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SYNTHESIS AND CHARACTERIZATION OF NOVEL PYRIDINIMINE-BASED SCHIFF BASE LIGANDS AND THEIR Cu (II) COMPLEXES FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

In this study, Cu (II) complexes with pyridinimine-based Schiff base tetradentate ligands were synthesized, by condensation of pyridine with substituted 3-formyl-2-hydroxy quinolines. These copper complexes were characterized using analytical techniques such as elemental analyses (CHN), spectral analyses (FTIR, ^{1}HNMR and ESR) and physicochemical studies (molar conductance and magnetic moment). FTIR spectra demonstrated coordination mode for the Schiff base ligand that behaves as tetradentate with Cu^{2+} ions. The elemental analysis by magnetic studies and ESR spectra suggested the distorted square-planar geometry for these complexes. Further, ESR spectra of the crystalline Cu (II) complexes showed covalent nature for the metal ligand bond. The molar conductance in dimethylformamide solution indicated that all complexes are non-electrolytes. Furthermore, these compounds were tested for their therapeutic potentials, using rat and mouse models. The Cu (II) complexes with pyridinimine-based Schiff base

tetradentate ligands exhibited potent anti-inflammatory, anti-arthritic and analgesic activities *in vivo*. In addition, the serum copper levels were significantly higher in rats, which received copper complexes. However, these copper complexes did not exhibit acute toxicity or deaths at the higher dose (800 mg/kg) tested in the present investigation. In conclusion, copper complexes-mediated anti-inflammatory activity might be through the stimulation of copper-dependent enzymes such as ceruloplasmin and super-oxide dismutase, both of which have free radical scavenging properties.

KEYWORDS: Schiff base, Cu (II) complex; pyridinimine, Spectral analysis, Biological evaluations.

INTRODUCTION

The coordination chemistry has emerged as an important research discipline, owing to the theoretical challenges, synthetic curiosities and the diverse potential biomedical applications of coordination compounds. It is reported that various metal complexes possess anticancer, antibacterial and anti-inflammatory activities.[1-6] The use of cisplatin in the treatment of certain types of cancer[7], D-penicillamine to treat Wilson's disease[8] and heavy metal toxicity[9], Co-labelled cyanocobalamine for the treatment of pernicious anemia[10] are some of the classical examples for the applications of coordination compounds in biomedicine. Many biomolecules are coordination compounds and use the coordination principles in their functioning. Hemoglobin and myoglobin – are biological oxygen carriers, cytochrome-c, the electron transport agent, metallo-enzymes carboxypeptidase A and carbonic anhydrase, cyanocobalamine, ceruloplasmin and chlorophyll are coordination compounds of biological importance.

Meth-Cohn et al.,[11] have reported the synthesis of 2-chloro-3-formyl quinolones, which on acid hydrolysis form 3-formyl-2-hydroxy quinolines. These compounds containing the functional groups -OH and –CHO at the appropriate positions on the ring may function as ligands for the synthesis of metal complexes. Further, the above products may also form Schiff bases, which function as ligands. Schiff bases have been known to posses many pharmacological activities such as tuberculostatic, fungicidal, anti-inflammatory, anti-tumour, antiviral and antimicrobial activities.[12–14] In view of various biomedical applications, it is worthwhile to synthesize a series of substituted 3-formyl-2-hydroxy quinolones and their Schiff bases to utilize them as ligands for the preparation of Cu (II) complexes. Henceforth, the substituted 3-formyl-2-hydroxy quinolines were used in the

synthesis of Schiff bases with pyridine. Further, Cu (II) complexes were synthesized using their Schiff bases and characterized by various physico-chemical methods. Finally, we determined the levels of copper in serum, anti-inflammatory, anti-arthritic and analgesic activities of these compounds and their Cu (II) complexes.

MATERIAL AND METHODS

All chemicals and reagents used in this study were of the analytical reagent grade and of highest purity available. Deionized water was used in all preparations and organic solvents including absolute ethanol, methanol and dimethylformamide (DMF) were spectroscopic pure from BDH. Type IV lambda carrageenan, and Freund's adjuvant containing *Mycobacterium butyricum* (1 mg/mL) were purchased from Sigma, St. Luis, MO, USA.

