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REGULAR ARTICLE

ANTIFUNGAL EFFECT OF COPPER(II)-PHENANTHROLINE COMPLEX WITH 5-CHLOROSALICYLIC ACID

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ABSTRACT

Transition metal complexes represent a novel group of drugs with potential therapeutic applications. A considerable attention has been given to the copper-based complexes with different type of organic ligands that are of crucial importance since they can finely tune the metal toxicity. In view of these findings we synthesised a bifunctional copper (II) – phenantroline complex with 5-Chlorosalicylic acid with the aim to test its potential antifungal activity using yeast *Saccharomyces cerevisiae* as a model organism. The prepared copper complex $[Cu(phen)(5\text{-}Clsal)(5\text{-}ClsalH_2)]_2$ dissolved in DMSO was applied in two following concentrations 30 μM and 60 μM. Parallel with these experiments, two concentrations of free 1,10-phenanthroline in DMSO corresponding to the quantities of the phenanthroline bound in complex were tested as a positive control variant. The results confirmed the toxic effect of copper complex as well as phenanthroline itself on the yeast growth in concentration manner. The effect of copper complex was stronger compared to that of phenanthroline. To assess fungicide or fungistatic effect of the tested substances, the spot assay was performed using the liquid cell culture with the lowest value of OD_{600} after 24 hours of incubation. Our results showed the differences in cell survival. While the lower concentrations of tested compounds exhibited fungistatic effect on the yeast cell culture, the higher concentrations showed the fungicidal activity.

Keywords: 1,10 – phenanthroline, 5-Chlorosalicylic acid, antifungal activity, *Saccharomyces cerevisiae*

INTRODUCTION

With the advancement in the field of bioinorganic chemistry the role of transition metal complexes as therapeutic compounds is becoming increasingly important. The use of transition metal complexes as therapeutic compounds has become more and more pronounced. Metal-based drugs represent a novel group of antimicrobial agents with potential therapeutic applications. With the advent of fungal isolates manifesting resistance to azole and polyene drugs there has been a requirement for new drugs with alternative modes of action or with the ability to increase the efficacy of existing prescription drugs **(Eshwika et al, 2004)**. A wide range of metal complexes are already in clinical use, and many of new metallodrugs, such as metal-mediated antimicrobial agents, and anticancer compounds are under investigation. However, development of transition metal complexes as drugs is not an easy task; considerable effort is required to get compounds with predicted properties **(Rafique et al., 2010; Cerchiaro, Ferreira, 2006)**.

For scientific researchers working in the field of bioinorganic chemistry, the copper represents one of the most interesting biometals for the preparation of new metal-based drugs with strong potential for therapeutic applications **(Duncan, White, 2012)**. Copper is an essential micronutrient that, thanks to its ability to undergo Cu(I) to Cu(II) redox cycling, plays a role in cellular redox reactions **(Jomová, Valko, 2011)**. However, as a redox active metal, copper can induce ROS formation being thus potentially harmful to living cells. This property of copper is basis for development of new copper-based drugs endowed with antimicrobial and antitumor activity. Compared to other transitional metals copper as an essential metal may be less toxic than non-essential ones.

Majority of the currently used antifungal drugs are active against *S. cerevisiae*, thus making it a suitable model for both drug development and the elucidation of the mechanisms underlying drug's action. *Saccharomyces cerevisiae* represents a practical and conventional system for studying the properties of antifungal compounds, not only against fungal human pathogens with which they are closely related (e.g., *Candida albicans*), but also with those that are evolutionarily more distant (e.g., filamentous fungi) **(Almedia et al., 2008)**.

The aim of this study was to (i) assess a possible antifungal activity of prepared copper(II)-phenanthroline complex containing 5-Chlorosalicylic acid using yeast *Saccharomyces cerevisiae* as a model organism and (ii) to determine the difference between the effects of complex compound containing phenanthroline and unbound phenantroline on the yeast growth.

