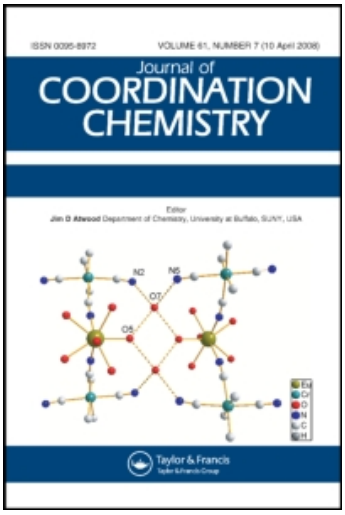

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Spectral characterization and anti-inflammatory activity of Schiff-base complexes derived from leucine and 2-acetylpyridine

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Schiff-base complexes $[ML{\cdot}nH_2OAc]mH_2O$ (where L = Schiff base derived from condensation of 2-acetylpyridine and leucine; M = Cu(II), Ni(II) or Co(II); $n = 0$–2 and $m = 3/2$–2) and $[ZnLOH]H_2O$ have been synthesized and characterized using elemental analyses, spectral analyses (UV-Vis, IR, ^{1}H NMR), conductance, thermal analyses, magnetic moments and QSAR analyses. The results showed that the ligand is mononegative tridentate coordinating the metal through pyridyl nitrogen, azomethine nitrogen, and carboxylate oxygen after deprotonation of the hydroxyl. Cu(II) forms square-planar and Ni(II) and Zn(II) form tetrahedral complexes, while Co(II) is octahedral. Prediction from quantitative structure activity relationship (QSAR) for anti-inflammatory activity in rats (% edema inhibition) has been made. The copper complex showed a significant analgesic and antirheumatic effect.

Keywords: Metal complexes; Leucine; 2-Acetylpyridine; Schiff base; Anti-inflammatory; Rheumatoid; Antioxidant

1. Introduction

Metal complexes derived from amino acid Schiff bases have received much attention because of possible biological activity. Metal complexes of Schiff-base phenolates with favorable cell membrane permeability have been exploited in cancer multidrug resistance [1] and tested as antimalarial agents [2]. Cu^{2+} salicylidine amino acid Schiff-base complexes exhibit antitumor activity [3]. Many articles reported antibacterial, antifungal, herbicidal activities for Schiff-base complexes [4–6]. Rheumatoid arthritis (RA) is a chronic multi-system disease of unknown etiology [7]. Although there are a variety of systemic manifestations, the characteristic feature of RA is persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution [7, 8]. Schiff bases are known to have anti-inflammatory activity; sets of Schiff bases with strong and long lasting anti-inflammatory activity against rat hind paw edema

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induced by carrageenan have been described [9]. Aromatic Schiff bases of 2,3-diaryl-1,3-thiazolidin-4-one derivatives have been prepared and tested for anti-inflammatory activity [10]. The accumulation of superoxide radicals in synovial fluid as a result of reduced extracellular superoxide dismutase activity has been suggested as a contributing factor in arthritis and other inflammatory diseases. The ability of transition metal complexes to scavenge extracellular superoxide radicals is known [11].

Research and patents report the anti-inflammatory activity of Cu(II)-amino acid complexes [12–16]; this work concerned Schiff bases derived from salicylaldehyde and its derivatives with amino acids [17–19] but, little work was done with Schiff bases derived from amino acids with ketonic compounds [20].

No work has been reported on Schiff bases derived from leucine and 2-acetylpyridine. In this paper the metal complexes of Cu(II), Co(II), Ni(II) and Zn(II) acetates with leucine and 2-acetylpyridine have been synthesized and characterized using spectral (UV-Vis, IR, ^{1}H NMR), thermal analyses, conductivity measurements and magnetic measurements. A QSAR study based on semi-empirical AM1 calculations for a series of Schiff bases predicts the anti-inflammatory activity in rats (% edema inhibition). The Cu(II) complex showed anti-inflammatory activity in rats (% edema inhibition), analgesic and antirheumatic effects.

