Monatsh Chem (2011) 142:487–494
DOI 10.1007/s00706-011-0462-1

ORIGINAL PAPER

Exploration on structure and anticonvulsant activity of transition metal complexes derived from an "end-off" compartmental bis-quinoxaline derivative with phthalazinyl-diazine as endogenous bridge

Srinivasa Budagumpi · Naveen V. Kulkarni · M. P. Sathisha · Sandeep P. Netalkar · Vidyanand K. Revankar · D. K. Suresh

Received: 10 February 2010 / Accepted: 14 February 2011 / Published online: 18 March 2011

Abstract An oligoquinoxaline derivative with phthalazine core has been prepared by condensation of 1,4-dihydrazinophthalazine with 2,3-dichloroquinoxaline in dry ethanol followed by acid hydrolysis. Classical endogenous bridging of phthalazine core with its diazine fragment was established in the transition metal(II) complexes derived from the ligand system by using various physicochemical and spectral techniques. The organic host acts as a hexadentate chelate with N_4O_2 donating sites for coordination towards later first-row transition metal ions. Complexes are in good agreement with the octahedral geometry and found to be 1:1 electrolytes. All synthesized compounds were screened for anticonvulsant activity in Wistar rats by using maximal electroshock method. The ligand, and Co(II) and Ni(II) complexes show appreciable suppression towards electroshock-induced seizures.

Keywords Quinoxaline · Phthalazine · Anticonvulsant · Octahedral · Maximal electroshock · Seizures

S. Budagumpi · M. P. Sathisha · S. P. Netalkar · V. K. Revankar (✉)
Department of Chemistry, Karnatak University, Pavate Nagar, Dharwad 580 003, Karnataka, India
e-mail: vkrevankar@rediffmail.com

S. Budagumpi
Department of Chemical Engineering, Pusan National University, Pusan 609-735, Republic of Korea

N. V. Kulkarni
Department of Chemistry, Technion - Israel Institute of Technology, 32000 Haifa, Israel

D. K. Suresh
Department of Pharmacology, Luqman College of Pharmacy, Gulbarga 585 102, Karnataka, India

Introduction

It is found in literature that 1,4-disubstituted phthalazine, quinoxaline, quinoline, and benzodiazepine derivatives are potent anticonvulsants, as they can strongly resist the unwanted evoked currents in the cerebella neurons growth in brain [1–5]. The central nervous system (CNS) is governed by inhibitory amino acids on the one hand, viz. gamma-aminobutyric acid (GABA), and excitatory amino acids on the other hand (glutamate). The ligand-gated ion channels (LGICs) encompass kainate, (*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and *N*-methyl-D-aspartic acid (NMDA) receptors. NMDA receptors are permeable to sodium, calcium, and potassium ions, following the direction of their natural gradient. The most characteristic feature of the NMDA receptor is its voltage-dependent regulation by magnesium, which means the channel pore can only be opened if partial depolarization has proceeded. It is evident from the reports that the mentioned compounds, especially 1,4-disubstituted phthalazine and benzodiazepine derivatives with quinoxaline substitutions, do act as AMPA/kainate receptor antagonists, hence becoming drugs of choice for epilepsy.

In particular, 3-substituted quinoline and quinoxaline derivatives are also known for epileptic activity [6], since their structures resemble reported anticonvulsants documented in the literature. In this work, we attempt to introduce a hydrazide fragment attached to the phthalazine ring, which is also an anticonvulsant agent, at 3-position of the quinoxaline ring to enhance the activity of the compounds formed.

The acyclic multinucleating ligands possessing two chelating arms and central donor bridging atom(s) are called "end-off" compartmental ligands. Diazine (–N=N–) bridge-containing end-off compartmental ligands are of

fundamental interest from the magnetochemistry point of view. Diazine bridges in some conjugated aromatic heterocyclic ligands can bring two metal centers into close proximity to form dinuclear complexes, and generate intramolecular magnetic exchange between the metal centers via the π-system of the heterocyclic ligand system [7–11]. This varies with the nature of the diazine ligands and the substituents present on the heterocycles. However, the nature of the exogenous bridging ion/group affects the magnetic exchange phenomena. Herein, we report the synthesis, characterization, electrochemistry, and anticonvulsant activity of later first-row transition metal(II) complexes derived from an end-off compartmental oligoquinoxaline derivative with phthalazine scaffold for endogenous bridging. We present the aspects of inorganic coordination chemistry that are of specific relevance to the metal ions in the field of medicine, especially as anticonvulsants.

