

## Synthesis, Physicochemical Investigation and Biological Studies of Zinc(II) Complexes with 1,2,4-Triazole Schiff Bases

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A series of metal complexes of Zn(II) have been synthesized with newly synthesized biologically active 1,2,4-triazole Schiff bases derived from the condensation of 3-substituted-4-amino-5-mercapto-1,2,4-triazole with 8-formyl-7-hydroxy-4-methylcoumarin. The structure of the complexes has been proposed in the light of elemental analyses, spectroscopic data (IR, UV-Vis, <sup>1</sup>H NMR and FAB-mass), thermal studies. Electrochemical study of the complexes is also reported. All the complexes are soluble to limited extent in common organic solvents but soluble to larger extent in DMF and DMSO and are non-electrolytes in these two solvent. All these Schiff bases and their complexes have also been screened for their antibacterial (*Escherichia coli*, *S. aureus*, *S. pyogenes*, *P. aeruginosa* and *Salmonella typhi*) and antifungal activities (*Aspergillus niger*, *Aspergillus flavus* and *cladosporium*) 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, Complexes

### INTRODUCTION

Coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities. The hydroxycoumarins are typical phenolic compounds and therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The coumarins display a remarkable array of biochemical and pharmacological actions, some of which suggest that, certain members of this group of compounds may significantly affect the function of various mammalian cellular systems [1].

It is well known that, N and S atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules. Metallo-organic chemistry is becoming an emerging area of research due to the demand for new metal-based antibacterial and antifungal compounds

[2,3]. The serious medical problem of bacterial and fungal resistance and the rate at which it develops has led to increasing levels of resistance to classical antibiotics [3-6]. The discovery and development of effective antibacterial and antifungal drugs with novel mechanism of action have become an urgent task for infectious diseases research programs [7]. Many investigations have proved that, binding of a drug to a metallic element enhances its activity and in some cases, the complex possesses even more healing properties than the parent drug [8]. Triazoles derivatives [9-12] are known to possess antibacterial, fungicidal, hypotensive and hypothermic activities. Metal complexes of 1,2,4-triazole derivatives have been extensively investigated and reported from our laboratory [13-17].

Jagannadha *et al.* have been synthesized and studied the synergistic behavior of the Schiff bases of 8-formyl-7-hydroxy-4-methyl-2H-1-benzopyran-2-one with aniline and p-methyl aniline and their interaction with Co(II), Ni(II), Cu(II) and Zn(II) have been also studied pH-metrically [18].

A survey of the literature reveals that, no work has

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been carried out on the synthesis of metal complexes with Schiff bases derived from 3-substituted-4-amino-5-mercapto-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin. These Schiff bases have donor sites with the OONS sequence and varied coordination abilities. This interesting nature of the Schiff bases has attracted our attention and aroused our interest in elucidating the structure of Zn(II) complexes with these bioactive Schiff bases (Fig. 1) on the basis of spectral, thermal, molar conductivities and these are also evaluated for their antibacterial and antifungal properties against various pathogenic bacterial strains using the minimum inhibitory concentration method.

## EXPERIMENTAL

### Materials

All chemicals and solvents used were of AR grade. Chlorides salts of metal(II) two ions and other pure reagents were purchased from Ranbaxy chemicals. 7-Hydroxy-4-methyl-coumarin was obtained from Acros Chemical Company. A series of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles was prepared as described in the literature [19-21].

### Synthesis of Schiff Bases [I-IV]

A mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazole and 8-formyl-7-hydroxy-4-methyl coumarin in 1:1 molar proportion in an alcoholic medium containing few drops of concentrated HCl was refluxed for 3-4 h [22]. The product separated is filtered, washed with alcohol and was recrystallized from EtOH.

### Synthesis of Zn(II) Complexes [1-4]

An alcoholic solution of Schiff base (I-IV) (1 mmol) was refluxed with 1 m mol of  $\text{ZnCl}_2 \cdot 2\text{H}_2\text{O}$  in ethanol on steam bath for 1 h. Then, to the reaction mixture 1 mmol of sodium acetate was added and was further refluxed for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol, ether and finally dried in vacuum over fused  $\text{CaCl}_2$ .

