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Original article

Synthesis, spectral characterization, in vitro antibacterial, antifungal and cytotoxic activities 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 synthesized biologically active 1,2,4-triazole Schiff bases derived from the condensation of 3-substituted-4-amino-5-mercapto-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin, which have been characterized by elemental analyses, spectroscopic measurements (IR, UV–vis, fluorescence, ESR), magnetic measurements and 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 DMF and DMSO. All these Schiff bases and their complexes have also been screened for their antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas 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

1. Introduction

Coumarins have long been recognized to possess anti-inflammatory [1], antioxidant [2], antithrombotic [3], antiallergic [4], hepatoprotective [4], antiviral [2] and anticarcinogenic [4] activities. The hydroxycoumarins are typical phenolic compounds and therefore act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants [5]. The coumarins display a remarkable array of biochemical and pharmacological actions [1–4]; the antitumor effects of coumarin and its major metabolite, 7-hydroxycoumarin, were tested in several human tumor cell lines [6]. Furthermore, cytotoxic effects of complexes of coumarin derivatives were examined on several neuronal cell lines [7].

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 [8,9]. The serious medical problem [9–12] of bacterial and fungal resistance and the rate at which it develops have led to increasing levels of resistance to classical antibiotics. 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 [13]. Many investigations have proved that binding of a drug to a metalloelement enhances its activity and in some cases, the complex possesses even more healing properties than the parent drug [14]. Triazole derivatives [15–18] are known to possess antibacterial, fungicidal, hypotensive and hypothermic activities. Metal complexes of 1,2,4-triazole derivatives have been extensively

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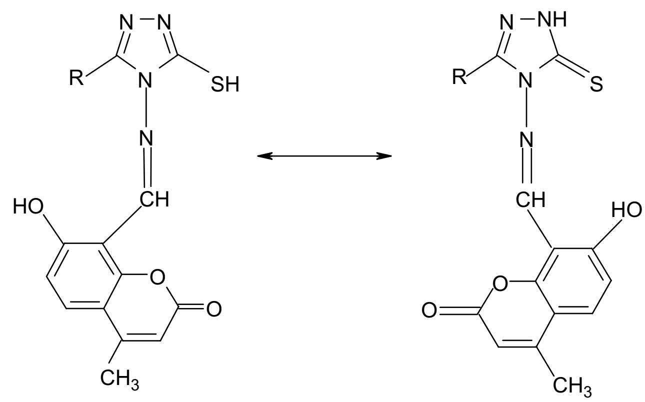

Fig. 1. Synthesized Schiff bases in tautomeric form.

investigated and reported from our laboratory [19–23]. Recently, a number of attempts have been made to obtain Co(II), Ni(II), Cu(II), Zn(II) complexes with the Schiff bases derived from cinnamaldehyde and 4-amino-3-ethyl-5-mercapto-*s*-triazole [24], and furfuraldehyde and 3-substituted-4-amino-5-mercapto-*s*-triazole [25]. These complexes have been isolated and characterized by elemental analysis, magnetic, spectral (IR, UV–vis, ^{1}H NMR, EPR) and thermal studies.

A survey of the literature reveals that no work has 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 nature of the Schiff bases (Fig. 1) has attracted our attention and aroused our interest in elucidating the structure of Co(II), Ni(II) and Cu(II) complexes. These are evaluated for their antibacterial and antifungal properties against various pathogenic bacterial strains using the minimum inhibitory concentration method.

2. Chemistry

2.1. Methods

2.1.1. Synthesis of Schiff bases [I–IV]

A series of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles were synthesized by reported methods [16,26,27]. A mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin [28] in 1:1 molar proportion in an alcoholic medium containing few drops of concentrated HCl was refluxed for 3–4 h. The product separated is filtered, washed with alcohol and recrystallized from EtOH.

2.1.2. Synthesis of Co(II), Ni(II) and Cu(II) complexes [1–12]

An alcoholic solution (25 ml) of Schiff base (**I–IV**) (1 mmol) was refluxed with 1 mmol of $CoCl_2 \cdot 6H_2O$/$NiCl_2 \cdot 6H_2O$/$CuCl_2 \cdot 2H_2O$ in 25 ml ethanol on steam bath for 1 h. Then, to the reaction mixture 2 mol of sodium acetate was added and reflux was continued for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol and ether and finally dried in vacuum over fused $CaCl_2$.

3. Pharmacology

3.1. In vitro antibacterial and antifungal assay

All the synthesized Schiff bases (**I–IV**) and their corresponding Co(II), Ni(II) and Cu(II) complexes (**1–12**) were screened in vitro for their biological activity by using five bacteria, namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Salmonella typhi* and three fungi, namely *Aspergillus niger*, *Aspergillus flavus* and *Cladosporium* by the reported method [29]. The stock solution (1 mg ml^{-1}) 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

Table 1
Antibacterial and antifungal results of Schiff bases (**I–IV**)

Compound	Conc. (μg ml^{-1})	Antibacterial activity (zone of inhibition in %)					Antifungal activity (zone of inhibition in %)		
		E. coli	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*S. typhi*	*A. flavus*	*Cladosporium*	*A. niger*
I	100	57	51	52	55	69	52	65	53
	50	47	39	39	49	57	–	61	–
	25	51	41	41	50	55	–	59	–
II	100	58	52	58	59	72	–	72	78
	50	52	43	47	47	62	–	62	68
	25	47	42	45	52	61	–	59	57
III	100	61	55	64	62	76	70	75	70
	50	58	42	57	57	67	65	67	63
	25	53	35	55	58	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 2
Antibacterial and antifungal results of Co(II), Ni(II) and Cu(II) complexes (**1**–**12**) and standard