Results and discussion

Chemistry

Magnetic moment and conductivity data for the complexes are given in Table 1. All complexes are insoluble in water, sparingly soluble in common organic solvents, and completely soluble in dimethylformamide (DMF), dimethyl sulfoxide (DMSO), and acetonitrile.

Table 1 Magnetic moment and conductance data of Co(II), Ni(II), Cu(II), and Zn(II) complexes

Complex	μ_{eff} (BM)	Molar conductance (mho cm^2 mol^{-1})
$[Co_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$	4.86	55.6
$[Ni_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$	2.47	58.1
$[Cu_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}2H_2O$	1.68	64.7
$[Zn_2L\mu(Cl)(H_2O)_4]Cl$	–	48.4

Infrared spectral studies

Pertinent infrared (IR) bands and their assignments are provided in Table 2. The IR spectrum of the ligand H_2L exhibits a broad secondary amine N–H stretching band in the region 3,190–3,350 cm^{-1}. This band is shifted to around 3,300 cm^{-1} in all complexes. This trend may be attributed to the coordination of the hydrazide –NH to the metal centers without deprotonation, and the same is further supported by the ^1H nuclear magnetic resonance (NMR) spectral study. A sharp band at 1,686 cm^{-1} in the free ligand is attributed to the lactam carbonyl of the quinoxaline core [12], which suffered a negative shift of about 12–23 cm^{-1} in all the complexes except Zn(II), confirming its involvement in the coordination. In case of the Zn(II) complex, there is no band above 1,637 cm^{-1}, and this trend may be attributed to the imidolization of the ligand. Furthermore, the imidol oxygen atom is coordinated to the metal center after deprotonation [13]. This was further confirmed by the disappearance of amide carbonyl stretching vibration band in the complex spectrum. The imidolized form of the ligand provides the more basic character in favor of forming stable complexes through deprotonation. A diazine stretching vibration band appeared at 1,630 cm^{-1} in the free ligand and suffered a shift of about 22–30 cm^{-1}, which unambiguously supports the classical endogenous diazine bridging between the metal centers [14]. A nonligand band in the region 423–471 cm^{-1} in the complexes is assigned to metal–nitrogen linkage [15].

^1H NMR spectral studies

The hexadentate ligand H_2L exhibits well-resolved peaks in DMSO-d_6 in the range 0–16 ppm, and the data are shown in Table 3. The spectrum shows a phthalazine –NH proton resonance at 11.9 ppm, quinoxaline –NH proton resonance at 11.5 ppm, and hydrazide –NH proton resonance at 4.1 ppm, and all three integrations correspond to two protons [16]. A conclusion drawn from the proton NMR study is the existence of a tautomeric form of the ligand in DMSO-d_6, as shown in Fig. 1. A set of multiplets

Table 2 Infrared spectral data of ligand H_2L and its complexes

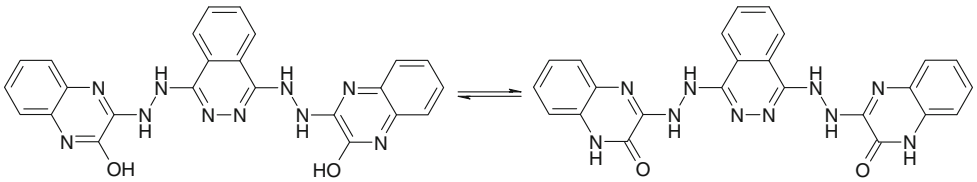
Compound	$\bar{\nu}$ (OH)	$\bar{\nu}$ (NH)	$\bar{\nu}$ (C=O) lactam	Phthalazine ring vibrations	$\bar{\nu}$ (M–N)
Ligand H_2L	–	3,196 b, 3,350	1,686	1,630	–
$[Co_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$	3,415	b, 3,200	1,673	1,652	s, 423
$[Ni_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$	3,429	b, 3,200	1,663	1,617	s, 450
$[Cu_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}2H_2O$	b, 3,420	b, 3,200	1,674	s, 1,600	s, 440
$[Zn_2L\mu(Cl)(H_2O)_4]Cl$	b, 3,420	b, 3,300	–	1,637	471

b broad band, *s* shoulder band

Table 3 ^{1}H NMR spectral data of the ligand H_2L and its Zn(II) complex (in ppm)

Compound	–NH (1- & 4-position)	–NH hydrazide	–NH quinoxaline	Aromatic
H_2L	11.9	4.1	11.5	7.1–8.6
$[Zn_2L\mu(Cl)(H_2O)_4]Cl$	11.8	7.6	–	6.8–7.3

Fig. 1 Tautomeric nature of the ligand H_2L in the solvent



in the region 7.1–8.6 ppm was observed for the aromatic protons. However, the spectrum of the Zn(II) complex shows the resonance of hydrazide –NH protons at a downfield region with the same integration number, which confirms the coordination of hydrazide –NH to the metal centers without deprotonation [17]. A peak due to quinoxaline –NH is absent in the complex spectrum, suggesting the formation of the imidol tautomer and subsequent coordination to the metal ions through the imidol oxygen atoms after deprotonation. Apart from this, there are no significant changes observed in the complex spectrum.