### Apparatus and Methods

The IR spectra of the Schiff bases and their Zn(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the  $4000\text{--}250\text{ cm}^{-1}$  region in KBr or CsI disks. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-

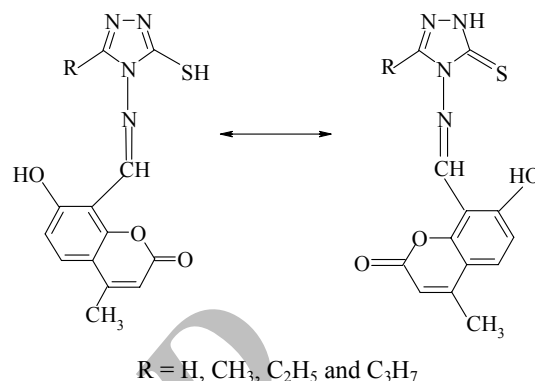


Fig. 1. Tautomeric structures of Schiff base.

spectrophotometer in the region of 200-1100 nm. The  $^1\text{H}$  NMR spectra of ligands were recorded in  $\text{CDCl}_3$  on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10 Am) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature and m-nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the +ve ion mode. The electrochemical studies of Zn(II) complexes were carried out on a CHI1110A-electrochemical analyzer (HCH Instruments, USA). Thermogravimetric analyses data were obtained from room temperature to  $1000\text{ }^\circ\text{C}$  at a heating rate of  $10\text{ }^\circ\text{C min}^{-1}$ , using a PERKIN-ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements were performed on an ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51. The metal contents were determined gravimetrically by a known method [23]. The results of elemental analyses, molar conductance and magnetic data are listed in Table 1.

## RESULTS AND DISCUSSION

All the Zn(II) complexes were stable in room temperature, non-hygroscopic, insoluble in water and many common organic solvents, infusible at high temperature and all were as polymeric in nature. The elemental analyses shown in Table 1 agree well with the formation of 1:1 stoichiometry of the  $\text{ML}_2 \cdot 2\text{H}_2\text{O}$  type. All the complexes are soluble in DMF and DMSO. The molar conductance values are too low to account for any dissociation in DMF indicating that, complexes are non electrolytic in nature.

**Table 1.** Elemental Analysis of Zn(II) Complexes and Their Molar Conductance Data

Comp. No.	Empirical formula	%M		%C		%N		%S		Molar conductance (Ohm <sup>-1</sup> cm <sup>-2</sup> mol <sup>-1</sup> )
		Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	
1	Zn(C <sub>13</sub> H <sub>8</sub> N <sub>4</sub> O <sub>3</sub> S).2H <sub>2</sub> O	16.19	16.21	38.87	38.90	13.94	13.96	7.95	7.98	28.14
2	Zn(C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> O <sub>3</sub> S).2H <sub>2</sub> O	15.61	15.66	40.44	40.48	13.45	13.49	7.68	7.71	26.12
3	Zn(C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>3</sub> S).2H <sub>2</sub> O	15.11	15.15	41.92	41.95	13.01	13.05	7.44	7.46	25.34
4	Zn(C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> S).2H <sub>2</sub> O	14.65	14.67	43.31	43.34	12.61	12.64	7.20	7.22	22.28

**Table 2.** Important Infrared Frequencies (in cm<sup>-1</sup>) of 3-Substituted-4-amino(8-formyl-7-hydroxy-4-methylcoumarin)-5-mercapto-1,2,4-triazole Schiff Bases

Ligand No.	$\nu(\text{NH})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	H-bonded -OH Stretching	$\nu(\text{C=C})$	$\nu(\text{SH})$	$\nu(\text{C-O})$ phenolic
I	3140	1700	1629	3260	1590	2730	1279
II	3147	1707	1631	3220	1600	2720	1285
III	3138	1705	1630	3250	1595	2725	1292
IV	3135	1710	1628	3265	1597	2720	1294

**Table 3.** Important Infrared Frequencies (in cm<sup>-1</sup>) of Zn(II) Complexes of 3-Substituted-4-amino (8-formyl-7-hydroxy-4-methylcoumarin)-5-mercapto-1,2,4-triazole Schiff Bases

Complex No.	$\nu(\text{OH})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{C-O})$ phenolic	$\nu(\text{M-N})$	$\nu(\text{M-S})$	$\nu(\text{M-O})$
1	3415	1685	1618	1345	447	365	376
2	3430	1683	1620	1348	463	367	375
3	3418	1680	1621	1349	448	364	379
4	3426	1689	1618	1347	476	368	384

## Infrared Spectra

The selected infrared spectra of the Schiff bases and their metal complexes along with their tentative assignments are reported in Tables 2 and 3. The infrared spectra of the Schiff bases show characteristic bands due to  $\nu(\text{NH})$  and  $\nu(\text{SH})$  around 3145 and 2700 cm<sup>-1</sup>, respectively [24]. Another band around 1100 cm<sup>-1</sup> is assigned to  $\nu(\text{C=S})$  [24]. These observations suggest that the Schiff bases exhibit thiol-thione tautomerism. The broad band at 3220-3270 cm<sup>-1</sup> and strong bands at 1705-1715, 1630-1625 and 1285 cm<sup>-1</sup> in the infrared spectra of the Schiff bases are assigned to H-bonded -OH stretching,  $\nu(\text{C=O})$  lactonic carbonyl [25],  $\nu(\text{C=N})$  and

phenolic  $\nu(\text{C-O})$  vibrations, respectively. The medium intensity band in the region 770-760 cm<sup>-1</sup> has been attributed to  $\nu(\text{C=S})$ . A medium band around 1055 cm<sup>-1</sup> is characterized for  $\nu(\text{O-C-O})$  corresponding to cyclic grouping.