Animals

Male albino rats (150-180 g, Charles Foster strain) and Swiss albino mice of either sex (18-25 g) were obtained from the Animal House, National College of Pharmacy, Shivamogga, Karnataka, India. The Schiff bases of substituted 3-formyl-2-hydroxy quinolines with pyridine and their Copper complexes were given orally as homogenized suspension in 0.5% sodium carboxymethyl cellulose using a feeding tube.

Instruments

The molar conductance of solid complexes in DMF (10^{-3} M) was measured using conductivity Bridge type CM-82. The elemental analyses of the separated solid chelates for C, H, N and S were performed in the micro analytical centers at Perfect Alloys Ltd., Shivamogga, Karnataka, India. The molar magnetic susceptibility of powder samples was measured by using Gouy balance method.[15, 16] Fourier transform infrared spectra (FTIR) were recorded on a Shimadzu-8300 FT-IR spectrophotometer in the region 4,000-400 cm^{-1} using KBr pellet method. The ^{1}H NMR spectra were recorded by Bruker-300 MHz in DMSO-d6 as solvent, where the chemical shifts were determined relative to the solvent peaks. The electron spin resonance spectra were also recorded on Cary 2390 spectrophotometer.

Synthesis of the Schiff base ligand

The Schiff bases were synthesized by refluxing a mixture of an equimolar amount of pyridine (0.01 M) and substituted 3-formyl-2-hydroxy quinolines, (a) 7-methoxy-2-hydroxy-3-formyl quinoline, (b) 6,7-dimethoxy-2-hydroxy-3-formyl quinoline and (c) 7-methyl-2-hydroxy-3-

formyl quinoline in methanolic medium on a water bath for 5–7 h. The solids separated were filtered, washed with methanol and dried at room temperature. The resulting products were recrystallized from methanol.

Synthesis of copper complexes

The ligands L_1-L_3 (0.01 M) were suspended in 100 mL of water, heated to 80–90°C and a solution of sodium hydroxide (0.1M) were added till a clear solution was formed, filtered and pH of the filtrates were adjusted between 6.8 to 7.2 with diluted acetic acid. The cupric chloride (0.005 M) dissolved in water was added to the above solutions of the ligands with constant stirring. The mixtures were refluxed for 2 h and then cooled at room temperature. The precipitates formed were filtered, washed with water, alcohol, and dried in vacuum over anhydrous calcium chloride. The melting/decomposition temperatures were determined.

The copper complexes were prepared by adding 50 mL of methanolic solution of cupric chloride (0.01 M) to slightly excess hot methanolic solution of the Schiff bases (1, 2, 3) in 1:2 metal-ligand ratio and refluxing the mixture on a water-bath for 4–6 h. The resulting colored complexes were filtered washed with methanol and petroleum ether and dried under reduced pressure over anhydrous $CaCl_2$ in a desiccator and further in an electric oven at 80°C. The melting/ decomposition temperatures were determined.

Anti-inflammatory activity

Anti-inflammatory activities of the Schiff bases and their complexes were carried out by carrageenan induced rat hind paw edema (acute inflammatory model).[17] Each group were assigned 6 rats, one of the groups that received vehicle alone served as control, while the other groups of animals, which received the test compounds or standard drug. The rats were dosed (10–100 mg/kg) orally with the test compound one hour before injection of 0.05 mL of 1% suspension of carrageenan into the sub plantar region of the rat hind paw. Additional groups were similarly treated with 10–100 mg/kg and 0.5% sodium carboxymethyl cellulose (vehicle controls). The volume of the injected paw was measured by water displacement in a plethysmograph immediately after carrageenan injection and again after 3 h. A mark was made at the lateral molecules and the foot was dipped to the same distance in to the arm of the plethysmograph.

Average edema volumes for test compound-treated and positive control rats were compared statistically with those of the vehicle treated control animals and expressed as the percent

edema inhibition, which was calculated using the formula. Edema inhibition (%) = 100 (1-Vt / Vc), where Vc is volume of the edema in the control group and Vt is volume of the edema in the treated group.

