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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

4-Aminoantipyrine-based Schiff-base transition metal complexes as potent anticonvulsant agents

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Abstract Metal complexes of Co(II), Ni(II), Cu(II), and Zn(II) with ligands (L^1H and L^2) derived from 4-aminoantipyrine, 2-hydroxy-3-formylquinoline, and isatin were synthesized and characterized by the elemental analysis, conductance measurements, magnetic susceptibility, and spectral analysis. The spectral data revealed that the ligands acted as a neutral tridentate, coordinating to the metal ion through the azomethine nitrogen, phenolic oxygen, and carbonyl oxygen of the 4-aminoantipyrine, 2-hydroxy-3-formylquinoline, and isatin molecule in ligands L^1H and L^2 only. Both the ligands and their metal complexes were studied for cyclic voltammetry studies. The ligand and the metal complexes were screened for their anticonvulsant activity, and it has been observed that the metal complexes are more potent than the ligands.

Keywords 4-Aminoantipyrine · Quinoline · Isatin · Anticonvulsant activity · MES

Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, inflicts more than sixty million people worldwide according to epidemiological studies (Loscher, 1998). In epilepsy, the normal pattern of neuronal activity becomes disturbed causing strange sensations, emotions and behaviors, or sometimes convulsions, muscle spasms, and loss of consciousness. The brain disorder in which clusters of nerve cells or neurons in the brain sometimes signal abnormally, which leads to the said disorders. The majority of antiepileptic drugs have been in use since 1985. They do not provide satisfactory seizure control in all the patients, and typically cause notable adverse side effects (Leppik, 1994; Al-Soud *et al*., 2003). Research to find more effective and safer antiepileptic drugs, is, therefore, imperative and challenging in medicinal chemistry. Antiepileptic drugs (AEDs) are important part of the treatment program for epilepsy, and the main aim of AEDs is to suppress them without inducing adverse side effects. A significant rising interest in the design of metal compounds as drugs and diagnostic agents is currently observed in the area of scientific study, appropriately termed medicinal inorganic chemistry (Thompson and Orvig, 2006). Investigations in this area focus mainly on the speciation of metal species in biological media, based on possible interactions of these metal ions with diverse biomolecules, in an effort to contribute to future development of new therapeutics or diagnostic agents (Timerbaev *et al.*, 2006). A wide range of metal complexes are already in clinical use and encourage further studies for new metallodrugs, such as metal-mediated antibiotics, antibacterial, antiviral, antiparasitic, radiosensitizing agents, and anticancer compounds; among which 4-aminoantipyrine and its complexes have been known to possess a variety of applications in biological, clinical, analytical, and pharmacological areas (Punniyamurthy *et al.*, 1995). Studies of a new kind of chemotherapeutic compounds are now attracting the attention of biochemists (Choi *et al.*, 1995; Katia *et al.*, 1996). The synthesized compounds were evaluated as anticonvulsant

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agents in experimental epilepsy models, i.e., maximal electroshock test (MES) and subcutaneous pentylenetetrazol (sc-PTZ) induced seizure in mice. They were also evaluated for neurotoxicity by the rotarod assay performed in mice. In the present investigation, we have undertaken a synthesis, characterization, and anticonvulsant evaluation of 4-aminoantipyrine-based Schiff-base metal complexes.

Experimental

All the chemicals used were of reagent grade, and the solvents were distilled before use. Carbon, hydrogen, and nitrogen analyses were carried out on a Thermo quest elemental analyzer, the results of which are presented in Table 1. The molar conductance measurements were made on an ELICO-CM-82 conductivity bridge with a cell having cell constant of 0.51 cm^{-1}. The magnetic measurements were obtained using Faraday balance at room temperature using $Hg[Co(SCN)_6]$ as calibrant. Electronic spectra of compounds in DMSO were recorded using VARIAN CARY 50 Bio UV–visible spectrophotometer. The IR spectra of ligands and their complexes were recorded as KBr pellets in the region 4000–400 cm^{-1} on Nicolet 170 SX FT-IR spectrometer. ^1H NMR spectra of ligands and zinc(II) complexes were recorded in DMSO-d_6 on Bruker 300 MHz spectrometer using TMS as an internal standard. The EPR spectra of copper(II) complexes were recorded at room temperature on Varian E-4 X-band spectrometer using TCNE as g-marker. The 4-aminoantipyrine, and isatin were purchased from Sd-fine chemicals. 2-Hydroxy-3-formylquinoline (Meth-Cohn and Narine, 1978) is prepared by the method reported earlier.