Compound	Conc. ($\mu g\ ml^{-1}$)	Antibacterial activity (zone of inhibition in %)					Antifungal activity (zone of inhibition in %)		
		E. coli	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*S. typhi*	*A. flavus*	*Cladosporium*	*A. niger*
1	100	58	55	61	58	70	58	67	59
	50	45	42	50	50	64	49	63	51
	25	48	39	47	49	66	47	63	48
2	100	67	–	69	63	75	60	–	69
	50	62	–	57	54	60	52	–	54
	25	60	–	52	55	62	50	–	48
3	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
4	100	68	61	73	80	80	80	95	78
	50	63	55	67	69	70	71	80	68
	25	60	47	66	72	74	67	83	57
5	100	64	63	50	52	71	52	78	73
	50	58	59	–	–	60	–	72	67
	25	52	53	–	–	59	–	71	59
6	100	67	69	55	55	78	58	85	75
	50	60	62	48	44	68	52	76	70
	25	59	62	41	39	68	47	73	61
7	100	71	70	59	58	80	64	81	70
	50	67	65	49	45	69	57	73	61
	25	68	66	43	46	70	51	71	52
8	100	74	71	–	60	83	66	80	66
	50	70	62	–	52	71	57	78	52
	25	67	60	–	50	70	52	73	48
9	100	58	59	52	49	68	51	60	68
	50	47	53	–	–	59	–	52	60
	25	46	52	–	–	54	–	51	59
10	100	59	–	59	–	72	54	58	70
	50	50	–	43	–	61	47	45	63
	25	49	–	41	–	57	41	42	60
11	100	68	70	63	62	76	58	69	68
	50	62	63	54	51	64	51	58	58
	25	59	61	52	46	60	48	52	51
12	100	71	73	64	65	80	–	79	62
	50	67	65	54	52	71	–	70	54
	25	65	64	53	48	65	–	75	50
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

suitably diluted with sterilized distilled water to get dilution of 100, 50 and 25 $\mu g\ ml^{-1}$. 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 Petri dishes 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 Petri dishes 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.

3.1.1. 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 $\mu g\ ml^{-1}$ concentrations along with standards at the same concentrations.

3.1.2. Pharmacology results

The microbial results are systematized in Tables 1 and 2 and Figs. 2 and 3. 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 activities. It is, however, known [30,31] 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

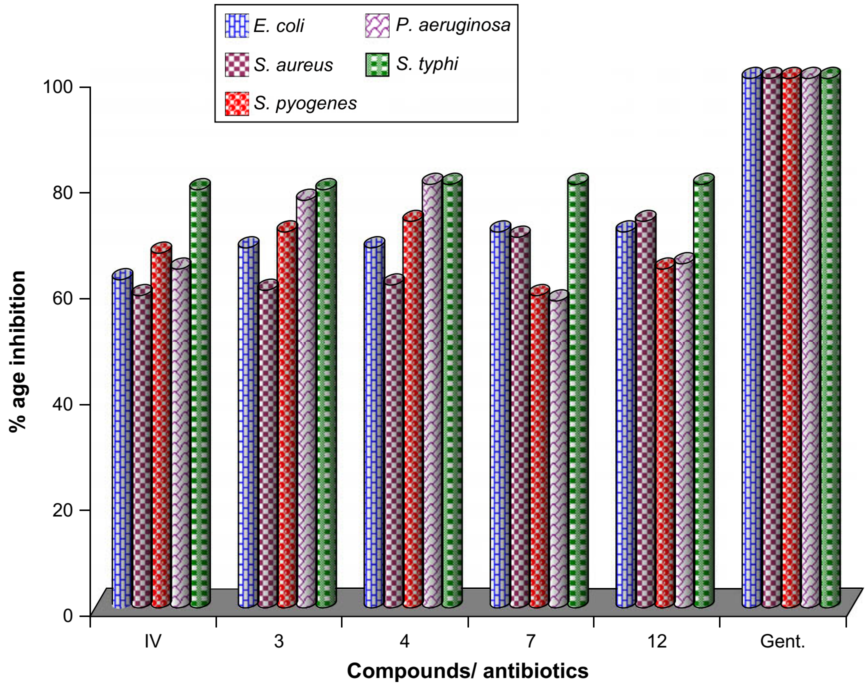

Fig. 2. In vitro antibacterial spectrum of compounds **IV**, **3**, **4**, **7**, **12**, and gentamycine (Std) at 100 μg ml^{-1} concentration.

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

In the case of bacteriological studies it was observed that some of the Schiff bases were found potentially active against all bacterial strains. 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, especially with *S. typhi*. Among these metal complexes compounds **3**, **4**, **7** and **12** show high activity against *S. typhi*.

In the case of antifungal activity, the results were compared with the standard drug (fluconazole). All Schiff bases showed activity against fungal species. 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

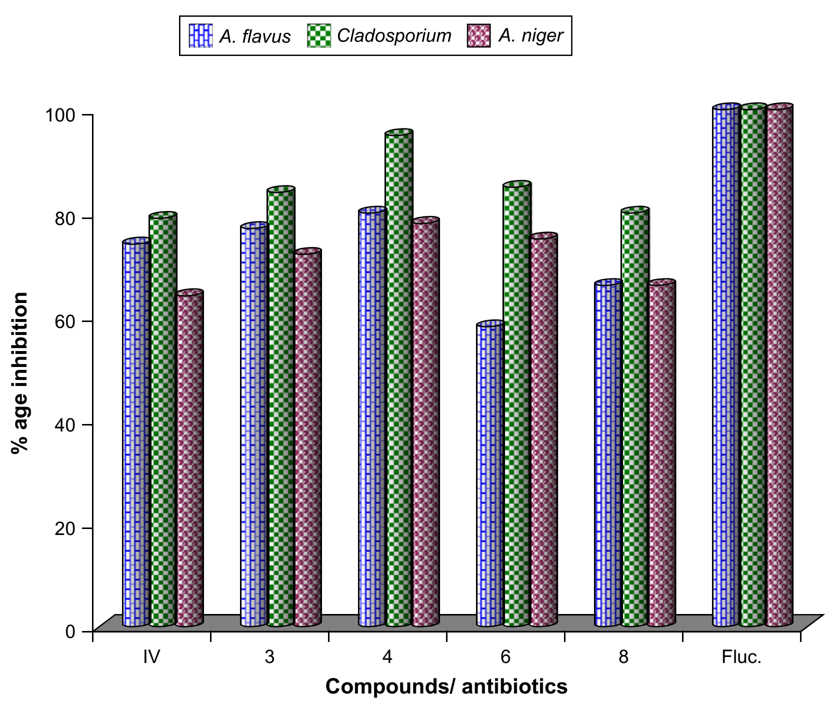

Fig. 3. In vitro antifungal spectrum of compounds **IV**, **3**, **4**, **6**, **8**, and fluconazole (Std) at 100 μg ml^{-1} concentration.

