03	14.14	14.17	27	3.22
7	Ni(C26H20N6O6).2H2O	9.65	9.67	51.41	51.43	2.27	3.29	13.82	13.85	26	3.15
8	Ni(C27H22N6O6).2H2O	9.42	9.45	52.16	52.19	3.51	3.54	13.50	13.53	23	3.23
9	Cu(C24H16N6O6).2H2O	10.87	10.89	49.33	49.35	2.71	2.74	14.37	14.39	28	1.68
10	Cu(C25H18N6O6).2H2O	10.61	10.63	50.19	50.21	3.00	3.01	14.03	14.06	18	1.80
11	Cu(C26H20N6O6).2H2O	10.37	10.39	51.01	51.02	3.26	3.27	13.71	13.74	26	1.72
12	Cu(C27H22N6O6).2H2O	10.14	10.16	51.78	51.79	3.50	3.52	13.41	13.43	19	1.65

Table II. The important infrared frequencies (cm^{-1}) of 3-substituted-4-amino (8-formyl-7-hydroxy-4-methylcoumarin)-5-hydrazino-1,2,4-triazole Schiff bases.

Ligand No.	ν (NH)	Lactone ν (C=O)	ν (C=N)	H-bonded -OH Stretching	ν (C=C)	Phenolic ν (C-O)
I	3130 brm 3071 m	1700	1625	2758	1590	1279
II	3125 brm 3068 m	1707	1630	2750	1600	1285
III	3115 brm 3058 m	1705	1615	2755	1595	1292
IV	3120 brm 3065 m	1710	1620	2752	1597	1294

IR spectra. The important IR spectral assignments of the ligands and their metal complexes are presented in Tables II & III.

In the Schiff bases, we observed a broad weak band with fine structure in the region 2758–2750 cm^{-1} and that can be attributed to the intramolecular H-bonded -OH. In addition to the above, a medium intensity broad band is observed at *ca.* 3130 – 3058 cm^{-1} in these Schiff bases is attributed to the ν (NH). The medium to high intensity band at *ca.* 1630 – 1615 cm^{-1} , is assigned to ν (C=N), that confirms the presence of the 8-formyl-7-hydroxy-4-methylcoumarin moiety. Medium intensity bands in the 1610 – 1590 cm^{-1} region are regarded as a combination of C=N of the triazole ring and aromatic C=C stretching vibrations. A high intensity band in the 1295 – 1279 cm^{-1} region with an additional band around 1600 – 1590 cm^{-1} is assigned to the phenolic ν (C-O) vibration. The band located at 1715 – 1700 cm^{-1} in all these Schiff bases attributed to ν (C=O) [32].

In case of Co(II), Ni(II) and Cu(II) complexes we observed the following changes. All the complexes exhibited a broad medium intensity band at *ca.* 3010 – 2990 cm^{-1} , which is ascribed to the ν (NH) vibration. A broad weak band with fine structure in the 2758 – 2750 cm^{-1} region, assigned to the H-bonded -OH in the Schiff bases is disappeared in the complexes. The high intensity band due to phenolic C-O appeared in the region 1295 – 1279 cm^{-1} in the

Schiff bases, appeared as a medium to high intensity band in the 1380 – 1370 cm^{-1} region in the complexes. These observations support the formation of M-O bonds *via* deprotonation. It mean that H-atoms of -OH groups have been replaced by the metal ion. The bands at 1505 – 1500 cm^{-1} in the Schiff bases are appeared in the 1520 – 1515 cm^{-1} region in the complexes this suggests the phenolic oxygen atoms are monodentate. The medium intensity band appeared around 1630 – 1615 cm^{-1} , due to ν (C=N) in Schiff bases, showed a lower shift by 10–20 cm^{-1} in these complexes. The low shift indicating that, the (C=N) groups of ligand are coordinated to the metal ion through nitrogen atoms. The band located at 1715 – 1700 cm^{-1} due to ν (C=O) in the Schiff bases are unaffected in the complexes indicating that the lactone Oxygens are not involved in the coordination. All these complexes exhibit a broad through band in the region 3440 – 3425 cm^{-1} indicates the presence of coordinated water molecule [33] and the additional two weaker bands in the region 800 – 750 and 720 – 700 cm^{-1} due to ν (-OH) rocking and wagging mode of vibrations, respectively [33]. The complexes showed a medium intensity band in the region 540 – 460 cm^{-1} assigned to ν (M-N) and 360 – 330 cm^{-1} assigned to ν (M-O) modes respectively.

Thus, the IR spectral data provide strong evidences for the complexation of the potentially tetradentate ligands.

Table III. The important infrared frequencies (in cm^{-1}) of Co(II), Ni(II) and Cu(II) complexes of 3-substituted-4-amino (8-formyl-7-hydroxy-4-methylcoumarin)-5-hydrazino-1,2,4-triazole Schiff bases.

