Anticonvulsant Activity and Toxicity Evaluation of Cu^{II} and Zn^{II} Metal Complexes Derived from Triazole-Quinoline Ligands

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Received February 23, 2010; accepted September 5, 2010; published online September 7, 2010

A novel Cu^{II} and Zn^{II} complexes of triazolo-quinoline derivatives were synthesized by *in situ* method and were characterized by the spectro-analytical methods and their pharmacological properties were evaluated. The compound C3 has exhibited promising anticonvulsant activity towards the electroshock induced seizures in Wistar rats and possesses low toxicity, providing a high safety profile.

Key words quinoline; triazole; anticonvulsant agent; maximal electroshock method

Epilepsy is an ever-present serious disease related to the neurological disorders. It is generally characterized by recurrent seizures that result from phasic changes in the firing properties of groups of neurons. The induction of a group of neurons into a pattern of burst-firing results in synchronized disruptive seizure discharge. The detailed studies reveals that, the excitatory and inhibitory neurotransmission as well as voltage-gated channels (that can be modulated selectively by natural or synthetic toxins) are the two major factors, that are found to be the crucial for seizure like activity.[1,2] The antiepileptic drugs, which are in efficacious clinical practice, act by inducing prolonged inactivation of the Na^+ channel, by blocking Ca^{2+} channel currents or by enhancing inhibitory γ-aminobutyrate (GABA)-ergic neurotransmission. Some of the other anticonvulsant agents act *via* a number of different mechanisms, which may include antagonism of glutamate-ergic neurotransmission.[3] However it is seen that, the majorities of antiepileptic drugs do not provide satisfactory seizure control in all patients and typically cause notable adverse side effects.[4,5] It is hence becomes a crucial and challenging task in the field of medicinal chemistry to find more efficient and safer antiepileptic agents.

In this regard, there are few attempts made, the organic moieties having anticonvulsant property were interacted with suitable metal ions and hence formed complexes are investigated for the anticonvulsant activity. Interestingly, a considerable enhancement of activity upon complexation has been observed,[6,7] which is certainly related to the increased lipophilicity upon complexation (which increases their permeability to the blood-brain barrier) and inertness of certain metal ligand linkages towards enzymatic degradation.[8,9]

Quinoline derivatives have been known to possess a variety of biological activities such as antitumor,[10] antimalarial,[11] antidepressant,[12] antiulcer[13] and cardiac stimulant.[14] Along with these, there are reports suggesting that the quinolines have exhibited a very good anticonvulsant activity in the maximum electroshock method test.[15] In this regard, so many attempts are made by incorporating various hetero cyclic groups to the quinoline core, in order to obtain compounds with better anticonvulsant activity.[16,17] Substitution of triazole or triazolone ring to the quinoline core has been found to increase receptor binding capacity and metabolic stability, which in turn enhances the anticonvulsant activity of the compound.[18,19] In the present research work, we have selected 2-hydroxy, 3-formyl quinoline as a precursor. The presence of oxygen functional groups in the quinoline core is expected to facilitate the elliptical activity[20] as well as complexation. Amino triazoles are incorporated to the precursor by means of Schiff base formation in the presence of Cu^{II} and Zn^{II} ions (these two are selected by considering the higher bioaccessiblity, as compared to the other transition metal ions).[21] The metal ions will certainly will fit the coordination cavity formed, and lead to the formation of *in situ* metal complexes. The compounds are investigated for acute toxicity and *in vitro* anticonvulsant activity in Wistar rats.

Results and Discussion

Chemistry. Analysis and Physical Measurements Estimation of the metal and chloride was carried out according the standard methods.[22] C, H, N and S analysis was carried out on Thermo Quest elemental analyzer. The molar conductivity measurements were made on ELICO-CM-82 conductivity bridge, with conductivity cell having cell constant $0.51\,cm^{-1}$. The magnetic susceptibility measurements were made on faraday balance at room temperature using Hg $[Co(SCN)_4]$ as calibrant. The ^{1}H-NMR spectra were recorded in DMSO-d_6 solvent on Bruker-300 MHz spectrometer at room temperature using tetra methyl silane (TMS) as internal reference. IR spectra were recorded in a KBr matrix using an Impact-410 Nicolet (U.S.A.) Fourier transform-infrared (FT-IR) spectrometer in 4000—400 cm^{-1} range. The electronic spectra of the complexes were recorded on a Hitachi 150-20 in the spectrophotometer range of 1000—200 nm. The ESR spectrum of the copper complex was carried out on a Varian E-4X-band electron paramagnetic resonance (EPR) spectrometer, using tetra cyanoethylene (TCNE) as the *g*-marker. The cyclic voltammetric studies were performed at room temperature in *N*,*N*-dimethyl formamide (DMF) under O_2 free condition using CH instruments Electrochemical analyzer, CHI-1110A (U.S.A.), comprising three electrode assembly of glassy carbon working electrode, platinum auxiliary electrode and Ag^+/AgCl reference electrode. Tetramethylammoniumchloride (0.01 M) was used as supporting electrolyte. The FAB mass spectra were drawn from JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon (6 kV, 10 mA) as the FAB gas and *m*-nitrobenzylalcohol as