Table 3
Elemental analyses of Co(II), Ni(II) and Cu(II) complexes and their magnetic and molar conductance data

Compound	Empirical formula	M%		C%		N%		S%		Molar conductance ($Ohm^{-1}\,cm^{-2}\,mol^{-1}$)	Mag. moments (μ_{eff} in BM)
		Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd		
1	$Co(C_{13}H_8N_4O_3S)\cdot 2H_2O$	14.90	14.92	39.48	39.50	14.17	14.18	8.08	8.10	28	4.72
2	$Co(C_{14}H_{10}N_4O_3S)\cdot 2H_2O$	14.39	14.41	41.05	41.08	13.68	13.69	7.81	7.83	26.12	4.84
3	$Co(C_{15}H_{12}N_4O_3S)\cdot 2H_2O$	13.90	13.93	42.55	42.56	13.22	13.24	7.54	7.56	25.34	4.95
4	$Co(C_{16}H_{14}N_4O_3S)\cdot 2H_2O$	13.45	13.48	43.92	43.94	12.80	12.82	7.30	7.32	22.28	4.86
5	$Ni(C_{13}H_8N_4O_3S)\cdot 2H_2O$	14.85	14.86	39.49	39.52	14.17	14.19	8.10	8.11	18.26	3.24
6	$Ni(C_{14}H_{10}N_4O_3S)\cdot 2H_2O$	14.33	14.36	41.10	41.11	13.69	13.70	7.80	7.82	20.18	3.26
7	$Ni(C_{15}H_{12}N_4O_3S)\cdot 2H_2O$	13.86	13.88	42.55	42.58	13.24	13.25	7.55	7.57	21.35	3.22
8	$Ni(C_{16}H_{14}N_4O_3S)\cdot 2H_2O$	13.42	13.44	43.95	43.96	12.80	12.82	7.31	7.33	24.12	3.28
9	$Cu(C_{13}H_8N_4O_3S)\cdot 2H_2O$	15.88	15.90	39.02	39.04	14.01	14.02	8.00	8.01	25.14	1.75
10	$Cu(C_{14}H_{10}N_4O_3S)\cdot 2H_2O$	15.33	15.36	40.60	40.62	13.51	13.54	7.71	7.74	26.45	1.83
11	$Cu(C_{15}H_{12}N_4O_3S)\cdot 2H_2O$	14.85	14.86	42.09	42.10	13.07	13.09	7.45	7.48	24.56	1.87
12	$Cu(C_{16}H_{14}N_4O_3S)\cdot 2H_2O$	14.37	14.39	43.46	43.48	12.65	12.68	7.23	7.25	23.27	1.90

compounds, especially with *Cladosporium*. All Schiff bases show high activity against *Cladosporium*. Schiff base (**II**) is inactive towards *A. flavus*. Compounds **3**, **4**, **6**, **8** and **11** show promising results.

The biological activity of the ligands exhibited a marked enhancement on coordination with the metal ions against all fungal strains. However, the metal complexes showed good antifungal activity against *A. niger*, *A. flavus* and *Cladosporium*. 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 their structures mainly possess an additional 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 for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduces the polarity [32] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [30] within 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 microorganism [31], thus destroying them more aggressively.

3.1.3. 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) by using the protocol of Meyer et al. [32]. Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm) filled with artificial seawater, 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 solution 100, 50 and 10 $\mu g\,ml^{-1}$ were transferred to nine vials (three for each dilutions were used for each test sample and 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 seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater 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 LD_{50} values [33].

In the case of cytotoxic activity it was observed that only Schiff bases **III** and **IV** and compounds **3**, **7**, **11**, and **12** displayed weak cytotoxic activity against *A. salina*, while the other compounds gave values of LD_{50} therefore can be considered non-cytotoxic.

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

Ligand	ν(NH)	Lactonil ν(C=O)	ν(C=N)	H-bonded −OH stretching	ν(C=C)	ν(SH)	Phenolic ν(C−O)
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 5
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-mercapto-1,2,4-triazole Schiff bases

Complex	ν(OH)	ν(C=O)	ν(C=N)	Phenolic ν(C−O)	ν(M−N)	ν(M−S)	ν(M−O)
1	3415	1685	1618	1345	447	365	376
2	3435	1681	1622	1342	456	368	378
3	3430	1683	1620	1348	463	367	375
4	3418	1680	1621	1349	448	364	379
5	3429	1688	1617	1342	450	370	380
6	3431	1690	1620	1345	458	369	381
7	3430	1685	1615	1350	463	371	379
8	3426	1689	1618	1347	476	368	384
9	3420	1685	1615	1351	449	348	382
10	3434	1687	1612	1348	475	346	385
11	3432	1690	1622	1346	480	347	381
12	3431	1689	1620	1353	446	345	378

Table 6
Ligand field parameters of Ni(II) complex with 3-substituted-4-amino(8-formyl-7-hydroxy-4-methycoumarin)-5-mercapto-1,2,4-triazole Schiff bases (**I–IV**)