In comparison with the spectra of the Schiff bases, all the complexes exhibit a downward shift (10-20 cm<sup>-1</sup>) of  $\nu(\text{C=N})$  indicating the participation of azomethine nitrogen in the coordination to the metal ion.

The high intensity band due to phenolic C-O appeared in the region 1285 cm<sup>-1</sup> in the Schiff bases appeared as a medium to high intensity band in the 1350 cm<sup>-1</sup> region in the

complexes. These observations support the formation of M-O bonds *via* deprotonation. So the H-bonded -OH groups have been replaced by the metal ion.

The deprotonation of the thiol group is indicated by the absence of a band in the metal complexes at  $2700\text{ cm}^{-1}$ , which is due to  $\nu(\text{S-H})$  of Schiff bases, indicating that the metal is also coordinated through sulfur atom. This is supported by the lower frequency shift appears around  $685\text{--}670\text{ cm}^{-1}$  in the metal complexes due to  $\nu(\text{C-S})$ .

The presence of coordinated water molecules in the complexes [24] are indicated by a broad band in the region  $3200\text{--}3500\text{ cm}^{-1}$  and two weaker bands in the region  $750\text{--}800$  and  $700\text{--}720\text{ cm}^{-1}$  due to  $\nu(\text{-OH})$  rocking and wagging mode of vibrations, respectively [26].

An interesting feature observed is the red shift in lactone  $\nu(\text{C=O})$  to the extent of about  $15\text{--}20\text{ cm}^{-1}$ , suggesting that, the metal is coordinated to the lactone oxygen [27]. This further supported by downward shift in  $\nu(\text{O-C-O})$  of the pyrrol ring. On the basis of IR data, it is concluded that, all the metal ions are coordinated to the azomethine nitrogen, phenolic oxygen, sulphur atom and lactone oxygen.

The new bands in  $375\text{--}350$  and  $450\text{--}480\text{ cm}^{-1}$  region in all the complexes are assigned to stretching frequencies of (M-O) and (M-N) bonds, respectively. The band in the region  $333\text{--}379\text{ cm}^{-1}$  of far IR-spectra is due to Metal-sulfur bond formation.

Thus the IR spectral data results provide strong evidences for the complexation of the potentially multidentate Schiff bases.

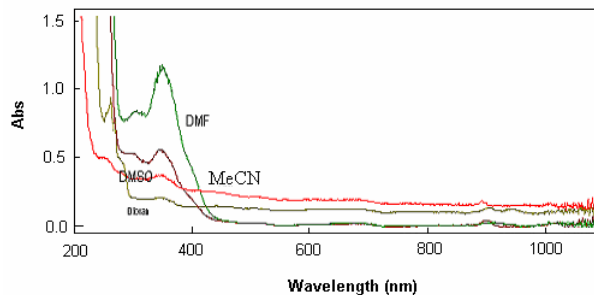
### <sup>1</sup>H NMR Spectral Studies

The <sup>1</sup>H 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. In addition to these signals, a sharp signal at 3.5 ppm is attributed to SH protons. These observations suggest that the Schiff bases exist in thiol-thione tautomerism. The signals around 2.84 ppm are due to methyl protons.

In the case of Zn(II) complex, the signal of azomethine protons of metal complexes was found at 9.12 ppm after complexations to the metal ion inferring co-ordination through the azomethine nitrogen atom of the ligands [28]. Disappearance of -SH protons in the spectra of complexes support the deprotonation of the thiol group.