Complex No.	ν (OH)	ν (C=O)	ν (C=N)	Phenolic ν (C-O)	ν (M-N)	ν (NH)	ν (M-O)
1	3440	1615	1380	1704	535	3010	376
2	3435	1707	1620	1377	540	3003	378
3	3430	1700	1605	1372	475	3006	375
4	3434	1705	1610	1379	480	2995	379
5	3429	1700	1614	1375	520	2998	380
6	3431	1707	1624	1374	510	3001	381
7	3430	1705	1607	1377	495	3000	379
8	3429	1710	1609	1373	485	3005	384
9	3427	1712	1613	1371	532	2995	382
10	3437	1707	1619	1370	515	2990	385
11	3432	1705	1604	1376	474	3008	381
12	3438	1704	1602	1372	460	3007	378

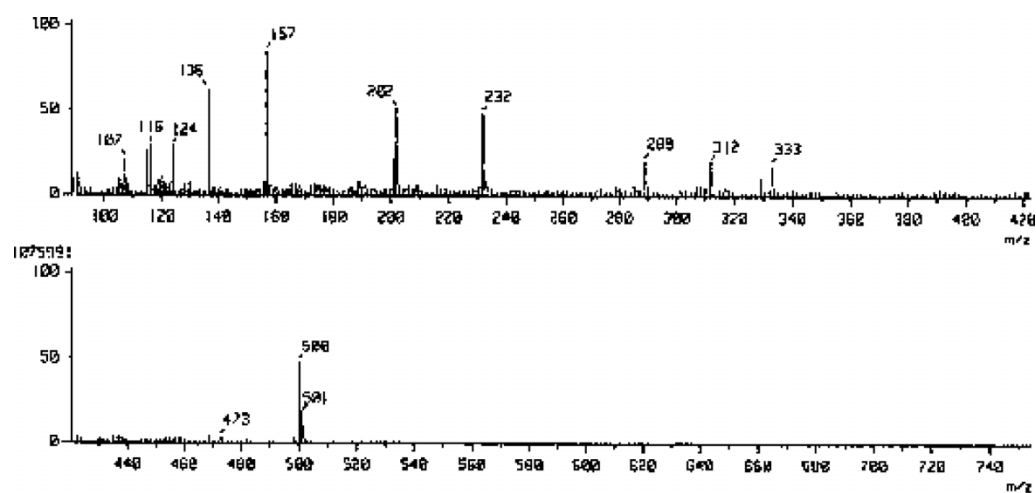


Figure 2. FAB-mass spectrum of Schiff base (II).

¹H NMR spectra. The ¹H NMR spectra of Schiff bases exhibit signals at 13.58, 10.21, 8.62 and 7.2–7.5 ppm due to –NH, phenolic OH, –CH = N and aromatic protons respectively. The signals around 2.84 ppm are due to methyl protons.

¹³C NMR Spectrum. The ¹³C NMR spectrum of Schiff base (II) exhibits the signals at 117.4, 119.2, 120.4, 128.1, 135.0 and 149.2 ppm are corresponding to aromatic carbons. The resonance at 11.6 ppm is due to aliphatic (–CH₃) carbon and the azomethine (–HC=N) carbon shows peak at 159.2 ppm.

FAB mass spectral studies. The FAB mass spectrum of Schiff base (II) is reproduced in Figure 2. The spectrum showed a molecular ion peak at *m/z*

500 which is equivalent to its molecular weight. The fragments in the spectrum leading to the formation of the species [C₂5H₂₀N6O6]⁺.

The FAB mass spectrum of Ni(II) complex (6) showed a molecular ion peak M⁺ at *m/z* 593, which is equivalent to its molecular weight of the Ni(II) complex (6) shown in Figure 3. The molecular ion peak fragmentation with the loss of two water molecules, gave a peak A₁ at *m/z* 557 due to the fragment ion [Ni(C₂5H₈N6O6)]⁺. Further, the fragment ion A₁ by the loss C₁₁H₇O₃ species gave a fragment ion A₂, at *m/z* 370. The fragment ion A₂ gave a fragment ion A₃, at *m/z* 183 by the elimination of another C₁₁H₇O₃. All these fragments leading to the formation of the species [Ni(L)]⁺ which undergoes demetallation to form the species [L + H]⁺ gave a fragment ion at *m/z* 498. All these fragmentation patterns are well observed in the FAB mass spectrum.