Complex	Transitions (cm^{-1})			ν_2 Calcd (cm^{-1})	Dq (cm^{-1})	B^1 (cm^{-1})	Distortion (%)	ν_1/ν_2	LSFE	μ_{eff} Calcd (BM)	β	β^0 (%)
	ν_1	ν_2	ν_3									
5	9794	15 838	26 297	15858.55	979.4	851.57	0.130	1.617	33.579	3.194	0.806	19.359
6	9854	15 842	26 261	15914.52	985.4	840.91	0.456	1.600	33.785	3.192	0.796	20.369
7	9577	15 597	26 421	15640.43	957.7	888.69	0.278	1.629	32.835	3.202	0.842	15.843
8	9642	15 682	26 140	15662.26	964.2	858.41	0.126	1.626	33.058	3.200	0.813	18.710

4. Results and discussion

The Schiff bases were soluble in some organic solvents. All the Co(II), Ni(II) and Cu(II) complexes were stable in room temperature, non-hygroscopic, insoluble in water and many common organic solvents, infusible at high temperature and all of them were polymeric in nature. The elemental analyses shown in Table 3 agree well with the formation of 1:1 stoichiometry of the type $ML\cdot 2H_2O$. All the complexes are sparingly soluble in common organic solvents but these complexes are soluble to a larger extent 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.

4.1. IR spectra

The selected IR spectra of the Schiff bases and their metal complexes along with their tentative assignments are reported in Tables 4 and 5.

The IR spectra of the Schiff bases show characteristic bands due to ν(NH) and ν(SH) at 3145 and 2700 cm^{-1}, respectively [34]. Another band at 1100 cm^{-1} is assigned to ν(C=S) [34]. These observations suggest that the Schiff bases exhibit thiol–thione tautomerism (Fig. 1). The broad band at 3220–3270 cm^{-1}, a strong band at 1705–1715, 1630–1625 and 1285 cm^{-1} in the IR spectra of the Schiff bases are assigned to H-bonded –OH stretching, ν(C=O) lactonic carbonyl [35], ν(C=N) and phenolic ν(C–O) vibrations, respectively. The medium intensity band in the region 770–760 cm^{-1} has been attributed to ν(C=S). A medium band around 1055 cm^{-1} is characterized for ν(O–C–O). In comparison with the spectra of the Schiff bases, all the complexes exhibit downward shift (10–20 cm^{-1}) of ν(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 at 1285 cm^{-1} in the Schiff bases appeared as a medium to high intensity band in the 1350 cm^{-1} 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 cm^{-1}, which is due to ν(S–H) of Schiff bases, indicating that the metal is coordinated through sulphur atom also. This is supported by the lower frequency shift which appears around 685–670 cm^{-1} in the metal complexes due to ν(C–S).

The presence of coordinated water molecules in the complexes [34] is indicated by a broad band in the region 3200–3500 cm^{-1} and two weaker bands in the region 750–800 and 700–720 cm^{-1} due to ν(–OH) rocking and wagging mode of vibrations, respectively [36].

An interesting feature observed is the red shift in lactone ν(C=O) to the extent of about 15–20 cm^{-1}, suggesting that the metal is coordinated to the lactone oxygen [37]. This is

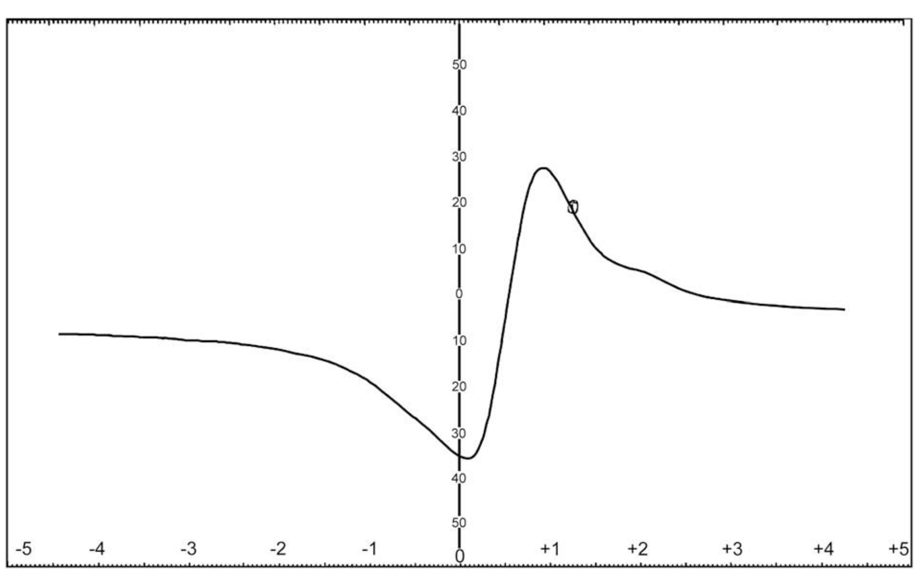

Fig. 4. ESR spectrum of Cu(II) complex (**10**).

