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Research Paper :

Syntheses and anti-inflammatory actvity of diphenyl-2, 2'-Dicarboxylic acid and its metal complexes

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ABSTRACT

Diphenyl amine-2,2'-dicarboxylic acid and its Cu(II), Ni(II), Co(II) and Zn(II) complexes have been synthesized and characterized by their elemental analyses, molecular weight determination, molar conductance, infrared and electronic spectra and magnetic measurements. The Zinc complex was tested by different methods for anti-inflammatory activity. It was found to be equipotent to Naproxen and Ibuprofen, though in higher dosed.

Key words : Metal complex, NSAID'S, Prostaglandins, Anti inflammatory agent

Inflammation is a tissue response involving physiological, morphological and biochemical changes. Several highly bioactive chemical mediators like histimine, 5-HT, kinins, interleukin-1, hydrolytic enzymes and prostaglandin are released during the dynamic process, making the process more complicated.

Unfortunately, none of the non-steroid anti-inflammatory drug (NSAID'S) is devoid of high incidence of gastric ulceration and side effects on kidney, lever, bone marrow and skin. The discovery of Ibuprofen [2-(4-iosphenyl) propionic acid] in early sixties, triggered a new trend in the research of a non-steroid substituted aryl carboxylic acid derivative. Though they were less gastric irritant than other NSAID'S but their long term use do lead to the undesirable side effects. Therefore, there is still a need for a NSAID effective of Park Davis chemists that N-aryl anthranilic acid derivatives exhibit potent oral anti-inflammatory activity in UV erythema assay further stimulated the interest in this area.

Copper is known to suppress inflammation and to possess antiulcer properties[1,2]. Lower levels of Zinc have been found in patient of rheumatoid arthritis. They respond to Zinc supplementation[3] and Zinc possess antiulcer activity[4]. Gold salts are well known to alter course of rheumatoid arthritis and metals like Iron, Manganese, Zinc and Copper also have been shown to be concerned with the synthesis of procollagen, proelastin, mucopolysaccharide etc. which are necessary for healing following tissue damage.

In the quest for better tolerated NSAID, we synthesized diphenylamine-2,2'-dicarboxylic acid (DPDC) and its metal complexes involving Cu(II), Ni(II), Co(II) and Zn(II) as metal ions. Their structures were established by using different physicochemical methods *viz.* elemental detection, molar conductance, molecular weight determination, electronic and infrared spectra and magnetic measurements. At the ligand and chelates were subjected to the primary screening against carrageenan induced rat oedema test. It was found that Zinc chelate was most active among all the compounds. Therefore, it was further investigated and the results are reported here.

MATERIALS AND METHODS

All the chemicals used were of analytical reagent grade.

Synthesis of Diphenyl amine-2,2'-dicarboxylic acid (DPDC) :

Diphenylamine-2,2'-dicarboxylic acid (Fig. 1) was synthesized by condensing equimolar amount of 2-chlorobenzoic acid with anthranilic acid in the presence

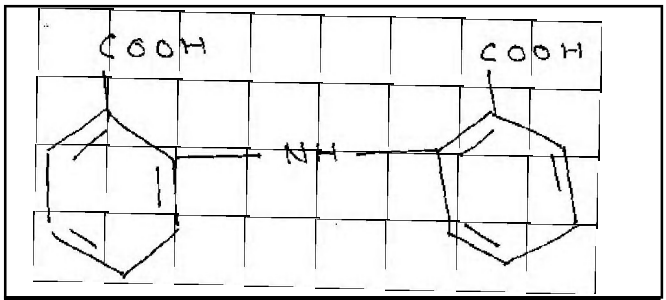

Fig. 1 : Diphenylenelamine-2,2'-dicarboxylic acid

of Copper oxide in slightly alkaline media. The compound was decolorized with activated charcoal on boiling. It was dried under vacuum.

Synthesis of metal complexes :

All the complexes were prepared by the improved method of Masumeci *et al.*[5].

The isolated compounds (Fig. 2) were tested for purity by running TLC. The infrared spectra of the DPDC and its metal complexes were recorded on a Perkin Elmer 521 spectrophotometer. Magnetic susceptibility measurements were carried out at room temperature using Guoy's balance. The electronic spectra were recorded in Dimethyl sulfoxide on a Shimadzu double beam spectrophotometer (UV-150-02 model). Molar conductance of the complexes were measured in their DMSO solution using the Toshniwal conductivity meter. Molecular weight of the compounds were determined by cryoscopic method. Thermogravimetric analysis has been carried out at Regional Sophisticated Instrumentation Center, Nagpur.