2. Experimental

2.1. *Reagents*

All the chemicals used were of analytical grade and used without further purification.

2.2. *Technique*

Carbon and hydrogen contents were determined at the micro analytical unit of Cairo University. Metal analyses were carried out by standard methods [21]. Molar conductance measurements of the complexes (10^{-3} M) in DMSO were carried out with a YSI model 32 conductivity bridge. Infrared spectra were determined using KBr discs on a Mattson 5000 FTIR spectrometer. Calibration was made with polystyrene film. Electronic spectra were recorded on a UV2 Unicam UV/Vis spectrometer using 1 cm Stoppard silica cells. A Shimadzu TGA-50H thermal analyzer was used to record simultaneously TG and DTG curves. The experiments were carried out in dynamic nitrogen (20 mL min^{-1}) with a heating rate of 10°C min^{-1} in the temperature range 25–1000°C using platinum crucibles. The sample sizes ranged from 4.65 to 10.52 mg. Highly sintered Al_2O_3 was used as a reference. The ^{1}HNMR spectrum of the Zn(II) complex in $CDCl_3$ was recorded on a Bruker Avance DRx-500 instrument.

2.3. *Preparation of metal complexes using a template reaction*

This general procedure was followed for all the complexes. An aqueous solution of leucine 0.01 mol in 10 mL water was added to 0.01 mol of 2-acetylpyridine in 10 mL ethanol. The metal ions were dissolved in minimum water (5 mL), then added to the

reaction mixture of the ligand dropwise with constant stirring and finally heated under reflux for 3h on a hot plate at 50°C. A fine precipitate of the solid complex formed, was filtered off, washed with ethanol-water mixture then diethyl ether and stored in a vacuum desiccator over anhydrous calcium chloride.

2.4. *Animals*

60 Sprague–Dawley rats (200–250 g) were obtained from the animal house of Mansoura Faculty of Medicine and fed on a standard rat chow and water *ad libitum*. Animal care and experiments were performed in accordance with the guidelines established by the *NIH Guide to the Care and Use of Laboratory Animals*, NIH publication no. 86–23.

Rats were housed under similar standard laboratory conditions. The animals were divided into two main groups (non arthritic control, arthritic group). In the arthritic group, all rats had been inoculated by the reagent of Collagen–Adjuvant arthritis into the left paw pad. Rats which developed right paw arthritic manifestations after 45 days were divided into four groups as follow, arthritic control, piroxicam treated, copper acetated treated and copper complex treated group. The compounds were given orally via gastric tube daily for seven days, 45 days after Freund's injection.

Induction of Collagen II-arthritis. This is a modified form of the previous models [22]. This model of Collagen–Adjuvant Arthritis [23, 24] is considered to be a representative of rheumatoid arthritis or ankylosing spondylitis in humans. Collagen II – Freund's adjuvant emulsion (0.1 mL) was injected intradermally into the left hind foot paw-pad of each rat (if no arthritis developed within 4 weeks, some of the animals were challenged by a second inoculation). After 45 days, the systemic arthritis developed in both hind paws [25]. Most of the previous compounds are insoluble in water. These were suspended in 0.5% sodium carboxymethyl cellulose [CMC]. The determined doses were injected intraperitoneally. The doses are calculated according to reference [26].

2.5. *Methods of measurement of joint inflammation and pain tolerance*

2.5.1. Measurement of joint inflammation right ankle periarticular edema scoring (rheumatoid index). Scoring was based on severity and extent of the erythema and edema of the periarticular tissue, and the enlargement, distortion or ankylosis of the joints. Its inflammation was graded from 1 to 4 [27]. Grading of 4 was when the joint was distorted and ankylosed, 3 when markedly enlarged, 2 when erythematous with edema, and 1 when normal [27]. Each of the six non-arthritic, non-treated rats had a score of 1. Right paw pad thickness and joint scoring were measured at the 7th day after starting drug treatment (45 days after complete Freund's adjuvant injection).