### Electronic and Fluorescence Spectral Studies

The UV-Vis spectra of Zn(II) complex in different

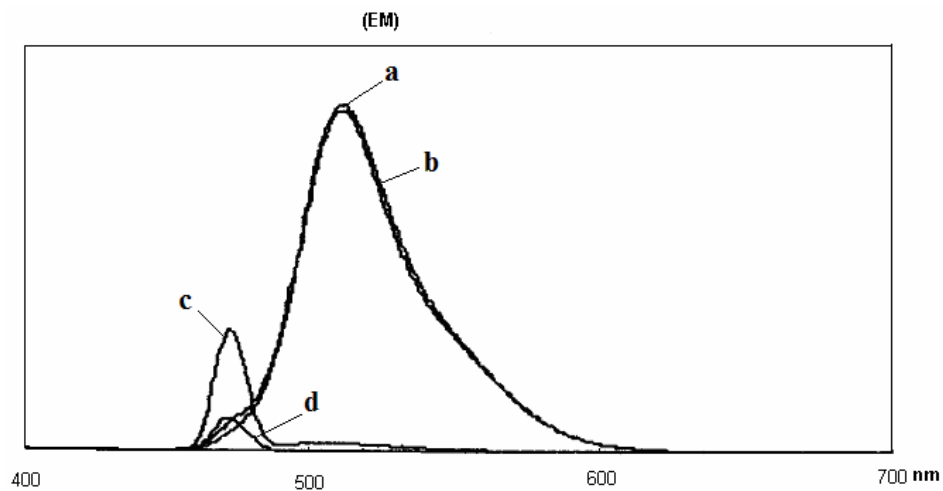


**Fig. 2.** UV-Vis spectra of Zn(II) complex in different solvents.

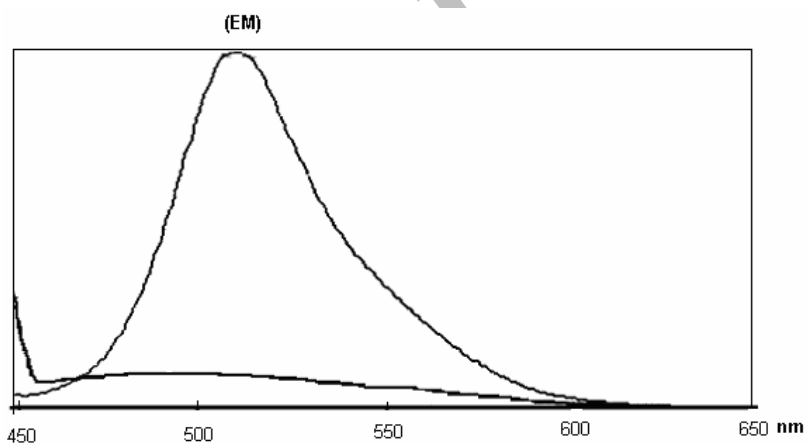
solvents are shown in Fig. 2. Maxima at 250 nm and 275 nm in the case of the ligand are due to  $\pi\text{--}\pi^*$  transitions. These bands are almost unchanged in the spectra of the complex. The ligand also shows a broad band at 335 nm with a shoulder at lower energy, due to the  $n\text{--}\pi^*$  transition associated with the azomethine linkage. This band in the complex has shown a bathochromic shift due to the donation of a lone pair of electrons to the metal and hence the coordination of azomethine [29,30].

The moderately intense broad band for the complex in the region  $350\text{--}390\text{ nm}$  is assigned to  $\text{S} \rightarrow \text{Zn(II)}$  ligand to metal charge transfer transition (LMCT). The LMCT band of the phenolate complex shows line broadening, with a tail running into the visible part of the spectrum. This may result from a phenolate to Zn(II) LMCT band being superimposed on the low energy side of  $\text{S} \rightarrow \text{Zn(II)}$  LMCT. The complex shows no appreciable absorptions in the region above 400 nm in all solvents, in accord with the  $d^{10}$  electronic configuration of the Zn(II) ion.

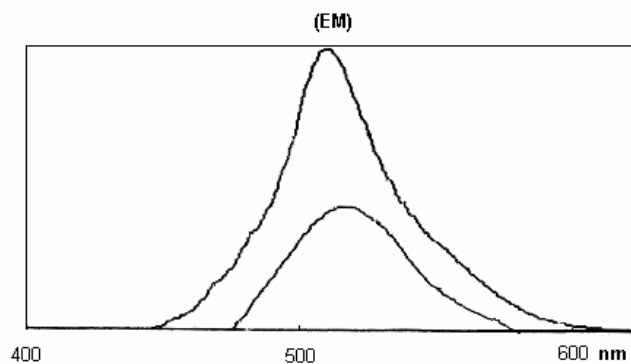
Fluorescence spectra of the Schiff base (II) and its Zn(II) complex are shown in Figs. 3–6. A strong emission band at 504 nm is observed, when excited with 350 nm radiation, at room temperature with sample concentration of 0.1 mM in DMF (Fig. 3). The free ligand shows a diminished fluorescent intensity upon complexation with diamagnetic zinc ion (Fig. 5). The emission is neither MLCT (metal-to-ligand charge transfer) nor LMCT in nature. We tentatively assign it to the intraligand ( $\pi\text{--}\pi^*$ ) fluorescence, since a similar emission is also observed for the free ligand, but with reduced intensity. It is known that electron lone pairs of nitrogen can quench the fluorescence of the moiety through photo-induced electron transfer [31–34]. Draining out of these pairs of electrons on to the metal orbital *via* complex formation causes a suppression of this fluorescence



