Synthesis, physiochemical studies, anti-inflammatory and analgesic activities of some metal complexes of Zr (IV) with amino acids.

Md. Anwar Hossain , Md. Chanmiya Sheikh , SK AL Zaheri Mahmud, Ronok Zahan , Md. Ashraful Alam

***Abstract*— New zirconium(IV) complexes were synthesized with 4,4'-bipyridine, imides and some amino acids in the solid form and characterized by elemental analysis, conductivity, magnetic moment measurement, FT- IR, ^{1}H-NMR, ^{13}C-NMR and FAB^{+} mass studies. For studying anti- inflammatory and analgesic activities of theses complexes some Swiss albino mice of 6-7 weeks old were taken. The test compounds of 20 mg/kg for each were selected throughout the research work. The anti-inflammatory activity of the test compounds was determined by 'carragenan induced mice paw edema inhibition' method. The analgesic activity was determined by 'acetic acid induced writhing' methods. These three compounds have showed positive effects as anti-inflammatory and analgesic agents.**

***Index Terms*— Zirconium, amino acids, 4,4'-bipyridine, anti-inflammatory and analgesic activity.**

I. INTRODUCTION

The coordination chemistry of Schiff bases derived from amino acids have been widely explored, though its use in supramolecular coordination chemistry remains largely unexplored. 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[1,2]. The vast literature on structural studies of amino acids complexes reveals some interesting features of their coordination behavior[3–8]. Metal complexes with Schiff base ligands have been receiving considerable attention due to the pharmacological properties of both ligands and complexes[9-12].The interest in the construction of Schiff base coordination complexes by reacting transition metal ions with bidentate has been constantly growing over the past years[13-15]. Many researchers have been conducted on Schiff base complexes, most of these complexes were found to be biologically active[16-19].

Pain has been defined as an unpleasant sensory and emotional experience associated with actual tissue damage, or described in terms of such damage[20,21] . Inflammation results in 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 inflammation[22]

The anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase pathway of arachidonic acid metabolism. Hence, for treating inflammatory diseases analgesic and anti-inflammatory agents are required[23]. Non steroidal anti-inflammatory drugs are the most clinically important medicine[24] but have some adverse effects [25] .

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Md. Anwar Hossain, Dept. of Chemistry, RUET, Bangladesh
Md. Chanmiya Sheikh, Dept. of Chemistry, Toyama University, Japan
SK AL Zaheri Mahmud, Mawson Institute, University of South Australia, South Australia-5095
Ronok Zahan, Dept. of Pharmacy, Rajshahi University, Bangladesh
Md. Ashraful Alam, [1]Dept. of Chemistry, RUET, Bangladesh

II. EXPERIMENTAL

A. Materials

Cystine(cys), Cysteine(cye), Succinimide(succ) and 4,4-Bipyridine(bpy) were obtained from the Sigma (USA). AnalaR grade zinc acetate, carrageenan reagent and diclofenac sodium were obtained from E Merck. They were used as supplied.

B. Synthesis of metal complexes

The three complexes [Zr(cys)(bpy)]- (1), [Zr(cye)(bpy)]- (2), [Zr(cys)(succ)]- (3) were synthesized by mixing an aqueous solution containing equimolar ratios of bpy (0□001 moles, 0□156 g) and cys (0□001 moles, 0□240 g), cye (0□001 moles, 0□121g), succ (0□001 moles, 0□□□□ g), which were added simultaneously and independently to equimolar concentrations of Zr(IV) chloride. Stoichiometric ratios of metal and ligands are dissolved in aqueous medium and are refluxed until the complex is precipitated, and if not, the pH of the solution mixture is changed to precipitate the complex. 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 elucidate the structure. All the metal complexes are stable to air and moisture and decompose at very high temperatures.

C. Elemental analysis and conductivity data

Carbon, hydrogen and nitrogen analyses were obtained from the micro analytical Heraeus Carlo Etba 1108 elemental analyser. Chloride analysis was carried out by Mohrs 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.

