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Original Article

Anticonvulsant and Antimicrobial Activity of Cu (II), Zn (II) and Co (II) Complex of Isatin 3-Glycine

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Abstract

The role of Cu, Zn and Co in human physiology is well documented. Isatin and glycine have inhibitory effects on central nervous system. To capitalize on these features metal complexes of isatin-3-glycine were prepared and evaluated for anticonvulsant activity. The Cu (II) complex was found to be most active among the compounds. The compounds were screened against *Staphylococcus aureus*, *Escherichia coli* and *Pseudononas aeruginosa*. Antimicrobial activity of ligand and its complexes were comparable to that of nitrofurantoin.

Keywords: Anticonvulsant activity; Antimicrobial activity; Isatin-3-glycine; Metal complex.
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1. Introduction

Isatin has a range of actions including mono amino oxidase inhibition, anticonvulsant and anxiogenic activities [1-4]. Glycine is one of the inhibitory neurotransmitters in central nervous system [5]. Further Cu, Zn and Co as metal ions play an important role in human physiology [6, 7]. Keeping in view the above facts, we have attempted synthesis and anticonvulsant action evaluation of Schiff base of isatin with glycine and their complexes with Cu (II), Zn (II) and Co (II). Compounds forming chelates with metal ions and metal complexes are widely reported with enzyme inhibition and antimicrobial activity [8]. Considering this, we have screened the title compounds against *Staphylococcus aureus*, *Escherichia coli* and *Pseudononas aeruginosa*.

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2. Materials and methods

2.1. Chemistry

Metal complexes of amino acids are formed by coordination through α-amino and α-carboxylate group [9]. The metal complexes were prepared following the reported method outlined in scheme 1 [10]. All reagents and solvents used were of general purpose grade. Melting points of pure compounds were determined in open capillary tubes using melting point apparatus (Sisco) and are uncorrected. Infrared spectra were recorded on Shimadzu-FT-IR-8400S spectrophotometer using KBr powder. Electronic spectra were recorded with the help of ultra violet (UV)-

visible double beam spectrophotometer (UV-1601, Shimadzu). The CHN elemental analysis was carried out using a Perkin Elmer-2400 elemental analyzer. The complexes were analyzed for their metal content by Aanalyst-200 atomic absorption spectrophotometer (Perkin Elmer).

2.1.1. Synthesis of isatin-3-glycine

Ethanolic solution of isatin (0.5 mol) was added to aqueous solution of glycine (0.5 mol). The mixture was heated under reflux for 1 h. The precipitate so obtained was filtered, washed and recrystallised from ethanol to produce isatin-3-glycine.

Isatin: IR (KBr; υ, cm^{-1}): 3217.37 (N-H-str), 1734.06 (C=O-str), 3053.42 (C-H-aromatic), 1332 (C-N -str) 1462 (C=C-str), 771.55 (C-H-out of plane bend (1,2-substituted benzene). Electronic spectra (nm): 419.

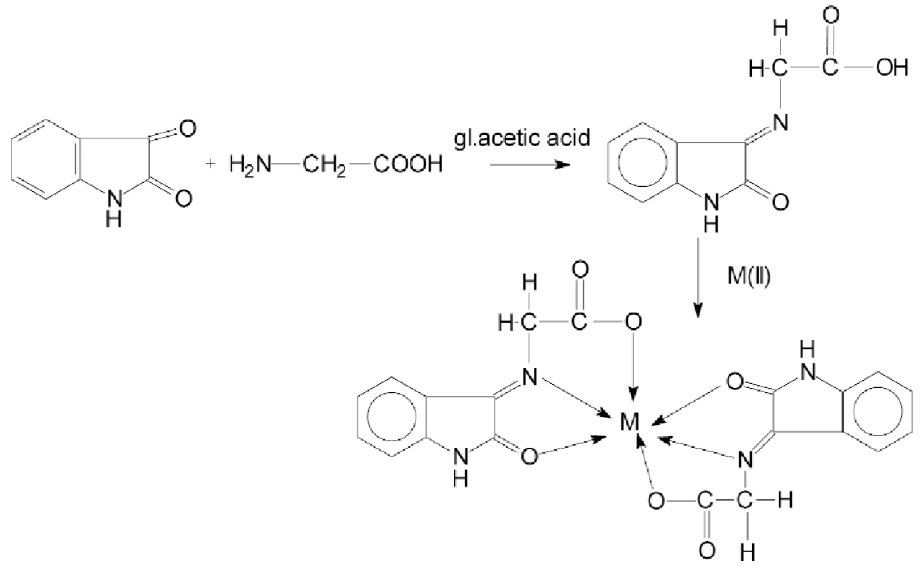
Ligand: Brick red. Yield: 71%, m.p.195.IR (KBr; υ, cm^{-1}): 3390.97 (O-H-str), 3238.59 (N-H-str), 1720.56 (C=O-str), 3074.63 (C-H-aromatic), 1620.26 (C=N-str) 1471.74 (C=C-str), 754.19 (C-H-out of plane bend (1,2-substituted benzene). Electronic spectra (nm): 487. CHN (%) Found: C: 58.1; H: 3.72; N: 13.5.$C_{10}H_80_3N_2$ requires C: 58.8; H: 3.92; N: 13.7.