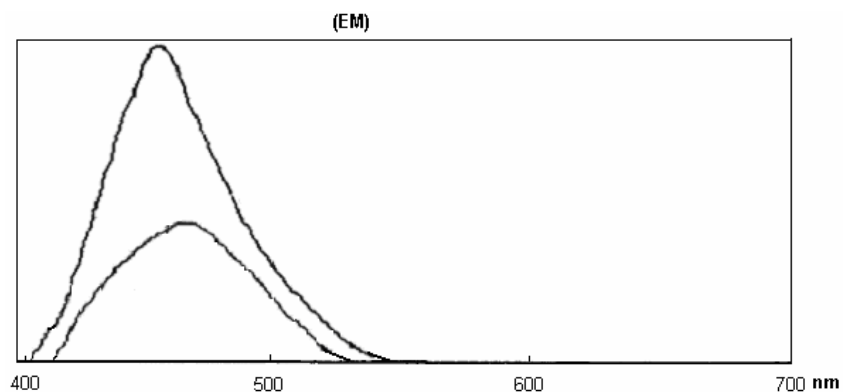
**Fig. 3.** Emission spectra of Schiff base (II) in DMF (a), DMSO (b), MeCN (c) and Dioxan (d).



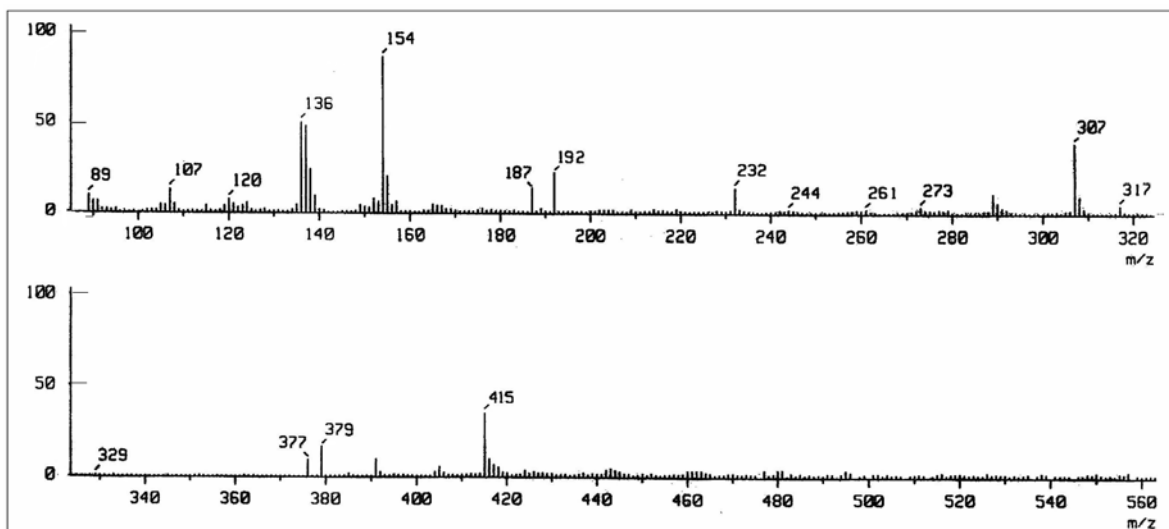
**Fig. 4.** Emission spectra of Schiff base (II) in pure DMF and in DMF with 2% NaOH (red shift).



**Fig. 5.** Emission spectra of Schiff base (II) and its Zn(II) complex (blue shifted) in DMF.



**Fig. 6.** Emission spectra of Schiff base (II) and its Zn(II) complex (blue shifted) in MeCN.



**Fig. 7.** FAB-mass spectrum of Schiff base (II)-Zn(II) complex.

quenching and therefore results in increase in fluorescence intensity [33]. The formation of metal chelates with metal ions, in general, also promotes fluorescence by increasing rigidity and minimizing internal vibrations. At higher concentration (1 mM), the fluorescence intensity decreases considerably due to self-quenching.

#### FAB-Mass Spectral Studies

The FAB mass spectrum of Schiff base (II) showed a molecular ion peak at  $m/z$  316 which is equivalent to its molecular weight. The fragments in the spectrum leads to the formation of the species  $[L+H]^+$ .