Electronic spectral studies

Electronic spectral data of the ligand H_2L and the complexes were recorded in DMSO. The spectrum of free ligand exhibits a distinguished band at 325 nm ascribed to the intraligand transitions, and this band remains in the spectra of complexes. The maxima appearing at 371 and 410 nm are due to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the azomethine fragments present in phthalazine, quinoxaline ring systems, and imidol chromophore. The band at 410 nm suffered blue-shift in the complexes, indicating the involvement of the diazine fragment of phthalazine core in the coordination. The electronic spectrum of the Cu(II) complex exhibits a medium-intensity broad band at 465 nm and a sharp band at 378 nm ascribed to the d–d transition and charge transfer transitions, respectively. Co(II) and Ni(II) complexes show medium-intensity bands at 553 (asymmetric) and 480, 576 nm assigned to the d–d transitions and witnessed the existence of octahedral ligand field around the metal ions [18, 19]. Both the spectra show sharp charge transfer bands in the region 320–370 nm. The Zn(II) complex shows a sharp charge transfer band at 463 nm.

Molar conductivity studies

The molar conductance value of all complexes was obtained at room temperature in DMSO solution at 10^{-3} mol dm^{-3} concentration (Table 1). The molar conductivity values of all complexes fall in the range 48.4–64.7 mho cm^2 mol^{-1}, which is in agreement with 1:1 electrolytic nature of the complexes [20].

Magnetochemistry

Room-temperature magnetic moment values of the paramagnetic complexes are tabulated in Table 1. The subnormal magnetic moment values reveal the operation of a moderate antiferromagnetic spin exchange interaction between the metal centers through the endogenous diazine bridging module present in the heterocyclic core. Although the diazine module brings the metal ions into close proximity, the more electronegative chloride as exogenous bridge affects the exchange interactions between the metal centers by causing a considerable drop in the electron density on the metal ions.

FAB mass-spectral studies

Copper and nickel complexes were characterized by the fast atom bombardment (FAB) mass-spectral technique using *m*-nitrobenzyl alcohol as the matrix material. The FAB mass spectra of the complexes exhibit molecular ion peaks at $m/z = 712$ and 693, respectively, which clearly suggests the monomeric nature of the complexes. The molecular ion observed in particular corresponds to the mass of the entire complex including bridging chloride and counter held chloride. The spectra exhibit some other peaks corresponding to the fragmented parts of the complexes. The presence of many peaks is due to the presence of isotopic atoms of both copper and chloride ions. By comparing other analytical and spectral data of the remaining complexes, it is evident that these are also monomeric, binuclear in nature.

Thermal studies

Complexes were studied for the thermal behavior over the temperature range 30–800 °C under nitrogen atmosphere. The thermogram of the copper(II) complex (shown in