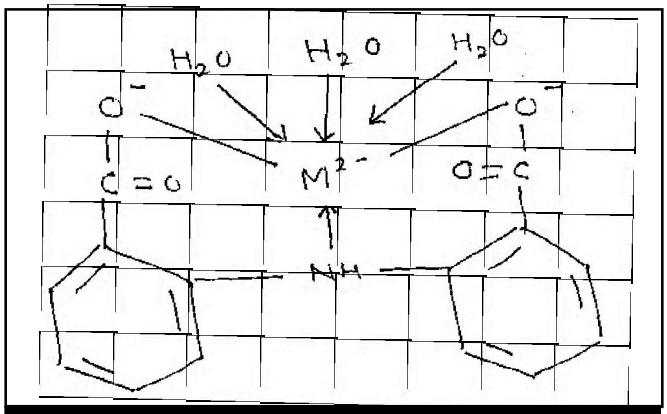

Fig. 2 : Structure of metal complex

Pharmacology :

The standard drugs and Zinc complex were administered subcutaneously as suspension in saline containing 1.4% Poly vinyl alcohol.

Carrageenan induced rat paw oedema test :

The anti-inflammatory action assessed according to the method of Winter *et al.*[6]. The standard drugs were Naproxen (Searl, India) and Ibuprofen (Boots, India). Overnight fasted rats (Wistar) of either sex weighing 140-160 g, were arranged in group of six each. Oedema was induced by injecting 0.1 ml of 1% carrageenan (Marine colloids Inc., USA) suspension in normal saline into the plantar aponeurosis of right paw. The paw volume was measured immediately and 4 hour after the injection of carrageenan by a volume differential meter (M 7101, Ugo Basile, Milan, Italy). The percentage inhibition was calculated.

Carrageenan induced rat paw oedema in adrenalectomized rats :

Male Wistar rats (140-160 g) were bilaterally adrenalectomized under light ether anesthesia by the method of Schultzer[7]. Water was replaced with normal saline for drinking. Two days after surgery the rats were divided into groups of six each. Oedema was induced by carrageenan and measured as in normal rats. The percentage inhibition was calculated.

Cotton pellet granuloma test :

Inhibition of granuloma tissue formation was assessed by the method of Winter and Porter[8]. Sterile cotton pellets (50 ± 1 mg) were implanted subcutaneously on either side of the midline dorsally under light anesthesia in male Wistar rats. The Zinc complex , Naproxen and Ibuprofen were administered each day for six days. On the 7th day the rats were sacrificed and the pellets were dissected out and dried to a constant weight at 80^{0}C. The mean weight of granulation tissue formed around each pellet of the group was calculated.

Adjuvant arthritis (Established) :

Male Wistar rats (160 ± 20 g) were injected with 0.1 ml of a fine suspension of Freunds adjuvant complete (Difco) into the plantar aponeurosis of right paw and the paw was left untreated for 14 days[9]. On day 14th, the rats which showed 45 to 55% oedema of the injected paw were grouped into 4 groups of 8 each. The Naproxen and Zinc complex were administered daily from 14th day to 28th day. The paw volume of both injected and uninjected paw was measured every alternate day using a water plethysmometer (M 7150 Ugo Basile, Milan, Italy).

The secondary lesions were assessed in the ear, forelimbs, hind limbs and tail. The following scale was used depending upon severity of lesion.

0 = normal; 1 = mild; 2 = moderate;
3 = severe; 4 = very severe;

The percentage inhibition was calculated.

Castor oil induced diarrhoea :

Effect of Zinc complex on castor oil (Amrutanjan Ltd., Hyderabad) induced fluid loss was assessed by the method of Awouters *et al.*[10]. Overnight fasted male (Charles Foster Strain) rats were used weighing 180 ± 20gm. Vehicle/drug administered SC 1/2 hour prior to 1

ml of castor oil orally. The treated rats were kept in groups of two in metabolic cages (Techniplast, Gazeda, Italy) for collection of castor oil induced gastrointestinal evacuation. Paper sheets of uniform weight were kept beneath each metabolic cage for fascal collection and was assessed at the end of 6 hours. The percentage inhibition was calculated. The rats were again used after fifteen days in the cross over test.