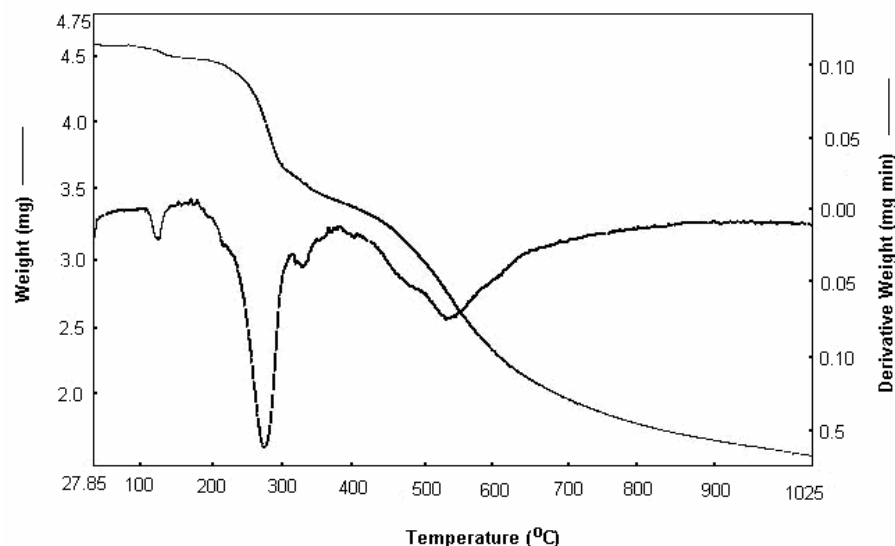
The FAB-mass spectrum of Zn(II) complex (2) has been depicted and reproduced in Fig. 7. The spectrum showed a

molecular ion peak  $M^+$  at  $m/z$  415 that is equivalent to its molecular weight  $[ML.2H_2O]^+$ . The molecular ion by the loss of two water molecules gave a fragment ion peak at  $m/z$  379.

All these fragments leading to the formation of the species  $[ML]^+$  which undergoes demetallation to form the species  $[L+H]^+$  at  $m/z$  314.

#### Thermogravimetric Study

The thermal behavior of all the complexes is almost the same. Hence, only the thermogravimetric studies of Zn(II)-Schiff base (II) complex were discussed (Fig. 8). As seen, the Zn(II)-Schiff base (II) complex decomposes gradually with the formation of metal oxide above 530 °C. The nature



**Fig. 8.** Thermogravimetric TGA/DTG curves of Zn(II) complex.

**Table 4.** Thermogravimetric Data of Zn(II) (2) Complexes of 3-Substituted-4-amino(8-formyl-7-hydroxy-4-methylcoumarin)-5-mercapto-1,2,4-triazole Schiff Base

Empirical formula	Decomposition temperature (°C)	%Weight loss		%Metal oxide		Inference
		Obsd.	Calcd.	Obsd.	Calcd.	
Zn(C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> O <sub>3</sub> S).2H <sub>2</sub> O	105-130	8.64	8.67	19.48	19.51	Loss of coordinated water molecules
	245-285	30.54	30.60			Loss of triazole moieties
	510-545	45.00	45.06			Loss of coumarin moieties

of proposed chemical change with the temperature range and the percentage of metal oxide obtained are given in Table 4.

The thermal decomposition of Zn(II) complexes takes place in three steps as indicated by DTG peaks around 130-170, 290-330 and 480-510 °C corresponding to the mass loss of two coordinated water molecules, triazole moiety and coumarin moiety, respectively.

The Freeman and Corroll procedure [35] was used to evaluate the kinetic parameters for the composition of Zn(II)-Schiff base (II) complex (*i.e.*, order of reaction,  $n$ , and energy of activation,  $E_a$ ) using a single experimental curve from the plot of  $\Delta \log(dw/dt)/\Delta \log W_r$  vs.  $1/\Delta \log T \cdot \Delta \log W_r$  (Fig. 9). The determined kinetic parameters are listed in Table 5.

## Electrochemistry

Electrochemical properties of the complexes were studied in DMF containing 0.05 M n-Bu<sub>4</sub>NClO<sub>4</sub> as the supporting electrolyte. A cyclic voltammogram of Zn(II) (Fig. 10) displays a reduction peak at -2.3214 V with a corresponding oxidation peak for Zn(I) at -1.9254 V. The peak separation of this couple ( $\Delta E_p$ ) is 0.396 V at scan rate of 50 mV s<sup>-1</sup> and increases with scan rate. The most significant feature of the Zn(II) complex is the Zn(II)/Zn(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 voltammetric responses with scan rate varying from 50 to 250 mV s<sup>-1</sup> gives the evidence for quasi-reversible one electron oxidation state. The ratio of cathodic

