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# Synthesis, Characterization and Biological Activity of Copper(II) Complexes with Phenylglyoxal bis-(Thiosemicarbazones)

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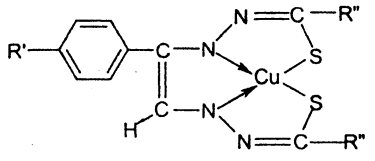
Six Cu(II) coordination compounds with a novel ligand 4-substituted phenylglyoxal **bis** (thiosemicarbazones) have been synthesized and characterized by various physico-chemical methods including elemental analysis, UV-visible and IR spectral studies. These compounds were tested for cytotoxic activity against *Ehrlich ascites* carcinoma (EAC) cells. In these experiments compounds have demonstrated cell growth inhibition activity. Compounds **1, 2, 3** and **4** have been screened for antimicrobial activity on *B. subtilis* and *E. coli.* They inhibited the bacterial growth considerably.

**Key Words: Copper(II) complexes, Phenylglyoxal bis(thiosemicarbazones)**

## INTRODUCTION

Chemotherapeutically thiosemicarbazones have assumed great importance in the recent years, as they possess antibacterial, antiviral and antitumour activities[1–4]. Various thiosemicarbazones exhibit antitumour activity by virtue of their *Thymidilate synthetase* enzyme inhibitory activity[5]. Complexing Cu(II) ion with the thiosemicarbazones increases their antitumour potency. Lipophilic character of these compounds accounts for the accumulation of Cu(II) ion inside the cells.

In present research studies we focused our attention on bis(thiosemicarbazone) moieties and their biological activity.




R' R"

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| | R′ | R″ |
|---|---|---|
| Compound **1** | —H | $-NH_2$ |
| Compound **2** | $-NO_2$ | $-NH_2$ |
| Compound **3** | -H | $-NH-CH_3$ |
| Compound **4** | $-NO_2$ | -NH—CH3 |
| Compound **5** | —H |  |
| Compound **6** | $-NO_2$ |  |

In this context, we have synthesized six Cu(II) complexes of phenyl glyoxal bis(thiosemicarbazones).

All the synthesized compounds were characterized by the UV-visible spectroscopy, IR spectroscopy and CHN analysis. Synthesized compounds were also tested for the antitumour activity (*in vivo*) against *Ehrlich ascites* carcinoma bearing mice. Antibacterial activity was evaluated against *B. subtilis* and *E. coli* by cup-plate method.

## EXPERIMENTAL

All reactants and solvents were synthetic reagent grade. Thiosemicarbazide was procured from Merck India Pvt. Ltd. and 4-methyl thiosemicarbazide was procured from Ranbaxy India Ltd. N-Morpholino thiosemicarbazide was prepared by standard method in our laboratory[6]. UV-Visible spectra were obtained with Elico SL-159 UV-Visible spectrophotometer. The IR spectra were recorded in the range of 4000–200 $cm^{-1}$ using Phillips mode Pye-Unicam SP-3-200 IR spectrophotometer. Elemental analysis (CHN) was performed with Carlo Erba 1106 elemental analyzer.

**Preparation of phenyl glyoxals:** Acetophenones were oxidized by refluxing equimolar concentrations of both acetophenone and selenium dioxide in aqueous dioxane for 8 h. In case of 4-nitro acetophenone the refluxing was performed for 24 h. The residue selenium was removed by filtration. Dioxane and water were removed by rotary evaporation technique, leaving a residue of crude phenyl glyoxal. This residue was added to boiling water, treated with activated charcoal and filtered. The filtrate was kept overnight to get hydrated crystalline phenyl glyoxal, which was collected subsequently by filtration[7,8].

**Preparation of ligands, phenyl gloxal bis(thiosemicarbazones):** Phenyl glyoxal bis-(thiosemicarbazones) were prepared by adding a solution of phenyl glyoxal hydrates (0.02 M) in 95% ethanol dropwise over 20 min, to solutions of the corresponding thiosemicarbazides (0.04 M) in a mixture of 30 mL 95% ethanol, 40 mL water and 4 mL concentrated hydrochloric acid. This mixture was further refluxed for 10 to 45 min, cooled to room temperature and the precipitates were filtered, washed with water and methanol and air dried.