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Table 1. General Information, Elemental Analysis and Molar Conductivity Data

Compounds	Molecular formula	Color	Yield in %	Elemental analysis (%) Calculated (Found)						Molar conductivity $Ohm^{-1} cm^2 mol^{-1}$
				C	H	N	S	M	Cl	
C1	$[Cu_2(C_{26}H_{24}N_{10}O_2S_2)]$	Dark brown	80	49.44 (50.05)	3.16 (3.25)	22.18 (22.46)	10.13 (10.02)	10.05 (9.84)	—	10.2
C2	$[Zn_2(C_{26}H_{24}N_{10}O_2S_2)]$	Yellow	76	49.29 (49.42)	3.15 (3.23)	22.08 (22.12)	10.10 (9.82)	10.33 (10.45)	—	9.5
C3	$[Cu_2(C_{24}H_{20}N_{10}O_2S_4)]$	Brown	79	43.14 (43.25)	2.39 (2.45)	20.97 (21.06)	19.17 (19.45)	9.51 (9.78)	—	9.6
C4	$[Zn_2(C_{24}H_{20}N_{10}O_2S_4)]$	Orange	76	43.01 (43.15)	2.38 (2.52)	20.91 (20.82)	19.11 (19.31)	9.78 (9.80)	—	10.3
C5	$[Cu(C_{12}H_{14}N_5OS_2)Cl(H_2O)_2]$	Dark brown	82	34.74 (34.86)	2.67 (2.79)	15.59 (15.48)	14.25 (14.28)	14.14 (14.26)	7.90 (7.78)	13.6
C6	$[Zn(C_{12}H_{14}N_5OS_2)Cl(H_2O)_2]$	Orange	80	34.58 (34.56)	2.66 (2.73)	15.52 (16.02)	14.19 (14.28)	14.52 (14.58)	7.89 (7.80)	12.8

matrix.

Molar Conductivity Measurements The molar conductance values of the complexes measured at room temperature in DMF solution with 10^{-3} mol/dm^3 concentration fall in the range 9.5 to 15.2 $ohm^{-1} cm^2 mol^{-1}$ indicating the non-electrolytic nature of the compounds.[23]

Infrared Spectroscopy Studies IR spectra of all complexes show a sharp band in the range 1631—1648 cm^{-1} assigned for the ν(C=N), which suggest the Schiff base formation.[24] Shift in the absorption frequencies observed was due to the participation of C=N group in coordination.[25] This is supported by the fairly lower values of ν(N–N) absorptions. Further M–N bands observed *ca.* 450 cm^{-1} in the spectra confirms the azomethine coordination. Absorptions around 2900—3000 cm^{-1} and 1300—1320 cm^{-1} which corresponds to –OH (Quinoline) stretching and bending vibrations respectively were not observed in any of the complexes.[26] In the case of C1 and C2, the hydroxy group is involved in toutomerisation, giving the quinolinone form to the ligand and the lactam oxygen is involved in the coordination, which is suggested by the sharp absorption bands around 1650—1660 cm^{-1} assignable to the lactam ν(C=O) and the new peak appeared around δ 13.10 ppm due to quinoline ring NH in the ^{1}H-NMR spectrum of C2.[27] In all other complexes hydroxyl group is coordinated upon deprotonation.[27] The higher frequency values of the ν(C–O) in the complexes counsel the same. The coupled vibrations among the thioamidic bands having the major contribution from ν(C=S) are generally observed around 1250 cm^{-1} and 750 cm^{-1}.[28] In case of C1 and C2 complexes, these absorptions have completely disappeared due to the thio-enolisation and subsequent coordination of sulphur through deprotonation.[28] In all other complexes the thioamidic bands have experienced a fall in the intensity and shift towards the lower energy side indicating the thioketo mode of coordination.[28] This can be well explored in the case of C3 and C4 complexes, where the intensity and the frequency of non-coordinating ν(C=S) can be compared with the coordinated one. Either of the two thioketo sulfur atoms participates in ligation and experiences decrees in the intensity and red shift, the other one will stay apart from the coordination and absorbs normally, this result in to splitting of the corresponding absorption bands. The thioketo mode of coordination is further supported by the presence of ν(N–H) absorptions around 3150—3200 cm^{-1} in the spectra. Further the ν(M–S) bands observed in the spectra evidences the sulfur coordination. The sulphhydryl ν(S–H) in the C5 and C6 complexes appears around 2500 cm^{-1} as a weak, broad signal whose presence is further confirmed by a signal at δ 5.88 ppm in ^{1}H-NMR spectra. However, the low intensity and the broadening of the bands may be attributed to the intermolecular hydrogen bonding. The broad band observed around *ca.* 3400 cm^{-1} in the case of C5 and C6 complexes was assigned to the ν(O–H) of coordinated water. The numerical values of the IR spectra are represented in Table 2.