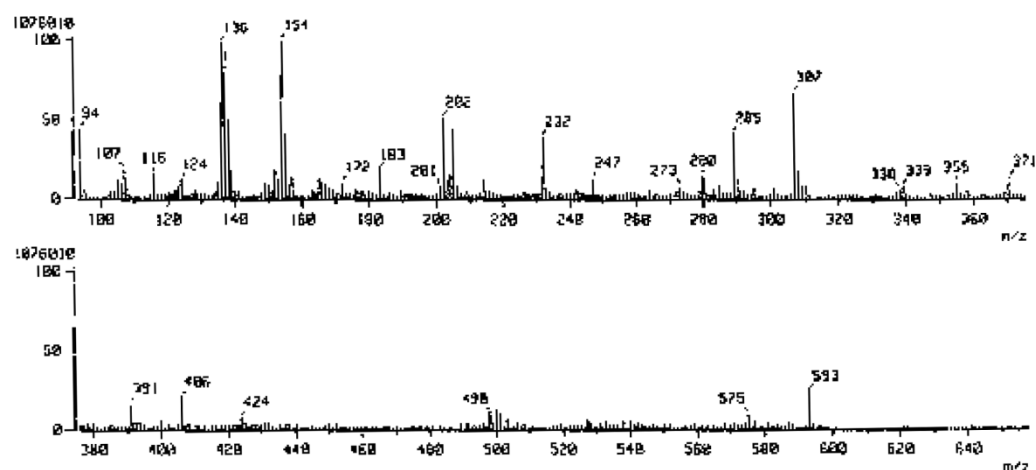


Figure 3. FAB-mass spectrum of Ni(II) (6) complex.

Table IV. Ligand field parameters of Ni(II) complexes with 3-substituted-4-amino (8-formyl-7-hydroxy-4-methylcoumarin)-5- hydrazino-1,2,4-triazole Schiff bases (I-IV)

Complex No.	Transitions (cm)			V_2 Cald. cm^{-1}	Dq (cm^{-1})	B^{-1} (cm^{-1})	% Distortion	V_1/V_2	LSFE	μ_{eff} Cald. (BM)	β	Do P%
	V_1	V_2	V_3									
5	9810	15835	26256	15867	981.00	846.21	0.203	1.614	33.63	3.193	0.801	19.86
6	9855	15785	26320	15928	985.50	845.54	0.898	1.602	33.78	3.192	0.801	19.93
7	9832	15766	26325	15904	983.20	948.92	0.873	1.604	33.71	3.193	0.804	19.61
8	9822	15844	26675	15963	982.20	878.18	0.784	1.613	33.65	3.193	0.832	16.83

Electronic absorption spectra. The cobalt(II) complexes showed two distinct bands in the region 9790–10000 and 18950–20661 cm^{-1} which are attributed to $T_{1g}(F) \rightarrow {}^4T_{2g}(F)(v_1)$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(v_3)$ transitions respectively and these are suggestive of octahedral geometry around the cobalt(II) ions [35,36]. The electronic spectra of nickel(II) complexes showed d-d bands in the region 9570–10000, 15597–15845 and 20492–27248 cm^{-1} . These are assigned [34] to the transitions $A_{2g}(F) \rightarrow {}^3T_{2g}(F)(v_1)$; $A_{2g}(F) \rightarrow {}^3T_{1g}(F)(v_2)$ and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(v_3)$ respectively, consistent with their well defined octahedral configuration [35,36]. The ligand field parameters [37] Dq, $[3, B', v_2/v_1]$ and LFSE have been calculated (Table IV). The electronic spectra of Cu(II) complexes show absorption band in the region 14540–14780 cm^{-1} is attributed to $T_g \leftarrow {}^2E_g$ transition indicative of distorted octahedral geometry [37, 38].

Magnetic studies. The magnetic moments obtained at room temperature are listed in Table I. The magnetic measurements for Co(II) and Ni(II) complexes showed magnetic moment values of 4.3–5.2 BM and 2.8–3.5 BM respectively, suggesting [40] consistency with their octahedral environment. The Cu(II) complexes show magnetic moments 1.75–1.87 BM which is slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [41].

ESR spectra of copper (II) complex (10). The ESR spectrum of copper (II) complex (10) has been studied, the g_{\parallel} and g_{\perp} values have been found to be 2.0404 and 2.15887 respectively. The g_{av} was calculated to be 2.1194. The Cu(II) complex shows reversed axial (compressed octahedral) with $g_{\parallel} < g_{\perp}$. The trend $g_{\parallel} < 2 g_{\perp}$ showed that, the electron is delocalised in d_z orbital of the ground state of Cu(II). In this case ($g_{\parallel} < g_{\perp}$) distortion occur by compression [42]. The parameter G, determined as $G = (g_{\parallel} - 2)/(g_{\perp} - 2)$ is found to be much less than 4 suggesting considerable interaction in the solid state [43].

Thermogravimetric analyses. The thermal decomposition of on respective Co(II) (2), Ni(II) (6) and Cu(II) (10) complexes takes place in three steps corresponding to the mass loss of two coordinated water molecules, one triazole moiety and two coumarin moieties.