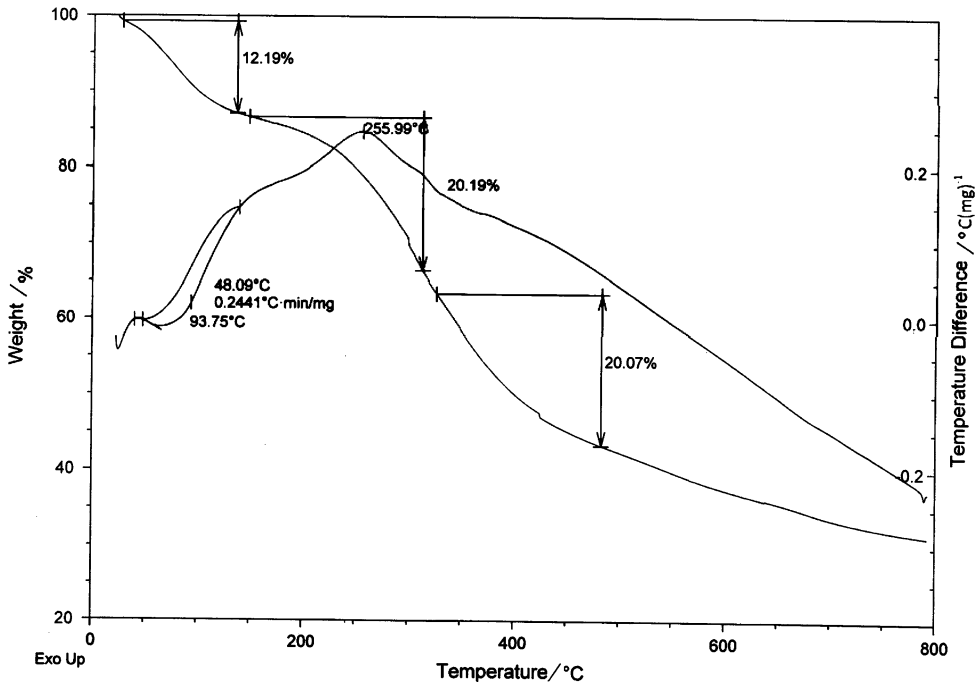


Fig. 2 Thermogram of the copper complex

Fig. 2) shows weight loss in three significant stages. Elimination of two coordinated and two crystal water molecules is evidenced by the appearance of weight loss of 12.2% in the temperature range 60–140 °C. Furthermore, the differential thermal analysis (DTA) curve shows two distinguished processes at 93.7 and 135 °C, corresponding to elimination of two crystal-held and two coordinated water molecules, respectively. In the next step, the weight loss of 20.2% in the temperature range 150–240 °C is assigned to the loss of four chloride ions as HCl, which is further evidenced by the appearance of an exothermic DTA signal at 256 °C. Finally, at the last stage, 20.1% weight loss occurred in the temperature range 375–500 °C, which is ascribable to the loss of the ligand part. Above 550 °C, a plateau was obtained, which corresponds to the formation of stable CuO.

Electrochemistry

The cyclic voltammograms of ligand and all complexes were recorded at room temperature in DMSO solution under oxygen-free conditions. An assembly of three electrodes consisting of a glassy carbon working electrode, platinum auxiliary electrode, and Ag/AgCl reference electrode was used to study the electrochemical behavior of the compounds with tetramethylammonium chloride (0.1 M) as supporting electrolyte. The electrochemistry of the compounds was studied in the potential range of −1 to +1 V at three different scan rates, viz. 0.05, 0.1, and 0.15 V/s.

Due to the presence of –NH fragments on either side of the phthalazine core, which makes these ligands electrochemically active, the cyclic voltammetric study of these ligands and complexes is of particular interest. The cyclic voltammogram of the ligand H_2L exhibits oxidation peaks at 0.01, 0.08, and 0.15 V and in the reverse scan reduction peaks are observed at −0.39, −0.45, and −0.53 V at the above-mentioned scan rates. The observed oxidation peak is attributed to the loss of hydrogen atoms as H^+, and the corresponding reduction is in good agreement with the regain of lost protons. Finally, the obtained redox behavior is a two-proton, two-electron transfer process. More or less only these peaks are observed in the cyclic voltammograms of the complexes, which clearly highlights the electrochemical inactivity of the metal complexes.

Pharmacology

Acute toxicity studies

Acute studies of the synthesized compounds in Wistar rats are very useful to decide the effective dose for administration. The compounds, viz. ligand H_2L, Co(II), Ni(II), Cu(II), and Zn(II) complexes, caused death of experimental rats above intraperitoneal doses of LD_{50} of 600, 500, 700, 800, and 500 mg/kg body weight of rat, respectively. This is an indication that the synthesized compounds cause toxicity at higher doses. According to the classification of Clarke and Clarke [21], substances with LD_{50} of 1,000 mg/kg body weight are regarded as safe or low-toxicity compounds.

Table 4 Effect of ligand H_2L and its Co(II), Ni(II), Cu(II), and Zn(II) complexes on maximal electroshock-induced convulsion in Wistar rats

Treatment/dose (mg/kg)	Time (s) in various phases of convulsion			
	Flexion	Extensor	Clonus	Stupor
Control, gum acacia 1% w/w	3.81 ± 0.30	12.0 ± 0.57	17.68 ± 1.44	222.0 ± 5.8
Phenytoin 20 mg/kg	3.10 ± 0.28	1.22 ± 0.12	5.22 ± 1.88	163.7 ± 6.11
Ligand H_2L 60 mg/kg	4.22 ± 0.41	2.27 ± 0.11	1.00 ± 0.33	175.5 ± 3.12
$[Co_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$ 50 mg/kg	5.00 ± 0.28	3.89 ± 0.31	1.85 ± 1.25	218.0 ± 4.89
$[Ni_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$ 70 mg/kg	6.00 ± 0.12	2.00 ± 0.35	1.25 ± 0.25	170.6 ± 4.11
$[Cu_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}2H_2O$ 80 mg/kg	4.12 ± 0.4	12.0 ± 0.58	13.62 ± 1.85	210.0 ± 2.22
$[Zn_2L\mu(Cl)(H_2O)_4]Cl$ 50 mg/kg	2.89 ± 0.19	11.0 ± 0.51	15.8 ± 1.89	210.0 ± 2.22

The administered dose to check the title activity in rats is one-tenth of the lethal dose.