D. Spectral analysis

D.1 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}.

D.2 *H-NMR spectra:* Deuterated solutions of complexes 1, 2 and 3 were prepared in 99□8% of $CDCl_3$. The pH of the solution was maintained at 5–6 by adding DCl solution. ^1H NMR spectra were recorded for the above complexes of 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.

D.3 *^{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 *FAB^+ mass spectra:*** FAB^+ mass spectra of the complexes were recorded using a JEOL SX-120 instrument.

III. ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

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

The anti-inflammatory activity[27] of the text compounds were determined using the carrageenan-induced mice paw edema inhibition method using 1.0% carrageenan solution. The test compounds were administered orally as suspensionsin 3% DMSO, 30 min before the ingection.

B. Analgesic activity. Acetic acid-induced writhing test

The analgesic activity of the test samples were studied[28] 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 group.

IV. RESULTS AND DISCUSSION.

Analytical data corresponding to the 1, 2 and 3 complexes are compiled 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 a 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 1) in DMSO correspond to non electrolytes for the complexes.

Table 1. Analytical and conductivity data of mixed ligand complexes of Zr(IV) with cystine, cysteine & succinimide and 4,4´-bipyridine. Found (Calcd) (%)

Complex	C	H	N	Metal	λM Ohm^{-1} cm^1mol^{-1}(in DMSO)
[Zr(cys)(bpy)] $ZrC_{16}N_4S_2O_4H_{18}$	39.75 (39.77)	3.72 (3.75)	13.25 (13.28)	18.88 (18.91)	9.5
[Zr(cye)(bpy)] $ZrC_{13}N_3S_1O_4H_{18}$	37.41 (37.42)	5.03 (5.05)	10.07 (10.9)	21.91 (21.94)	11
[Zr(cys)(succ)] $ZrC_{10}N_3S_2O_6H_{13}$	28.24 (28.27)	3.53 (3.55)	9.90 (9.92)	21.55 (21.57)	10