Antiarithritic activity by chronic inflammatory method

Adjuvant arthritis was induced in groups containing six rats each by subcutaneous injection of 0.13 mL of Freud's adjuvant containing dead *M. butyricum* (1 mg/ml) in liquid paraffin into the plantar surface of the rat hind paw.[18] The paw volumes were measured on the first day and eighteenth day with a plethysmograph. The test compounds and phenylbutazone were given at a dose of 33 mg/kg/day for 18 days, beginning on one day before the adjuvant injection. An additional group was treated similarly with 3 mg/kg indomethacin. Edema volumes for the test compounds treated and positive control groups were compared with those of the vehicle treated control group. The results were expressed as percent edema inhibition.

Analgesic activity

This method was based on acetic acid-induced writhing in mice.[19] Groups of six mice each were dosed with the test compounds or with aspirin, at a dose of 100 mg/kg. p.o., 1 h before intraperitoneal injection of 0.6% acetic acid (10 mL/kg). Mice were observed for the total number of writhes for 15 min beginning 5 min after the acetic acid injection, and the total numbers of writhes were recorded. The mean number of writhes for each group was calculated and compared statistically with the vehicle treated control group. Results were expressed as the percent inhibition of the number of writhes, which was calculated by using formula, percent inhibition = 100(1-Wt / Wc), where Wc is the number of writhes for the control group and Wt is the number of writhes for the treated group.

In vivo toxicity studies

Acute toxicity of the compounds was determined in mice (18–25 g), by administering the compounds orally at 800 mg/kg dose. The animals were observed for their death over a period of seven days.

ESTIMATION OF SERUM LEVELS OF COPPER

Method 1

Sera from the rats used in anti-arthritic study were used for copper estimation. On the day 8, blood was collected in clean dry test tubes from retro bulbar venous plexus of the eye, by

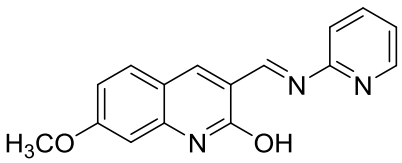
means of capillary, under anaesthesia. The collected blood was kept in dark for 30 minutes to ensure clotting and then centrifuged at 2500 rpm for 15 min to separate the serum. The separated serum was transferred to clean dry sample tubes and stored in the refrigerator. Further, the serum levels of copper were estimated according to the method described by Khalifa et al.[20] Briefly, the standard solution containing 1-12 µg of copper were placed in 50 mL conical flasks and volume made up in each instance to 50 mL with 0.2N HCl. A control flask containing HCl alone was included. Iron (III) chloride ammonium thiocynate reagent (3 mL) was added to each flask and incubated in thermostatistically controlled water bath at 40°C for 5 min. Further, thiosulphate (0.04N) was warmed to the same temperature (40°C) and added to the flasks from a rapid delivery 5 mL pipette and the time noted as zero time. The flasks were then swirled gently, and the time taken for completion of reduction of iron (III) chloride, as indicated by disappearance of the red color of iron (III) thiocyanate was recorded. The difference between two recorded times (control and standard) is the reaction time. Standard reference graph was prepared by plotting the reaction times against concentration of copper.

Method 2

Copper was extracted from blood serum, by the method described by Gubler, et al.[21] Briefly, to 0.5 mL of serum contained in a test tube, 1 mL of 2N HCl was added, stirred and allowed to stand for 10 min. To this, 1.0 mL of 20% trichloracetic acid solution was added and stirred and allowed to stand for another 10 min. The supernatant (1 mL) was made up to 5 mL with 0.2N HCl and the reduction time was determined at 40°C as described earlier. The copper content in 1 mL of supernatant was determined from standard reference graph and copper content per mL of serum was calculated.