MATERIAL AND METHODS

Synthesis of copper complex

The copper complex was prepared similarly to previously described method (Ranford et al., 1993). The 5-Chlorosalicylic acid (1.0 mmol) was slowly added to an ethanol solution of copper(II) acetate (1.0 mmol) with 1,10-phenantroline (1.0 mmol) under stirring. The total volume of reaction mixture reached 50 ml and the resulting solution turned to green. The reaction mixture was stirred at ambient temperature. The precipitate (product) was filtered off under vacuum and dried at room temperature. The mother liquid was left to crystallize at ambient temperature and the crystal suitable for X-ray structure determination was separated and dried. The X-ray and EPR characterization of the complex was already briefly presented (Jomová et al., 2011).

Yeast strain and growth conditions

Yeast strain of *Saccharomyces cerevisiae* BY 4741 comes from EUROSCARF collection. Cells were grown in YPD medium containing 1 g of yeast extract (HiMedia, India), 2 g of peptone (Serva, Germany) and 2 g of glucose (Lachema, Czech Republic) per 100 mL of distilled water. For a solid medium, 2 g of agar (Serva, Germany) were added. Single colony of yeast cell was inoculated in 10 mL of YPD medium and incubated at 30 ºC for 16 h under continual shaking at 150 rpm. The cells from overnight pre-culture were transferred to 50 mL of fresh YPD media, so that the culture reached the optical density value OD_{600} corresponding to circa 2.10^8 cells/mL. The culture served as inoculum for all tests done.

Determination of antifungal activity

The antifungal activity of copper complex and unbound phenanthroline was tested on yeast cells inoculated in liquid or solid medium. Both substances, the copper complex and phenanthroline were separately dissolved in DMSO and added to the prepared inoculated liquid media to final concentrations 30 mM, 60 mM and 31 mM, 61 mM, respectively. Cells were incubated with shaking at 30 ºC and the cell growth was assessed by measuring of optical density of the culture at 600 nm at regular time intervals within 24 hours of cultivation. Each measurement was done in triplicate and data represent means values. To determine the fungicide or fungistatic effect of tested compounds, 5 µL of the yeast cell culture with the lowest value of OD_{600} after 24 hours of incubation was spotted onto YPD agar plates. The growth was allowed for 48 hours at 30 ºC.

RESULTS AND DISCUSSION

In this study we prepared the Cu(II) complex compound containing 1,10 - phenanthroline and 5-Chlorosalicylic acid with the aim to investigate its possible antifungal effect. The scientific confirmation of the biological activity of phenanthroline and metal – phenanthroline complexes, which represent novel highly active anti-fungal agents, was described by **Coyle et al. (2004)**. **Kumar et al. (2008).** These authors reported that the complexes have also been widely utilized as foot printing agents of both proteins and DNA, probes of the dimensions of the minor groove of duplex structures, and identifiers of transcription start sites. Based on these arguments, different copper compounds containing diverse ligands have been prepared and studied as potential therapeutic agents.

The prepared green crystal of copper complex was studied by means of the X-ray spectroscopy which revealed a dimeric structure of the compound $[Cu(phen)(5\text{-Clsal})(5\text{-ClsalH}_2)]_2$ **(Jomová et al., 2011)** (Fig 1). It has been found that binuclear copper (II) complex containing 1,10 – phenanthroline bind strongly with DNA and cleave it more effectively than their corresponding monomeric complexes **Kumar et al. (2008)**.