Prostagladins (PG's) estimations :

Prostaglandins were extracted in the inflammatory exudate by the method of Higgs and Salman[11]. The exudate was transferred to a graduated tube and treated with 5.0 ml of absolute ice cold acetone. It was stirred and centrifuged at 0^0C. The supernatant liquor, after addition of 2 volumes of n-hexane was stirred and centrifuged. The lower aqueous layer was acidified to pH 3.5 with citric acid. The PG's were extracted into ethyl acetate. The ethyl acetate layer was evaporated to dryness under reduced pressure and reconstituted in Kreb's solution for the bio assay. PG's were bio assayed on rats fundus strip[12]. PGE_2(Sigma) was used as standard and the contents were estimated by matching assay.

Analgesic activity :

Analgesic activity was assessed in prescreened mice using acetic acid (BDH)[13] or Phenyl quinone (Sigma)[14]. The prescreened Swiss albino mice were divided into three groups. The mice were fasted for sixteen hours before the start of the experiment. The mice were given Ibuprofen or Zinc complex 1/2 hour before the injection of acetic acid (50 mg/kg) or Phenylquinone (2mg/kg) intraperitoneally. The data were reported as all or none *i.e.* number of writhing per minute for each mice treated with vehicle or respective treatment groups. The number of writhing movements shown by each mice was counted for 20 minutes using manually operated digital counter. The percentage inhibition was calculated.

Arachidonic acid induced mortality in mice :

The test was conducted as per method of Kohler *et al.*[15]. Arachidonic acid (Sigma) solution was administered into the tail vein in a volume of 10 ml/kg in Swiss albino mice. For determining the inhibitory activity of Zinc complex or naproxen or vehicle, these were administered SC to groups of 5 mice 1 hour before arachidonic acid challenge. The percentage mortality and percentage protection in each group was noted 24 hours after arachidonic acid challenge.

Ulcerogenic test :

Experiments were carried out in 24 hours fasted male and female (non pregnant) rats (Charles Foster Strain) weighing between 140-175 g. Phenylbutazone was used for comparison. Water was allowed *ad libitum* before and during the experiment. Diphenyl amine-2,2'-dicarboxylic acid or Zinc complex or Phenylbutazone was given orally as a suspension in saline containing 1.4 % poly vinyl alcohol and sacrificed 6 hours after the treatment. After opening the abdomen the stomach was removed, cut open along the greater curvature, washed and examined under stereoscopic binocular microscope (Meopta) for scoring the lesions under blind conditions[16]. The lesions were scored as follows :

0 = No Lesion; 1 = Haemorrhagic effusion;
2 = Mucosal ulceration (< 2/3 area);
3 = Deep ulceration; 4 = Perforated ulcers;
The ulcerogenic index (UI) was calculated as follows:

$$\text{Ulcerogenic index(UI)} = \frac{\text{ADU x \% RU}}{100}$$

where ADU - Average degree of ulceration
% RU - % of rats with ulcer.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevA

Elemental analysis, molecular weight determination and conductance studies :

Elemental analysis and molecular weight of the compounds have been determined and found in agreement with the theoretical values. These data show the presence of three water molecules in the complexes. The presence of water molecules were further confirmed by thermal dehydration and infrared spectra of the complexes. The low conductance value (0.9-1.8 ohm^{-1} cm^2 mol^{-1}) indicated their non electrolytic nature due to the charge neutralization of the metal ion with ligand.

Electronic spectra and magnetic measurements :

The magnetic moments of Cu(II), Ni(II) and Co(II) complexes calculated from the corrected magnetic susceptibility and electronic spectra have been discussed.

Copper complex :

The magnetic moment value of the Copper complex (1.86 B.M.) indicates octahedral geometry[17]. This was further confirmed by the electronic spectra. It shows only one band in the region 13793 cm^{-1} due to the $^2E_g \rightarrow {}^3T_{2g}$

transition suggesting the distorted octahedral geometry[18].

Nickel complex :

The effective magnetic moment of nickel complex (3.11 B.M.) suggests an octahedral geometry of the complex. The geometry was further supported by electronic spectra. The bands observed at 10190 cm^{-1}, 17200 cm^{-1} and 26000 cm^{-1} were probably due to the three spin allowed transitions from $^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P), respectively in an octahedral environment.

Cobalt complex :

Cobalt complex was supposed to have octahedral geometry as confirmed by their magnetic moment value (4.87 B.M.). Three bands observed in the electronic spectra of the Cobalt complex at 7400 cm^{-1}, 17760 cm^{-1} and 20150 cm^{-1}, respectively which could be due to the three $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^4A_{2g}$ (F) and $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) transition, respectively.