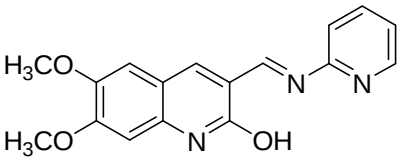
Characterization of Schiff base ligands

Ligand 1: (7-methoxy-2-hydroxy-3-formyl quinoline)pyridinimine



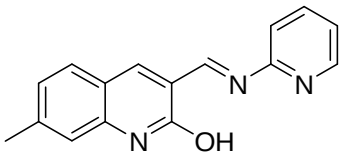
Colour; Light yellow, mp: 222-224°C, Formula: $C_{16}H_{13}N_3O_2$, FTIR (KBr): 1616 cm^{-1} (azomethine C=N), 2726 cm^{-1} (intramolecular OH), 1268 cm^{-1} (phenolic C-O), 3446 cm^{-1} (phenolic OH). ^1H NMR (in ppm): δ 3.72 (s, 3H, $-OCH_3$), 7.01-7.75 (m, 8H, Ar-H), 10.29 (s, 1H, CH of imine), 11.87 (s, 1H, OH, H-bonded). MW: 279.3, Elemental analysis: Cald: C, 73.37; H, 5.07; N, 10.07; Found: C, 73.31; H, 5.11; N, 10.06: Yield: 62%.

Ligand 2: (6,7, dimethoxy-2-hydroxy-3-formyl quinoline)pyridinimine



Colour: Yellow, mp: 224-227°C Formula: $C_{17}H_{15}N_3O_3$, FTIR (KBr): 1614 cm^{-1} (azomethine C=N), 2718 cm^{-1} (intramolecular OH), 1268 cm^{-1} (phenolic C-O), 3442 cm^{-1} (phenolic OH). Elemental Analysis: Cald: C, 70.12;H,5.23; N,9.09; Found: C, 69.89; H,5.22; N,9.11; Yield: 63%.

Ligand 3: (7,-methyl-2-hydroxy-3-formyl quinoline)pyridinimine



Colour: Pale yellow mp: 224-226°C Formula: $C_{16}H_{13}N_3O$, FTIR (KBr): 1610 cm^{-1} (azomethine C=N), 2724 cm^{-1} (intramolecular OH), 1263 cm^{-1} (phenolic C-O), 3438 cm^{-1} (phenolic OH). Elemental Analysis: Cald: C, 77.84; H,5.38; N,10.68; Found: C, 77.82; H, 5.40; N,10.69; Yield: 57%.

Statistical analysis

All determinations were carried out in triplicates and expressed as mean values ± SD and results at $P<0.05$ were considered statistically significant. Data were statistically analyzed by Mann-Whitney's multiple comparison analysis or Student t test. The spectral analyses were using OriginPro 8.0 (OriginLab, USA) software.

RESULTS AND DISCUSSION

Synthesis of Schiff bases (ligands 1, 2, 3) and their copper complexes (4, 5, 6).

Substituted 3-formyl–2-hydroxy quinolines (1-3) were used as starting material and Schiff bases were prepared by refluxing ligands (1-3) with 2-aminopyridine. The products were obtained in good yield ranging from 55-65%. The analytical and physical data including molecular weights, elemental analysis, colour, percent of yield and molar conductance are presented in **Table 1** and **2**. The C, H and N analysis of substituted 3-formyl–2-hydroxy quinolines (1-3) were in good agreement with the calculated values (±04% theoretical value) and all the three Schiff bases are coloured (pale yellow to yellow), insoluble in water, however soluble in methanol. The ligands (1, 2, 3) reveled IR bands at 1640±15 cm^{-1} (azomethine C=N), 2800-2700 cm^{-1} (intramolecular H-bonded OH), 1260±10 cm^{-1} (phenolic C-O). The other broad band at 3440±15cm^{-1} can be assigned to phenolic O-H stretching vibrations. The ^{1}HNMR spectral data of ligand 1 showed a singlet at δ 10.29 ppm due to the CH proton of imines. The OH proton (H bonded intramolecular with imine N) observed as a

broad singlet at δ 11.87 ppm. The multiplet observed at δ 7.01 - 7.75 ppm corresponds to eight aromatic protons.