Anticonvulsant studies

The effects of the synthesized compounds against electroshock-induced seizures in Wistar rats in different stages of convulsion are tabulated in Table 4. The maximal electroshock method (MES) was performed to find the anticonvulsant activity of the ligand H_2L and its later first-row transition metal(II) complexes. The anticonvulsant efficacy of the novel 1,4-disubstituted phthalazine derivatives was evaluated 30 min after intraperitoneal (i.p.) administration against maximal electroshock-induced seizures in Wistar rats, which has been recognized as an excellent model for screening novel anticonvulsant agents.

The extensor phase of convulsion, where the hind limb extensor tone was observed in the rats, and the abolition of hind limb extension were taken as the parameters of anticonvulsant activity of the tested compounds. Remarkable suppression of episodes of neurological dysfunction arising from abnormal synchronous activity of neurons was noted in case of the ligand H_2L and its Co(II) and Ni(II) complexes. From the results it was observed that these compounds show much shorter time for the rats to recover in the hind limb extensor phase of the convulsion. The internal standard phenytoin took 1.22 ± 0.12 s for the recovery, whereas H_2L and its Co(II) and Ni(II) complexes took 2.27 ± 0.11, 3.89 ± 0.31, and 2.00 ± 0.35 s, respectively. However, the remaining compounds allowed sufficient time for the recovery in the extensor phase.

Finally, in clonus and stupor phase, the animals were almost inactive. However, pupils were sacrificed in the latter two cases and recovered in the former cases. It is concluded from the anticonvulsant studies that H_2L and its Co(II) and Ni(II) complexes were active against the maximal electroshock-induced seizures in Wistar rats whereas the remaining were not. It is predicted from the comparison data that the mode of action of the novel compounds is similar to that of the standard phenytoin, which suppresses seizures by direct action on membrane "stabilization" (usually by action on fast Na^+ channels). It is very difficult to determine the mode of action and the therapeutic efficacy of the synthesized compounds at this level, which can be explored only after a comprehensive study.

Conclusions

Later first-row transition metal(II) complexes of an oligoquinoxaline derivative having a phthalazinyl diazine module as endogenous bridge have been prepared and characterized by various spectral and analytical techniques. The ligand acts as a hexatopic neutral and dibasic chelate towards Co(II), Ni(II), Cu(II), and Zn(II) (tentative structures of the complexes are shown in Fig. 3). These diazine-bridged complexes were found to be octahedral and 1:1