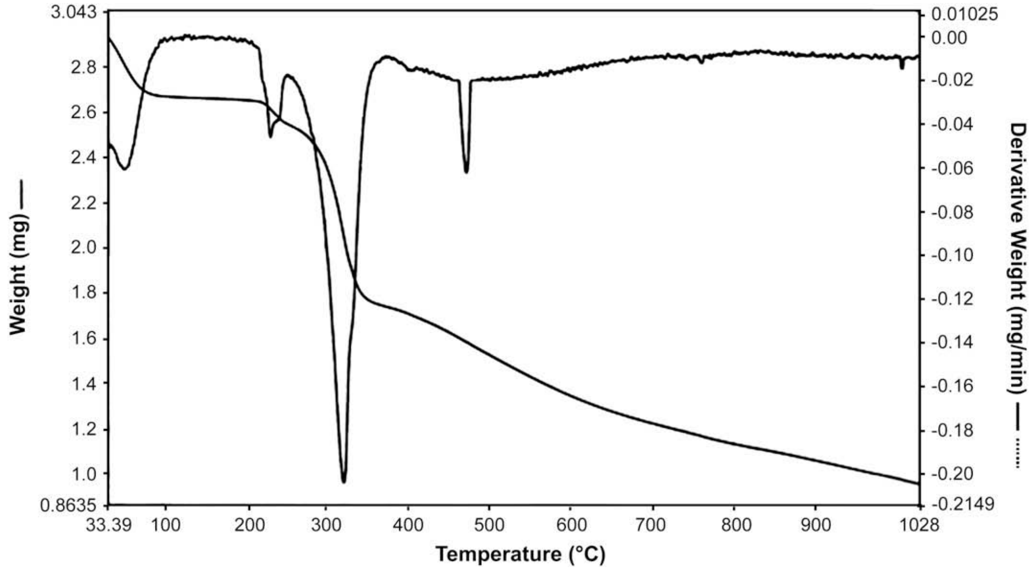

Fig. 5. Thermogravimetric (TGA/DTG) curves of Cu(II) complex (**10**).

further supported by downward shift in ν(O–C–O) of the coumarin ring [38]. 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 the region of 375–350 and 450–480 cm^{-1} in all the complexes are assigned to stretching frequencies of (M–O) and (M–N) bonds, respectively. The band in the region 333–379 cm^{-1} of far IR spectra is due to metal–sulphur bond formation.

Thus the IR spectral data results provide strong evidences for the complexation of the potentially tetradentate Schiff bases and also suggests that the complexes exist in the solid state as polymeric structure with bonding of metal(II) likely to both the deprotonated phenolic oxygen and lactose carbonyl oxygen.

4.2. ^{1}H NMR spectra

The ^{1}H NMR spectra of Schiff bases exhibit singlet at 13.58, 10.21, 8.62 ppm and multiplet at 7.2–7.5 ppm due to –NH [39], phenolic OH, –CH=N [40] and aromatic protons, respectively. In addition to these signals, a sharp signal at 3.5 ppm is attributed to SH protons [19]. These observations suggest that the Schiff bases exist in thiol–thione tautomerism. The singlet around 2.84 ppm is due to methyl protons [40].

4.3. Electronic spectra

The cobalt complexes exhibited two distinct bands in the region 9790–10 000 and 18 950–20 661 cm^{-1} which may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1) and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3) transitions, respectively, and these are suggestive of octahedral geometry around the cobalt(II) ions [41,42]. The electronic spectra of nickel complexes showed d–d bands in the region 9570–10 000, 15 597–15 845 and 20 492–27 248 cm^{-1}. These are assigned [41] to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3), respectively, consistent with their well-defined octahedral configuration [41,42]. The ligand field parameters [43] Dq, β, B',

Table 7
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-mercapto-1,2,4-triazole Schiff base (**II**)

Empirical formula	Decomposition temperature (°C)	Weight loss (%)		Metal oxide (%)		Inference
		Obsd	Calcd	Obsd	Calcd	
$Co(C_{14}H_{10}N_4O_3S)\cdot 2H_2O$	205–235	8.77	8.80	18.30	18.32	Loss of coordinated water molecules
	310–325	31.05	31.06			Loss of triazole moieties
	490–485	45.71	45.73			Loss of coumarin moieties
$Ni(C_{14}H_{10}N_4O_3S)\cdot 2H_2O$	210–228	8.79	8.81	18.25	18.28	Loss of coordinated water molecules
	320–330	31.04	31.07			Loss of triazole moieties
	485–495	45.74	45.76			Loss of coumarin moieties
$Cu(C_{14}H_{10}N_4O_3S)\cdot 2H_2O$	200–230	8.70	8.71	19.20	19.23	Loss of coordinated water molecules
	290–330	30.68	30.71			Loss of triazole moieties
	480–510	45.20	45.22			Loss of coumarin moieties

ν_2/ν_1 and LFSE have been calculated (Table 6). The electronic spectra of Cu(II) complexes showed absorption band in the region 14 540−14 780 cm^{-1} attributed to $^2T_g \leftarrow {}^2E_g$ transition indicative of distorted octahedral geometry [44,45].

4.4. Magnetic studies

The magnetic moments obtained at room temperature are listed in Table 3. The magnetic measurements for Co(II) and Ni(II) complexes showed magnetic moment values of 4.3−5.2 and 2.8−3.5 BM, respectively, suggesting [46] consistency with their octahedral environment. The Cu(II) complexes show magnetic moments, 1.75−1.87 BM, slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [47].

4.5. ESR spectra of copper(II) complex (10)

The ESR spectrum of copper(II) complex (**10**) with ligand (**2**) has been studied and depicted in Fig. 4. The $g_{\|}$ and $g_{\perp}$ values have been found to be 2.04351 and 2.15835, respectively. The g_{av} was calculated to be 2.12007. The Cu(II) complex shows reversed axial (compressed octahedral) with $g_{\|} < g_{\perp}$. The trend $g_{\|} < g_{\perp}$ showed that the electron is delocalised in d_z^2 orbital of the ground state of Cu(II). In this case ($g_{\|} < g_{\perp}$) distortion occurs by compression [48]. The parameter G, determined as $G = (g_{\|} - 2)/(g_{\perp} - 2)$, is found to be much less than 4 suggesting considerable interaction in the solid state [49].

4.6. Thermal studies

The thermal behavior of all the complexes is almost same. Hence, only Co(II) (**2**), Ni(II) (**6**) and Cu(II) (**10**) complexes were discussed.

The Cu(II) complex (**10**) has been reproduced in Fig. 5. Co(II) (**2**), Ni(II) (**6**) and Cu(II) (**10**) complexes decompose gradually with the formation of metal oxide above 530 °C. The nature of proposed chemical change with the temperature range and the percentage of metal oxide obtained are given in Table 7. The thermal decomposition of Co(II) (**2**), Ni(II) (**6**) and Cu(II) (**10**) complexes takes place in three steps as indicated by DTG peaks around 200−230, 290−330 and 480−510 °C corresponding to the mass loss of two coordinated water molecules, triazole moiety and coumarin moiety, respectively.