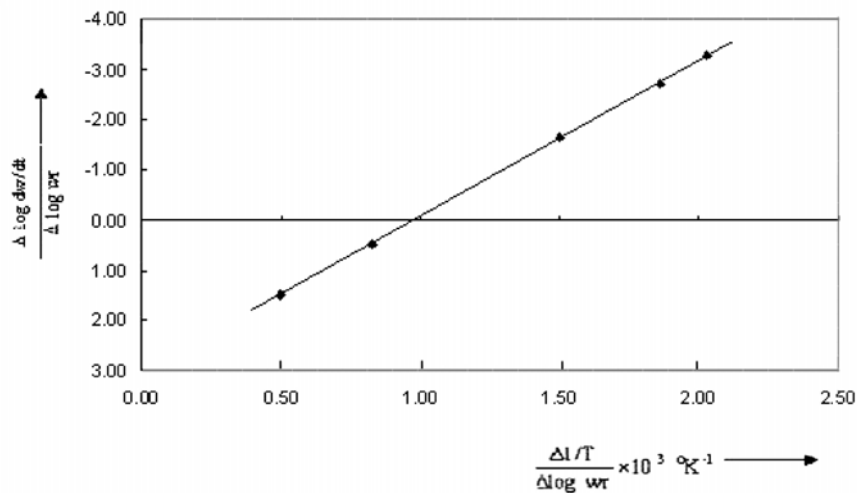


Fig. 9. Kinetics of thermal decomposition of Zn(II) complex.

Table 5. Kinetic Parameters for Thermal Decomposition of Zn(II) Complex

Empirical formula	$\Delta \log(dw/dt)/\Delta \log W_r$	$10^3/\Delta \log T \cdot \Delta \log W_r$ (K <sup>-1</sup> )	<i>n</i>	<i>E<sub>a</sub></i> (kcal mol <sup>-1</sup> )
Zn(C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> O <sub>3</sub> S).2H <sub>2</sub> O	-3.28	2.026	0.98	14.43
	-2.72	1.859		
	-1.64	1.501		
	0.48	0.827		
	1.49	0.498		

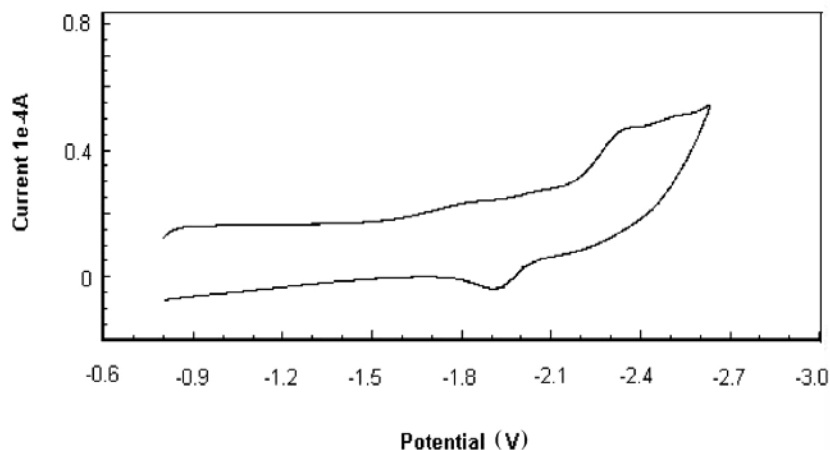


Fig. 10. Cyclic voltammogram of Zn(II) complex.

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 [36].

### Biological Activities

***In vitro* antibacterial and antifungal assay.** All the synthesized Schiff bases (I-IV) and their corresponding Zn(II) complexes were screened *in vitro* for their biological



activity by using five bacteria, namely *E. coli*, *S. aureus*, *S. pyogenes*, *P. aeruginosa* and *Salmonella typhi* and three fungi namely *A. niger*, *A. flavus* and *Cladosporium* by the reported method [37]. The stock solution ( $1 \text{ mg ml}^{-1}$ ) of the test chemical was prepared by dissolving 10 mg of the test compound in 10 ml of DMF. The stock solution was suitably diluted with sterilized distilled water to get dilutions of 100, 50 and  $25 \text{ } \mu\text{g ml}^{-1}$ . Control for each dilution was prepared by diluting 10 ml of solvent instead of stock solution with sterilized distilled water.

The bacteria were sub-cultured in agar medium. The petridishes were incubated for 24 h at  $37^\circ\text{C}$ . 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  $37^\circ\text{C}$ . 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 used.

**Minimum inhibitory concentration (MIC).** Compounds showing promising antibacterial/antifungal activity were selected for minimum inhibitory concentration studies. The minimum inhibitory concentration was determined by assaying at 100, 50 and  $25 \text{ } \mu\text{g ml}^{-1}$  concentrations along with standards at the same concentrations.

**In vitro antibacterial and antifungal results.** The microbial results are systematized in Tables 6 and 7. The antibacterial and antifungal studies suggested that all the Schiff bases are biologically active and their Zn(II) complexes showed significantly enhanced antibacterial and antifungal activities. It is, however, known [38,39] 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 presence of metal ions) may be the possible reasons for the increased activity.

In the case of bacteriological studies, it was observed

that the ligand shows a highly activity toward *E. coli*, *S. typhi* and *S. pyogenes* and moderately active towards *P. aeruginosa* and *S. aureus*. The complexes (2) and (4) show highly activity towards *E. coli*, *S. aureus* and *S. pyogenes*. The other complexes are moderately active or some inactive towards *S. pyogenes* and *P. aeruginosa* at minimum concentration.