Preparation of the ligands

The ligand L^1H was prepared by boiling the mixture of 4-aminoantipyrine (2.03 g, 0.01 mol) and 2-hydroxy-3-formylquinoline (1.73 g, 0.01 mol) in ethanol (40 ml). The hot solution was allowed to cool to room temperature; yellow solid compound was formed (Scheme 1).

The ligand L^2 was prepared by the method reported earlier (Sengupta and Gupta, 1982), and the product formed in this case was orange-colored amorphous solid (Scheme 1).

Preparation of complexes

Ethanolic solution of metal(II) chloride {$CoCl_2·6H_2O$ (0.237 g, 0.001 mol), $NiCl_2·6H_2O$ (0.237 g, 0.001 mol). $CuCl_2·2H_2O$ (0.170 g, 0.001 mol) and $ZnCl_2$ (0.136 g, 0.001 mol)} was added with stirring to an ethanolic solution of the ligand {(L^1H (0.358 g, 0.001 mol), L^2 (0.332 g, 0.001 mol)}, and refluxed at water bath temperature for 3–4 h. Then, the obtained complexes were filtered off, washed with ethanol, and dried under vacuum over P_2O_5.

Pharmacology

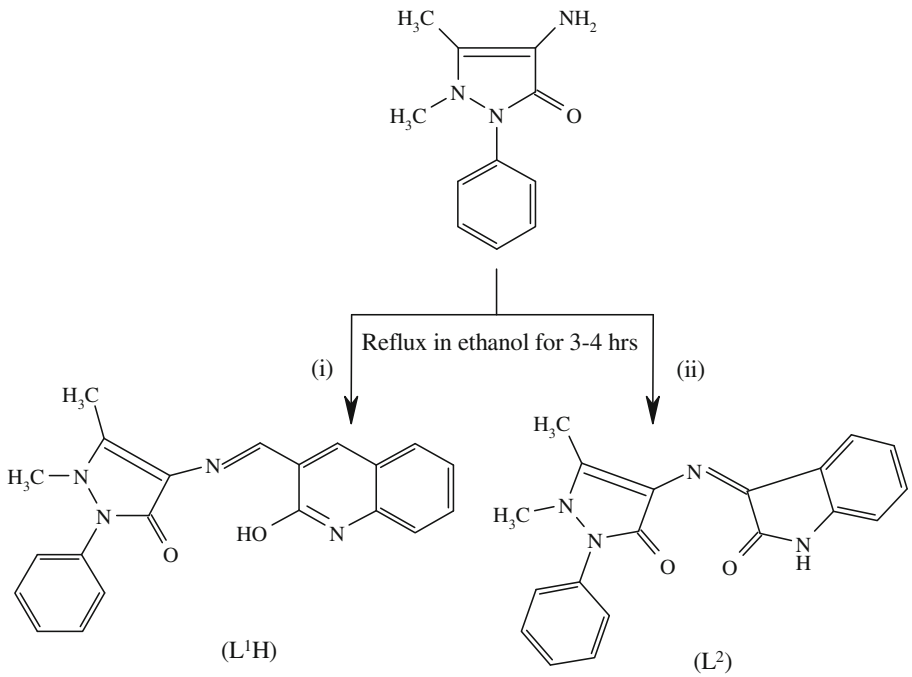
Animals for the investigation

Wister rats of either sex weighing between 180 and 200 g were used in the present investigation with prior permission from the Institutional Animal Ethics Committee (IAEC). Animal studies were performed as per the rules and regulations of CPCSEA. The animals were acclimatized to the experimental room having temperature

Table 1 Analytical, conductivity, and magnetic data for the ligands and their complexes