^{1}H-NMR Studies The ^{1}H-NMR spectra of zinc complexes were recorded and the numerical values were tabulated in Table 3. In the case of C2 peak observed at δ 10.2 ppm was attributed to azomethine proton. The low field shift, as compared to the normal value evidences the azomethine coordination.[26] The peak at δ 8.73 ppm is attributed to C_3–H of quinoline ring and the other aromatic protons were resonated in the range δ 7.26—7.89 ppm. The presence of a peak, at δ *ca.* 13.10 ppm assignable to quinoline ring N–H reveals the quinolinone form of the ligand in the complex. The Absence of any peak around δ 5.00 ppm attributable to the –SH of triazole, indicates the coordination of the sulphur via deprotonation. The methyl protons of triazole were resonated at δ *ca.* 2.06 ppm.

In the C4 complex the azomethine proton was resonated at δ 10.54 ppm and the quinoline ring C_3–H was observed at δ 8.82 ppm. Two sister peeks appeared at δ 13.27 and 13.06 ppm were due to the triazole N–H protons suggesting the thioketo form of the ligand in the complexes.[28] A slight difference in the chemical shift value of either of the N–H can be correlated to the participation of one thioketo sulphur in the coordination. The coordination of thioketo group offers a change in chemical environment of the adjacent N–H proton, hence it experiences little downfield shift as compared to the other N–H proton which is adjacent to the non-coordinated thioketo sulphur. The aromatic proton resonances were observed around δ 7.36—7.98 ppm.

The C6 complex exhibits peaks at δ 4.00, 5.85 and 8.40 ppm attributed to methylene, mercapto and quinoline ring C_3–H protons respectively. The appearance of resonance peak corresponding to the sulphhydryl proton with expected

Table 2. IR Spectral Data in cm^{-1}

Compound	ν(O–H) Water	ν(N–H) Triazole	ν(C=N) Azomethine	ν(C=N) Triazole ring	ν(C=S) Thioamide		ν(S–H)	ν(C–O)	ν(N–N)	ν(M–N)	ν(M–S)
C1	—	3168 m	1635 b[a]	1560 m	1264 m	709 m	—	1380 m	978 m	464 s	422 s
C2	—	3185 m	1640 b[a]	1561 s	1215 m	711 m	—	1382 m	995 m	468 s	420 s
C3	—	3171 b	1644 s	1556 m	1220 m 1289 s	720 m 760 s	—	1421 s	1008 m	464 s	432 s
C4	—	3200 b	1633 s	1551 s	1226 m 1264 s	718 m 765 s	—	1419 s	986 m	476 s	425 m
C5	3420 b	3295 b	1648 s	1556 m	1225 m	699 m	2450 m	1400 s	990 m	466 s	425 m
C6	3391 b	3196 b	1643 s	1557 m	1242 m	702 m	2420 m	1399 s	985 m	470 s	430 s

b, road; s, strong; m, medium. *a*) Broadened due to the strong absorption of lactam group around 1650—1660 cm^{-1}.

Table 3. ^{1}H-NMR Spectral Data

Compound	Chemical shift values in δ ppm							
	Triazole ring N**H**	Azomethine –**H**C=N	Quinoline ring C_3–**H**	Aromatic protons	Sulphhydryl CH_2–S**H**	Methylene –C**H**$_2$–	Coordinated **H**$_2$O	–C**H**$_3$
C2[a]	—	10.20	8.73	7.26—7.89	—	—	—	2.06
C4	13.27[b] 13.06[b]	10.54	8.81	7.36—7.98	—	—	—	—
C6	13.72[b]	12.09	8.40	7.20—7.66	5.88[b]	4.01	3.04	—

a) Peak observed around δ 13.10 ppm is assigned to quinoline ring NH. *b*) Disappeared on D_2O exchange.

chemical shift value suggests non-participation of group in the coordination.[28] The azomethine proton has experienced high de-shielding effect upon coordination and resonated at δ 12.01 ppm. The triazole ring N–H was resonated at δ 13.72 ppm and the broad signal around δ 3.00 ppm indicates the water coordination. The NMR monitored studies reveals that the dimerisation of metal complexes is accompanied with the broadening of signals due to the superimposition of two or more resonances corresponding to the stereochemically equivalent units.[29]