2.5.2. Pain tolerance measurement right paw pad pressure tolerance (Analgesimeter: Ugo Basile, Italy). For assessment of the analgesic activity of the used drugs, pressure was applied by the analgesimeter on the rat pad of the right paw. The pressure was increased gradually (a certain number of grams per second until the rat either squeaks or tries to withdraw its limb). The force of pressure was continuously monitored by a pointer moving along a linear scale. Increased pressure tolerance of drug treated rats

indicates analgesic activity of the administered drug [27, 28]. This measurement of pressure tolerance was done at the 7th day of drug treatment (45 days after complete Freund's adjuvant injection).

2.5.3. Proximal joint (right ankle) mobilization tolerance (pain scoring). This mobilization tolerance was graded from 1 to 4. Degree 1 corresponds to tolerance of complete flexion 90°; degrees 2, 3 and 4 correspond to increasing degrees of maltolerance according to a rat hind limb withdrawal, squeaking and when the flexion becomes painful. Degree 4 corresponds tosqueaking with just initiation of flexion. Each of the six non-arthritic, non-treated rats had a score of 1 [27]. This measurement of mobilization tolerance was done at the 7th day of drug treatment (45 days after complete Freund's adjuvant injection).

2.6. *Measurement of antioxidant activity*

Serum malondialdehyde (MDA) as lipid peroxidation was measured by the thiobarbituric acid (TBA) test [29, 30]. The sample under test is treated with TBA at low pH, and a pink chromogen is measured. In the TBA reaction, one molecule of MDA reacts with two molecules of TBA with production of a pink pigment with an absorption maximum at 532–535 nm [30].

2.7. *Measurement of serum copper*

The copper content in serum is estimated by atomic absorption spectroscopy (AAS) without pretreatment [31].

2.8. *Measurement of Rheumatoid Factor (RF)*

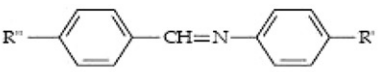
Rheumatoid Factor (RF) was estimated using sheep erythrocytes sensitized with rabbit gamma-globulin, Rose *et al.* [32] related hemaglutination by human serum to the disease rheumatoid arthritis.

2.9. *Statistical analysis*

Results are expressed as mean ± standard error. Multiregression analysis (one way ANOVA, Newman–Keuls and *F*-test) were used for correlating physicochemical descriptors to the edema inhibition through QSAR and analysis of the pharmacological data. Mann Whitney for average ± S.E was used for comparing different groups; statistical difference was considered significant at p-value < 0.05 [33].

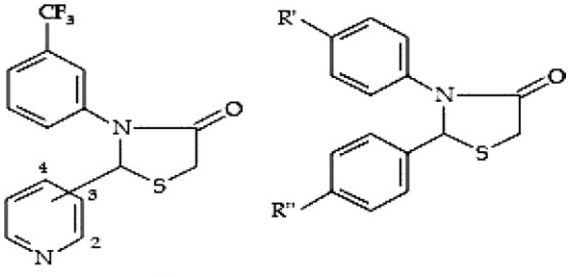
2.10. *Quantitative structure activity relationship (QSAR)*

QSAR analyses to predict promising drugs saves cost and time. In this work QSAR equations have been elaborated to select compounds of Schiff bases of high anti-inflammatory activity. From the literature, Schiff bases (figure 1), the



R′ = SO_2NH_2, **R'' = F 1, CH_3 2**
= SO_2CH3, **F 3, CH_3 4**

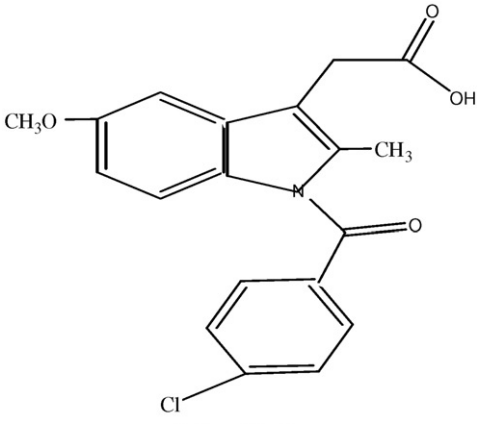
Figure 1. Schiff base derivatives as anti-inflammatory agents.