Further, all three complexes are soluble in DMF and DMSO. The metal to ligand ratio in the complexes is found to be 1:2. The synthesized complexes are brown coloured, non-hygroscopic and stable in air. The conductance of these complexes was determined at 10^{-3}M concentration in DMF. The observed conductance is in the range of 14.81-18.72 Ω^{-1} cm^2 mol^{-1}. The low molar conductance values indicate that all these complexes are non-electrolytes, and practically no interchange reaction occurs between the solvent and the dissolved complexes. In addition, the magnetic moments measured for the complexes 4-6 show μ_{eff} values in the range of 1.72-1.88 BM. These values are in agreement with the μ_{eff} values reported by Nakamoto et al.,[22] for distorted square planar geometry copper (II) complexes, Further, the electronic spectra is in favor of the distorted square planar geometry.

Table 1. Analytical data of copper (II) complexes.

Number assigned	Complex	Mol. Formula	Mol. Weight	Metal Found (Calcd)	C % Found (Calcd)	H % Found (Calcd)	N % Found (Calcd)
4	Cu $(L_1)_2$	$C_{34}H_{26}N_6O_4Cu$	618.15	10.20 (10.28)	66.12 (66.06)	4.21 (4.24)	9.08 (9.06)
5	Cu $(L_2)_2$	$C_{36}H_{30}N_6O_6Cu$	678.20	9.35 (9.37)	63.71 (63.76)	4.41 (4.46)	8.22 (8.26)
6	Cu $(L_3)_2$	$C_{34}H_{26}N_6O_2Cu$	586.15	10.88 (10.84)	69.66 (69.67)	4.44 (4.47)	9.51 (9.56)

Table 2. Physical data of copper (II) complexes.

Number assigned	Complex	Color	% Yield	MP/DP (^{0}C)	Molar conductance $\Omega^{-1}cm^2mol^{-1}$	$^{\mu}$eff (BM)
4	Cu $(L_1)_2$	Brown	58	245	16.34	1.83
5	Cu $(L_2)_2$	Brown	57	239	18.67	1.81
6	Cu $(L_3)_2$	Brown	61	243	14.81	1.79

Fourier transforms infrared spectra (FTIR)

The characteristic IR spectral analysis of the ligands and complexes are presented in **Table 3**. The comparison of these bands revealed that the ligand band at 1650 ± cm^{-1} (azomethine, C=N) shifted to lower frequency 5-10 cm^{-1} with decreased intensity indicating its co-ordination.[23] The ligand band at 1260 ± cm^{-1} (phenolic C-O) suffered a positive shift by 10–15 cm^{-1} in the complexes, suggesting the co-ordination of oxygen of the Schiff bases.[22] The participation of phenolic O-H group in complexation has been further confirmed by the disappearance of the ligand band at 2800-2900 cm^{-1} (phenolic O-H, H-bonded: -O-H- N=C-),

upon complexation. The new bands at 515±10 cm^{-1} in the metal complexes have been assigned to Cu-O bond.

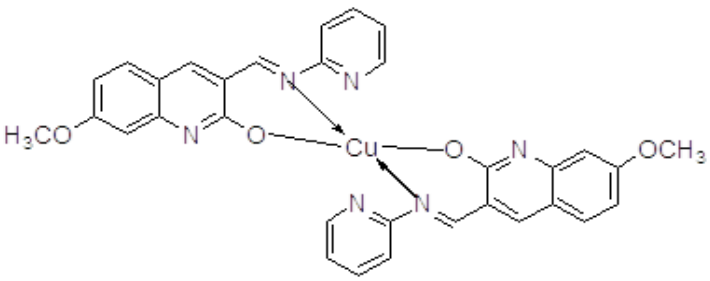
Table 3. Infrared spectral data (cm^{-1}) of Schiff bases of substituted 3-formyl-2-hydroxy quinolines with pyridine and their copper complexes.