Electronic Spectral Studies All the complexes exhibit electronic transitions around 260—315 nm, assigned to the intra ligand $\pi\rightarrow\pi^*$ transitions. A broad band at *ca.* 370 nm with a shoulder on low energy side is observed due to $n\rightarrow\pi^*$ transition associated with azomethine linkage.[30] The peak observed near 400 nm in all the complexes with ε *ca.* 25000 l cm^{-1} mol^{-1} is assigned to S→M ligand to metal change transfer transition (LMCT). The Cu^{II} complexes, C1, C3 and C5 exhibit absorptions around 480 and 680 nm with low ε values (ε *ca.* 100 l cm^{-1} mol^{-1}) assignable to the d–d transitions. Generally Cu^{II} complexes can adopt square-planar, square-pyramidal, trigonal-bipyramidal, octahedral and tetrahedral geometries, which, except for the first, are generally distorted from the idealized structures. The d–d spectra shown by these coordination geometries are distinctive only in the case of the tetrahedral environment where the absorptions occur at much lower energies and generally show, well separated absorption peaks; in case of all other distorted geometries, spectrum shows closely spaced absorption manifolds. Hence it is difficult to predict the accurate structure for the complexes only on the basis of electronic spectral analysis. However the octahedral geometry is assigned in the present case by considering the magnetic and analytical studies. Zn^{II} complexes, C2, C4 and C6 show absorptions around 400 nm, which are corresponding to intra ligand transitions.

Magnetochemistry All the three copper complexes exhibit the effective magnetic moments (μ_{eff}) in the range 1.62 to 1.68 BM at 303 K (1.68, 1.62 and 1.65 BM for C1, C3 and C5 respectively), which indicates an octahedral geometry. Fairly lesser effective magnetic moment values are the indicative of higher covalancy of Cu–S bond and lower spin–orbit coupling of sulphur.[31]

EPR Studies The solid state X-band EPR spectra of copper complexes exhibit isotropic intense broad signal with g_{iso} values 2.04, 2.03 and 2.05 respectively, with no hyperfine splitting in $g_{\|}$ or $g_{\perp}$ region. No half field absorption which is an index of metal–metal interaction was observed. This type of spectra was reported earlier for the complexes bearing large organic ligand substituents having considerable covalent character for metal–ligand bonds.[32] Similar comparison is made between S-bonded and O-bonded complexes, a decrease of 0.144 in g_{eff} values for S-bonded complexes was observed when compared with the O-bonded complexes. It is concluded that the EPR parameters are dependent on the coordinating atoms. This type of behavior has been observed for Schiff-base complexes and is attributed to (a) higher covalancy of Cu–S compared to Cu–O bond and (b) higher spin–orbit coupling constants for Sulphur than for Oxygen. Both factors reduce the spin–orbit contribution of the Cu(II) ion to the *g*-tensor, decreasing the *g*-value.[33]

Mass Spectral Analysis The elemental and analytical data suggest the molecular formula $[Cu_2(C_{26}H_{24}N_{10}O_2S_2)]$, $[Cu_2(C_{24}H_{20}N_{10}O_2S_4)]$ and $[Cu(C_{12}H_{14}N_5OS_2)Cl(H_2O)_2]$ for the copper complexes respectively, which is supported by the FAB mass spectral analysis. The peaks at highest *m*/*z* value can be assigned to the molecular ion by certainty, with the aid of consistent isotopic pattern. Peaks observed at *m*/*z* 632, 668 and 449 for the complexes are in consistent with the molecular mass of the complexes, representing the dimeric structures for first two copper complexes and monomeric

structure for the later. Apart from this, the mass spectra show some other peaks, which are due to molecular cations of various fragments of complex. The proposed structures assigned are represented in Fig. 1 (the representative FAB mass spectra are given in Fig. 2).

Thermal Studies The thermogram of C1 shows a plateau region up to 200 °C, indicating the absence of any water molecule or chloride in the complex. The disintegration of complex starts at 200 °C, the weight loss of 4.94 and 19.70% in the range 200—400 °C is corresponding to the decomposition ligand part. The DTA studies indicate the exothermic nature of the decomposition. The final product is found to be metal oxide. The copper complex C5 also exhibits three step decomposition pattern with 7.97% weight loss around 100—130 °C and 8.04% weight loss at around 190—280 °C corresponding to the liberation of two water molecules and chloride respectively. The differential thermal analysis (DTA) curve indicates respectively the endothermic and exothermic nature of the two processes. The continuous weight loss in the high temperature range is due to the ligand fragmentation, which ends up with a plateau region due to the stable metal oxide formation.