5 - 8 **9 - 12**

R′ = SO_2NH_2, **R'' = F, CH_3**
= SO_2CH_3, **F, CH_3**

Figure 2. Thiazolidinone derivatives as anti-inflammatory agents.



Indomethacin

Figure 3. Indomethacin as anti-inflammatory agent.

thiazolidinone derivatives (figure 2) and indomethacin (figure 3) were screened for anti-inflammatory activity [10], giving a database in which anti-inflammatory activity in rats (% edema inhibition) for 11 derivatives of Schiff bases were measured. Table 1 represents the biological activities at concentration (200 mg kg^{-1}) of the investigated derivatives [10].

Physicochemical properties (descriptors) of the investigated chemical compounds (figures 1 and 2) are obtained by using Hyperchem version 7 [34].

Table 1. Theoretical (calculated by equation (1)) and anti-inflammatory activity in rats (% edema inhibition) (experimental) degrees of inhibition from [10].

Compound	Anti-inflammatory activity in rats (% edema inhibition)	Anti-inflammatory activity in rats (% edema inhibition) calculated
1	14	16.10
2	8	5.76
3	59	50.39
4	44	50.38
5	37	33.75
6	0	−0.45
7	8	20.88
8	25	15.40
9	26	23.94
10	52	49.51
11	26	34.15
Indometh.	88	88.01

$R^2 = 0.9334$, $P < 0.031$, $F = 8.006$.

Table 2. Calculated descriptors for **1**–**12**.

Compound	Volume[a]	Hydration energy[b]	log *P*	Dipole *x*[c]	Dipole *y*[c]	Molar refractivity[d]	Total energy[b]
1	817.90	−12.20	2.82	−0.40	−0.90	68.00	−125.40
2	850.00	3.30	3.15	0.13	−1.30	72.80	−113.00
3	833.60	−6.80	3.08	0.14	1.54	68.40	−123.00
4	878.10	−6.00	3.40	0.03	4.25	73.21	−111.00
5	861.00	−4.80	6.95	−1.24	−3.20	74.76	−164.00
6	912.50	−9.60	7.02	−1.63	−5.43	75.10	−164.00
7	871.00	−1.90	7.02	−1.60	−5.10	75.10	−163.70
8	949.30	−8.80	9.41	−0.40	−5.30	85.90	−159.60
9	954.8	−9.20	9.74	3.70	−1.66	90.70	−148.00
10	968.60	−6.00	9.67	−3.96	−4.50	86.30	−157.00
11	979.60	−5.50	10.00	1.42	−3.66	91.10	−145.60
Indometh.	1045.00	865.50	9.25	−0.46	−4.50	88.60	−165.50

[a]$Å^3$; [b]$Kcal\,mol^{-1}$; [c]Debye; [d]$cm^3\,mol^{-1}$.

These descriptors include the volume, hydration energy, logarithm of partition coefficient and dipole moment on X–Y directions dmx and dmy, with the semi-empirical AM1 method of calculation (table 2).