Number assigned	Ligand/ Complex	Azomethine C=N	Intra molecular H-bond	Phenolic C-O	Phenolic O-H	Cu-N
1	L_1	1654	2726	1268	3446	-
2	L_2	1654	2718	1268	3442	-
3	L_3	1650	2724	1263	3428	-
4	Cu (L_1)2	1647	-	1272	3401	519
5	Cu (L_2)2	1646	-	1276	3392	522
6	Cu (L_3)2	1641	-	1277	3396	513

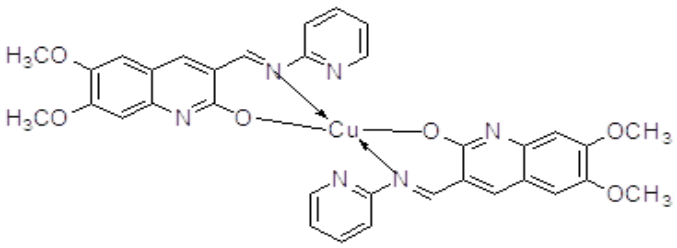
Electron spins resonance spectra (ESR)

ESR spectra of the complexes were recorded for poly crystalline samples at room temperature. The g value obtained from the spectra is shown in **Table 4.** From the observed g values $g_{11} > g_1 > g_m$ (2.00277), it is evident that the unpaired electron lies predominantly in the d_x^2-$_y^2$ orbital with the possibility of some mixing of d_z^2 because of low symmetry.[24] The $g_{11} <$ 2.3 indicates the covalent nature[25] for the metal ligand bond. The values $g_{11} > g_1$ in these complexes suggests the distorted square-planar geometry.[24] Based on these experimental data, the general chemical structures assigned for the copper (II) complexes (4, 5, 6) with ligands are shown below.

Copper complex* 4: *(7-methoxy-2- hydroxy-3-formyl quinoline) pyridinimine



Copper complex* 5: *(6,7-dimethoxy-2- hydroxy-3-formyl quinoline) pyridinimine



Copper complex 6: (7-methyl-2- hydroxy-3-formyl quinoline) pyridinimine

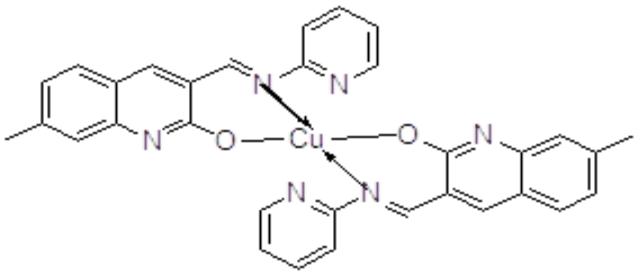


Table 4. Electron paramagnetic resonance spectral data of copper (II) complexes.

Number assigned	Complex	g_{11}	$g_{\perp}$	g_{av}
4	Cu (L_1)2	2.276	2.0704	2.1379
5	Cu (L_2)2	2.270	2.0705	2.1382
6	Cu (L_3)2	2.272	2.0703	2.1384

Anti-inflammatory activity

Anti-inflammatory activities of the Schiff base ligands (1, 2, 3) and their copper complexes (4, 5, 6) were carried out by using carrageenan-induced edema model. The copper complexes exhibited significant anti-inflammatory activity as shown in **Table 5 and 6**. It is interesting to note that the complexes 4 and 5 exhibited similar extent of edema inhibition (~80%) when compared to phenylbutazone (73.68%) and ibuprofen (76.31%) at 100 mg/kg doses. Further, the complexes 4-6 tested at lowest dose (10 mg/kg) exhibited edema inhibition (~37-42%) comparable to that of ibuprofen (39.47%). However, all these complexes showed slightly lesser edema inhibition compared to that of ibuprofen at 50 mg/kg doses. Based on their chemical structures, the presence of electron-withdrawing methoxy groups in complexes 4 and 5 might have contributed to the increased potency of anti-inflammatory activity.

Table 5. Anti-inflammatory activity of Schiff bases of substituted 3-formyl-2-hydroxy quinolines with pyridine and their copper complexes.