In the case of antifungal activity, it was observed that the ligand show moderate activities against *A. flavus*, *A. niger* and high activity towards *Cladosporium*. In the case of complexes, the complexes (2) and (3) show high activity against *A. niger*, *Cladosporium* and *A. flavus*. in case of *A. flavus* and *A. niger* some complexes are inactive at minimum concentration.

**In vitro cytotoxicity.** The synthesized Schiff bases and their Zn(II) complexes were screened for their cytotoxicity (brine shrimp bioassay) using protocol of Meyar *et al.* [40]. Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish ( $22 \times 32 \text{ cm}$ ) 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  $10 \text{ } \mu\text{g ml}^{-1}$  were transferred to 9 vials (three for each dilutions were used for each test sample and  $\text{LD}_{50}$  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  $\text{LD}_{50}$  values [41].

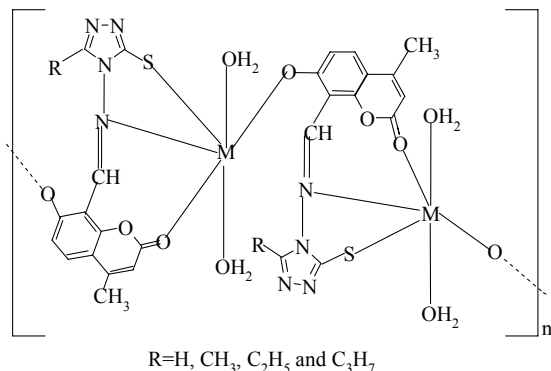
All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay). From the data recorded in Table 8, it was observed that the compounds (II), (IV) and complex (2) displayed potent cytotoxic activities as  $\text{LD}_{50} = 7.439 \times 10^{-4}$ ,  $8.974 \times 10^{-4}$  and  $8.945 \times 10^{-4} \text{ M ml}^{-1}$ , respectively, against *Artemia salina*, while all other compounds were almost inactive for this assay.

**Table 6.** Anti Bacterial and anti Fungal Results of Schiff Bases (I-IV)

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>P. aeruginosa</i>	<i>S. typhi</i>	<i>A. flavus</i>	<i>Cladosporium</i>	<i>A. niger</i>
I	100	57	50	52	55	69	52	65	53
	50	47	-	39	49	57	-	61	-
	25	51	-	41	50	55	-	59	-
II	100	58	52	58	59	72	60	72	62
	50	52	43	47	47	62	49	62	58
	25	47	42	45	52	61	-	59	-
III	100	61	55	64	52	76	70	75	70
	50	58	42	57	47	67	65	67	63
	25	53	-	55	-	63	62	68	60
IV	100	62	59	67	64	79	74	79	67
	50	60	54	60	59	71	69	73	62
	25	59	49	55	57	65	68	74	57
Standard	100	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100	100

**Table 7.** Anti Bacterial and anti Fungal Results Zn(II) Complexes (1-4) 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>P. aeruginosa</i>	<i>S. typhi</i>	<i>A. flavus</i>	<i>Cladosporium</i>	<i>A. niger</i>
1	100	60	55	61	58	70	58	67	59
	50	55	42	50	50	64	49	63	51
	25	48	39	47	49	66	-	63	-
2	100	68	60	71	77	79	77	84	72
	50	63	54	64	68	62	70	77	67
	25	58	49	60	70	61	69	80	65
3	100	76	70	59	58	80	64	81	70
	50	67	65	-	-	69	57	73	61
	25	68	66	-	-	70	51	71	52
4	100	73	75	69	60	83	66	80	66
	50	67	70	58	52	71	47	78	52
	25	60	67	49	50	70	-	73	-
Standard	100	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100	100



**Fig. 11.** Octahedral structure of Zn(II) complexes.

## CONCLUSIONS

The synthesized 3-substituted-4-amino(8-formyl-7-hydroxy-4-methylcoumarin)-5-mercapto-1,2,4-triazole Schiff bases act as tetradentate Schiff bases. The metals are coordinated to azomethine nitrogen, lactonyl oxygen, phenolic oxygen and sulfur atom. The analytical, IR, UV-Vis,  $^1H$  NMR, FAB-mass and thermal studies confirm the bonding of Schiff bases to metal ions. Electrochemical study of Zn(II) complex can provide the degree of the reversibility of one electron transfer reaction and they have a quasi-reversible character. All Schiff bases found to be potentially active towards all microbial strains. The complexes (2) and (4) showed high activities against all bacterial strains and the complexes (2) and (3) showed promising results against all fungal strains.

All the complexes are insoluble in water and fusible at higher temperatures and are polymeric in nature [42,43].

All these observations put together lead us to propose the structure shown in Fig. 11 for the resulting  $ML_2H_2O$  complex.

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