

Synthesis And Characterization Of Some Metal Complexes Of Zn(II) With 1,10- Phenanthroline And Some Amino Acids: Anti-Inflammatory And Analgesic Activities Of Its Complexes.

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ABSTRACT: New mixed ligand complexes of Zn(II) with 1,10- Phenanthroline and some amino acids were prepared in the solid form and characterized by elemental analysis, conductivity, magnetic moment measurement, FT-IR, ¹H-NMR, ¹³C-NMR and FAB+ mass studies. To examine anti-inflammatory and analgesic activities of its complexes, some Swiss albino mice of 05-07 weeks old were taken. One dose of the test compounds of 10 mg/kg was selected throughout the research work. The anti-inflammatory activity of the test compounds were determined by 'carragenan induced mice paw edema inhibition' method, and however, the analgesic activity was determined by 'acetic acid induced writhing' methods. These four compounds showed positive effects as anti-inflammatory and analgesic agents. Anti-inflammatory and analgesic activities of the test compounds at 50 mg/kg were quite comparable to those of standard drugs at 10 mg/kg.

Keywords: Zinc, amino acids, 1,10-Phenanthroline, anti-inflammatory and analgesic activity, ¹H-NMR, ¹³C-NMR and FAB+ mass

1. INTRODUCTION

The amino acid, histidine, is the most frequently occurring ligand of zinc in the active centre of zinc containing enzymes¹. 1,10-Phenanthroline is a classic ligand in coordination chemistry, which couples versatility in metal ion binding with peculiar properties of its complexes. Both catalytic and structural zinc in enzymes are always coordinated by at least one cysteine and histidine donor^{2,3}. Of the metal ligating amino acids bearing N, O and S containing donors in their side chains, cysteine and histidine are the most prominent ones for zinc^{4,5}. However, the importance of zinc for stabilization of protein loops in enzymes, zinc fingers etc., has generated new interest in the field of Zn coordination chemistry⁶⁻¹². Inflammation causes the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation¹³. Frequently, most of anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism. Hence, for treating inflammatory diseases analgesic and anti-inflammatory agents are required¹⁴. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicines¹⁵ but severe adverse effects¹⁶ and tolerance and dependence induced by opiates, use of these drugs have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs are needed as alternatives to NSAIDs and opiates.

2. Experimental

2.1: Materials

1,10-Phenanthroline (Phen), Cysteine (Cys), Cystine (Cye), Histidines (His) and DL-Leucine (Ieu) were obtained from the Sigma (USA). AR grade zinc acetate, carrageenan reagent and diclofenac sodium were obtained from E Merck. They were used as supplied.

2.2 Synthesis of metal complexes

The amino acids (0.001 mole) were deprotonated by KOH (0.001 mole) in aqueous medium until the pH of the solution was 8-9. Then, aqueous solution of 1,10-Phenanthroline (0.001) was prepared which were added simultaneously and independently to equimolar concentrations of zinc acetate. The synthesized complexes were found to be insoluble in the commonly known organic solvents. Consequently, the following physical measurements and analysis were carried out to check the purity and elucidation the structure. All the metal complexes are stable to air and moisture and decompose at very high temperatures(>300°C).

2.3 Elemental analysis and conductivity data

Carbon, hydrogen and nitrogen analyses were obtained from the microanalytical Heraeus Carlo Erba 1108 elemental analyser. Chloride analysis was carried out by Mohr's method. The metal contents were estimated from these solutions on an atomic absorption spectrometer, Perkin-Elmer 23380. Conductivity of metal complexes was measured in freshly prepared DMSO solutions and obtained using a Digisun Digital conductivity bridge (model: DI-909) and a dip type cell calibrated with KCl solution.

2.4 Spectral analysis

2.4a IR spectra: The IR spectra were recorded (as KBr discs) on infrared spectrophotometers, Shimadzu IR-435, and Perkin-Elmer FTIR in the region 4000–400 cm⁻¹.

2.4b ¹H-NMR spectra: Deuterated solutions of complexes 1, 2, 3 and 4 were prepared in 99.98% of CDCl₃. The pH of the solution was maintained at 5–6 by adding DCl solution. ¹H NMR spectra were recorded for the above complexes of

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concentration 5×10^{-2} mol dm^{-3} at room temperature on a Varian Gemini 200/MHz pulsed FT NMR spectrometer. TMS was used as the internal standard.