Number assigned	Ligand/Complex	Edema volume (mL ±SD)	Edema inhibition (%)
1	L1	0.30±0.03	18.92
2	L2	0.31±0.02	13.51
3	L3	0.34±0.05	19.05
4	Cu (L1)2	0.08±0.02	80.95*
5	Cu (L2)2	0.07±0.03	81.08*
6	Cu (L3)2	0.21±0.05	50.00*
	Phenylbutazone	0.10±0.03	73.68*
	Ibuprofen	0.09±0.04	76.31*

Edema volume was measured 3h after carrageenan injection and expressed as mean±SD (n = 6). The percent edema inhibition was calculated by comparing edema volume with the respective vehicle-treated controls. *Statistically significant (P<0.05, Mann-Whitney).

Table 6. Anti-inflammatory activity of copper complexes at different doses.

Number assigned	Complex	Edema Volume		Edema inhibition (%)			ED50 mg/kg
		10 mg/kg	50 mg/kg	10 mg/kg	50 mg/kg	100 mg/kg	
4	Cu (L_1)2	0.24 (0.05)	0.20 (0.06)	36.84*	47.36*	80.95*	21.38
5	Cu (L_2)2	0.23 (0.05)	0.18 (0.06)	39.47*	47.36*	81.08*	17.98
6	Cu (L_3)2	0.22 (0.04)	0.18 (0.03)	42.10*	52.63*	50.0*	56.23
	Ibuprofen	0.23 (0.05)	0.16 (0.05)	39.47*	57.89*	74.35*	27.54

Edema volume measured 3h after carrageen injection and expressed as mean±SD. Control edema volume = 0.38 (0.06); Percent edema inhibition calculated by comparing volume with that of the respective vehicle treated control animals. *Statistically significant (P<0.05, Mann-Whitney).

Anti-arthritic activity

Anti-arthritic activity of complexes 4, 5 and 6 were evaluated by using adjuvant-induced arthritis model. As shown in **Table 7**, all these complexes showed significant anti-arthritic activity. The complexes 4 and 5 demonstrated ~61-64% anti-arthritic activity, which is comparable to phenylbutazone (52.77%) and indomethacin (58.33%).

Table 7. Anti-arthritic activity of complexes (4, 5, 6) by adjuvant induced arthritics rats.

No.	Complex	Edema Volume (mL±SD)	Edema inhibition (%)[b]
	Control	0.36 (0.03)	-
4.	Cu $(L_1)_2$	0.14 (0.04)	61.11*
5.	Cu $(L_2)_2$	0.13 (0.04)	63.88*
6.	Cu $(L_3)_2$	0.19 (0.06)	47.22*
	Phenylbutazone	0.17 (0.05)	52.77*
	Indomethacin	0.15 (0.04)	58.33*

Given p.o., at a dose of 33 mg/kg (indomethacin 3 mg/kg) once daily for 18 days starting from one day prior to adjuvant injection. Edema volume measured on 18th day and expressed as mean ± standard deviation. Percent edema inhibition calculated by comparing volume with that of the respective vehicle treated control animals. * Statistically significant (P<0.05, Mann-Whitney).

Analgesic activity

The methoxy copper complexes 4 and 5 with significant anti-inflammatory activity exhibited ~40% analgesic activity, as measured by writhing inhibition. Further, the analgesic activity of these complexes were improved when compared to their ligands. However, the analgesic activity of these complexes were found to be less potent than the standard drug aspirin at 100 mg/kg dose tested. Moreover, the copper complex 6 that showed reduced anti-inflammatory activity in carrageenan-induced edema model was also found to possess lower analgesic activity (**Table 8**).

Table 8. Analgesic activity of Schiff bases of substituted 3-formyl-2hydroxy quinoline with pyridine (1, 2, 3), their copper complexes. (4, 5, 6).