QSAR is based on the chemical descriptors delivered from table 2. Also, equation (1) had been obtained from these data using multiregression statistical calculations.

$$\begin{aligned}(\%\ \text{edema inhibition}) = (&-0.392337\ \text{volume}\\ &+ 0.21811\ \text{hydration energy}\\ &+ 81.4709\ \log P - 16.87842\ \text{refractivity}\\ &+ 4.5935\ \text{total energy}\\ &- 2.723408\ \text{dipole}\ Y + 6.2645068\ \text{dipole}\ Z\\ &+ 1838.2085) \end{aligned} \quad (1)$$

The degree of the validity of equation (1) was measured via different tools, one based on calculating the biological activity (anti-inflammatory activity in rats as % edema inhibition). The data obtained are compared with the experimental values [10] (table 3). For comparison, table 1 compares the results obtained experimentally and calculated by using equation (1).

The regression coefficient ($R = 0.9334$) and degree of freedom ($F = 8.003$ and $P < 0.031$) reflect validity of the proposed equation. The most important descriptor affecting the percentage of anti-inflammatory activity in rats (% edema inhibition) is the hydration energy (t-value < 0.836 and p-value < 0.001) [35]. From the calculations, [CuLAc] (figure 4) was found to be the best.

3. Results and discussion

3.1. *Characterization of metal complexes*

The analytical data and some physical properties of the metal complexes are collected in table 3.

The complexes are stable in air, soluble in water and common organic solvents except the Ni(II) complex which is insoluble in water. The low molar conductivity values in DMF and DMSO ($\approx 10\,\Omega^{-1}\,cm^2\,mol^{-1}$) suggest the nonconducting nature of these complexes [36]. The presence of leucine and 2-acetylpyridine in the metal complexes has

Table 3. Analytical data and some physical properties of the complexes.

Compound	M.P. (°C)	Color	Found (Calcd)		
			C	H	M
[CuLAc]	>300	Dark brown	51.0(50.6)	5.7(5.6)	17.5(17.8)
[NiLAc]·$2H_2O$	>300	Blue	46.8(46.5)	7.0(6.2)	14.9(15.1)
[CoL(H_2O)$_2$Ac]·$3/2H_2O$	220	Brown	43.2(43.4)	6.8(6.5)	14.2(14.3)
[ZnLOH]H_2O	>300	White	46.5(46.8)	6.5(6.0)	19.0(19.5)

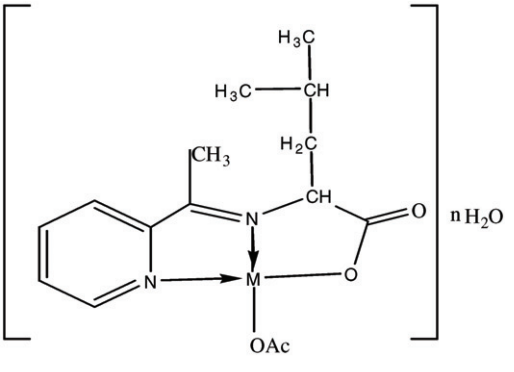


Figure 4. M = Cu(II) or Ni(II) and $n = 0$–2.

been confirmed by the spot test technique [37] and TLC after hydrolyses of the metal complexes. Water of hydration has been determined from TGA measurements.

3.2. *IR spectra*

The most important IR assignments of the metal complexes (table 4) have been determined by careful comparison of the spectra of leucine and 2-acetylpyridine. IR spectra of all complexes show the disappearance of bands at 3057 and 1698 cm^{-1} assigned to $\nu_{as}(NH_3^+)$ group of leucine and ν(C=O) group of 2-acetylpyridine, respectively. A new band at $\approx$1650 cm^{-1} assigned to the azomethine group (C=N*) appears in the spectra of all complexes. This indicates condensation between the amino group of leucine and the carbonyl group of 2-acetylpyridine in formation of the Schiff base.