**Preparation of phenyl glyoxal bis-(thiosemicarbazones) copper(II) complexes:** These complexes were synthesized by adding cupric acetate (0.0025 M) in 40 mL of water dropwise over 10 min with stirring to a refluxing solution of phenyl gloxal bis-(thiosemicarbazones) (0.0025 M) in 300 mL of methanol. This mixture was further refluxed for 20 min and allowed to cool very slowly, kept for 1 to 2 days under stirring. These complexes formed were then collected by filtration, washed with methanol and air-dried.

**Cytotoxic activity:** Weighed amounts of synthesized compounds were triturated with 0.5% of carboxymethyl cellulose (Sigma Co.) in 0.9% of normal saline, so that the required amount of chelates were present in 0.5 mL of drug suspension. Fresh suspensions were prepared everyday. Female adult Swiss albino mice, 7 to 9 weeks of age, weighing 20 to 25 g, were used throughout these studies. They were housed in polypropylene cages and were given standard mouse pellet and water *ad libitum*. *Ehrlich ascites* cacrinoma (EAC) cells were procured from the Department of Radiobiology, Kasturba Medical College, Manipal, Karnataka, India. These cells were maintained intra-peritonially by serial transplantation in adult female Swiss albino mice. Animals were inoculated intraperitonially with $2 \times 10^5$ EAC cells per mouse to 42 female Swiss Albino mice (7 groups of six each) so as to develop ascites tumor; after 24 h of tumor transplantation six groups of these animals were treated with a dose of 20 mg/kg body weight of synthesized compound, except for compound **1** which was given at a dose of 2 mg/kg body weight (the dose regimen were calculated after preliminary toxicity studies). A control group was treated with the same volume of saline (0.9%) containing 0.5% carboxymethyl cellulose. Treatment was given for five alternate days. Mean survival time (MST) for each group was noted. Survival time for each treated group was compared with the control group by using the following formula.

$$\text{Increase of life span} = \left[\frac{\text{MST of treated group}}{\text{MST of control group}} \times 100\right] - 100$$

**Antibacterial activity:** Test solutions (1 mg/mL) of compounds **1** to **4** were made with dimethyl formamide. Test organisms *B. subtilis* and *E. coli* were obtained from Natural Remedies Pvt. Ltd., Bangalore, and Muller Hinton Agar Media procured from Hi-Media India Ltd. Then 30 mL of Muller Hinton Agar Media (Hi-Media) was poured into sterile petridishes under aseptic conditions and were allowed to settle. After settling they were inoculated with test organism by cotton swab method [9]. Inoculated plates were bored by standard 8 mm diameter borer; 0.1 mL (100 mg) of test samples of copper(II) thiosemicarbazones were introduced into the respective bores. They were incubated at 35 to 37°C for 24 h and zones of inhibition were measured after incubation period. Experiments were performed in triplicate and mean values are reported. Ciprofloxacin (50 mcg/mL) was used as the standard.

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS

| Compound No. | m.p. | m.f. | m.w. | Analysis %, found (calcd.) | | |
|---|---|---|---|---|---|---|
| | | | | C | H | N |
| 1 | 213 | $C_{10}H_{10}N_6S_2Cu$ | 342 | 35.13 (34.86) | 2.95 (3.01) | 24.58 (24.63) |
| 2 | 220 | $C_{10}H_9N_7O_2S_2Cu$ | 387 | 31.04 (31.23) | 2.34 (2.20) | 25.34 25.72 |
| 3 | 242 | $C_{12}H_{14}N_6S_2Cu$ | 370 | 38.96 (38.76) | 3.81 (3.80) | 22.72 (23.18) |
| 4 | 235 | $C_{10}H_{13}N_7O_2S_2Cu$ | 416 | 34.73 (34.99) | 3.14 (3.33) | 23.63 (24.12) |
| 5 | 271 | $C_{18}H_{24}N_6O_2S_2Cu$ | 482 | 44.85 (44.02) | 4.60 (4.52) | 17.43 (17.32) |
| 6 | 284 | $C_{18}H_{21}N_7O_4S_2Cu$ | 527 | 41.02 (41.78) | 4.01 (4.24) | 18.60 (18.53) |