Electrochemical studies. Electrochemical properties of Cu(II) and Co(II) complexes have quasi-reversible character and can provide a rough evaluation of the degree of the reversibility This establishes the electrode process as diffusion controlled [44].

Fluorescence studies. The emission spectra of the Schiff bases and their complexes were investigated in various solvents such as DMF, DMSO, MeCN and dioxan (Table V & VI).

Biological results

Antimicrobial results. The microbial results are systematized in Tables VII & VIII and shown in Figures 4 & 5. The antibacterial and antifungal studies suggested that, all the Schiff bases were found to be biologically active and their metal(II) complexes showed significantly enhanced antibacterial and antifungal activity. It is, however, known [49,54] that, chelation tends to make the Schiff bases act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of bacteria and fungi more than the parent Schiff bases. It is suspected that, factors such as solubility, conductivity, dipole moment and cell permeability mechanism (influenced by the

Table V. Emission spectral data of Schiff base (II)

Solvents	Schiff base (II) (EX)	(EM)
DMSO	438.0 (438)	503.0 (472.0)
DMF	440.0 (440)	504.0 (480.4)
MeCN	446.0 (446)	494.8 (464.0)
Dioxan	442.0 (442)	468.0 (471.4)

The values in the parenthesis are these observed after the addition of 2% NaOH solution.

Table VI. Emission spectral data of Co(II) (2), Ni(II) (6) and Cu(II) (10) complexes

Solvents	Complexes	(EX)	(EM)
DMSO	Co(II)	353.0 (410.2)	475.0 (465.2)
	Ni(II)	461.0 (488.4)	487.6 (469.2)
	Cu(II)	389.2 (432.0)	444.6 (455.6)
DMF	Co(II)	445.0 (410.2)	470.8 (464.8)
	Ni(II)	460.0 (488.4)	487.6 (467.4)
	Cu(II)	412.0 (432.0)	440.2 (460.0)
MeCN	Co(II)	353.6 (410.2)	304.4 (462.0)
	Ni(II)	470.0 (488.4)	580.6 (470.5)
	Cu(II)	373.2 (432.0)	429.0 (462.4)
Dioxan	Co(II)	243.2 (410.2)	285.6 (466.2)
	Ni(II)	244.0 (488.4)	286.2 (471.2)
	Cu(II)	244.0 (432.0)	285.2 (464.8)

presence of metal ions) may be the possible reasons for the increase in activity.

In the case of bacteriological studies, the results were compared with the standard drug (Gentamycine). It was observed that, some of the Schiff bases were found potentially active against all bacterial strains. Compound (IV) shows high activity against all bacterial strains especially with *P.aeruginosa* and *E. coli*, were as metal(II) complexes (1–12) of these Schiff bases (I–IV) were also screened against the same bacterial strains. It was evident that, overall potency of the uncoordinated compounds was enhanced on coordination with metal ions. Among these metal complexes, Ni(II) and Cu(II) complexes exhibited high activity.

In case of antifungal activity, the results were compared with the standard drug (Fluconazole). All Schiff bases were show high activity against fungal species. Compounds (I) and (III) show very high activity, an interesting feature is that the compound (III) shows high activity even more than the standard against *A. Niger*, However, the Co(II), Ni(II) and

Cu(II) complexes (1–12) of these Schiff bases showed much enhanced activity as compared to the uncoordinated compounds, especially with *Cladosporium*. All Schiff bases show high activity against all three fungal species, in some cases, the complexes show more activity in MIC.

It was evident from the data that this activity significantly increased on coordination. This enhancement in the activity may be rationalized on the basis that the presence of C=N bond. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity, since the enzymes which require these groups of their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduces the polarity [29] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [49–53] with in the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the micro-organism [48] thus destroying them more aggressively. The minimum inhibitory concentration 10 µg/mL was shown by compound 4 against *S. aureus*, *A. Niger* and compound 10 against *A. Flavus*. Compound II shown MIC 10 µg/mL⁻¹ against *P.aeruginosa* and *A. Flavus*. In all other cases, the compounds exhibited MICs ranging from 10 µg/mL – 100 µg/mL against all the microbial strains some of are given in Table IX.

Cytotoxic bioassay. All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay). It is evident from the data recorded in Table X that, only five compounds (4), (6), (8), (10) and (12) displayed potent cytotoxic activity as LD₅₀ = 8.945 × 10⁻⁴,

Table VII. Anti bacterial and anti fungal results of Schiff bases (I-IV)

Compd.	Conc.(ug ml-1)	Antibacterial activity (Zone of inhibition in %)				Antifungal activity (Zone of inhibition in %)		
		E. coli	S. aureus	S. pyogenes	P.aeruginosa	A. flavus	Cladosporium	A. niger
I	100	50	45	48	74	75		
	50	40	–	–	58	74	66	61
	25	35	–	–	55	68	50	52
II	100	58	59	52	60	88	82	78
	50	50	47	43	52	71	67	40
	25	43	36	38	47	45	52	–
III	100	55	57	59	69	70	76	131
	50	48	46	50	57	100	88	120
	25	40	39	41	50	90	71	54
IV	100	61	60	57	63	74	72	80
	50	53	52	46	56	79	67	74
	25	47	46	38	48	77	54	71
Standard	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100