The Schiff base is a mononegative tridentate ligand to Cu(II), Co(II), Ni(II) and Zn(II) through pyridyl nitrogen, azomethine nitrogen and carboxylate oxygen after deprotonation of the hydroxyl, consistent with the following observations. Positive shifts are observed for the bands 1560–1570, 630–670, 375–400 and 330–353 cm^{-1} assigned to νC=C + νC=N, $Q_{i\cdot p.}$, $Q_{o\cdot p.}$ and νM–N (py), respectively [38] and the bands observed in the regions 1625–1655, 715–770 and 563–583 cm^{-1} assigned to νC=N*, δCOO^-, γCOO^-, respectively [36]. New bands are observed in the regions 395–448 and 465–530 cm^{-1} assigned to νM–N and νM–O [38]. The spectra of Cu(II), Co(II) and Ni(II) complexes show two bands in the regions 1466–1471 and 1210–1250 cm^{-1} assigned to $\nu_{as}CH_3COO$ and ν_sCH_3COO; the difference between these two bands indicates monodentate coordination in case of Cu(II) or Ni(II) and bidentate coordination in case of Co(II) [39]. The spectrum of the Zn(II) complex shows a band at 1064 cm^{-1} assigned to δ(M–OH) group [20].

3.3. *Thermal analyses*

The thermal analyses (T.G and D.T.G) of the complexes were carried out from 25 to 800°C. The estimated mass losses were computed based on the TG results and the calculated mass losses were computed using the results of microanalyses.

Thermal analyses curves (TGA and DTG) of $[CoL(H_2O)_2Ac]\cdot 3/2H_2O$ show decomposition in the temperature range 34–117°C from loss of one and half molecule of water of hydration (6.3% close to 6.5% theoretical). The second step of decomposition lies in the temperature range 118–246°C, corresponding to loss of coordinated water, 8.1% which is close to the calculated 8.7%. The next step corresponds to the loss of acetate at 248–337°C, 14.7% which is close to the

Table 4. The most important IR bands of the complexes.

Compound	$\nu C=N^*$	νC=C + νC=N	ν_sCOO	ρCOO	γCOO	Ring skeletal	νM–O	νM–N	νM–Npy
$[CuLAc]\cdot 2H_2O$	1655	1568	1620	570	403	669	480	431	342
$[NiLAc]\cdot 2H_2O$	1630	1570	1600	563	400	631	470	395	330
$[CoL(H_2O)_2Ac]\cdot 3/2H_2O$	1650	1560	1620	575	405	670	465	420	352
$[ZnLOH]H_2O$	1625	1575	1610	583	429	660	530	448	353

calculated 14.6%. The last step in the temperature range 337–501°C corresponds to decomposition of the Schiff base and loss of $C(CH_3)NCHCH_2CH(CH_3)_2CO_2$. The estimated mass loss of this step is 36.5% (calculated 38.2%). The residue is CoPy with estimated mass of the residue 31.0%, close to the calculated 33.0%.

Thermal analyses curves (TGA and DTG) of $[NiLAc] \cdot 2H_2O$ show a decomposition step in the temperature range 40–120°C corresponding to loss of two water molecules of hydration (8.4% close to 9.3% calculated). The second step in the temperature range 150–400°C corresponds to decomposition of the ligand.

3.4. *Electronic spectra and magnetic moments*

The electronic spectrum of the Co(II) complex in DMSO shows bands at 16666 and 19230 cm assigned to $^4T_{1g} \rightarrow {}^4A_{2g}(P)$ (ν_2) and $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ (ν_3), respectively in octahedral geometry [38]. The spectrum also shows a band at 23696 cm^{-1} assigned to LMCT [40].

The ligand field parameters (Racah B parameters = 827, the nephelauxetic parameter $\beta = 0.85$, where $\beta = B$ complex/B free ion and $10D_q = 9100\,cm^{-1}$) fall in the range suggested for octahedral Co(II) [40].

The magnetic moment value (4.9 B.M.) supports octahedral Co(II).

The electronic spectrum of $[NiLAc] \cdot 2H_2O$ in DMSO shows a band at 15479 cm^{-1} corresponding to $^3T_1 \rightarrow {}^3T_1P$ (ν_3), suggesting tetrahedral geometry of Ni(II) [40]. The magnetic moment value (3.5 B.M.) lies in the range for tetrahedral Ni(II) [40].