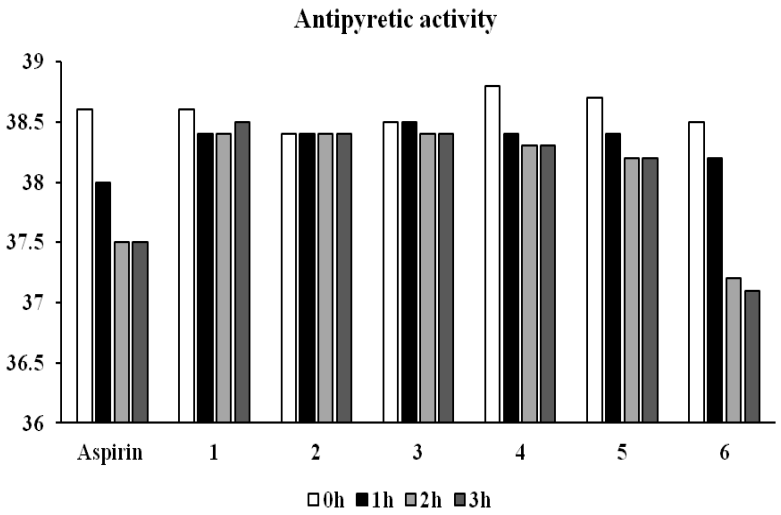
No.	Ligand/Complex	No. of writhes in 15 min (±S.D.)[a]	Writhing inhibition in (%)[d]
1.	L_1	47(7)[c]	28.36
2.	L_2	48(8)[c]	25.00
3.	L_3	50(8)[b]	25.37
4.	Cu (L_1)2	39(11)[b]	41.79*
5.	Cu (L_2)2	38(9)[c]	40.60*
6.	Cu (L_3)2	43(11)[b]	35.82*
	Aspirin	30(11)[b]	55.22*

Number of writhes in 15 min beginning 5 min after acetic acid injection, and expressed as mean ± standard deviation. Control no of writhes = 67 (7); Control number of writhes = 64 (8); Percent writhing inhibition calculate by comparing number of writhes with that of the respective vehicle treated control animals. * Statistically significant ($P<0.05$, Student‘t’ test).

Antipyretic activity

The antipyretic activity of Schiff bases and their copper complexes were evaluated by yeast-induced pyrexia in rats. None of these compounds decreased pyrexia induced by yeast (**Table 9**). Aspirin, the standard drug decreased the temperature of 1.1°C in 3 h, whereas 0.1°C decrease was observed in the controls.

Table 9					
Fig 5					
		0h	**1h**	**2h**	**3h**
	Aspirin	38.6	38	37.5	37.5
	1	38.6	38.4	38.4	38.5
	2	38.4	38.4	38.4	38.4
	3	38.5	38.5	38.4	38.4
	4	38.8	38.4	38.3	38.3
	5	38.7	38.4	38.2	38.2
	6	38.5	38.2	37.2	37.1



Acute toxicity studies

The biologically active copper complexes 4 and 5 were tested for their acute toxicity in mice. No deaths were seen even at 800 mg/kg doses, following over a period of seven days (data not shown).

Estimation of serum levels of copper

Sera obtained from the rats were subjected to the estimation of copper levels. The average serum levels of copper in normal rats were found to be143 µg/100 mL. The vehicle-treated group showed a slight increase of 5.6% in serum levels of copper when compared to the sham controls. The rats treated with copper complexes 4 and 5 (33 mg/kg/day) showed 30.48% increase in serum levels of copper, while the rats that received phenylbutazone (33 mg/kg/day) showed a 45.05% fall in serum copper levels (**Table 10**).

Table 10. Effect of Cu (II) complexes on serum levels of copper.

No.	Complex	Dose mg/kg/day	Mean reaction time (sec)	Serum Copper level µg/day	Percent increment (%)
	Normal	-	45.0	143.0	-
	Control	Vehicle	44.0	150.0	5.6
4.	Cu $(L_1)_2$				
5.	Cu $(L_2)_2$	33	41.0	186.0	30.48
	Phenylbutazone	33	55.0	78.0	-45.05

CONCLUSIONS

Taken together, the copper complexes of Schiff bases with significant anti-inflammatory activity exhibited marked increases in serum levels of copper. It is well-known that the free radicals and superoxide anions are involved in the events leading to inflammation.

Furthermore, copper-containing enzyme superoxide dismutase, the scavenger of superoxide anions, requires copper ions for its activity. Therefore, a possible mechanism by which these complexes execute anti-inflammatory activity might be via stimulation of synthesis of copper-dependent enzymes such as ceruloplasmin and superoxide dismutase, both of which possess free radical scavenging properties.

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