Table VIII. Anti bacterial and anti fungal results of Co(II), Ni(II) and Cu(II) complexes (1–12) and standard

Compd.	Conc ($\mu\text{g ml}^{-1}$)	Antibacterial activity (Zone of inhibition in %)				Antifungal activity (Zone of inhibition in %)		
		<i>E.coli</i>	<i>S.aureus</i>	<i>S.pyogenes</i>	<i>Paeruginosa</i>	<i>A.flavus</i>	<i>Cladosporium</i>	<i>A. niger</i>
1	100	45	55	62	70	86	69	49
	50	–	48	54	64	81	58	69
	25	–	–	42	52	80	50	60
2	100	68	62	70	68	91	71	52
	50	54	0	59	61	80	63	79
	25	41	55	54	52	77	51	78
3	100	79	75	68	76	89	82	80
	50	67	68	59	71	78	71	82
	25	53	59	51	64	71	58	83
4	100	72	76	70	79	94	85	85
	50	55	70	60	72	84	77	86
	25	40	61	53	67	79	66	80
5	100	75	79	69	78	84	76	82
	50	58	70	61	70	77	62	87
	25	40	65	52	64	70	59	79
6	100	78	82	72	81	89	83	70
	50	62	74	68	75	81	72	84
	25	52	67	60	70	72	58	70
7	100	70	71	68	80	79	78	62
	50	62	64	57	72	71	67	88
	25	48	52	60	66	64	60	82
8	100	80	73	71	73	76	82	88
	50	72	61	64	62	69	70	82
	25	69	50	59	54	62	61	70
9	100	52	66	65	82	88	77	80
	50	42	59	52	72	79	69	72
	25	38	52	48	69	78	58	67
10	100	68	73	69	85	94	83	89
	50	54	64	50	76	90	72	73
	25	50	60	42	70	91	64	67
11	100	72	78	77	80	89	78	90
	50	63	70	70	71	80	64	81
	25	58	67	61	62	80	59	74
12	100	75	79	73	76	90	84	85
	50	66	63	62	64	82	75	72
	25	60	57	57	56	85	65	67
Standard	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100

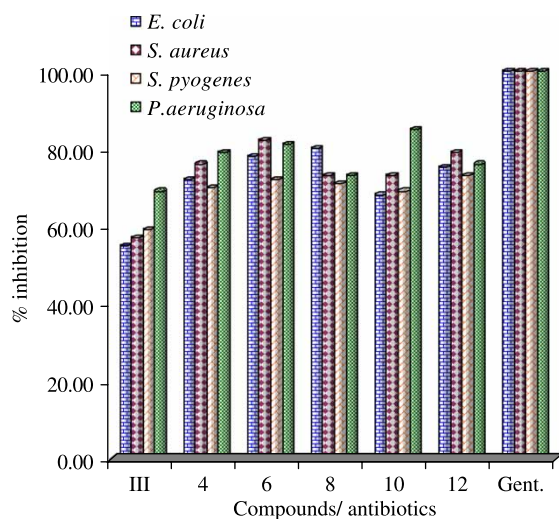
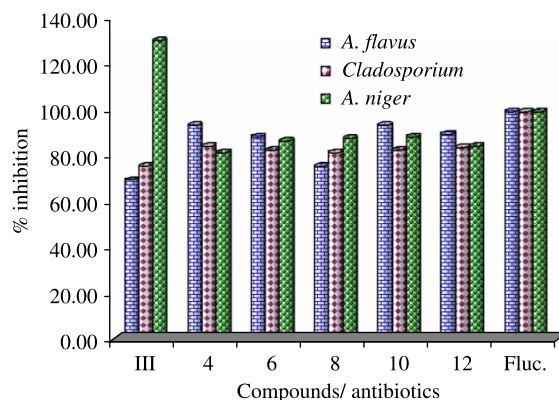
Figure 4. In vitro antibacterial spectrum of compounds III, 4, 6, 8, 10, 12, Gentamycin (Std.) at 100 μgml concentration.Figure 5. In vitro antifungal spectrum of compounds III, 4, 6, 8, 10, 12, Flucanazole (Std.) at 100 μgml concentration.

Table IX. Minimum inhibitory concentration MIC (ug/mL) results for some compounds.