2.4c ^{13}C -NMR spectra: The ^{13}C -NMR spectra were recorded in CDCl_3 and DMSO-d_6 using TMS as internal standard with Bruker 500 MHz high resolution NMR spectrometer

2.4d Magnetic susceptibilities: Magnetic susceptibilities of the Zn complexes were recorded at room temperature on a Faraday balance (CAHN-7600) using $\text{Hg}[\text{Co}(\text{CNS})_4]$ as the standard. Diamagnetic corrections were made by using Pascal's constants¹⁷

2.4e FAB^+ mass spectra: FAB^+ mass spectra of the complexes were recorded using a JEOL SX-120 instrument.

3. Anti-inflammatory and Analgesic activity of the test compounds.

3.1. Anti-inflammatory activity: Carrageenan induced paw edema test in mice

The anti-inflammatory activity¹⁸ of the test compounds were determined using the carrageenan-induced mice paw edema inhibition method applying 1.0% carrageenan solution as the phlogistic agent. The test compounds were administered orally as suspensions in 3% DMSO, 30 min before the injection of the phlogistic agents

3.2 Analgesic activity. Acetic acid-induced writhing test

The analgesic activity of the test samples were studied¹⁹ using acetic acid-induced writhing model in mice. Swiss albino mice of either sex were divided into control, standard and different test groups contains four mice in each.

4. Results and discussion.

Analytical data corresponding to the 1, 2, 3 and 4 complexes are reported in table 1. It may be seen from the table that the complexes are in equimolar stoichiometric 1 : 1 : 1 ratio. The presence or absence of chloride ions in the above complexes was determined by Mohr's method. No evidence was found for the presence of acetate ions in the coordination sphere of the complexes. The conductivity values (table 2) in DMSO correspond to non electrolytes for the complexes²⁰

Table 1. Analytical, conductivity and magnetic data for Zn(II) complexes. Found (Calcd) (%)

Complex	C	H	N	Metal	μ_{eff} (B.M.) (Temp.K)
[Zn(Cys)(Phen)]	55.61 (55.63)	3.47 (3.50)	6.18 (6.20)	14.10 (14.12)	Diamagnetic 3.14
[Zn(Cye)(Phen)]	65.33 (65.35)	3.33 (3.35)	7.81 (7.83)	18.05 (18.08)	Diamagnetic 3.13
[Zn(His)(Phen)]	54.85 (54.87)	5.71 (5.74)	12.12 (12.15)	18.57 (18.59)	Diamagnetic 3.34
[Zn(leu)(Phen)]	62.34 (62.36)	3.37 (3.39)	7.85 (7.87)	17.15 (17.18)	Diamagnetic 3.35

Table-2: Physical properties of the complexes

No	Name of the complexes	Color	Melting points $^{\circ}\text{C}$	Molar conductance ($\text{ohm}^{-1}\text{cm}^2 \text{mole}^{-1}$)
01	[Zn(Cys)(Phen)]	White powder	295-297(d)	14
02	[Zn(Cye)(Phen)]	Grey white	290-292(d)	14
03	[Zn(His)(Phen)]	White powder	>300 (d)	13
04	[Zn(leu)(Phen)]	White powder	>300 (d)	13