Zinc complex :

On the basis of elemental analyses, thermal analyses, infrared spectra, molar conductance and molecular weight determination data, Zinc complexes was proposed to have an octahedral geometry.

Infrared spectra :

For the sake of brevity, only shifted or altogether new peaks appearing in the spectra of metal chelates have been discussed. Diphenyl amine-2,2'-dicarboxylic acid shows a band around 3250 cm^{-1} which is shifted to the lower frequency region in the case of their complex, suggesting coordination through N of -NH group. The infrared spectra showing band at 1680 cm^{-1} which is shifted to the lower frequency region in the metal complexes, confirm the coordination of the ligand to the metal ion through carboxylic acid moiety[20]. The presence of coordinated watermolecules in the complexes is revealed by stretching modes occurring at 3500-3600 cm^{-1} and bending modes at 1580 cm^{-1}. The appearance of the band at 480-490 cm^{-1} (M-N) AND 405-420 cm^{-1} (M-O) support the coordination through N and O donor sites of the ligand[21].

Pharmacology :

Anti inflammatory activity :

In a quest for safe, effective and better tolerated NSAID, we synthesized Diphenyl amine-2,2'-dicarboxylic acid and their metal complexes. The compounds were primarily screened in an acute model of inflammation *i.e.* carrageenan induced rat oedema test. It was found that Zinc complex inhibited 40 to 52 % oedema in a dose level of 12.5 to 100 mg/kg (Table 1) and it was almost equipotent with Ibuprofen in normal and adrenalectomized rats (Table 2). However, it was approximately 28% less

Table 1 : Effect of Zinc complex on carrageenan induced rat paw oedema

Pretreatment (Dose mg/kg SC)	Paw volume Mean ± SE	Percentage inhibition
Vehicle	1.03 ± 0.05	41.75
Naproxen(0.5)	0.60 ± 0.04	55.33
(1.0)	0.46 ± 0.06	57.28
(2.0)	0.44 ± 0.04	63.10
(4.0)	0.38 ± 0.03	75.72
(8.0)	0.25 ± 0.04	
Vehicle	1.27 ± 0.05	-
Zinc complex(12.5)	0.76 ± 0.02	40.15
(25.0)	0.68 ± 0.03	46.45
(50.0)	0.76 ± 0.05	47.24
(100.0)	0.61 ± 0.04	51.96

n = 6 in each group

Table 2 : Effect of Zinc complex in normal and Adrenalectomized rats against carrageenan induced raw paw oedema

Pretreatment (Dose mg/kg SC)	Normal rats		Adrenalectomized rats	
	Paw volume in mean ml ± SE	% inhibition	Paw volume in mean ml ± SE	% inhibition
Vehicle	1.30 ± 0.40	-	1.02 ± 0.20	-
Copper complex (50)	0.60 ± 0.03	53.84	0.60 ± 0.25	41.20
Ibuprofen (50)	0.62 ± 0.04	52.30	0.58 ± 0.40	43.20

n = 6 in each group

Table 3 : Effect of zinc complex on cotton pellet granuloma in rats

Pretreatment (Dose mg/kg SC)	Weight of dry Granuloma mg ± SB	% Inhibition
Control	250.4 ± 5.60	-
Copper complex(50)	218.0 ± 5.60	13.00
Naproxen(25)	205.3 ± 4.00	18.00
Ibuprofen(50)	203.8 ± 5.30	18.61

n = 8 in each group

Table 4 : Effect of Zinc complex on adjuvant arthritis (established) test in rats

Pretreatment (kg SC)	Days							
	0 14th	2 16th	4 18th	6 20th	8 22th	10 24th	12 26th	14 28th
Control	1.41±0.12	1.89±0.15	1.85±0.12	1.89±0.09	1.84±0.12	1.87±0.12	1.82±0.17	1.70±0.16
Zinc complex (25)	1.40 ±0.11	1.72±0.15	1.64±0.12	1.49±0.13	1.37±0.13	1.65±0.15	1.38±0.15	1.33±0.16
p	-	*	*	*	*	*	*	*
% inhibition			9.00	11.30	18.10	25.50	11.80	24.20
Naproxen (25)	1.39±0.12	1.32±0.12	1.07±0.09	0.98±0.07	0.95±0.08	0.77±0.06	0.86±0.08	0.87±0.07
p		**	***	***	***	***	***	***
% inhibition	-	-	30.14	42.20	46.10	48.40	58.80	42.70
Naproxen (25)	1.32±0.12	1.20±0.14	1.03±0.08	0.95±0.06	0.85±0.08	0.70±0.08	0.73±0.07	0.73±0.10
p	-	**	***	***	***	***	***	***
% inhibition	-	-	36.50	44.30	47.80	53.80	62.60	59.90

n= 8 in each group

*, ** and *** indicate significance of values at P=0.005, 0.001, 0.01

effective in subacute cotton pellet granuloma test for inflammation (Table 3) and was approximately 50% effective as compared to 4 mg/kg Naproxen in chronic test of inflammation *i.e.* adjuvant arthritis established (Table 4).