Electrochemistry The electrochemical behaviour of the complexes was investigated in room temperature by following the cyclic voltammetric experiments. The copper and zinc complexes are scanned in the potential range of +1.0 to −1.0 V with different scan rates (0.05, 0.1 and 0.15 V/s). All the three Cu^{II} complexes posses redox behaviour in the present experimental conditions indicating their biocompatibility.[34] While the zinc complexes do not show any electrochemical response over the working potential range indicating that electrochemical activity exhibited by copper complexes is purely metal based. The voltammograms are shown in Fig. 3 and numerical results are compiled in Table 4. All the copper complexes show a single peak in the range 0.29—0.46 V, in the anodic potential scan, which corresponds to $Cu^{II} \rightarrow Cu^{III}$, oxidation reaction. During the backward scan complexes exhibit a cathodic peak in the range −0.18 to −0.31 V corresponding to the reduction of metal species, $Cu^{III} \rightarrow Cu^{II}$ (in case of C5 reduction occurs in the range 0.34 to 0.37). The redox couple Cu^{II}/Cu^{III} shows a high value of ΔE_p, separation between the cathodic and anodic peak potentials ($E_{pa} - E_{pc}$) which is greater than 60 mV (which varies with scan rate) indicating the quasi-reversible nature of the redox process.[35] However C5 which has a lower ΔE_p value than other two complexes, shows decrease in the peak potential separation with lowering of scan rate, this may be assigned to the reversibility of system with very slow rate. Moreover, the total electrochemical behaviour of the complexes can be rationalized in terms of flexibility and the size of the coordination cavity in the complexes and the geometric requirements and the size of the metal ions in the different oxidation states.[36]

Pharmacology. Acute Toxicity Toxicity test results indicated that compounds C2 and C6 showed no toxicity at the dose of 400 mg/kg, whereas compounds C3 (500 mg/kg), C5 (600 mg/kg), C4 (700 mg/kg) and C1 (800 mg/kg) exhibit even better non-toxic nature, suggesting that they did not cause ataxia or other toxic reactions and thus possessed high safety profile.

Anticonvulsant Activity Maximal electroshock method (MES) was performed to evaluate the antiepileptic property of the prepared compounds. In the present pharmacological investigation, the anticonvulsant activity exhibited by the compounds (administered doses were given in Table 5) was compared with the Phenytoin drug, which is used as an internal standard in anticonvulsant study at the dose of 20 mg/kg body weight. MES induced convulsions in animals represent grand mal type of epilepsy, which resemble petit mal type of convulsions in man. Time (in seconds) at various phases of convulsion is tabulated in Table 5.

Flexion, the first phase attained by the Wistar rats immediately after delivering the electroshock of 150 mA through a pair of earclip electrodes. At this phase, the recurrent seizures were occurred in the rats by the appearance of neck jerking. The group of rats administered with phenytoin has recovered in 3.00±0.30 s. The copper encapsulated compounds C1, C3 and C5 have shown better action in this phase with petite recovery time as compared to the respective zinc complexes, C2, C4 and C6. Among which the compound C3 exhibits high anti-convulsion effect. We believe, the presence of free thione group which not only increases the lipophilicity but also enhance the receptor binding affinity of compound is responsible for the higher anticonvulsant activity. The free sulphhydryl group in C5 is however expected to behave in the similar fashion, providing a good anticonvulsion effect, but the presence of coordinated water molecules might have decreased the hydrophobicity of compound, which reduces the interacting assets of compound, leading to the decreased activity.

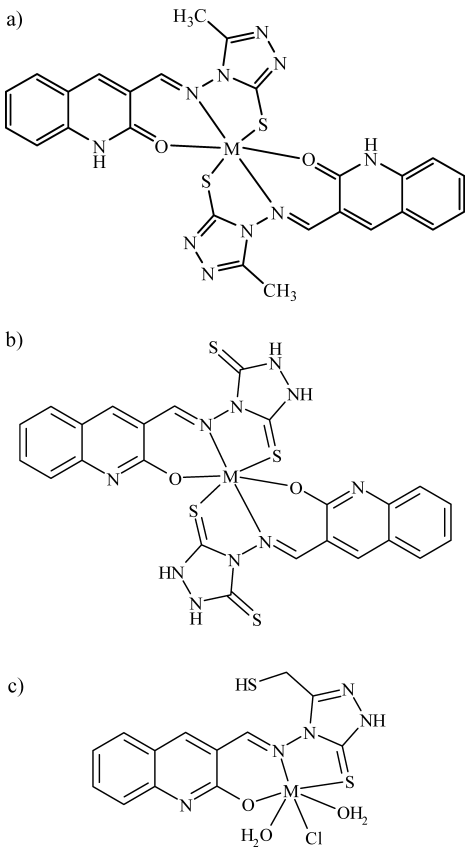

Fig. 1. Proposed Structure for Complexes

a) Structure of C1 and C2, where M=Cu and Zn. b) Structure of C3 and C4, where M=Cu and Zn. c) Structure of C5 and C6, where M=Cu and Zn.