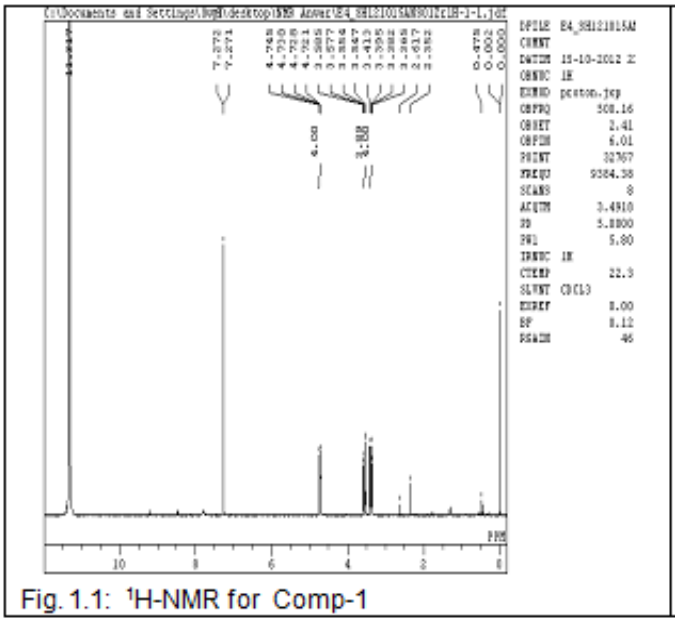

Fig. 1.1: ^{1}H-NMR for Comp-1

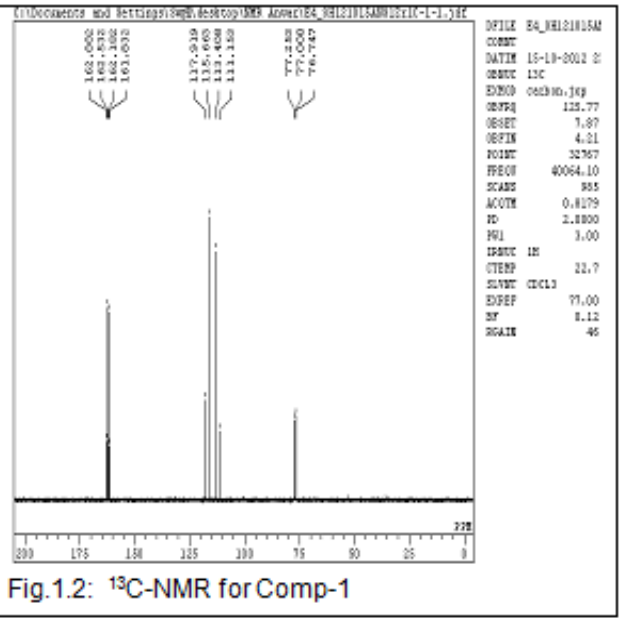

Fig.1.2: ^{13}C-NMR for Comp-1

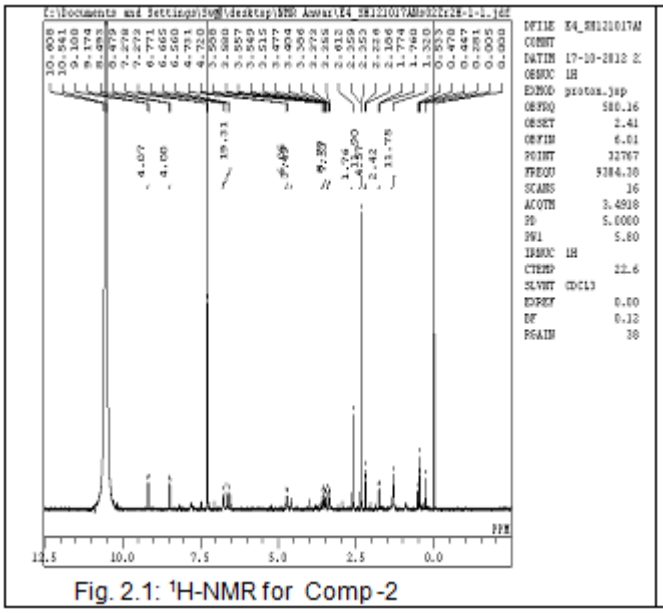

Fig. 2.1: ^{1}H-NMR for Comp-2

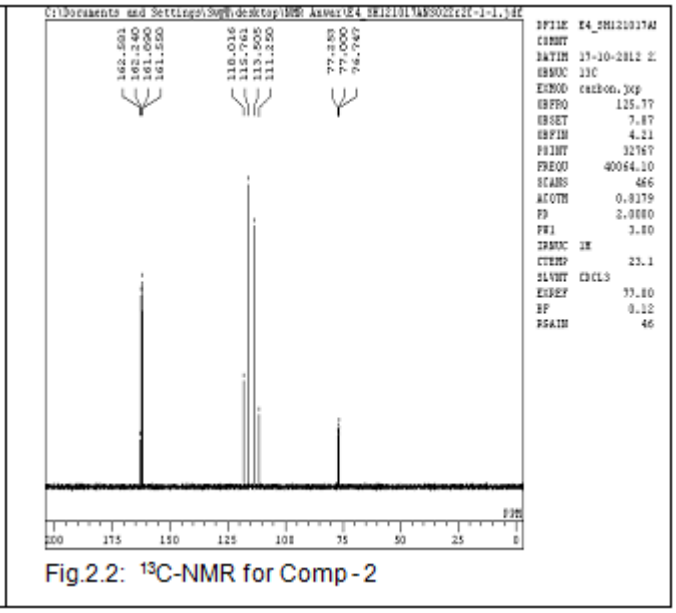

Fig.2.2: ^{13}C-NMR for Comp-2

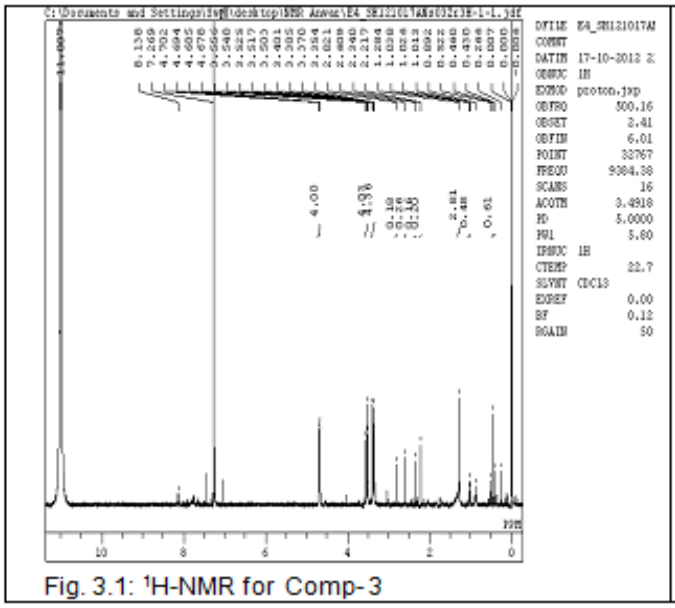

Fig. 3.1: ^{1}H-NMR for Comp-3

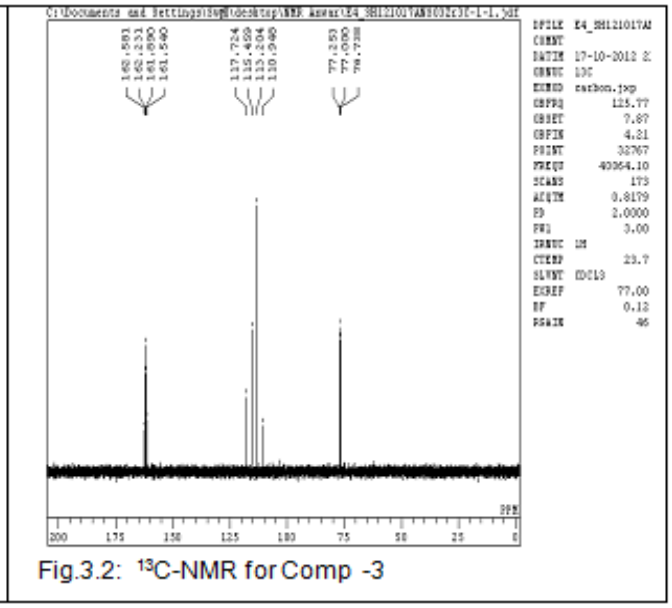

Fig.3.2: ^{13}C-NMR for Comp -3

A. Characterization of compound [Zr(cys)(bpy)]

Colour, reddish brown; m.p. 245-247 °C (d); IR (KBr): v(NH), 3424; v(C=O), 1621; vasym(COO), 1585; vsym(COO), 1337; bend(OH), 1193; v(Zr←N), 539; v(Zr-O), 504 cm^{-1}. ^{1}H NMR(Varian Gemini 500 MHz pulsed FT NMR): δ 3.36 (q, 4H,-CH_2), 3.55 (d, 4H, -CH), 4.72 (s, 2H, -NH), 7.27 (m, Ar-H). ^{13}C NMR (500 MHz, $CDCl_3$): δ; 76.74 (-CH-), 111, 113, 115, 117 (Aromatic carbons), 161(C=N), 162 (C=O) ppm, MS (ESI LCQ-MS): m/z; 459.0, 307.2, 289.2, 154.1, 136.1, 107.0, 89.0, 77.0. Found (Calcd)(%) for $ZrC_{16}N_4S_2O_4H_{18}$: C 39.75, H 3.72, N 13.25. Found: C 39.77, H 3.75, N 13.28.