Table-3: IR, ¹H-NMR, ¹³C NMR and mass data for Zn(II) complexes

Comp.	Spectral data
01	IR (KBr): $\nu(\text{OH})$, 3069; $\nu(\text{C}=\text{O})$, 1613; $\nu_{\text{asym}}(\text{COO})$, 1553; $\nu_{\text{sym}}(\text{COO})$, 1389; bend(OH), 1155; $\nu(\text{Zn-N})$, 518; $\nu(\text{Zn-O})$, 409 cm^{-1} . ¹ H NMR(Varian Gemini 500 MHz pulsed FT NMR): δ : 2.3 (s, 2H), 7.4 (t, 4H, Ar-H), 7.5 (m, 4H, Ar-H), 7.6 (m, 4H, Ar-H), 8.5 (d, 1H, Ar-H), 9.3 (d, 1H, NH) ppm. ¹³ C NMR (500 MHz, CDCl_3): δ : 77 (-CH-), 110, 112, 117, 118 (Ar.C), 120, 125, 127, 128, 130, 145, 165 (C=O) ppm. MS (ESI LCQ-MS): m/z; 460, 308, 288, 154, 136, 108, 89.
02	IR (KBr): $\nu(\text{OH})$, 3067; $\nu(\text{C}=\text{O})$, 1660; $\nu_{\text{asym}}(\text{COO})$, 1587; $\nu_{\text{sym}}(\text{COO})$, 1398; bend(OH), 1138; $\nu(\text{Zn-N})$, 576; $\nu(\text{Zn-O})$, 533 cm^{-1} . ¹ H NMR: δ : 7.3 (s, 7H, Ar-H), 7.72 (m, 6H, Ar-H), 7.90 (m, 6H, Ar-H), 8.54 (m, 1H, Ar-H) 9.20 (m, 1H, Ar-H) ppm. ¹³ C NMR, δ : 78 (-CH-), 110, 112, 115, 117(Ar. C), 130, 133, 165(C=N), 173(C=O) ppm. m/z; 383, 348, 307, 289, 217, 192.
03	IR (KBr): $\nu(\text{OH})$, 3298; $\nu(\text{C}=\text{O})$, 1616; $\nu_{\text{asym}}(\text{COO})$, 1507; $\nu_{\text{sym}}(\text{COO})$, 1367; bend(OH), 1175; $\nu(\text{Zn-N})$, 545 cm^{-1} . ¹ H NMR, δ : 1.81 (s, 6H, -CH ₃), 1.83 (s, 3H-CH ₂), 4.45 (s, 3H), 7.20 (s, 7H, Ar-H). ¹³ C NMR (500 MHz, CDCl_3): δ : 20, 22, 24 (CH ₃ , CH ₂), 39, 55, 75, 111(Ar. C), 161, 162(C=N) ppm, m/z; 459, 307, 289, 154, 136, 107, 89, 57.
04	IR (KBr): $\nu(\text{OH})$, 3068; $\nu(\text{C}=\text{O})$, 1659; $\nu_{\text{asym}}(\text{COO})$, 1585; $\nu_{\text{sym}}(\text{COO})$, 1396; bend(OH), 1138; $\nu(\text{Zn-N})$, 574; $\nu(\text{Zn-O})$, 532 cm^{-1} . ¹ H NMR: δ : 7.35 (s, 6H, Ar-H), 7.75 (m, 6H, Ar-H), 7.91 (m, 7H, Ar-H), 8.5 (m, 1H, Ar-H) ppm. ¹³ C NMR, δ : 77 (-CH-), 110, 113, 115, 117(Ar. C), 130, 133, 162(C=N), 173 (C=O) ppm. m/z; 381, 345, 307, 289, 192, 107.

¹H-NMR spectra: They were identified with the help of literature data²¹.

4.2 Magnetic susceptibility

Magnetic susceptibility was recorded at room temperature on Faraday balance and the magnetic moments of complexes 1 - 4 were found 3.14 – 3.46 BM suggest that the central metal ion is in high spin configuration and tetrahedral in geometry.

4.3.a Anti-inflammatory activity: Carrageenan induced paw edema test in mice

The anti-inflammatory activity¹⁸ of the test compounds were determined using the carrageenan-induced mice paw edema inhibition method employing 1.0% carrageenan solution as the phlogistic agent. The test compounds were administered orally as suspensions in 3% DMSO, 30 min before the injection of the phlogistic agents, at dose level of 10 mg/kg(p.o) body weight. Diclofenac sodium was used as a standard at a dose level of 10 mg/kg(p.o) body weight. 3% DMSO served as a control. Groups of four Swiss albino mice of either sex were used in each experiment. The volume of paw edema was measured with the help of

plethysmograph by mercury displacement method at 0 h (immediately after injection of carrageenan). Then, the volume of paw edema was observed at 1, 2, 3 and 4 h. The results are presented in Table (4).

4.3.b Analgesic activity. Acetic acid-induced writhing test

The analgesic activity of the test samples were studied¹⁹ using acetic acid-induced writhing model in mice. Swiss albino mice of either sex were divided into control, standard and different test groups contains three mice in each. The control group received 3% DMSO and standard group was treated with diclofenac sodium at a dose level of 10 mg/kg (p.o.). Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.6% acetic acid but diclofenac sodium was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 30 min. The results are given in table (5)

Table 4. Anti-inflammatory activity of the test compounds by carrageenan induced paw edema in mice.

Volume of hinds paw edema.

Compounds	Dose (mg/kg)	0.5 hr	1 hr	2 hr	3 hr
Comp-1	10	0.33	0.31	0.27	0.19
Comp-2	10	0.34	0.31	0.27	0.20
Comp-3	10	0.33	0.30	0.26	0.19
Comp-4	10	0.35	0.32	0.26	0.21

No. of mice in each group was three. Table 4 shows that Volume of hinds paw edema decreases with time. In the carrageenan-induced mice paw edema test (Table 4) for acute inflammation, the test compounds at doses of 10 mg/kg showed the volume of paw edema decreases with time. Which has almost the same effect to the Standard drug diclofenac sodium.