No	Compounds	Elemental analysis (%) found/calculated					Molar conductance in λ_M mho nbsp;cm^2 mol^{-1}	Magnetic moment in μ_{eff} BM
		C	H	N	M	Cl		
L^1H	$(C_{21}H_{18}N_4O_2)$	70.10/70.39	4.97/5.02	15.13/15.64	–	–	–	–
C1	$[Co(C_{21}H_{16}N_4O_2)_2]·2H_2O$	62.01/62.15	4.14/4.43	13.16/13.81	7.02/7.26	–	7.8	5.19
C2	$[Ni(C_{21}H_{16}N_4O_2)_2]·2H_2O$	61.98/62.17	4.15/4.44	13.19/13.81	7.11/7.22	–	7.4	3.13
C3	$[Cu(C_{21}H_{16}N_4O_2)_2]·2H_2O$	61.21/61.80	4.1/4.41	13.63/13.73	7.24/7.78	–	10.1	1.96
C4	$[Zn(C_{21}H_{16}N_4O_2)_2]·2H_2O$	61.18/61.72	3.96/4.40	13.54/13.71	7.51/7.89	–	8.2	Diamagnetic
L^2	$(C_{19}H_{16}N_4O_2)$	68.11/68.67	4.53/4.81	16.55/16.86	–	–	–	–
C5	$[Co(C_{19}H_{16}N_4O_2)Cl_2H_2O]·H_2O$	45.13/45.88	3.91/4.02	11.15/11.26	11.45/11.85	13.97/14.08	13.4	5.13
C6	$[Ni(C_{19}H_{16}N_4O_2)Cl_2H_2O]·H_2O$	45.23/45.91	3.94/4.02	11.09/11.27	11.41/11.80	13.99/14.09	16.5	3.09
C7	$[Cu(C_{19}H_{16}N_4O_2)Cl_2H_2O]·H_2O$	45.09/45.46	3.89/3.98	11.07/11.16	12.31/12.66	13.67/13.95	14.8	1.91
C8	$[Zn(C_{19}H_{16}N_4O_2)Cl_2H_2O]·H_2O$	44.98/45.37	3.91/3.98	10.97/11.14	12.36/12.83	13.54/13.93	11.3	Diamagnetic

Scheme 1 Schematic presentation of preparation of ligands



(i) 2-Hydroxy-3-formylquinoline
(ii) Isatin

23 ± 2°C, controlled humidity conditions, and 12:12-h light and dark cycle. The Wister rats were housed in sterile Plexiglas transparent cages containing sterile paddy husk as bedding material with maximum of four animals in each cage. The rats were fed on autoclaved standard rat food pellets and water ad libitum.

Acute toxicity study (Kulkarni, 1981)

For screening any synthesized compound for any of its pharmacological properties, it is customary to carryout acute toxicity study to determine the safe effective dose of the synthesized compound. Wister rats of either sex weighing between 180 and 200 g were starved for 18 h before the experiment. The animals were divided into the group of eight each, after recording their body weights. The tested sample solutions of suitable concentration in 1% gum acacia were administered orally in different groups. Initially all the test samples were administered with 12.5 mg/kg body weight; if all the animals survived with this dose, then the samples were tested at higher dose range viz., 25, 50, 100, 200, and 400 mg/kg, and if the test samples caused 100% death at this dose, the lower dose range was treated as LD_{50} dose. Finally, the lethal dose fixed for the reporting compounds is 700 mg/kg for ligands L^1H, and L^2 and their complexes. The administered dose is the one-tenth of the threshold dose.

Testing of compounds for anticonvulsant activity against maximal electroshock generic seizures in Wister rats (Kulkarni, 1981)

Previously weighed and numbered Wister rats were categorized into eight groups each consisting of four rats. One group was used as control and the other for the phenytoin drug treatment, and the remaining six for the novel sample treatment. The drug phenytoin, control, and test samples in gum acacia were administered orally to the respective group of animals. Corneal earclip electrodes were placed on the cornea of the rats, and 150 mA of electric current was applied for 0.2 s by means of electroconvulsiometer to all groups. Each animal was placed into individual plexiglas transparent case and observed for 30-min. duration (in seconds), in various phases of convulsion, viz., tonic flexion, tonic extensor, clonic convulsions, and stupor was noted. All the experimental groups were compared with the respective control treated with vehicle.

Statistical analysis

Values are expressed as mean ± SEM, statistical differences between means were determined by performing one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered as significant difference in the present study.

