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Studies on antimicrobial activity of semicarbazones and their metal complexes

M. RAMA CHARY, N. PADMAJA, M. RAVINDER and S. SRIHARI*

Department of Chemistry, Kakatiya University, Warangal - 506 009 (India).

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ABSTRACT

A few semicarbazone ligands and their Co(II), Cu(II),Pd(II) and Hg(II) complexes have been screened for antimicrobial activity against some Gram (+ve) and Gram(-ve) bacterial and fungal species. The metal complexes are found to have higher activity than the ligands. Further, the metal complexes exert differential activity against the organisms studied and the results obtained are discussed.

Key words: Metal-Semicarbazone complexes, antimicrobial activity.

INTRODUCTION

Semicarbazones constitute a special class of organic compounds owing to their chemical and biological importance. These compounds which are potential chelating agents endowed with diverse ligating behavior[1,2] have been reported to possess a wide spectrum of medicinal properties[3-6]. The biological activity of these compounds has been attributed to their ability to chelate trace metal ions and, in many a case, the metal ion association exerts a synergistic effect on the activity of the free ligands[7-8]. In this paper, we report the results on the antimicrobial screening of semicarbazone ligands namely 2-aminonicotinaldehyde semicarbazone (ANSC), 3–formyl chromone semicarbazone (FCSC), 2-hydroxy-3-methoxybenzadehyde semicarbazone (HMBSC) and 2, 4-dihydroxyacetophenone semicarbazone (DPSC) and their Co(II), Cu(II), Pd(II) and Hg (II) complexes against gram (+ve) bacterium – Bacillus subtilis, gram (-ve) bacterium – Escherichia coli and fungus – Aspergillus niger.

EXPERIMENTAL

Preparation of solutions

The test solutions of the samples were prepared in dimethylformamide. The standard antibiotics benzyl penicillin and streptomycin sulphate were used as standards for antibacterial screening and nistatin was used as a standard for antifungal screening.

All the samples under present investigation were dissolved in DMF to give a final concentration of 1 mg/ml. The antibacterial standards were dissolved in sterile distilled water. The antifungal standard was dissolved in buffered 70% propanol.

Preparation of inoculum and nutrient medium[9,10]

Nutrient broth (P^H – 7.2) was used for the preparation of inoculum of bacteria. The composition of broth was peptone 5.0 g, Beef extract 1.5 g, Yeast extract 1.5 g and distilled water 1000 ml. Nutrient agar containing 1.5 % of agar in addition to the composition of nutrient broth was used for the preparation of medium for antibacterial screening.

For antifungal screening, inoculum was prepared by transferring a loopful of stock culture (Glucose – 110 g, Peptone – 10 g, Agar – 20 g distilled water upto 1000 ml) to a 125 ml Erlenmayer flask containing 80 ml of Sabouraud's broth. The composition of inoculum broth is same as that of stock culture with the exception of agar. The

inoculum flask was incubated for 18 hours at 25^{O} C and stored at 50^{O} C.

Preparation of plates

For bacterial screening the agar medium was sterilized by autoclaving at 121^{0} C (15 lb/Sq.In) for 15 minutes. About 25 ml of the molten medium was poured in each of the sterilized petri dishes. About 0.5 ml of 24 hours old broth cultures of different strains of bacteria were added to the respective petri dishes. The contents of petri dishes were mixed thoroughly. After solidification of the medium, four cups (diameter 8 mm) were made with the help of a sterile borer at equal distances.

For antifungal activity, the corning sterile petri plates were used for investigation. About 20 ml of previously inoculated Sabouraud's agar medium was poured in it. After solidification of the medium , four cups (diameter 8 mm) were made with the help of a sterile borer at equal distances.

Accurately measured 0.1 ml of samples and 0.1 ml of standard were added into the cups and labeled accordingly. The plates kept undisturbed in a cool place for one hour to allow the solutions to diffuse into the medium. The plates were then kept for incubation at 37^{O} C for 24 hours.

For antifungal screening each cup in petri plate was loaded with 0.1 ml of respective solutions. The plates were kept undisturbed in a cool place for 2 hours to allow the solutions to diffuse into the medium and then incubated at 25°C for 24 hours.

Measurements of Activity

The presence of a definite zone of inhibition surrounding the cups indicated antimicrobial activity. The diameter of the zone of inhibition was recorded. The experiments were performed atleast in triplicate.