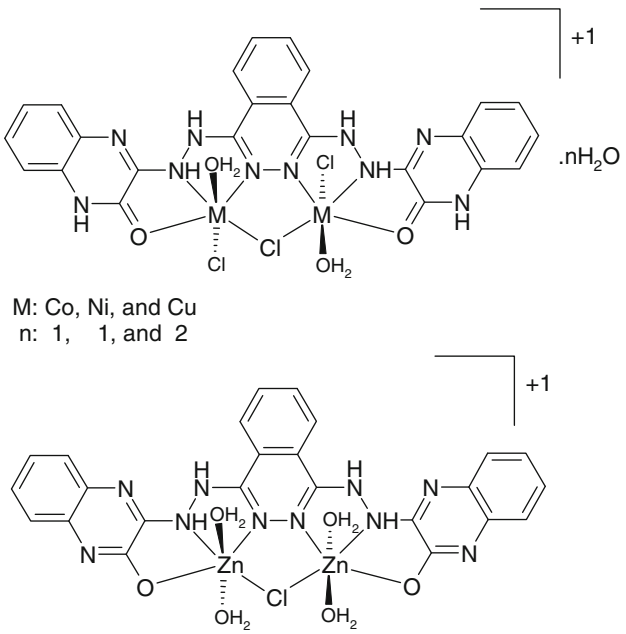


Fig. 3 Proposed structures of the complexes

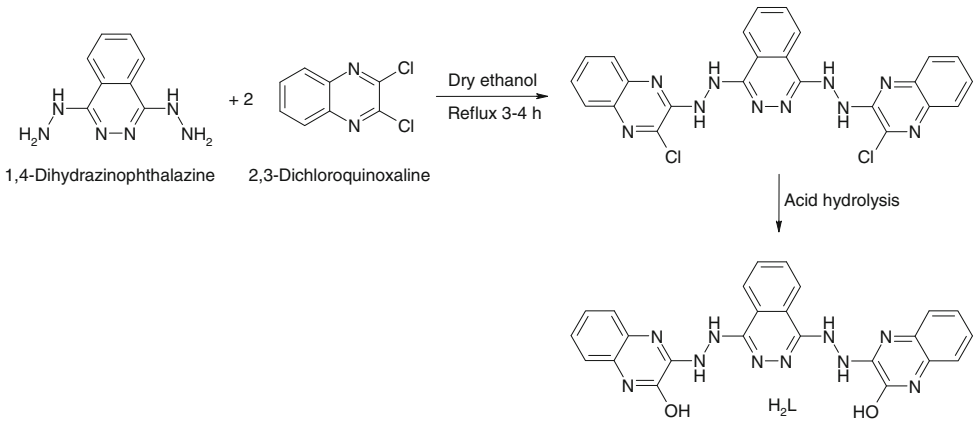


Fig. 4 Preparation of the end-off compartmental ligand

electrolytes in nature. Ligand H_2L was found to be electrochemically active in the working potential range, whereas the derived complexes exhibit only ligand-based electrochemical signals. These novel compounds were tested for the anticonvulsant activity, since they structurally mimic standard anticonvulsants reported in the literature. Among tested samples, ligand, and Co(II) and Ni(II) complexes showed strong suppression of seizures caused by the maximal electroshock method in Wistar rats.

Experimental

Analysis and physical measurements

Estimation of the metal(II) ions was carried out according to standard methods. Molar conductivity measurements were carried out using an ELICO-CM-82 conductivity bridge. Carbon, hydrogen, and nitrogen were estimated using a Thermoquest CHN analyzer; obtained results agreed favorably with calculated values. Magnetic susceptibility measurements were carried out using a Faraday balance at room temperature using $Hg[Co(SCN)_4]$ as reference. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker 300-MHz spectrometer at room temperature using tetramethylsilane (TMS) as internal reference. IR spectra were recorded in a KBr matrix using an Impact-410 Nicolet (USA) Fourier-transform infrared (FT-IR) spectrometer in the 4,000 to 400 cm^{-1} range. The electronic spectra of the complexes were recorded using a Hitachi 150–20 spectrophotometer in the range of 1,000 to 200 nm. The electron spin resonance (ESR) spectrum of the copper complex was measured using a Varian E-4X-band EPR spectrometer, using tetracyanoethylene (TCNE) as the g-marker. Thermogravimetry (TG) and DTA measurements of the complexes were recorded in nitrogen atmosphere using a Universal V2.4F TA instrument with heating rate of 10 °C/min, keeping the final temperature at 800 °C. Cyclic voltammetric studies were performed at room temperature in DMF under O_2-free conditions using a CH Instruments CHI-1110A (USA) electrochemical analyzer. FAB mass spectra were drawn from a JEOL SX 102/DA-6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas.

Chemistry

All chemicals used for the present investigation were of reagent grade. The solvents were dried and distilled before use according to literature methods. Preparation of 2,3-dichloroquinoxaline [22] and 1,4-dihydrazinophthalazine [23] was carried out with slight modifications according to reported methods.

1,4-[Di(2-hydroxy-3-quinoxalinyl)hydrazino]phthalazine-(H_2L) ($C_{24}H_{18}N_{10}O_2$)

Dry ethanolic solution (20 cm^3) containing 0.19 g 1,4-dihydrazinophthalazine (0.001 mol) was added to 0.394 g 2,3-dichloroquinoxaline (0.002 mol) in 20 cm^3 dry ethanol, and the mixture was stirred for 30 min and refluxed for about 4 h at water bath temperature. The separated pale-orange solid was filtered and washed with ethanol. It was then recrystallized from ethyl acetate and stored under vacuum. Yield: 0.367 g (72%); m.p.: 227–229 °C.

To the ligand precursor (0.514 g, 0.001 mol) in 5 cm^3 ethanol, 20 cm^3 4 M hydrochloric acid was added and refluxed at 100–110 °C for about 6 h. Then the solid was filtered, washed with ethanol, and recrystallized from ethyl acetate. Yield: 0.301 g (63%); m.p.: 276–277 °C; FT-IR (KBr disc): $\bar{\nu}$ = 3,350, 3,196 (NH), 1,686 (C=O), 1,630 (phthalazine ring) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 11.9 (s, 2H, –NH at 1- and 4-position of phthalazine), 11.5 (s, 2H, –NH quinoxaline), 7.1–8.6 (m, 12H, aromatic), 4.1 (s, 2H, –NH hydrazide) ppm; ^{13}C NMR (DMSO-d_6): δ = 160.9 (amide), 143.1 (3-quinoxaline), 139.1 (1,4-phthalazine), 135, 131.2, 129.1 (quinoxaline aromatic

region), 122.3, 119, 115.1, 107.1 (phthalazine aromatic region) ppm.