Result and discussion

IR spectral study

The IR spectra providing valuable information regarding the nature of the functional group coordinated to the metal atom are presented in Table 2. The ligands (L^1H, and L^2) showed broad bands around 1715, and 1703 cm^{-1}, respectively, which are attributed to the ν(C=O) of antipyrine molecule. A sharp band at 1680 cm^{-1} is assigned to the ν(C=O) of isatin system in case of ligand L^2. Upon complexation, the ν(C=O) of the antipyrine molecule has shifted to lower frequency region, suggesting that carbonyl group is coordinated to the metal ion. The carbonyl-stretching frequency of the isatin moiety at 1680 cm^{-1} has been lowered by 60–70 cm^{-1} in the spectra of (C5–C8) complexes. This clearly indicates the coordination of the carbonyl group to the metal ion. The spectra of both the ligands (L^1H and L^2) show the characteristic ν(>C=N) bands in the region 1570–1500 cm^{-1}. In the spectra of the complexes, this band appears at lower frequency region indicating the coordination of the azomethine nitrogen atom to the metal ion (Guofa *et al.*, 1990). The ligand acts as tridentate-chelating agent coordinated to the metal ion via the one nitrogen ν(>C=N) and two oxygen atoms. Moreover, ligand L^1H and L^2 have shown sharp bands at 3456 and 3140 cm^{-1} which are the characteristic features of the -OH and ring –NH of quinoline and isatin molecule, respectively. Owing to the hydrated nature of the complexes, it is difficult to observe the absence and the presence of the (–OH) and ring (–NH) of quinoline and isatin molecule in all the complexes. Presence of band around 3400 cm^{-1} in all the complexes is the indication of coordinated/lattice celled water molecule.

Table 2 IR spectral data of the ligands and their complexes in cm^{-1}

Compound	ν(C=N) (azomethine)	ν(C=O) (antipyrine)	ν(C=O) isatin	ν(O–H)
L^1H	1596	1703	–	3419
C1	1566	1659	–	3441
C2	1559	1640	–	3448
C3	1568	1646	–	3444
C4	1560	1645	–	3440
L^2	1607	1714	1680	–
C5	1565	1691	1617	3441
C6	1547	1692	1614	3431
C7	1560	1697	1619	3432
C8	1562	1697	1619	3431

^1H NMR study

^1H NMR analysis of the ligands (L^1H and L^2) and their zinc complexes (C4 and C8) were carried out in DMSO d_6 solvent. Spectrum of the ligand (L^1H) shows the peaks around 10.1 and 8.8 ppm, which are ascribed to the –OH and the azomethine protons, respectively. The signal due to azomethine proton exhibits a downfield shift in its zinc (C4) complex, suggesting the coordination of azomethine nitrogen atom to the metal ion. Similarly, zinc (C4) complex displays the absence of quinoline –OH proton, which indicates the coordination of –OH group to the metal atom via deprotonation. In the case of ligand (L^2), peak appearing at 11.1 ppm is due to the presence of the ring (–NH) of isatin moiety. However,, the ring (–NH) of isatin molecule retained in the spectrum of its zinc (C8) complex is evidence for its non-involvement in the complexation. The peak resonating around 6–8 ppm and 2–3 ppm in both the ligands and their zinc complexes are attributed to the aromatic proton and methyl proton, respectively.

Molar conductivity

Molar conductivities of the complexes were measured in DMSO solution with 10^{-3} M concentration. All the complexes show molar conductance value in the range 7.4–16.5 mho cm^2 mol^{-1} (Table 1). These low conductance values suggest that the complexes are non-electrolytic in nature (Geary, 1971).

Electronic spectra

The electronic spectra of the ligands (L^1H and L^2) display the bands around 260 and 370 nm, which are assigned to the π–π^* and n–π^* transitions, respectively. The n–π^* transitions are accompanied with the (>C=N–) azomethine group of the ligands; in complexation, low shift of this band is the indication of the coordination of azomethine nitrogen to the metal ion. The cobalt complexes (C1 and C5) have shown the band around 425 nm which is attributed to the LMCT. In addition to this, complex (C1) displays the band at 593 nm assigned to d–d transition. In the same way, the nickel complexes (C2 and C6) exhibiting the bands around 565 and 573 nm are supportive of the data for the assigned geometry of the complexes. For the copper (C3 and C7) and zinc (C4 and C8) complexes, bands observed around 430–460 nm are due to the LMCT transition only.

EPR spectral study

EPR studies of paramagnetic transition metal(II) complexes yield information about the distribution of the

unpaired electrons and, hence, about the nature of the bonding between the metal ion and its ligands. The EPR spectra of Cu(II) complexes (C3 and C7) were recorded at room temperature. Complexes exhibit isotropic signal with the g value observed to be 2.06 and 2.08, respectively, with an absence of hyperfine splitting.

FAB mass spectra

In the present investigation, the FAB mass spectra of both the Cu(II) complexes (C3 and C7) have been recorded. The molecular ion peaks observed for both the complexes are found to be 816 and 506, respectively, which indicates that the complexes are monomeric in nature.