Compd.	Antibacterial activity (Zone of inhibition in %)				Antifungal activity (Zone of inhibition in %)		
	E. coli	S. aureus	S. pyogenes	P.aeruginosa	A. flavus	Cladosporium	A. niger
III	15	15	20	10	10	15	20
4	20	10	25	25	15	25	10
6	25	15	25	15	15	25	15
8	15	25	25	25	20	25	20
10	25	20	25	20	10	20	20
12	15	25	20	20	15	15	20

8.839×10^{-4} , 7.135×10^{-4} , 7.022×10^{-4} and 8.759×10^{-4} M/mL, respectively, against *Artemia salina*, while all other compounds were almost inactive for this assay.

Conclusion

The Synthesized Schiff base act as tetradentate ligand through the coordination of azomethine nitrogen and phenolic oxygen atoms to the metal ion. The bonding of ligand to metal ion was confirmed by the analytical, IR, electronic, magnetic, ESR, FAB mass, thermal and electrochemical studies. From fluorescence data, it confirms the involvement of phenolic oxygen in coordination with the metal ion.

In biological results it confirms that, all the Schiff bases are biologically active and their metal(II) complexes are shown more promising activity then the Schiff bases.

All compounds are slightly soluble common organic solvents and soluble in DMF and DMSO. All these observations put together lead us to propose the following structures shown in Figure 6 in which, the complex having the stoichiometry of the type $ML_2 \cdot 2H_2O$ ($M = Co(II), Ni(II)$ and $Cu(II)$).

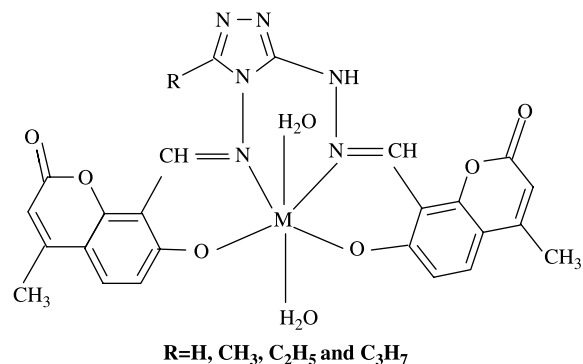


Figure 6. Proposed structure of Metal(II) complexes.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table X. Brine shrimp bioassay data of the ligands (I–IV) and their metal (II) complexes 1–12.

Compound	LD ₅₀ (moles/mL)
I	4.517×10^{-3}
II	6.324×10^{-3}
III	5.842×10^{-3}
IV	6.247×10^{-3}
1	2.430×10^{-3}
2	2.459×10^{-3}
3	1.742×10^{-3}
4	8.945×10^{-4}
5	2.750×10^{-3}
6	8.839×10^{-4}
7	1.764×10^{-3}
8	7.135×10^{-4}
9	1.751×10^{-3}
10	7.022×10^{-4}
11	2.299×10^{-3}
12	8.759×10^{-4}

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Table S1. Thermogravimetric data of Co(II) (2), Ni(II) (6) and Cu(II) (10) complexes of 3-substituted-4-amino (8-formyl-7-hydroxy-4-methylcoumarin)-5-hydrazino-1,2,4-triazole Schiff base (II)

Empirical Formula	Decomposition temperature oC	%Weight loss.		Metal Oxide %		Inference
		Obsd	Calcd.	Obsd.	Calcd.	
Co(C ₂₅ H ₁₈ N ₆ O ₆).2H ₂ O	105–135	6.05	12.62	6.07	12.64	Loss of coordinated water molecules Loss of triazole moieties Loss of coumarin moieties
	330–365	20.90	20.91			
	440–480	63.05	63.08			
Ni(C ₂₅ H ₁₈ N ₆ O ₆).2H ₂ O	110–140	6.04	6.07	12.57	12.60	Loss of coordinated water molecules Loss of triazole moieties Loss of coumarin moieties
	325–355	20.89	20.92			
	435–472	63.09	63.10			
Cu(C ₂₅ H ₁₈ N ₆ O ₆).2H ₂ O	220–230	6.00	6.02	13.30	13.31	Loss of coordinated water molecules Loss of triazole moieties Loss of coumarin moieties
	300–330	20.73	20.75			
	455–473	62.57	62.59			

SUPPLEMENTARY MATERIAL

Thermogravimetric analyses. TG and DTG studies were carried out for some of the complexes. These complexes decompose gradually with the formation of respective metal oxide above 500°C. The nature of proposed chemical change with the temperature range and the percentage of metal oxide obtained are given in the Table S1. The thermal decomposition of on respective Co(II) (2), Ni(II) (6) and Cu(II) (10) complexes takes place in three steps as indicated by DTG peaks around 105–135, 330–365 and 440–480°C of Co(II) and Ni(II) and around 220–230, 300–330 and 455–473°C of Cu(II) corresponding to the mass loss of two coordinated water molecules, one triazole moiety and two coumarin moieties respectively.