B. Characterization of compound [Zr(cye)(bpy)]

Colour, brown; m.p. >300 °C (d); IR (KBr): v(NH), 3401; v(C=O), 1635; vasym(COO), 1582; vsym(COO), 1310; bend(OH), 1156 ; v(Zr←N), 541; v(Zr-O), 463 cm^{-1}. ^{1}H NMR(Varian Gemini 500 MHz pulsed FT NMR): δ; 2.17(s, 12H -CH_2) 2.35 (s, 4H- OH_2) 4.72 (s, 2H, -NH), 7.3 (m, Ar-H). ^{13}C NMR (500 MHz, $CDCl_3$): δ; 76.74(-CH-), 111, 113, 115, 118 (Aromatic carbons), 161.55(C=N), 162.58 (C=O) ppm, MS (ESI LCQ-MS): m/z. 459.0, 307.2, 289.2, 154.1, 136.1, 107.0, 89.0, 65.0. Found (Calcd)(%) for $ZrC_{13}N_3S_1O_4H_{18}$: C 37.41, H 5.03, N 21.91. Found: C 37.42, H 5.05, N 21.94.

C. Characterization of compound [Zr(cys)(succ)]

Colour, brown; m.p. 247-248°C (d); IR (KBr): v(NH), 3435; v(C=O), 1622; vasym(COO), 1584; vsym(COO), 1338; bend(OH),1193; v(Zr←N), 539; v(Zr-O), 519 cm^{-1}. ^{1}H NMR(Varian Gemini 500 MHz pulsed FT NMR): δ; 2.17(s, 12H -CH_2) 2.35(s, 4H-CH_2) 4.72 (s, 4H,-NH),7.3 (m, Ar-H). ^{13}C NMR (500 MHz, $CDCl_3$): δ; 76.74 (-CH-), 111, 113, 115, 117 (Aromatic carbons), 161(C=N), 162 (C=O) ppm, MS (ESI LCQ-MS): m/z 425, 307.2, 289.2, 154.1, 136.1, 107.0,

89.0, 65.0 . Found (Calcd) (%) for $ZrC_{10}N_3S_2O_6H_{13}$: C 28.24, H 3.53, N 9.90 Found: C 28.28, H 3.55, N 9.92

IR- data: They were identified with the help of research work[26]

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

The anti-inflammatory activity of the text compounds were determined using the carrageenan-induced mice paw edema inhibition method employing 1.0% carrageenan solution. The test compounds were administered orally as suspensionsin 3% DMSO, 30 min before the ingection at dose level of 20 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 (2).

B. Analgesic activity. Acetic acid-induced writhing test

The analgesic activity of the test samples were studied 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. The control group received 3% DMSO and standard group was treated with diclofenac sodium at a dose level of 20 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 intraperitonially 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 (3).

Table 2. Anti-inflammatory activity of the test compounds by carrageenan induced paw edema in mice.

Volume of hinds paw edema.

Comounds	Dose (mg/kg	0.5 hr	1 hr	2 hr	3 hr
Comound-1	20	0.44	0.34	0.26	0.19
Comound-2	20	0.43	0.33	0.28	0.20
Comound-2	20	0.33	0.31	0.27	0.20

No. of mice in each group were three. Table 2 shows that Volume of hinds paw edema decreases with time.

In the carrageenan-induced mice paw edema test (Table 2) 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 3. Effects of test compounds on acetic acid induced writhing test in mice.

Acetic acid induced writhing method.

No.of compounds	Dose (mg/kg	No. of mice	No. of writhing before administration	No. of writhing after administration
Comound-1	20	03	13	10
Comound-2	20	03	12	10
Comound-3	20	03	10	08

Table 3 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.

V CONCLUSION

On the following experimental observation, it was found that the complexes were octahedral in geometry and however, coordination of 4,4´-bipyridine through N^- and amino acids were COO^- and NH_2 to Zr(IV). Anti- inflammatory and analgesic activities of the test compounds at 50 mg/kg were quite comparable to those of standard drugs at 20 mg/kg.

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