Magnetic study

The experimentally determined room temperature magnetic susceptibilities of the complexes are presented in Table 1. For the cobalt complexes, C1 and C5, magnetic moment values are found to be 5.19 and 5.13 B.M respectively, which corresponds to the presence of three unpaired electron, and evidences the octahedral geometry of both the complexes. The magnetic moment of the nickel complexes, C2 and C6, are observed to be 3.13 and 3.09 B.M, respectively, which are attributed for the high-spin configuration and show the octahedral environment around Ni^{+2} ion in both the complexes. Copper complexes, C3 and C7, have shown magnetic moment values, 1.96 and 1.91 B.M., respectively, corresponding to the presence of one unpaired electron.

TG study

Thermal behaviors of (C6 and C7) complexes having composition $[Ni(C_{19}H_{16}N_4O_2)Cl_2\ H_2O]\cdot H_2O$ and $[Cu(C_{19}H_{16}N_4O_2)Cl_2\ H_2O]\cdot H_2O$, respectively, have been studied in the temperature range 40–900°C with heating rate of 10°C min^{-1} in a nitrogen atmosphere, where both the complexes decompose in three steps. In the first step of decomposition, weight loss observed in the temperature range around 97–102°C is attributed to the loss of lattice-celled water molecule in each case. The dehydrated complexes (C6 and C7) continued to lose their weight in the temperature range around 170°C, which corresponds to the loss of one coordinated water molecule. Third step illustrates the weight loss of ligand moiety which is observed around temperature range 400–600°C. Above 600°C temperature, the plateau was obtained, which indicates the formation of a respective metal oxide. Weight losses from the TG agrees well with the theoretical calculations.

Cyclic voltammetry

Cyclic voltammetry is a highly versatile electro-analytic technique. In recent years, it has become as the most popular technique for studying electrochemical reactions. The electrochemical behaviors of ligands and their complexes have been investigated in DMSO solution. Only copper(II) complex (C7) found to be redox active in the potential ranging from −1.0 to 1.0, with the scan rates of 0.5, 1.0, and 1.5 V, is studied (Fig. 1). Copper complex $[Cu(C_{19}H_{16}N_4O_2)Cl_2H_2O]\cdot H_2O$ exhibit well-defined quasireversible redox peak corresponding to the formation of $Cu^{II} \rightarrow Cu^{III}$ with anodic peak at 0.320 V (*Epa*), followed by the respective cathodic waves in the reverse scan at 0.625 V (*Epc*) due to $Cu^{III} \rightarrow Cu^{II}$ reduction. The values of *Epa* and *Epc* of the quasireversible Cu^{II}/Cu^{III} couple were only little affected in the scan-rate variation studies, suggesting that there is no change in the quasireversibility. The peak separation between *Epa* and *Epc* (ΔEp) found to be greater than 59 mV supports the quasireversibility of the redox couple, corresponding to the one-electron process.

Pharmacology study

Maximal electroshock method (MES) is used as the preliminary method for anticonvulsant studies of the synthesized compounds, viz., L^1H, L^2, and (C3, C4, C7, C8) at 70 mg/kg body weight of the Wister rats. The pharmacological data of these compounds are presented in Table 3. Information presented in Table 3 represents a thorough and detailed presentation of test results obtained at specific times of seizure induction in all the phases—I, II, III, and IV of convulsion. The satisfactory anticonvulsant actions of the above said compounds are quite long and comparable to phenytoin which is used as the standard anticonvulsant drug, at the dose of 20 mg/kg body weight. Flexion

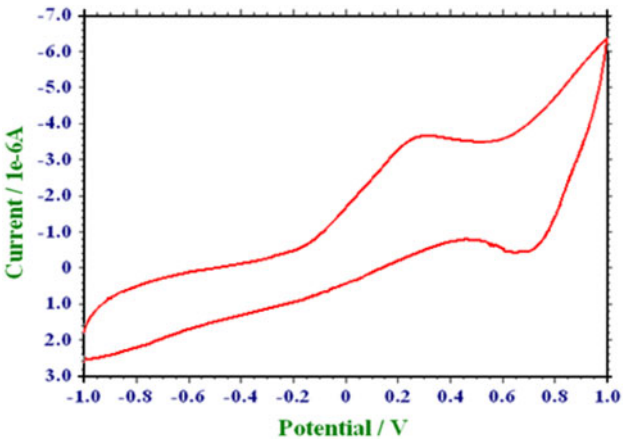


Fig. 1 The cyclic voltammetry of the complex C7

Table 3 Anticonvulsant activity (MES test) data of ligands, and their copper and zinc complexes