Table 5. Effects of test compounds on acetic acid induced writhing test in mice.**Acetic acid induced writhing method.**

No. of comp.	Dose (mg/kg)	No. of mice	No. of writhing
Comp- 1	10	Three	7
Comp- 2	10	Three	15
Comp- 3	10	Three	20
Comp- 4	10	Three	14

Table 5 shows the effect of the test compounds on acetic acid-induced writhing in mice. The oral administration of test compounds significantly inhibited writhing response induced by acetic acid in a dose dependent manner.

Conclusion:

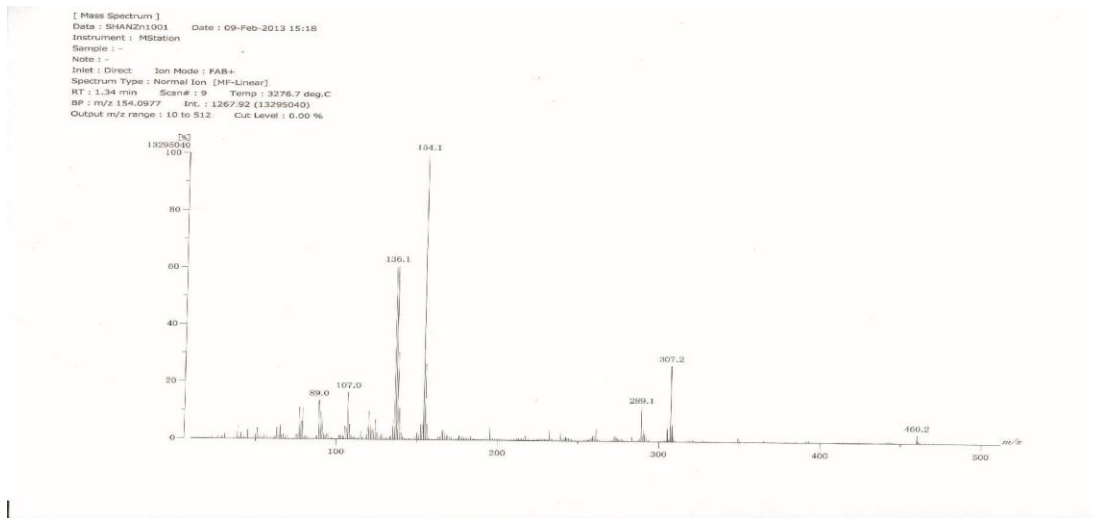
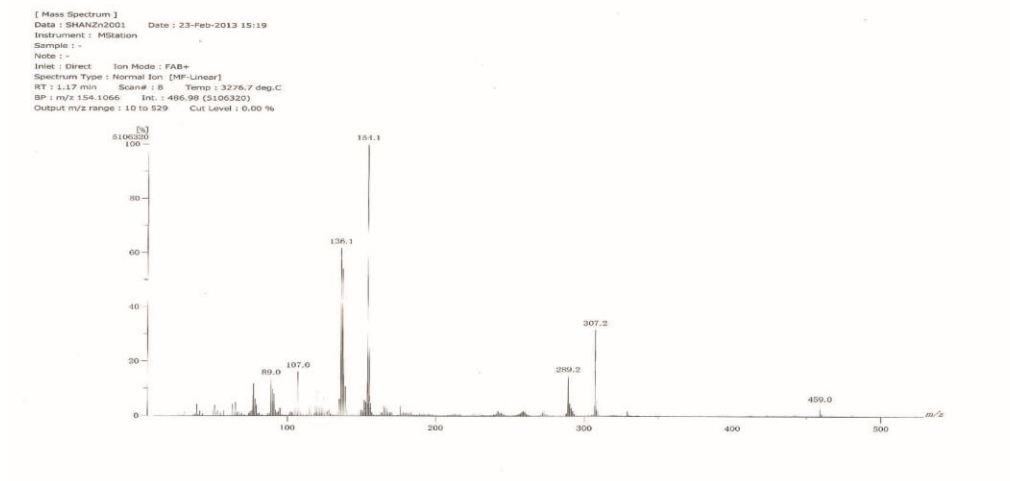
It was observed that the following complexes were tetrahedral in geometry and coordination no. was four, and however, anti-inflammatory and analgesic activities of the test compounds at 50 mg/kg were quite comparable to those of standard drugs at 10 mg/kg.

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**Fig. 4.1:** FAB+ mass spectra for comp-1**Fig. 4.2:** FAB+ mass spectra for comp-2