Acta Poloniae Pharmaceutica – Drug Research, Vol. 61 No. 2 pp. 123–125, 2004

ISSN 0001-6837
Polish Pharmaceutical Society

ANTIMICROBIAL EFFECT OF Cu(II) COMPLEXES CONTAINING OXIME LIGANDS

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Abstract: The antibacterial, antifungal and antitubercular activity of Cu(II) complexes was studied. All the complexes have been screened against *Staphylococcus aureus, Salmonella typhi, Candida albican, Aspergillus niger, Saccharomyces cerevisiae* and $H_{37}Rv$ and found to be more toxic than the parent ligand. The activity increased in the order Cu(5–methyl–2,3–hexanedione dioxime)$_2$ < Cu(5–methyl–3–oximino–hexan–2–one–hydrazone)$_2$ < Cu(5–methyl–3–oximino–hexan–2–one–phenylhydrazone)$_2$.

Keywords: antibacterial; antifungal; antitubercular activity; metal complexes; biological strains

The literature survey reveals that Schiff base ligands are excellent coordinating ligands and can exhibit variety of structure in their metal complexes. Schiff bases and their copper complexes are known for their biological importance as fungicides (1, 2) and bactericides (3). Some of the copper complexes are known to possess anti–inflammatory (3) and anticancer (4) properties. Metals are known to display antimicrobial activity. The activity of metallic solutions has been examined from various points of view. Generally, the toxicity of metals increases with atomic weight (5). In addition to atomic weight, toxicity of metals for various organisms have been shown to be related to electronegativity of metallic ions (6) and stability of metal chelates (7). In recent years, inorganic compounds and organometals have again come to the forefront of interest from the biological point of view. In 1956, Horsfall (6) found fungicidal properties of $CuSO_4 \cdot 5H_2O$. Sprowls and Poe (8) studied the inhibiting effect of copper(II) chloride, nitrate and sulphate on *Staphylococcus aureus* and *Salmonella typhi*. An antimicrobial effect was observed for copper(II) 8–hydroxyquinolinate (9). In order to make it commercially profitable, several scientists have tried to replace 8–hydroxyquinoline with another suitable ligand.

EXPERIMENTAL

For the purpose of systematic diagnosis, oxime based Schiff bases were selected and their Cu(II) complexes were synthesised. The present work is animed at the study of antibacterial, antifungal and antitubercular activity of Cu(II) complexes of oxime based Schiff bases.

The following oxime based Schiff bases Cu(II) compounds were studied: Cu(HMHDDO)$_2$, Cu(MOHOH)$_2$ and Cu(MOHOP) where H_2MHDDO = 5–methyl–2,3–hexanedione dioxime, HMOHOH = 5–methyl–3–oximino–hexan–2–one–hydrazone, HMOHOP = 5–methyl–3–oximino–hexan–2–one–phenylhydrazone. The complexes were prepared as per the literature (10,11). The synthesis of metal complexes were carried out by a general method of synthesis. An aqueous solution of Cu(II) chloride (0.01 mmole) was mixed with constant stirring with an ethanolic solution of ligands (0.02 mmole) and the mixture was stirred vigorously. The pH of the solution was adjusted to 6–6.5 by using 1:1 ammonia solution. The green coloured crystals of the complex were obtained, which were filtered, washed with warm water followed by 50% ethanol and dried *in vacuo*.

The antimicrobial activity of the compounds under investigation were studied for the two types of bacterial species *Staphylococcus aureus* and *Salmonella typhi*. For antifungal activity three species were used, namely *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae*, while for antitubercular activity $H_{37}Rv$ was used. The media used for the determination of minimum inhibitory concentration (MIC in μg/ml) of the microorganisms were as follows:

Antibacterial:	Mueller Hinton
Antifungal:	Sabouraud broth
Antitubercular:	Middle brook. 7H9 Broth base/Middlebrook OADC Growth Supplement.