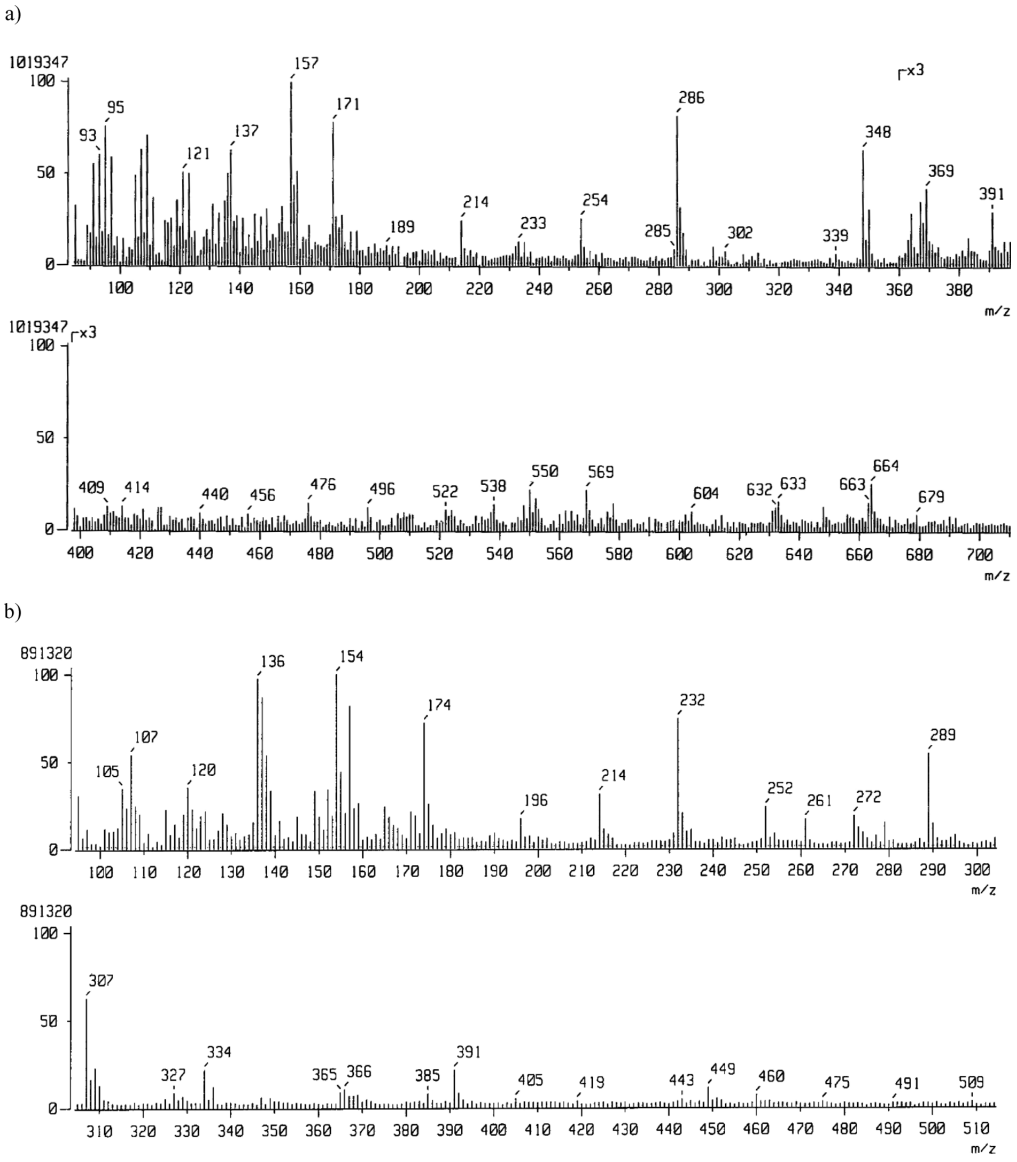

Fig. 2. FAB Mass Spectra of Copper Complexes
a) FAB mass spectra of C3. b) FAB mass spectra of C5.

In the second, extensor phase the hind limb extensor tone was observed and the abolition of hind limb extension was taken as the parameter of anticonvulsant activity.[36,37] The standard Phenytoin administered rats took 1.00 ± 0.00 s to get recover. The time–course of the anticonvulsant activity (for abolition of hind limb extensor) of synthesized complexes is little greater, as compared to that of standard used, as they reach the maximum value of 4.62 ± 0.71 to 10.9 ± 0.7 s.