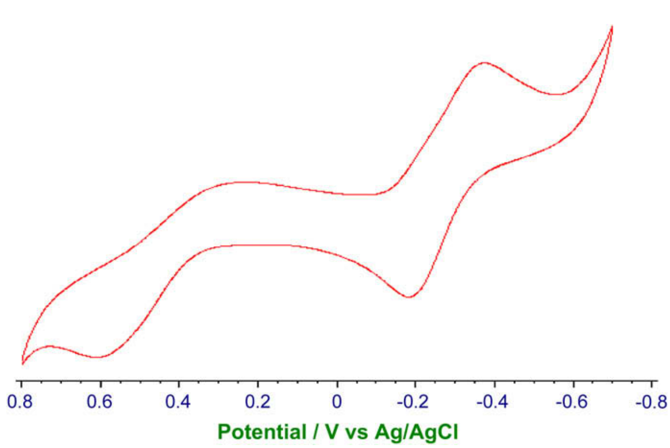

Fig. 6. Cyclic voltammogram of Cu(II) complex (**10**).

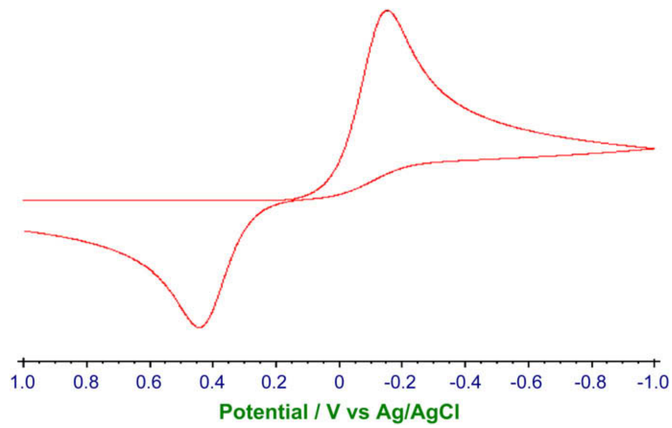

Fig. 7. Cyclic voltammogram of Ni(II) complex (**6**).

4.7. Electrochemistry

Electrochemical properties of the complexes were studied on a CHI1110A-Electrochemical analyzer in *N*,*N*-dimethyl formamide (DMF) containing 0.05 M n-Bu_4NClO_4 as the supporting electrolyte. A cyclic voltammogram of Cu(II) (**10**) (Fig. 6) radical displays a reduction peak at $E_{pc} = 0.2673$ V and again it reduced to Cu(I) and displays a reduction peak at $E_{pc} = -0.3733$ V, respectively, with a corresponding oxidation peak (Cu(I) radical) at $E_{pa} = -0.1822$ V and $E_{pa} = 0.6027$ V for Cu(II), respectively. The peak separation of this couple (ΔE_p) is 0.33 and 0.191 V at 0.05 V 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 voltammetric responses with the scan rate varying 50−250 mV/s give 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 [50].

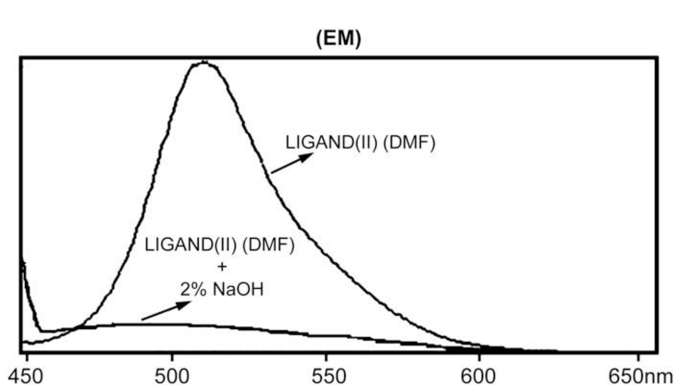

Fig. 8. Emission spectra of Schiff base (**II**) in DMF and red shift of Schiff base (**II**) in DMF with 2% NaOH.

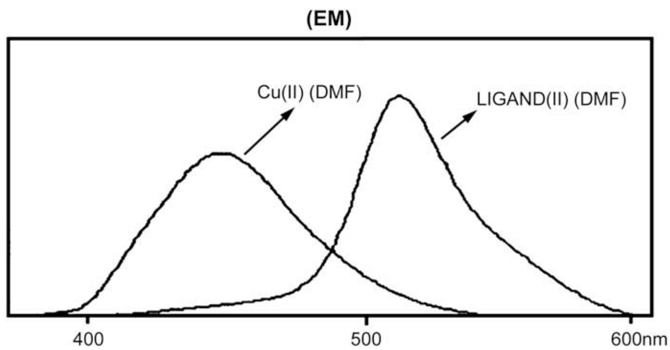

Fig. 9. Emission band of Schiff bases (**II**) and Cu(II) complex (**10**) in DMF solution.

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 Ni(II) (**6**) complex (Fig. 7) exhibits a reduction peak at $E_{pc} = 0.152$ V with a direct re-oxidation peak at $E_{pa} = 0.443$ V corresponding to the formation of Ni(II)/Ni(I) couple. The peak separation of this couple (ΔE_p) is 0.29 V. This Ni(II) complex also have a quasi-reversible character as the separation in peak potential is higher than 59 mV and the peak currents rise with increasing $\nu^{1/2}$. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility.

4.8. Fluorescence studies

The emission spectra of the Schiff bases derived from 3-substituted-4-amino-5-mercapto-1,2,4-triazole and 8-formyl-7-hydroxy-4-methylcoumarin and their complexes were investigated in various solvents such as DMF, DMSO, MeCN and dioxan.