Preparation of the complexes

The complexes of the ligand H_2L with Co(II), Ni(II), Cu(II), and Zn(II) were prepared by refluxing the respective metal chlorides (0.002 mol) in 50 cm^3 ethanol with ligand (0.478 g, 0.001 mol) for about 3–4 h at water bath temperature. In case of cobalt and zinc complexes, pH of the reaction mixture was raised by addition of alcoholic NH_3 to provide the basic condition for complexation. The obtained complexes were separated by filtration under suction and dried over anhydrous $CaCl_2$.

Diaquadichloro-μ-chloro-[1,4-[di(2-oxato-3-quinoxalinyl)hydrazino]-phthalazinhexayl]dicobalt chloride hydrate ($[Co_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$) ($C_{24}H_{22}Cl_4Co_2N_{10}O_5$)
Yield 92.3%, m.p.: >280 °C; FT-IR (KBr disc): $\bar{\nu}$ = 3,415 (OH), 3,200 (broad, NH), 1,673 (C=O), 1,652 (phthalazine ring), 423 (M–N) cm^{-1}; magnetic moment: 4.86 BM; molar conductance: 55.6 ohm cm^2 mol^{-1}.

Diaquadichloro-μ-chloro-[1,4-[di(2-oxato-3-quinoxalinyl)hydrazino]-phthalazinhexayl]dinickel chloride hydrate ($[Ni_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}H_2O$) ($C_{24}H_{22}Cl_4N_{10}Ni_2O_5$)
Yield: 89.7%, m.p.: >280 °C; FT-IR (KBr disc): $\bar{\nu}$ = 3,429 (OH), 3,200 (broad, NH), 1,663 (C=O), 1,617 (phthalazine ring), 450 (M–N) cm^{-1}; magnetic moment: 2.47 BM; molar conductance: 58.1 ohm cm^2 mol^{-1}.

Diaquadichloro-μ-chloro-[1,4-[di(2-oxato-3-quinoxalinyl)hydrazino]-phthalazinhexayl]dicopper chloride dihydrate ($[Cu_2L\mu(Cl)Cl_2(H_2O)_2]Cl{\cdot}2H_2O$) ($C_{24}H_{24}Cl_4Co_2N_{10}O_6$)
Yield: 96.2%, m.p.: >280 °C; FT-IR (KBr disc): $\bar{\nu}$ = 3,420 (broad, OH), 3,200 (broad, NH), 1,674 (C=O), 1,600 (phthalazine ring), 440 (M–N) cm^{-1}; magnetic moment: 1.68 BM; molar conductance: 64.7 ohm cm^2 mol^{-1}.

Tetraaqua-μ-chloro-[1,4-[di(2-hydroxy-3-quinoxalinyl)-hydrazino]-phthalazinhexayl]dizinc chloride ($[Zn_2L\mu(Cl)(H_2O)_4]Cl$) ($C_{24}H_{24}Cl_2N_{10}O_6Zn_2$)
Yield: 93.7%, m.p.: >280 °C; FT-IR (KBr disc): $\bar{\nu}$ = 3,420 (broad, OH), 3,300 (broad, NH), 1,637 (phthalazine ring), 471 (M–N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 11.8 (s, 2H, –NH at 1- and 4-position of phthalazine), 7.6 (s, 2H, –NH hydrazide), 6.8-7.3 (m, 12H, aromatic) ppm; molar conductance: 48.4 ohm cm^2 mol^{-1}.

Pharmacology

Animals selected for the present study
Wistar 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 23 ± 2 °C, controlled humidity conditions, and 12:12 hour light:dark cycle. The Wistar rats were housed in sterile transparent Plexiglass cages containing sterile paddy husk as bedding material with a maximum of four animals in each cage. The rats were fed on autoclaved standard rat food pellets and water ad libitum.

Acute toxicity study [24, 25]
Acute toxicity studies and the determination of dose were performed according to the Organization for Economic Cooperation and Development (OECD-420). It is customary to carry out an acute toxicity study to determine the safe and effective dose of the novel compounds, since their toxicity is unknown. Wistar rats were starved for 18 h prior to the experiment. The animals were divided into groups of eight each after recording their body weight. Test sample solutions of suitable concentration in 1% gum acacia were administered orally in different groups. Initially all test samples were administered with 12.5 mg/kg body weight; if all animals survived with this dose, then the samples were tested at higher dose range, viz. 25, 50, 100, 200, and 400 mg/kg. If the test samples caused 100% death at this dose, the lower dose range was treated as the LD_{50} dose. The administered dose is one-tenth of the threshold dose in the present study.