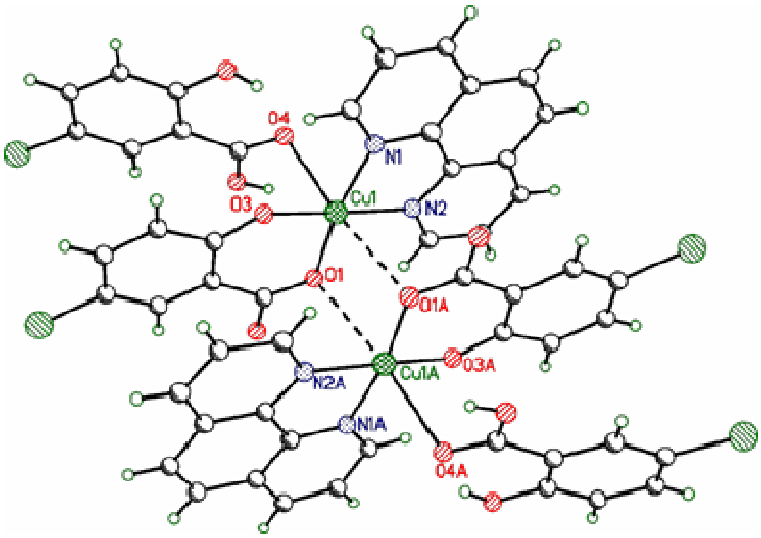


Figure 1 Structure of complex [Cu(phen)(5-Clsal)(5-ClsalH$_2$)]$_2$ **(Jomová et al., 2011)**

Our results showed that growth of *S. cerevisiae* in media supplemented with both used concentrations of [Cu(phen)(5-Clsal)(5-ClsalH$_2$)]$_2$ was inhibited in concentration manner indicating an antifungal activity of the Cu(II) complex (Fig 2). However, similar results were observed in parallel experiments with phenanthroline as a positive control variant.

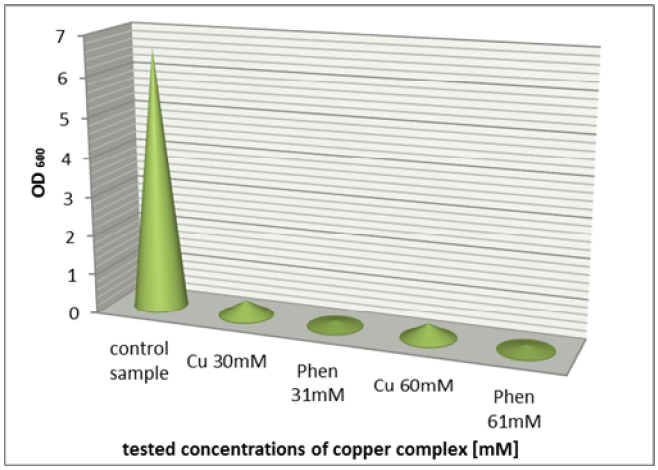
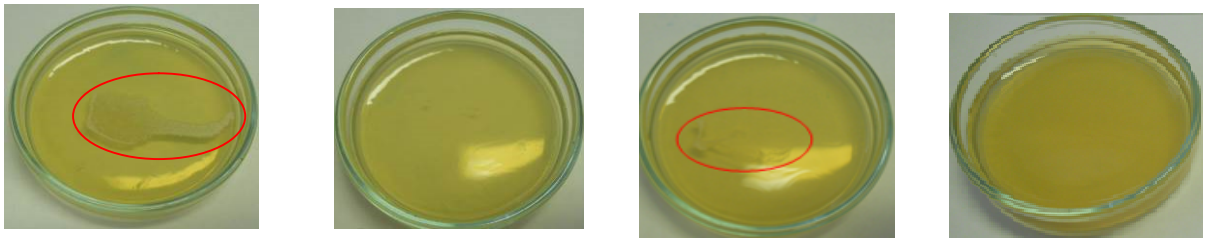


Figure 2 The growth of yeast culture after 24 hours cultivation in the presence of two different concentrations of copper complex and two different concentrations of phenanthroline solutions

Tests for fungistatic / fungicidal activity of the used compounds performed on the YPD agar plates were evaluated by the presence of colonies. The results suggest fungicidal effect of the higher concentrations of both substances on the growth of *S. cerevisiae* (Fig 3).



a) 30 mM Cu complex b) 60 mM Cu-complex c) 31 mM Phen d) 61 mM Phen

Figura 3 YPD agar plates inoculated by the yeast culture with inhibited growth

It is scientifically explored that 1,10-phenanthroline and metal–phenanthroline complexes display fungicidal and fungistatic activity, disrupt mitochondrial function and induce oxidative stress. The effect of these drugs on the structure of yeast and mammalian cell organelles and the integrity of cellular DNA was examined by **Coyle et al. (2004)**. All drugs induced extensive changes to the internal structure of yeast cells such as retraction of the cytoplasm, nuclear fragmentation and disruption of the mitochondrion. Moreover, phenanthroline and metal–phenanthroline complexes have the potential to induce apoptosis in fungal and mammalian cells.