Electrochemical studies

Electrochemical properties of the complexes were studied on a CHI1110A-Electrochemical analyzer in *N,N*-dimethyl formamide (DMF) containing 0.05 M *n*-Bu₄NClO₄ as the supporting electrolyte. A cyclic voltammogram of Cu(II) (10) (Figure S1) radical displays a reduction peak at $E_{pc} = 0.1792V$ and again it reduced to Cu(I) displays a reduction peak at $E_{pc} = -0.3248V$ respectively, with a corresponding

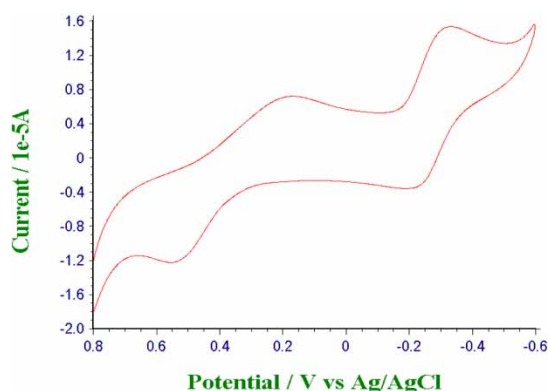


Figure S1. Cyclicvoltammogram of Cu(II) (10) complex.

oxidation peak (Cu(I) radical) at $E_{pa} = -0.2254V$ and $E_{pa} = 0.5451V$ for Cu(II) respectively. The peak separation (ΔE_p) of this couple is 0.33V and 0.191V respectively at scan rate 0.05V and increases with scan rate. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility of one electron transfer reaction. The analyses of cyclic voltametric responses with the scan rate varying 50 to 250 mV/s gives the evidence for quasi-reversible one electron oxidation state. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates. This establishes the electrode process as diffusion controlled [44].

The separation in peak potentials increases at higher scan rates. These characteristic features are consistent with the quasi-reversibility of Cu(II)/Cu(I) couple.

The Co(II) (2) complex (Figure S2) exhibits a reduction peak at $E_{pc} = 0.1592V$ with a direct re-oxidation peak at $E_{pa} = 0.5704V$ corresponding to the formation of Co(II)/Co(I) couple. The peak separation of this couple (ΔE_p) is 0.41V. The Ni(II) (6)

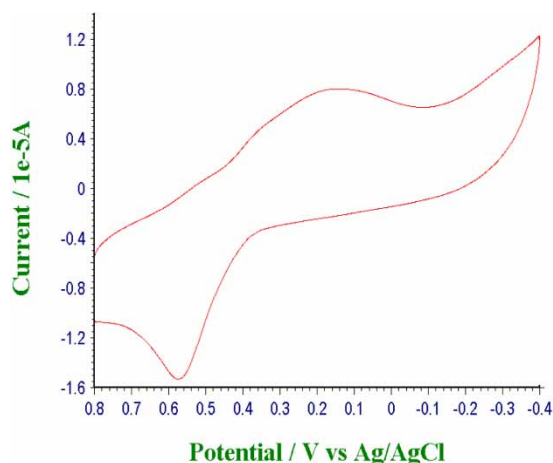


Figure S2. Cyclicvoltammogram of Co(II) (2) complex.

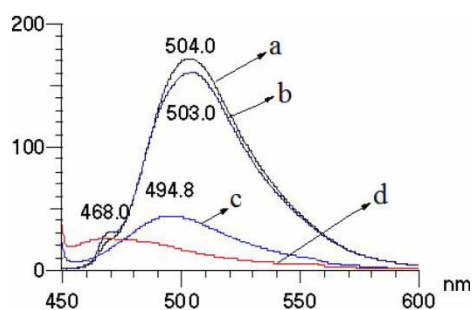


Figure S3. Emission spectrum of Schiff base (II) in DMF (a), DMSO (b), MeCN (c) and Dioxan (d).

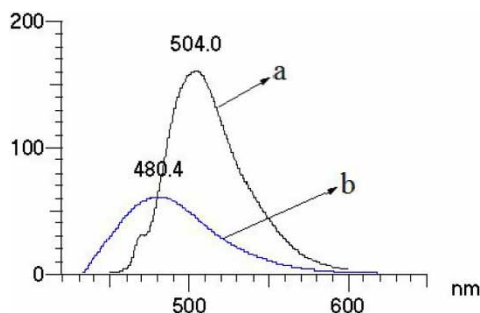


Figure S4. (a) Emission spectra of Schiff base in DMF. (b) Emission spectra (red shift) of Schiff base in DMF with 2% NaOH.

complex exhibits a reduction peak at $E_{pc} = -0.7895V$ with a direct re-oxidation peak at $E_{pa} = -0.6507V$ corresponding to the formation of Ni(II)/Ni(I) couple. This Co(II) and Ni(II) complex also have a quasi-reversible character as the separation in peak potential are higher than 59 mV and the peak currents rise with increasing v . The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility.