The electronic spectrum of [CuLAc] in DMF shows a broad band at 19600 cm^{-1} assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions in a square-planar geometry [41]. The band at ca. 30.000 cm^{-1} is a charge-transfer, probably LMCT. The high magnetic moment value (1.9 B.M.) of the square-planar complex compared with the observed values for the distorted octahedron may be taken as additional evidence for the presence of a square-planar geometry.

3.5. *^1H NMR*

The ^1H NMR spectrum of the Zn(II) complex in $CDCl_3$ shows signals at 1.5 (triplet), 1.2–1.4 (multiplets), 1.8 (doublet-doublet) and 2.2 p.p.m. (singlet) assigned to the protons of CH, $CH(CH_3)_2$, CH_2 and CH_3 groups, respectively. The multiplets in the 7.3–8.8 p.p.m. region are assigned to the protons of pyridine and the singlet at 3.5 p.p.m. to OH group.

3.6. *Anti-inflammatory effect of [CuLAc]*

Animal treatment by the copper ion in the form of copper acetate and [CuLAc] induced an anti-inflammatory effect detectable by the 7th day big joint inflammation (table 5).

The [CuLAc] treated group appeared to have a significant reduction in right pad thickness by the 7th day as compared with that of the arthritic non-treated group (table 5) and is more active than copper acetate. This behavior is in accord with

Table 5. Anti-inflammatory effect and analgesic effect of Cu–acetate, piroxicam and [CuLAc] on adjuvant-collagen arthritis in rats ($m \pm$ S.E).

Group	Dose (mg 200g^{-1}) according to Paget's table[164]	Rheumatoid index $n^{\Psi} = 12$	Analgesic effect	
			Pain tolerance $n = 12$	Analgesemeter gm^{-1} $n = 12$
Non-arthritic non-treated	[SCMC] Solvent	1.52 ± 0.09	1.00 ± 0.00	1.00 ± 0.0
Arthritic non-treated	[SCMC] Solvent	3.63 ± 0.17*	2.25 ± 0.07*	2.00 ± 0.11*
$Cu(Ac)_2 \cdot 2H_2O$	0.22	$2.31 \pm 0.07^{+}$	$2.13 \pm 0.04^{+}$	$2.20 \pm 0.08^{+}$
Piroxicam	0.36	$1.90 \pm 0.0.06^{+}$	$1.88 \pm 0.09^{+}$	1.83 ± 0.11
[CuLAc]	0.41	$1.45 \pm 0.03^{+}$	2.33 ± 0.01	$1.00 \pm 0.06^{+}$

*$P < 0.05$ *vs.* non-arthritic control and arthritic non treated.
$^{+}P < 0.05$ *vs.* arthritic control and treated groups.
Ψ *n*: number of rats.
SCMC 0.5% sodium carboxymethyl cellulose.

Table 6. Influence of copper acetate, piroxicam and [CuLAc complex on MDA, copper content of serum (μg mL^{-1}) and rheumatoid factor in adjuvant collagen of rheumatoid arthritis model in rats ($m \pm$ S.E).

Group	MDA (nmol mL^{-1})	Cu (μg mL^{-1})	RF (IU mL^{-1})
Non-arthritic non-treated	2.29 ± 0.29	1.1 ± 0.06	134 ± 6.86*
Arthritic non-treated	3.87 ± 0.52***	0.75 ± 0.03**	630 ± 141***
Cu $(Ac)_2 \cdot 2H_2O$	$2.44 \pm 0.14^{+}$	$0.585 \pm 0.03^{+}$	$400 \pm 44^{++}$
Piroxicam	$2.55 \pm 0.30^{++}$	$0.955 \pm 0.03^{++}$	$333 \pm 44^{+++}$
[CuLAc]	$0.6 \pm 0.19^{+++}$	$0.89 \pm 0.01^{++}$	$387 \pm 51^{+++}$