Treatment/dose mg/kg	Time (seconds) in various phases of convulsion			
	Flexion	Extensor	Clonus	Stupor
Control	3.16 ± 0.28	12 ± 0.80	19.0 ± 3.0	220 ± 5.0
Gum acacia 1% W/W				
Phenytoin	3.10 ± 0.5	1 ± 0.01	5.1 ± 4.6	170 ± 15.5
20 mg/kg				
Ligand L^1H	3.19 ± 0.4	11 ± 0.8	20.0 ± 0.6	215 ± 7.0
70 mg/kg				
Copper (C3) complex	3.03 ± 0.4	5.0 ± 0.7	4.8 ± 6.2	156 ± 15.0
70 mg/kg				
Zinc (C4) complex	3.01 ± 0.6	7.0 ± 0.3	3.9 ± 7.3	142 ± 12.5
70 mg/kg				
Ligand L^2	2.6 ± 0.4	11 ± 0.5	20.0 ± 0.8	205 ± 9.0
70 mg/kg				
Copper (C7) complex	3.05 ± 0.8	5.5 ± 0.4	4.6 ± 4.0	152 ± 8.0
70 mg/kg				
Zinc (C8) complex	3.0 ± 0.2	6.5 ± 0.7	4.2 ± 5.0	145 ± 4.0
70 mg/kg				

is the first phase of convulsion when 150 mA alternating current was delivered for 0.2 s via corneal electrodes to the Wister rats. At this phase, the recurrent seizures occurred in the rats indicated by the appearance of neck jerking. The time taken by the group of rats administered with phenytoin to get recovered is 3.10 ± 0.5, which is almost same in the case of all the tested samples. Abolition of the hind limb tonic extensor component indicates that the test compound's ability to inhibit MES-induced seizure is observed in the extensor phase. The samples, C3, C4, C7, and C8, have taken the shorter time period at this phase compared with the ligands (L^1H and L^2). Finally, the animals were almost inactive in clonus and stupor phase, even they cannot move. However, the animals recovered in case of standard phenytoin drug.

Conclusion

In this research article, we have presented the syntheses of Schiff base ligands and their later first-row transition metal(II) complexes. The structures of the ligands and their metal complexes were confirmed by various spectral and elemental analyses. The ligand-to-metal (L:M) stoichiometry is found to be 2:1 and 1:1 in case of L^1H and L^2, respectively (Fig. 2). The present study suggests the coordination of the ligands to the metal ions in N, O, O fashion. All the complexes are found to be non-electrolytic in nature with octahedral geometry. The prepared compounds were evaluated for pharmacological properties and have exhibited promising anticonvulsant activity toward the electroshock-induced seizures in Wistar rats. The

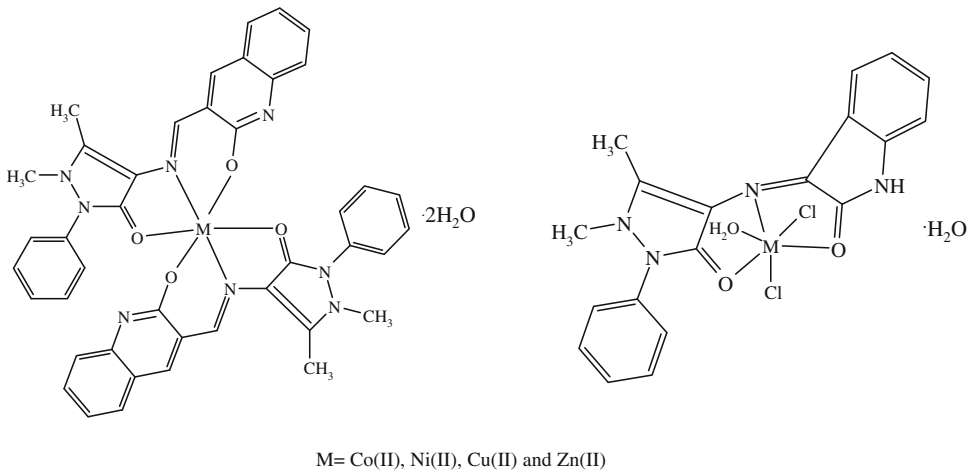


Fig. 2 The tentative structure of the complexes

complexes have shown better activity as compared to their ligands.

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