Testing of compounds for anticonvulsant activity against maximal electroshock-induced seizures in Wistar rats [24, 25]
Previously weighed and numbered Wistar rats were categorized into seven groups, each consisting of four rats. One group was used as control, one for phenytoin drug treatment, and the remaining five for the novel sample treatments. 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 an electroconvulsiometer to all groups. Each animal was placed into an individual transparent Plexiglass case and was observed for 30 min (in seconds), and various phases of convulsion, viz. tonic flexion, tonic extensor, clonic convulsions, and stupor, were noted. All experimental groups were compared with the respective control.

Statistical analysis
Values are expressed as mean ± standard error of the mean (SEM), and statistical difference between means was determined by performing one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered as indicating significant difference in the present study.

Acknowledgments The authors thank the Department of Chemistry and USIC, Karnatak University, Dharwad for spectral and analytical facilities. Recording of FAB mass spectra (CDRI Lucknow) is gratefully acknowledged. S.B. thanks the University Grants Commission, New Delhi, India for awarding a Research Fellowship in Science for Meritorious Students.

References

1. Sarro GD, Gitto R, Russo E, Ibbadu GF, Barreca ML, Luca LD, Chimirri A (2005) Curr Top Med Chem 5:31
2. Chimirri A, Sarro GD, Quartarone S, Barreca ML, Caruso R, Luca LD, Gitto R (2004) Pure Appl Chem 76:931
3. Grasso S, Sarro GD, Sarro AD, Micale N, Zappala M, Puja G, Baraldi M, Micheli CD (2000) J Med Chem 43:2851
4. Gitto R, Orlando V, Quartarone S, Sarro GD, Sarro AD, Russo E, Ferreri G, Chimirri A (2003) J Med Chem 46:3758
5. Pelletier JC, Hesson DP, Jones KA, Costa AM (1996) J Med Chem 39:343
6. Larsen PK, Ebert B, Lund TM, Osborne HB, Slok KA, Johansen TN, Brehn L, Madsen U (1996) Eur J Med Chem 31:515
7. Klingelea J, Decherta S, Meyer F (2009) Coord Chem Rev 253:2698
8. Schneider CJ, Cashion JD, Moubaraki B, Neville SM, Batten SR, Turner DR, Murray KS (2007) Polyhedron 26:1764
9. Chandrasekhar V, Thirumoorthi R, Azhakar R (2007) Organometallics 26:26
10. Eisenwiener A, Neuburger M, Kaden TA (2007) Dalton Trans 218
11. Dey SK, Abedin TSM, Dawe LN, Tandon SS, Collins JL, Thompson LK, Postnikov AV, Alam MS, Müller P (2007) Inorg Chem 46:7767
12. Annigeri SM, Sathisha MP, Revankar VK (2007) Transition Met Chem 32:81
13. Budagumpi S, Kulkarni NV, Kurdekar GS, Sathisha MP, Revankar VK (2010) Eur J Med Chem 45:455
14. Kuzelka J, Mukhopadhyay S, Spingler B, Lippard SJ (2004) Inorg Chem 43:1751
15. Saha NC, Saha A, Butcher RJ, Chaudhuri S, Saha N (2002) Inorg Chim Acta 339:348
16. Budagumpi S, Sathisha MP, Kulkarni NV, Kurdekar GS, Revankar VK (2010) J Incl Phenom Macrocycl Chem 66:327
17. Budagumpi S, Kurdekar GS, Kulkarni NV, Revankar VK (2009) J Incl Phenom Macrocycl Chem. doi:101007/s10847-009-9701-z
18. Lever ABP (1968) Inorganic Electronic Spectroscopy. Elsevier, Amsterdam
19. Sathisha MP, Shetti UN, Revankar VK, Pai KSR (2008) Eur J Med Chem 43:2338
20. Geary WJ (1971) Coord Chem Rev 7:81
21. Clarke EGC, Clarke ML (1977) Veterinary Toxicology. Cassed and Collier, London
22. Cucino RF, Pandey RK (2005) J Org Chem 70:5344
23. Reynolds GA, Van Allen JA, Tinker JF (1959) J Org Chem 24:1205
24. Mishra AK, Dandiya PC, Kulkarni SK (1973) Indian J Pharmacol 5:449
25. Kulkarni SK (1981) Arch Int Pharmacodyn 252:124