It is reported that inhibitors of prostaglandin synthesis are effective in inhibiting caster oil induced diarrhoea (Awouters et al[10]). Prostaglandins are synthesized throughout the gut to sustain peristaltic activity which is inhibited by NSAID's[22]. Zinc complex also inhibited prostaglandin synthesis induced by riecinoleic acid, a major component of castor oil (Table 5). This was further substantiated by the fact that Zinc complex inhibited (30.4%) prostaglandin E_2 like substances in inflammatory exudate (Table 6). Zinc complex did not prevent acetic acid or Phenylquinone induced writhing in mice as compared to 50 mg/kg Ibuprofen which protected 25% and 80% writhing, respectively (Table 7 and 8), primarily indicating that Zinc complex do not have an analgesic activity. However, other methods to evaluate its analgesic activity are in progress. It has been observed that I.V. injection of arachidonic acid leads to formation of either prostaglandin's or thromboxane A_2 which induces platelet thrombi and constriction of pulmonary vessels[23]. Zinc complex in 50 mg/kg dose protected the mice (20%) against arachidonic acid induced mortality (Table 9).

Gastrointestinal ulceration is well known side effect and is the common reason for rejecting an active anti-inflammatory compound. Therefore, it is necessary in the very early stages of a screening program for new anti inflammatory compound to assess the incidence and severity of gastric ulceration. Zinc complex had very low ulcerogenic index and therefore can be said to have low

Table 5 : Effect of Zinc complex on castor oil induced diarrhea in rats

Pretreatment (Dose mg/kg SC)	Mean evacuation in gm ± SE in 6 hours	% Inhibition
Control	6.10 ± 0.16	-
Zinc complex(50)	4.62 ± 0.38	25

n = 15 in both groups

Table 6 : Effect of Zinc complex on PGE_2 like substances in the inflammatory exudate

Pretreatment (Dose mg/kg SC x 3 days)	PGE_2 like substance mg/kg ± SE	Percentage
Control	40.80 ± 0.35	-
Zinc complex(50)	28.40 ± 0.37	30.40
Naproxen(25)	15.50 ± 0.28	62.00

n = 8 in each group

Table 7 : Effect of Zinc complex on 0.5 % acetic acid induced writhing in mice

Pretreatment (Dose mg/kg SC)	Writhing Mean ± SE	% Protection
Control	11.00 ± 1.60	-
Ibuprofen(50)	8.20 ± 1.50	25
Zinc complex(50)	11.33± 0.91	nil

n = 6 for each group

Table 8 : Effect of zinc complex on 0.02 % Phenylquinone induced writhing test in mice

Pretreatment (Dose mg/kg SC)	Writhing Mean ± SE	% Protection
Control (n=5)	14.00 ± 1.20	-
Ibuprofen(n=5)	1.600 ± 0.20	80
Zinc complex(n=5)	20.00± 4.60	nil

Table 9 : Effect of zinc complex on arachidonic acid induced mortality in mice

Pretreatment (Dose mg/kg SC)	No. of mice dead/total	% mortality	% Protection
Control	5	100	-
Ibuprofen(50)	4	80	20
Copper complex (50)	0	0	100

n = 5 in each group

incidence of gastric irritation common to most of NSAID's(Table 10).

Detailed studies suggest that zinc complex imparted mild increase in spontaneous activity and respiration. It has also induced passivity while the compound had no effect on piloerection, crouching gait, diuresis and body temperature.

Table 10 : Ulcerogenic potential of Diphenyl amine-2,2'-dicarboxylic acid and its zinc complex

Pretreatment (Dose mg/kg SC)	ADU	%RU	UI
Vehicle	0	0	0
DPDO (100)	1.5	80	0.6
Zinc complex(100)	2.0	100	2.0
Oxyphenbutazone(100)	3.0	100	3.0

n = 10 in each group

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