CONCLUSION

Our preliminary results indicate an antifungal activity of prepared copper complex compound $[Cu(phen)(5\text{-}Clsal)(5\text{-}ClsalH_2)]_2$. Though the effect of free 1,10 – phenanthroline on the yeast growth was slightly stronger, copper- phenanthroline complexes may represent a novel group of antifungal agents which, in combination with other ligands, may provide the drugs with desired properties. Given their distinct mode of action compared to conventional anti-fungal drugs their use as therapeutic agents appears to be promising.

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REFERENCES

ALMEIDA, B. – SILVA, A. – MESTIQUITA, A. – MARQUES, B. – RODRIQUES, F. – LUDOVICO, P. 2008. Drug – induced apoptosis in yeast. In *Biochimica et biophysica acta*, p. 1436 – 1448.

AVERY, S. V. – HOWLETT, N. G. – RADICE, S. 1996. Copper Toxicity towards *Saccharomyces cerevisiae*: Dependence on Plasma Membrane Fatty Acid Composition. In *Applied and environmental microbiology,* vol. 62,1996, no. 11, p. 3960 – 3966

CERCHIARO, G. – FERREIRA, A. M. 2006. Oxindoles and copper complexes with oxindole – derivatives as potential pharmacological agents, In *Journal of the Brazilian Chemical Society*, vol. 17, no. 8, p. 1473 – 1485.

COYLE, B. - KINSELLA, P. - MCCANN, M. - DEVEREUX, M. - O´CONNOR, R. - CLYNES, M. - KAVANGH, K. 2004. Induction of apoptosis in yeast and mammalian cells by exposure to 1,10 phenanthroline metal complexes. In *Toxicology in Vitro*, vol 18, p. 63 – 70.

DUNCAN, C. – WHITE, A. R. 2012. Copper complexes as therapeutic agents, In *Metallomics*, vol. 4, p. 127 – 138.

ESHWIKA, A. - COYLE, B. - DEVEREUX, M. - MCCANN, M. - KAVANAGH, K. 2004. Metal complexes of 1,10-phenanthroline-5,6-dione alter the susceptibility of the yeast *Candida albicans* to Amphotericin B and Miconazole. In *BioMetals,* vol. 17, p. 415 – 422.

JOMOVÁ, K. - ČONGRÁDYOVÁ, A. – MONCOĽ, J. – LAWSON, M. – VALKO, M. 2011. Copper(II)-phenantroline complexes with derivates of salicylic acid. (Structure and EPR spectroscopy)., In *XXIII. International Conference on Coordination and Bioinorganic Chemistry : New Trends in Coordination, Bioinorganic, and Applied Inorganic Chemistry,* p. 34 – 40.

JOMOVÁ, K. – VALKO, M. 2011. Advances in metal-induced oxidative stress and human disease. In *Toxicology,* vol. 283, p. 65-87.

KUMAR, R. S. – ARUNACHALAM, S. – PERIASAMY, V. S. – PREETHY, C. P. – RIYASDEEN, A. – AKBARSHA, M. A. 2008. DNA binding and biological studies of some novel water – soluble polymer – copper (II) – phenanthroline complexes, In *European journal of medicinal chemistry*, vol.43, p. 2082 – 2091.

RAFIQUE, S. – IDREES, M. – NASIM, A. – AKBAR, H. – ATHAR, A. 2010. Transition metal complexes as potential therapeutic agents, In *Biotechnology and molecular biology reviews,* vol. 5, no. 2, p. 38 – 45.