4.8.1. Emission spectra

The Schiff bases characterized by an emission band around 511 nm in DMF, DMSO, MeCN and dioxan 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 480 nm in DMF, DMSO, MeCN and dioxan solutions. The changes clearly indicate that proton transferred (H-bonded ion pair) species exist in equilibrium [51] and also we observed the λ_{max} of the Schiff bases undergoes red shift in DMF, DMSO, MeCN and dioxan solutions due to the hydrogen bond formation (Fig. 8) [52].

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-mercapto-1,2,4-triazole. The Co(II), Ni(II) and Cu(II) complexes were characterized by the emission band around 450 nm and it is observed that the emission band of Schiff bases around 510 nm disappeared because of the interaction of the phenolic oxygen with the metal ion (Fig. 9). 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 [53,54]. Magnetic perturbation, redox activity, etc., have been invoked [54] in the past to rationalize fluorescence quenching by transition metal ions. But in the case of Cu(II) complexes we could observe the enhancement of fluorescence in MeCN solution.

5. Conclusion

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 sulphur atom. The analytical, IR, ESR, electronic, magnetic, and thermal studies confirm the bonding of Schiff bases to metal ions. Electrochemical study of Cu(II) and Ni(II) complexes can provide the degree of the reversibility of one electron transfer reaction and they have a quasi-reversible character. All Schiff bases were found potentially active

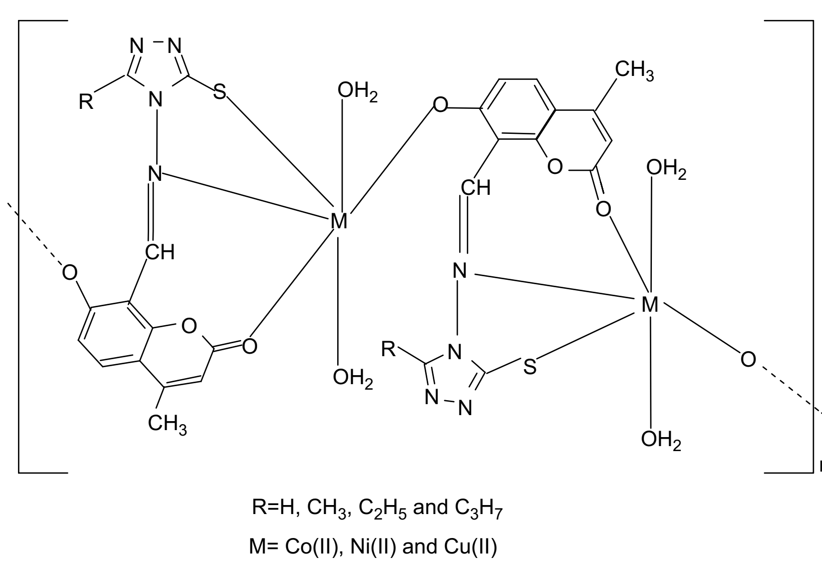

Fig. 10. Proposed structure of metal(II) complexes.

towards all microbial strains. Bacterial studies of the metal(II) complexes **3**, **4**, **7** and **12** and the fungal studies of complexes **3**, **4**, **6**, **8** and **11** show promising results.

The IR data, taken together with the insolubility of the complexes in water and as all the complexes are fusible at higher temperatures, suggest that they exist in the solid state as polymeric structures with bonding of metal(II) likely to both the deprotonated phenolic oxygen and lactone carbonyl oxygen [55–57].

All these observations put together lead us to propose the following structure (Fig. 10) in which the metal(II) complexes exhibit coordination number six on the basis of magnetic and electronic spectral data having the stoichiometry of the type $(ML\cdot 2H_2O)_2$.

6. Experimental protocols

6.1. Chemistry

All chemicals and solvents used were of AR grade. All metal(II) salts were used as their chlorides. Further remaining pure reagents were purchased from Ranbaxy Chemicals. The metal contents were determined gravimetrically by the known method [58]. The results of elemental analyses, molar conductance and magnetic data are listed in Table 1.

The IR spectra of the Schiff bases and their metal complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region in KBr disks. 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 Schiff bases were recorded in DMSO 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. Molar conductivity measurements were recorded on an 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 moments were carried out by Faraday balance.