2.1.2. Synthesis of ML_2 $(AcO)_2$

A hot aqueous solution of metal acetate (0.04 mol) was added to an ethanolic solution of the ligand (0.08 mol) and the mixture was heated under reflux for 1 h. The reaction mixture was concentrated to a small volume. On cooling the complex precipitated which was filtered off, washed with ethanol and dried in a desiccator. Other compounds were synthesized in a similar manner.

[Cu L_2 $(AcO)_2$]: Brown. Yield: 65%, m.p. 337-339. IR(KBr; υ, cm^{-1}): 3227.02 (N-H-str), 1707.06 (C=O-str), 3074.63 (C-H-aromatic), 1618.33 (C=N-str) 1602.90 (COO^- asymmetric str), 1396.51(COO^- symmetric str), 1471.74 (C=C-str), 754.19 (C-H-out of plane bend (1,2-substituted benzene) 489.94 (N-M str), 536.23 (O-M str). Electronic spectrum (nm): 254.5, 260.5, 334.5. Cu (%); Calc. (Found): 13.52(12.8). CHN (%) Found: C: 51.4; H: 3.1; N: 11.68. $C_{20}H_{14}0_6N_4Cu$ requires C: 51.18; H: 2.98; N: 11.92.

[Zn L_2 $(AcO)_2$]: Violet. Yield: 58 %, m.p.



Scheme 1. Synthesis process for $ML_2(AcO)_2$.

Table 1. Anticonvulsant and antimicrobial activities of metal complex of isatin.

	Anticovulsant activity		Antimicrobial activity		
Compd.	**Onset of convulsion (s)**	**Incidence (%)**	***E. coli***	***P. aeruginosa***	***S. aureus***
Ligand	348.20±18.66*	66.2	19.00±0.66	18.70±2.30	16.50±2.68
$CuL_2(AcO)_2$	386.20±22.66	33.2	18.80±1.72	14.50±3.40	15.45±1.30
$ZnL_2(AcO)_2$	284.00±12.73*	83.0	21.70±1.40	23.50±2.60	20.30±0.70
CoL_2Cl_2	379.00±8.68*	49.8	22.50±2.20	17.60±1.50	23.40±1.35
Control	165.30±3.340	100	-	-	-
Diazepam	Absent	0	-	-	-
Nitrofurantoin	-	-	21.4±2.65	14.3±0.6	16.7±1.5

Anticonvulsant values are expressed as mean±SEM. n=6 in each group. One way ANOVA, F= 123.86, df = 4, 35.*p< 0.05. In antimicrobial activity '-' indicates no zone of inhibition. All the values are mean standard deviation of three determinations. Values showed significant difference from solvent control at p<0.01.

293-295. IR (KBr; υ, cm^{-1}): 3227.02 (N-H-str), 1707.06 (C=O-str), 3074.63 (C-H-aromatic), 1618.33 (C=N-str) 1388.79 (COO^- symmetric-str), 1471.74 (C=C-str), 754.19 (C-H-out of plane bend (1, 2-substituted benzene). CHN (%) Found: C: 51.34; H: 3.15; N: 12.12. $C_{20}H_{14}0_6N_4Zn$ requires C: 50.91; H: 2.96; N: 11.87.

$[CoL_2Cl_2]$: Red. Yield: 52 %, m.p. 325-328. IR (KBr; υ, cm^{-1}): 3227.02 (N-H-str), 1707.06 (C=O-str), 3074.63 (C-H-aromatic), 1618.33 (C=N-str) 1471.74 (C=C-str), 1602.90(COO^- asymmetric-str),1398.44 (COO^- symmetric-str), 754.19 (C-H-out of plane bend(1,2-substituted benzene) 493.79 (N-M-str), 543.94 (O-M-str). Electronic spectrum (nm):276,520nm. Co (%); Calc. (Found):12.66 (12.25). CHN (%) Found: C: 51.78; H: 3.24; N: 12.32. $C_{20}H_{14}0_6N_4Co$ requires C: 51.62; H: 3.01; N: 12.05.