All the activities were assayed by a serial dilution technique (12). The antibacterial, antifun-

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Table 1. Biological anctivity (MIC) in μg/ml of the compounds

Compound	Antibacterial activity		Antifungal activity			Antitubercular activity
	a	b	c	d	e	f
H_2MHDDO	100	100	50	200	200	200
Cu(HMHDDO)$_2$	100	50	25	100	50	100
HMOHOH	100	25	50	200	100	200
Cu(MOHOH)$_2$	100	12.5	25	100	50	100
HMOHOP	100	25	50	200	200	200
Cu(MOHOP)$_2$	50	12.5	25	50	100	100

a. *S. typhi;* ***b.*** *S. aureus;* ***c.*** *C. albicans;* ***d.*** *A. niger;* ***e.*** *S. cerevisiae;* ***f.*** *m–tuberculosis ($H_{37}Rv$)*

gal activities of the complexes along with the ligands were tested at different concentrations against selected microorganisms by growth in liquid culture. Equal volumes of species inoculum were inoculated in tubes containing media, followed by the addition of different concentrations of complexes. Each complex was examined at five different concentrations 200, 100, 50, 25 and 12.5 μg/ml. For each concentration five replications were used with suitable control (without complex) and after inoculation, tubes were incubated at 37°C. The MIC was measured after 24 h in case of antibacterial activity, 48 h for antifungal activity and 10 days for the antitubercular activity. The solvent used was dimethyl formamide in all the cases. The standard used for antibacterial, antifungal and antitubercular activity was ampicillin, nystatin and ethambutol, respectively. The standard MIC was 0.01 μg/ml against *S. aureus* and 1 μg/ml for *S. typhi* in case of ampicilin, 1.56–12.5 μg/ml for *nystatin* and 0.1 μg/ml for ethambutol against the selected microorganisms.

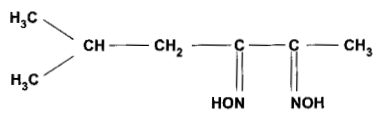

Figure 1. 5–methyl–2,3–hexanedionedioxime.

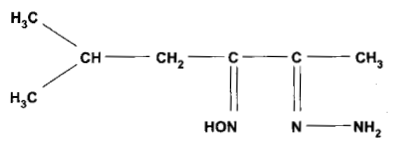

Figure 2. 3–hydroxyimino–5–methyl–2–hexanone hydrazone.

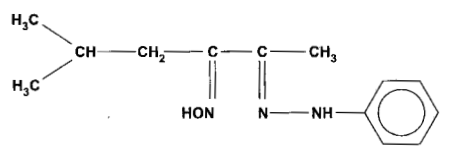

Figure 3. 3–hydroxyimino–5–methyl–2–hexanophenylhydrazone.

RESULTS AND DISCUSSION

It is clear from the bactericidal screening data (Table 1) that the complexes are more toxic in comparison with the ligands at each screening concentration. The trend of growth inhibiting was found to be in the order Cu(HMHDDO)$_2$ < Cu(MOHOH)$_2$ < Cu(MOHOP)$_2$. The efficiency of growth inhibition is in the order of the substituted functional group as hydroxylamine hydrochloride < hydrazine hydrate < phenylhydrazine moiety (Figures 1, 2 and 3). The presence of bulky substituents may be responsible for the enhancement of such biological activity. However, the hydrazine containing ligands show more activity in many cases (13–16).

All the Cu(II) complexes were more active, due to the faster diffusion of the complexes through cell membrane. Moreover, the faster diffusion of complexes may probably be due to the following reasons:

I. The smallest ionic radius of Cu(II) ion facilitates the faster diffusion.

II. High stability and the solubility of all complexes in lipid may cause faster diffusion (13).

Acknowledgements

The present authors wish to express their deep gratitude to Director of the Haffkine Institute of Research, Training and Testing, for providing antimicrobial study as well as Director of the Institute

of Science, Mumbai (India) for making research facilities available.

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Received: 31.01.2003