Finally, in the last two phases of convulsion, Clonus and Stupor, where the animals were almost inactive, so that they even cannot move.[37] The compound C3, exhibits a very good activity in both of two stages, as the administered animals were recovered within a short period. The potency of C3 as an anticonvulsant is found to be same as the Phenytoin drug, a standard used, in these two convulsion phases. Apart from this, the compound C2 shows a bit promising activity in the clonus phase of convulsion with recovery time of 4.9 ± 6.0 s. The other compounds do not show significant activity in these two stages and the animals are hence sacrificed.

It is well known that the Phenytoin drug acts to reduce the unwanted, runaway brain activity seen in seizure by decreasing the electrical conductance among brain cells by stabilizing the inactive state of voltage gated sodium channels. We believe our prepared compounds follow the same mechanism in order to cure the tonic–clonic seizures. Triazole ring with the sulphur functionality is expected to enhance the receptor binding ability of molecule and hence stabilizing the inactive state of voltage gated sodium channels. Further, the anti-epileptic activity of molecule is found to be dependent upon its orientation, functional groups present and the nature of the metal ion. The detailed SAR studies are necessary to explore the therapeutic efficacy of the molecules.

Conclusion

New Cu^{II} and Zn^{II} complexes of triazolo-quinoline derivatives were prepared by *in situ* method. The ligands behave as tridentate monobasic with O, N and S donor atoms. Both Cu^{II}

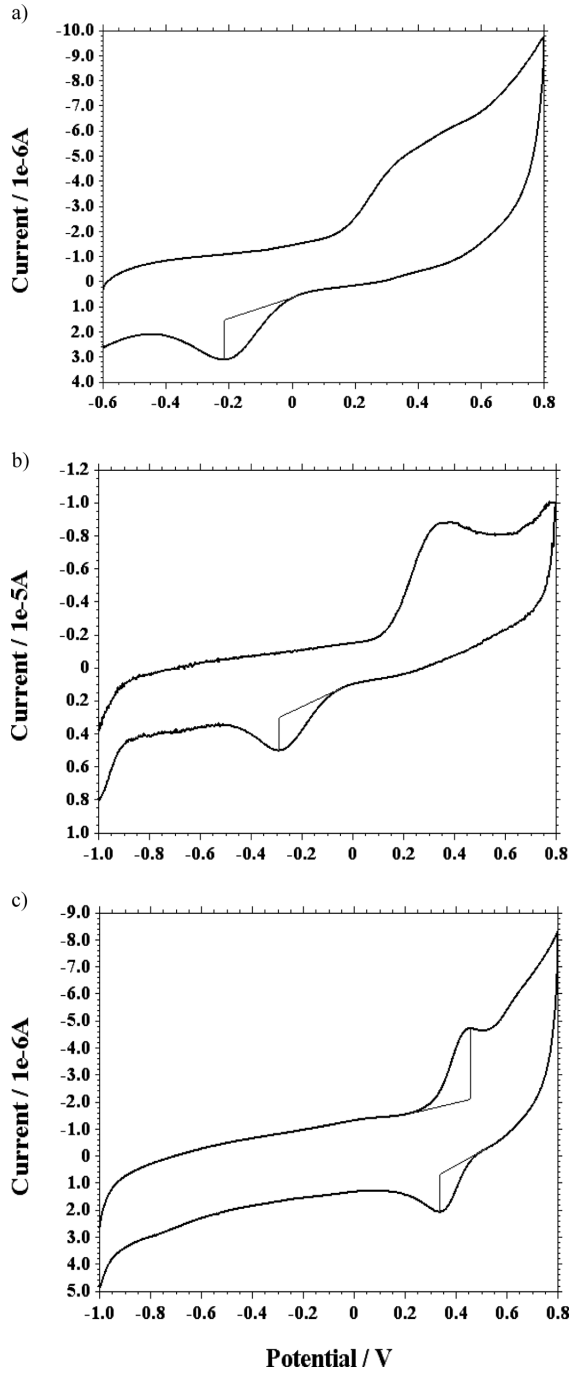

Fig. 3. Cyclic Voltammogram of Copper Complexes at the Scan Rate of 0.1 V/s

a) Voltammogram of C1. b) Voltammogram of C3. c) Voltammogram of C5.

and Zn^{II} complexes were found to be nonelectrolytes and have octahedral geometry. The spectro-analytical investigations have supported the presumed structures of compounds. The copper complexes exhibit redox behaviour in the applied potential range which is purely metal based. The prepared compounds were evaluated for pharmacological properties and have exhibited promising anticonvulsant activity towards the electroshock induced seizures in Wistar rats and possess low neurotoxicity, providing a high safety profile. The higher activity exhibited by the compound C3 as compared to phenytoin drug (standard drug used) is correlated to the structural features of compound. The detailed studies are suggested in order to understand the exact structure–activity relationship and therapeutic efficacy of the molecule.