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References

[1] L. Pochet, C. Doucet, M. Schynts, N. Thierry, N. Boggeto, B. Pirotte, K.Y. Liang, B. Masereel, P. de Tulio, J. Delarge, M. Reboud-Ravaux, J. Med. Chem. 39 (1996) 2579.
[2] X. Shao, D.H.L. Ekstrand, R. Bhikhabhai, C.F.R. Kallander, J.S. Gronowitz, Antiviral Chem. Chemother. 8 (1997) 149–159.
[3] B. Rendenbach-Muller, R. Schelcker, M. Traut, H. Weifenbach, Bioorg. Med. Chem. Lett. 4 (1994) 1195.
[4] S. Kostova, P. Raleva, Genova, R. Argirova, Bioinorg. Chem. Appl. (2006) 1–9.
[5] Irena Kostova, Curr. Med. Chem. – Anti-Cancer Agents 5 (2005) 29–46.
[6] U.S. Weber, B. Steffen, C. Sigers, Res. Commun. Mol. Pathol. Pharmacol. 99 (1998) 193–206.
[7] I. Kostova, I. Manolov, M. Karaivanova, Arch. Pharm. 334 (2001) 157–162.
[8] A. Scozzafava, C.T. Supuran, J. Med. Chem. 43 (2000) 3677.
[9] S.A. Rice, M. Givskov, P. Steinberg, S. Kjelleberg, J. Mol. Microbiol. Biotechnol. 1 (1999) 23–31.
[10] J.G. Lara, M. Masalha, S.J. Foster, Drug Discov. Today 10 (2005) 643–651.
[11] G.D. Wright, Chem. Biol. 7 (2000) R127–R132.
[12] J. Travis, J. Potempa, Biochim. Biophys. Acta 14 (2000) 35–50.
[13] H.J. Smith, C. Simons, Proteinase and Peptidase Inhibition: Recent Potential Targets for Drug Development, Taylor and Francis, London, 2001.
[14] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1999.
[15] Ya Al-Sound, M.N. Al-Dweri, Na Al-Masoudi, Il Farmaco 59 (10) (2004) 775–783.
[16] Hp Michael, C. Dines, J. Mol. Struct. 705 (2004) 177–187.
[17] Z.H. Chohan, Synth. React. Inorg. Met.-Org. Chem. 34 (2004) 833.
[18] Z.H. Chohan, A. Scozzafava, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 17 (4) (2002) 261–266.
[19] S.A. Patil, B.M. Badiger, S.M. Kudari, V.H. Kulkarni, Transition Met. Chem. 8 (1983) 238.
[20] B.M. Badiger, S.A. Patil, S.M. Kudari, V.H. Kulkarni, Rev. Roum. Chim. 31 (1986) 849.
[21] A.Y. Naik, S.D. Angadi, V.H. Kulkarni, Oriental J. Chem. 10 (1994) 23.
[22] M.S. Yadawe, S.A. Patil, Transition Met. Chem. 22 (1997) 220.
[23] P.G. Avaji, B.N. Reddy, P.S. Badami, S.A. Patil, Transition Met. Chem. 31 (2006) 842–848.
[24] A. Kumar, G. Singh, R.N. Handa, S.N. Dubey, Indian J. Chem. 38A (1999) 613–617.
[25] A.K. Sen, G. Singh, K. Singh, R.K. Noren, R.N. Handa, S.N. Dubey, Indian J. Chem. 36A (1997) 891–894.
[26] D. Heng-Shan, Q. Bin, W. Kun, W. Qing-Lian, Z. Zi-Yi, Magn. Reson. Chem. 38 (2000) 210–212.
[27] S.S. Papakonstantinou-Garuofalia, E. Tani, O. Todoulou, A. Papadaki-Valiraki, E. Filippators, E.D. Clercq, P.N. Kourounakis, J. Pharm. Pharmacol. 50 (1) (1998) 117–124.
[28] E. Spath, M. Pailer, Chem. Ber. 68 (1935) 940.
[29] A.K. Sadana, Y. Miraza, K.R. Aneja, O. Prakash, Eur. J. Med. Chem. 38 (2003) 533–536.
[30] Z.H. Chohan, C.T. Supuran, A. Scozzafava, J. Enzyme Inhib. Med. Chem. 19 (1) (2004) 79–84.
[31] Z.H. Chohan, M. Praveen, Appl. Organomet. Chem. 15 (2001) 617–625.
[32] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin, Planta Med. 45 (1982) 31.
[33] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Am. J. Clin. Pathol. 45 (1966) 493.
[34] G. Singh, P.A. Singh, K. Singh, D.P. Singh, R.N. Handa, S.N. Dubey, Proc. Natl. Acad. Sci. Ind. 72A (2002) 87–94.
[35] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81.
[36] P.R. Shukla, V.K. Singh, A.M. Jaiswal, J. Narain, J. Indian Chem. Soc. 60 (1983) 321–324.
[37] V.J. Tyaga Raju, Vilas Ranbaore, Vasudha Atre, M.C. Ganorkar, J. Indian Chem. Soc. Vol. (LIX) (Feb. 1982) 199–203.
[38] F.D. Lewis, S.V. Barancyk, J. Am. Chem. Soc. 111 (1989) 8653–8661.
[39] M.S. Gunthkal, T.R. Goudar, S.A. Patil, Oriental J. Chem. 16 (2000) 151–154.
[40] Sayaji Rao, Asian J. Chem. 17 (2005) 2663–2668.
[41] A.B.P. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, 1984.
[42] S.M.E. Khalil, Chem. Pap. 54 (1) (2000) 12–18.
[43] R.S. Drago, Physical Methods in Inorganic Chemistry, Reinhold Publishing Corporation, New York, 1968.

[44] C.H. Krishna, C.M. Mahapatra, K.A. Dash, J. Inorg. Nucl. Chem. 39 (1977) 1253.
[45] B.K. Rai, Mukesh Kumar, J. Indian Coun. Chem. 20 (2003) 22.
[46] C.J. Balhausen, Introduction to Ligand Fields, McGraw Hill, New York, 1962.
[47] B.K. Patel, M.M. Patel, Indian J. Chem. 29 (1990) 90−92.
[48] R.C. Rossenberg, A.C. Root, P.K. Bernstein, H.B. Gray, J. Am. Chem. Soc. 97 (1975) 2092.
[49] B.T. Hathaway, Struct. Bonding 14 (1973) 60.
[50] A.-J. Bard, L.-R. Izatt (Eds.), Electrochemical Methods: Fundamentals and Applications, second ed. Wiley, New York, 2001.
[51] S. Mukherjee, Indian J. Chem. 26 (1987) 1002.
[52] H. Beens, K.H. Grellmann, M. Gurr, A.H. Weller, Discuss. Faraday Soc. 39 (1965) 183.
[53] A.W. Czarnik, Chem. Biol. 2 (1995) 423.
[54] A.W. Varnes, R.B. Dodson, E.L. Wehry, J. Am. Chem. Soc. 94 (1972) 946.
[55] T. Kaliyappan, S. Rajagopan, P. Kannan, J. Appl. Polym. Sci. 91 (2003) 494−500.
[56] G.S.V. Kumar, B. Mathew, J. Mac. Sci. 41 (2004) 1037−1050.
[57] S.C. Bernadette, A.E. Denise, Kevin Kavanagh, Malachy McCann, Andy Noble, Bhumika Thati, Maureen Walsh, Inorg. Chim. Acta 359 (2006) 3976−3984.
[58] A.L. Vogal, A Text Book of Quantitative Chemical Analysis, fifth ed. Addison Wesley Longman, London, 1999.