***$P < 0.001$ *vs.* non-arthritic control.
$^{+}P < 0.05$ *vs.* arthritic control. $^{++}P < 0.01$. $^{+++}P < 0.001$.

observations of many researchers [12] that anti-inflammatory activity of Cu-complexes is due to the physicochemical properties of the complex rather than its constituents [42]. The anti-inflammatory action of Cu-complex may result from redox activity of copper, in particular the ability of Cu to scavenge the highly reactive pro-inflammatory superoxide radical anion O_2^- [43, 44].

3.6.1. The analgesic effect of [CuLAc]. [CuLAc] has a concomitant near normal degree of big joint manipulation tolerance and normal numeric pad pressure tolerance by the 7th day (table 5). The results are in accordance with the reported results for complexation of NSAIDs with Zn(II), Cd(II) and Pt(II) ions to produce safer NSAIDs [45].

3.6.2. The effect of [CuLAc] on serum copper level. Serum copper level (1.1 ± 0.04) in non-treated rats with carrageenan induced pleurisy was in accord with the reported level of serum copper in normal rats 1.13 μg mL^{-1} [46] (table 6).

The observed lowering effect of copper acetate could be explained by ability of acetate with copper in the blood to be excreted in urine. Table 6 shows that our compound and piroxicam have similar effects on amount of copper.

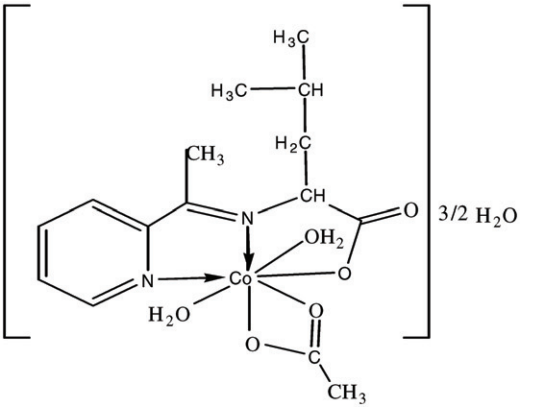


Figure 5. Suggested structure of $[CoL(H_2O)_2Ac] \cdot 3/2H_2O$.

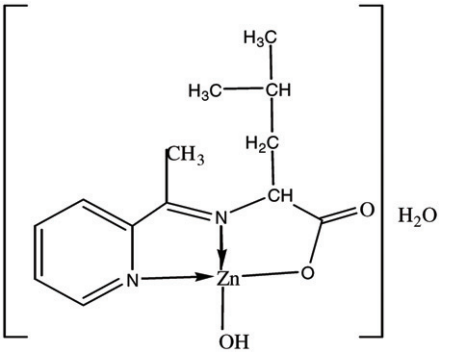


Figure 6. Suggested structure of $[ZnLOH] \cdot H_2O$.

3.6.3. Antioxidant effect of copper complex. Copper acetate and piroxicam have antioxidant activity as indicated by their lowering effect on serum malonaldehyde level (table 6) as compared to copper acetate treated groups of rats subjected to collagen-adjuvant. The antioxidant activity of piroxicam had been proved by Pipe *et al.* [47].

The mechanism operating for the antioxidant activity of the generated copper acetate complex and piroxicam in the body involves redox cycling at the metal center.

The anti-inflammatory improvement of [CuLAc] was clearly observed from data obtained when RF was measured in the treated group (table 6).

4. Conclusion

Based on analytical and spectrochemical studies, the Schiff-base ligand is a mononegative tridentate ligand through pyridyl nitrogen, azomethine nitrogen and carboxylate oxygen with the following geometries, Cu(II) complex square-planar, Co(II) octahedral, Ni(II) and Zn(II) tetrahedral.

The pharmacological activity of Cu(II) complex has been studied and compared with the commercial product piroxicam.

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