Fluorescence Studies

The emission spectra of the Schiff bases derived from 3-substituted-4-amino-5-hydrazino-1,2,4-triazole and 8-formyl-7-hydroxy-4-methyl coumarin and their

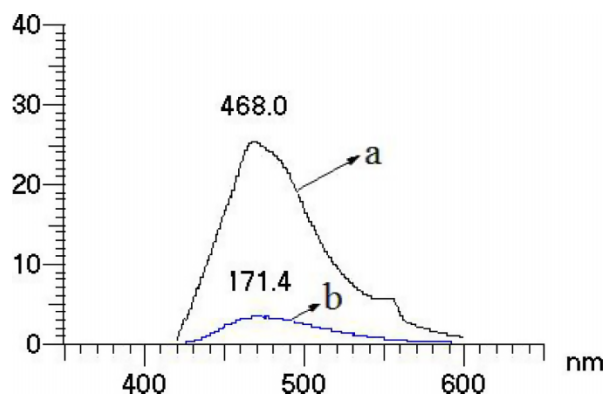


Figure S5. (a) Emission spectra of Schiff base in Dioxan. (b) Emission spectra (blue shift) of Schiff base in Dioxan with 2% NaOH.

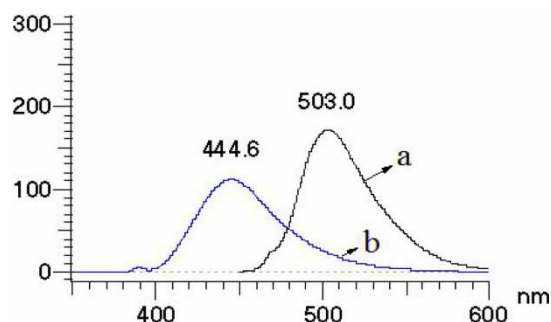


Figure S6. (a) Emission spectra of Schiff base in DMSO. (b) Emission spectra of Cu(II) complex in DMSO.

complexes were investigated in various solvents such as DMF, DMSO, MeCN and dioxan and the results are given in the Table V & VI. *Emission spectra of schiff base (II)*. The Schiff bases were characterized by an emission band around 503, 504, 494.8 and 468 nm in DMSO, DMF, MeCN and dioxan respectively (Figure S3) is due to the formation of phenoxide anion and cleavage of the imine bond is observed in the Schiff bases. Upon addition of aqueous alkali (2% NaOH) to all the above prepared solutions, we observed the band at 472, 480.4, 464 and 471.4 nm in DMSO, DMF (Figure S4), MeCN and Dioxan (Figure S3) solutions respectively (Table VI). The changes clearly indicate that, proton transferred (H-bonded ion pair) species exist in equilibrium [45] and also we observed the X_{max} of the Schiff bases undergoes red shift in DMSO, DMF, and MeCN and blue shift in Dioxan solutions is due to hydrogen bond formation [46].

Emission spectra of Co(II) (2), Ni(II) (6) and Cu(II) (10)

We have also studied the emission spectra of the Co(II), Ni(II) and Cu(II) complexes with 3-methyl-4-amino(8-formyl-7-hydroxy-4-methylcoumarin)-5-hydrazino-1,2,4-triazole (Table VII). The emission spectra of the Cu(II) in DMSO and Co(II), Ni(II) and Cu(II) complexes of dioxan solution is reproduced in Figure S4 and S5 respectively. In case of DMF solution the Co(II), Ni(II) and Cu(II) complexes were

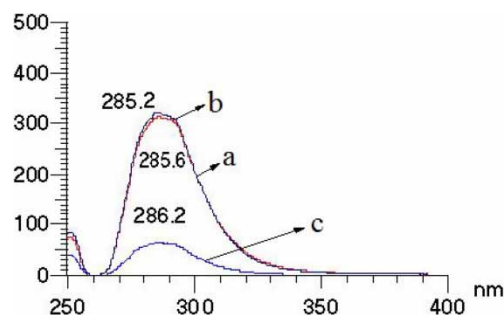


Figure S7. Emission spectra of Cu(II) (a), Co(II) (b) and Ni(II) (c) complexes in Dioxan.

characterized by the emission band around 470.8, 487.6 and 440.2 nm respectively. It is observed that, the emission band of Schiff bases around 504.0 nm disappeared because of the interaction of the phenolic oxygen with the metal ion. There was decrease in intensity of fluorescence of Co(II), Ni(II) and Cu(II) complexes in all prepared solutions. In all other

previous studies, it has been reported that, transition metal ions decrease the fluorescence quite effectively [47,48]. Magnetic perturbation, redox activity, etc., has been invoked [48] in the past to rationalize fluorescence quenching by transition metal ions. But, in case of Ni(II) complexes we could observed the enhancement of fluorescence in MeCN solution.