Table 4. Cyclic Voltammetry Reults

Complex	Scan rate (V/s)	E_{pa} (V)	E_{pc} (V)	ΔE_p [a] (V)	I_{pc}/I_{pa}
C1	0.15	0.40	−0.22	0.62	0.80
	0.1	0.38	−0.20	0.58	0.81
	0.05	0.36	−0.18	0.54	0.85
C3	0.15	0.32	−0.31	0.63	0.82
	0.1	0.30	−0.28	0.58	0.83
	0.05	0.29	−0.26	0.55	0.84
C5	0.15	0.46	0.34	0.12	0.89
	0.1	0.45	0.36	0.09	0.90
	0.05	0.44	0.37	0.07	0.92

a) ($\Delta E_p = E_{pa} - E_{pc}$).

Table 5. Effect of 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	3.18±0.32	11±0.60	18.70±2.5	216±6.0
Phenytoin 20 mg/kg	3.00±0.30	01±0.00	4.8±4.8	160±20.5
C1 80 mg/kg	3.12±0.6	09±0.60	16±2.8	210±5.6
C2 40 mg/kg	4.1±1.07	4.62±0.71	4.9±6.0[a]	172±3.0
C3 50 mg/kg	3.0±0.8[a]	5.80±0.2	5.5±4.0[b]	168±6.0[b]
C4 70 mg/kg	3.60±0.7	9.0±0.7	15.0±2.8	210±5.0
C5 60 mg/kg	3.09±0.42	10.9±0.7	17.0±3.0	214±6.8
C6 40 mg/kg	4.50±1.08	4.68±0.3	12.0±3.8	214±5.0

a) Good activity. *b*) Significant activity.

Experimental

Chemistry. Materials and Methods All the chemicals used were of reagent grade and the solvents were dried and distilled before use according to the standard procedures. The preparations of 2-hydroxy-3-formyl quinoline[38] aminotriazoles[39] were carried out according to the earlier reports. Metal chlorides were used for the complex formation.

General Methodology for Preparation of Compounds To the hot ethanolic solution of 2-hydroxy-3-formyl quinoline (0.01 M, 1.73 g in 100 ml), 0.1 M solution of metal(II) chloride ($CuCl_2 \cdot H_2O$, 1.70 g/$ZnCl_2$, 1.30 g in 100 ml ethanol) is added, with stirring. After the thorough mixing, a solution of 0.01 M amino-triazole in ethanol (3-methyl-5-thione-4-amino-1,2,4-triazole (1.31 g), 3,5-thione-4-amino-1,2,4-triazole (1.48 g), 3-methyl-sulfhydryl-4-amino-5-mercapto-1,2,4-triazole (1.52 g)) is added dropwise to the mixture with continuous stirring. The mixture is then refluxed over steam bath for 2 h to obtain the complex in solid form. Compound isolated by filtration, is then washed with hot alcohol and dried (Yield and MP of each complexes are provided in Table 1).

Pharmacology. Animals for the Investigation Male and female Wistar rats weighing about 180—200 g were used in the present analysis with prior permission from 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 normal temperature (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 4 animals in each cage. The rats were fed on autoclaved standard rat food pellets and water *ad libitum*.

Acute Toxicity Test[40] Whenever a chemical substance is administrated to biological system, different types of interactions can occur and a series of dose–related responses result. In most cases these responses are desired and useful, but there are a number of other effects which are not advantageous. These may or may not be harmful to the patients. Hence in the pharmacological evaluation of any synthesized or isolated compound, it is customary to

carryout acute toxicity study to determine the safe effective dose of the novel compound. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals).

Wistar rats of either sex weighing between 180—200 g were starved for 18 h prior to the experiment. The animals were divided in to the group of eight each, after recording their body weight. The 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 the animals survived with this dose, then the samples were tested at higher dose range *viz.*, 25, 50, 100, 200, 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 800, 400, 500, 700, 600 and 400 mg/kg respectively for C1, C2 C3, C4, C5 and C6 complexes. The administered dose is the one tenth of the threshold dose.

Analysis of Anticonvulsant Activity of Compounds against Maximal Electroshock Generic Seizures in Wistar Rats[40] Previously weighed and numbered Wistar rats were categorized in to seven groups each consisting of 4 rats. The first group was labeled as control and the second one was used for the phenytoin drug (antiepileptic drug used as standard in the present analysis) treatment and remaining five groups are used for treatment with the prepared compounds. 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 was observed for 30 min. Time (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±S.E.M., statistical difference 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.

Acknowledgments The authors thank Department of Chemistry and USIC, Karnatak University, Dharwad for the spectral facility. Recording of FAB-mass spectra (CDRI Lucknow) are gratefully acknowledged. Further, the authors (Naveen V. Kulkarni) thank Karnatak University, Dharwad for providing Nilekani fellowship.

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