Dose dependent activity of compounds

Based on antimicrobial profiles, further studies were made to find out dose dependent activity of the selected compounds. Four different concentrations of test samples (0.5,1.0, 2.0 and 3.0 mg/ml) and four concentrations of standard drugs (50,100,200 and 500 µg/ml) were employed in assessing the extent of antimicrobial activity of the compounds. Accurately measured 0.1 ml of the test and standard solutions were placed in cups prepared in seeded agar petri dishes as described earlier. The petri plates were left undisturbed in a cool place for one hour to allow proper diffusion and then incubated at 37° C for 24 hours in case of bacteria and at 25° C for 48 hours in case of fungus. After the incubation period, the diameter of zone of inhibition was measured with antibiotic zone reader and the experiments were carried out in triplicate.

The activities of the compounds are compared with those of the respective standards. The results are presented in Table Nos. 1 and 2.

RESULTS AND DISCUSSION

The antimicrobial screening of the ligands and their metal complexes has been first carried out on Bacillus subtilis (Gram +ve) and Escherichia coli (Gram –ve) bacteria and Aspergillus niger (fungus) to find out the activity spectrum of the compounds. A zone of inhibition of 20 mm or above has been considered as a significant activity.

It is observed that none of the ligands is associated with considerable antimicrobial activity, while the metal complexes screened possess higher activity than the ligands. The complexes viz. Co-HMBSC, Cu-ANSC, Pd-FCSC and Hg- DPSC have been associated with significant activity.

The results in the table indicate that the activities of the complexes against the bacteria and fungus studied are comparable with small variation either way.

The set of four complexes that is significantly active against B. substilis, E.coli and A.niger at the concentration 1mg/ml (Table -1) has been subjected to quantitative study (Dose-dependent studies) against the same organisms employing four different concentrations i.e., 0.5,1.0,2.0 and 3.0 mg/ml in DMF. The concentrations of the standards that have been used correspond to 50,100,200 and 500 µg/ml and the results obtained are presented in tables 2 A-2C.

The results in the Tables 2A-2C indicate that the compounds are associated with the least activity at the lowest concentration. Most of the compounds screened show jump in activity when

the concentration is increased from 0.5 mg/ml to 1.0 mg/ml. And thereafter, as the concentration is raised, the activity remains the same or the increase, if there is any, is only marginal. These observations recommend the optimum concentration of the compounds for activity to be 1 mg/ml.

From the results it is known that the metal complexes exert more of antimicrobial activity than the ligands indicating that the metals are actually in action[11,12]. It is assumed that the role of ligands associated with the metal ion in a complex is to make the metal ion fat soluble. Once the complex makes its way across the cell membrane, depending on the environment, it undergoes dissociation releasing the metal ion and enabling it to exert its toxic effect. The differential activity associated with the complexes may be traced to this.

Table 1: Antimicrobial activity of the metal complexes

S. No.	Complex	Concentration : 1 mg/ml in DMF Zone of inhibition (in mm)		
		Bacillus subtilis	***Escherichia coli***	***Aspergillus niger***
1.	Co-HMBSC	20 (10)	25 (13)	20 (10)
2.	Cu- ANSC	26 (10)	20 (10)	21 (13)
3.	Pd-FCSC	26 (10)	20 (10)	20 (10)
4.	Hg-DPSC	20 (10)	22 (10)	24 (13)

The values in parentheses correspond to those of the ligands

Table 2(a): Quantitative antimicrobial activity of the metal complexes Organism: Bacillus subtilis, standard: Benzyl penicillin

S. No.	Compound	Zone of inhibition in mm			
		A	B	C	D
1.	Co-HMBSC	20	20	20	10
2.	Cu-ANSC	24	22	20	12
3.	Pd-FCSC	27	27	26	15
4.	Hg-DPSC	28	28	20	14
5.	Standard	24	21	20	16

Table 2(b): Quantitative antimicrobial activity of the metal complexes Organism: Escherichia col, Standard : Streptomycin sulphate

S. No.	Compound	Zone of inhibition in mm			
		A	B	C	D
1.	Co-HMBSC	23	22	20	14
2.	Cu-ANSC	33	33	25	19
3.	Pd-FCSC	24	22	20	13
4.	Hg-DPSC	24	23	22	20
5.	Standard	26	24	20	16

Table 2(c): Quantitative antimicrobial activity of the metal complexes Organism: *Aspergillus niger*, Standard : Nistatin

S. No.	Compound	Zone of inhibition in mm			
		A	B	C	D
1.	Co-HMBSC	21	20	20	13
2.	Cu-ANSC	22	22	20	16
3.	Pd-FCSC	22	22	21	14
4.	Hg-DPSC	25	25	24	20
5.	Standard	25	20	18	17

* Test solution

A = 3 mg/ml	A = 500 µg/ml
B = 2 mg/ml	B = 200 µg/ml
C = 1 mg/ml	C = 100 µg/ml
D = 0.5 mg/ml	D = 50 µg/ml

(* for Tables 2 A, 2B and 2C)

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