TABLE-2
UV-VISIBLE AND IR SPECTRAL BANDS ($cm^{-1}$) OF SYNTHESIZED COMPOUNDS

| Compound | $\lambda_{max}$ (nm) | ν(N—H) | ν(C=N) | ν(C—S) | ν(C—$NO_2$) |
|---|---|---|---|---|---|
| 1 | 303 | 3280 | 1600 | 1180 | — |
| 2 | 320 | 3300 | 1610 | 1110 | 1320 |
| 3 | 306 | 3310 | 1520 | 1180 | — |
| 4 | 330 | 3400 | 1510 | 1170 | 1350 |
| 5 | 311 | — | 1500 | 1190 | — |
| 6 | 312 | — | 1600 | 1150 | 1320 |

TABLE-3 CYTOTOXICITY ACTIVITY OF COMPOUNDS 1–6 ON *EHRILCH ASCITES* TUMOR BEARING MICE

| Sl. No. | Group | MST (Days) | T/C × 100 | ILS |
|---|---|---|---|---|
| 1 | Control | 13.5 | — | — |
| 2 | Compound-1 | 27.2 | 201.48 | 101.48 |
| 3 | Compound-2 | 24.3 | 180.00 | 80.00 |
| 4 | Compound-3 | 28.8 | 213.00 | 113.00 |
| 5 | Compound-4 | 23.0 | 170.37 | 70.30 |
| 6 | Compound-5 | 20.8 | 154.07 | 54.07 |
| 7 | Compound-6 | 21.8 | 161.48 | 61.48 |

MST: Mean survival time; T: Treated animal MST;
C: Control animal MST; ILS: Increase in life span

TABLE-4
ANTIBACTERIAL ACTIVITY OF COMPOUNDS **1, 2, 3** AND **4** ON *B. SUBTILIS* AND *E. COLI.*

| Compounds | Zone of inhibition (mm) | |
|---|---|---|
| | *B. subtilis* | *E. coli* |
| Compound-**1** | 25 | 29 |
| Compound-**2** | 18 | 20 |
| Compound-**3** | 21 | 21 |
| Compound-**4** | 20 | 24 |
| Standard (Ciprofloxacin) | 30 | 30 |

## RESULTS AND DISCUSSION

The elemental analysis experimental values are inconsistent with calculated values.

The UV-visible spectra of ligands have shown $\lambda_{max}$ in the region of 322 to 351 nm. While spectra of copper complexes have shown hypsochromic shift in their $\lambda_{max}$ and an additional peak in visible region. These changes may be attributed to formation of coordinate bond between non-bonded pair of electrons in the ligand nitrogen with copper(II) ion[10]

The IR spectra of compounds **1, 2, 3** and **4** exhibit absorption bands in region of 3400–3300 $cm^{-1}$, these bands were assigned to ν(N—H). Absence of these bands in the spectra of **5** and **6** was due to presence of tertiary nitrogen[11].The absence of absorption bands in the region of 1800–1600 $cm^{-1}$ of all ligand spectra indicates absence of carbonyl group. The strong bands in the 1690–1470 $cm^{-1}$ in the spectra of all complexes were assigned to ν(C=N) stretching[12].

The medium intensity band in the region 1250–1020 $cm^{-1}$ was assigned to ν(C—S). Nitro group containing complexes exhibited an additional band in the region of 1390–1250 $cm^{-1}$ due to ν(C—$NO_2$)[13].

**Biological activity:** Compounds **1, 2, 3** and **4** have shown antibacterial activity in concentration of 1 mg/ml. These compounds showed more antibacterial activity against *E. coli* than *B. subtilis.* This indicates the effectiveness of compounds against gram –ve organisms than on gram +ve organisms. Amongst all the compounds screened, compound **1** showed maximum activity against both *E. coli* and *B. subtilis.* Antibacterial activity of complexes may be due to diffusion of the metal chelates as a whole through organism due to its lipophilic character.

All the synthesized compounds considerably delayed the onset of tumour development and the life spans of the treated EAC induced animals extended. Amongst the compounds screened, compound **1** and **3** showed maximum cytotoxic activity, compound **5** showed least cytotoxic activity. The two compounds with electron withdrawing group on aromatic ring showed less cytotoxic activity.

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