2.2.1. Evaluation of anticonvulsant activity

Anticonvulsant study [11] was carried out following the protocols of animal ethical committee at pharmacology laboratory of University Department of Pharmaceutical Sciences, Utkal University approved by (registration no-990/c/06/CPCSEA) committee for the purpose of control and supervision of experiments on animals, Ministry of Environment and Forest (India). Swiss albino male mice, weighing 20-25 g were randomly arranged in different (6 in each) groups. Test compounds were suspended in Tween 80 (0.2%) (Sigma, USA) and were given i.p. in dose of 10 mg/kg body mass. Dosing volume was 0.2 ml / 20 g. Diazepam was dissolved in water in 2% concentration and used as a reference standard. Pentylenetetrazole (PTZ, Sigma) was dissolved in water in 2% concentration. The solutions were suitably diluted. Pentylenetetrazole (80 mg/kg) was given i.p. 30 min. after the test compounds or Diazepam (4 mg/kg). The animals were observed for convulsions. Animals, those showed no convulsions within one hour after PTZ injection are considered to be protected (Table 1).

2.2.2. Evaluation of antimicrobial activity

The *in vitro* screening was carried out using one gram-positive bacterium (*Staphylococcus aureus,*) and two gram-negative bacteria (*E. coli, P. aeruginosa*) by disc diffusion method [12-14]. The compounds were dissolved in dimethyl formamide (6%), which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. A solution of concentration 30 mg/ml was made for each test compounds and finally sterilized by filtration using 0.45 µm Millipore filters. The sterile discs (Hi-media, 6 mm in diameter) were impregnated with 10 µl of the test solutions (300 µg/disc) and placed in inoculated agar. Nitrofurantoin (300 µg/disc) was used as standard drug. The control was prepared using dimethyl fomamide. The inoculated plates were incubated at 37 °C for 24 h. The antibacterial activity of test

compounds against the bacterial strains is given in Table 1 as zone of inhibition.

2.3. Statistical analysis

The results are expressed as mean±S.E.M. The differences between control and the groups was determined using the one way analysis of variance (ANOVA) followed by Dunnett's test with 5% significance level ($p<0.05$). The results obtained in antimicrobial evaluation are expressed in mean standard deviation of three determinations.

3. Results and discussion

The IR spectra of the free ligand showed a broad band around 3230 cm^{-1} that can be attributed to NH stretching vibration of isatin. The position of this band remains at nearly the same frequency in spectra of the metal complexes implying the un-coordination of this group. Bands at 1720 and 1620 cm^{-1} in the ligand due to C=O and C=N stretching frequencies shift towards lower values (1670-1685 and1600-1610 cm^{-1}) in all complexes indicating that the carbonyl oxygen atom of the isatin residue and the azomethine nitrogen atom are coordinated. A band at 1360-1380 cm^{-1} in the spectra of complexes is assigned to symmetric stretching vibration of carboxyl group of amino acid, suggesting the involvement of this group in the complex formation. It can be deduced, from the above argumentation, that the ligand (isatin-3-glycine) behaves as a monobasic tridentate chelating agent towards the central M (II) via the three bonding sites of the azomethine nitrogen atom, carbonyl oxygen atom of the isatin residue and the negatively charged oxygen atom of the amino acid carboxyl groups as shown in Scheme 1. The Co (II) complexes exhibit two bands at 520 and 276 nm in the electronic spectra which are in accordance with Co (II) high-spin octahedral geometry.

All of the compounds delayed the onset of convulsion in a dose dependent manner (Table 1). All of the animals showing convulsion died with in 40 min. The incidences of convulsion indicate the percentage of animals exhibiting convulsion. The isatin-3-glycine and its metal complexes with Cu (II), Zn (II) and Co (II) increased onset of convulsion, significantly ($p<0.01$) compared to the control. As expected the Cu (II) complex exhibited maximum anticonvulsant action. Complexation with Zn (II) seems to have decreased the anticonvulsant property of the ligand. The activity was enhanced on complexation with Cu (II) and Co (II). However, the complexes did not provide full protection against convulsion.

The antimicrobial actions of title compound were comparable to that of nitrofurantoin. Complexation with metals does not seem to enhance the antimicrobial action of the ligand, significantly. However, the action of CoL_2Cl_2 was found to be better against *S. aureus* and *E. coli*. The zone of inhibition for ZnL_2Cl_2 was better against *P. aeruginosa*. This study can be extended to investigate the toxicity and pharmacokinetic aspects to get clear insight into the therapeutic utility of these compounds.

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