6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
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Improvement of antifungal activity and therapeutic profile of fluconazole by its complexation with copper(II) and zinc(II) ions. Complex characterization and antimicrobial activity studies

Nevena Lj. Stevanović[1,*], Ivana Aleksic[2], Jakob Kljun[3], Darko P. Ašanin[4], Tina P. Andrejević[1], Jasmina Nikodinovic-Runic[2], Iztok Turel[3], Miloš I. Djuran[5] and Biljana Đ. Glišić[1]

[1] University of Kragujevac, Faculty of Science, Department of Chemistry, R. Domanovića 12, 34000 Kragujevac, Serbia;
[2] University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, 11042 Belgrade, Serbia;
[3] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Department of Chemistry and Biochemistry, Večna pot 113, SI-1000, Ljubljana, Slovenia;
[4] University of Kragujevac, Institute for Information Technologies Kragujevac, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia;
[5] Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000 Belgrade, Serbia.
Corresponding author: nevena.stevanovic@pmf.kg.ac.rs

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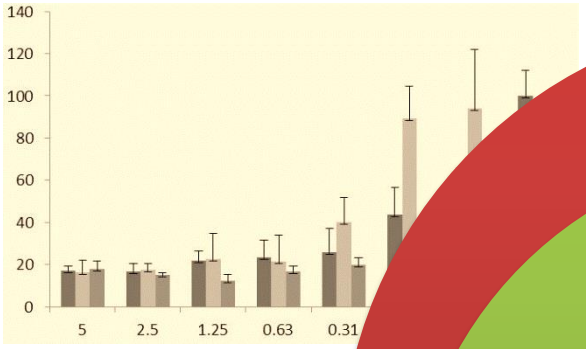
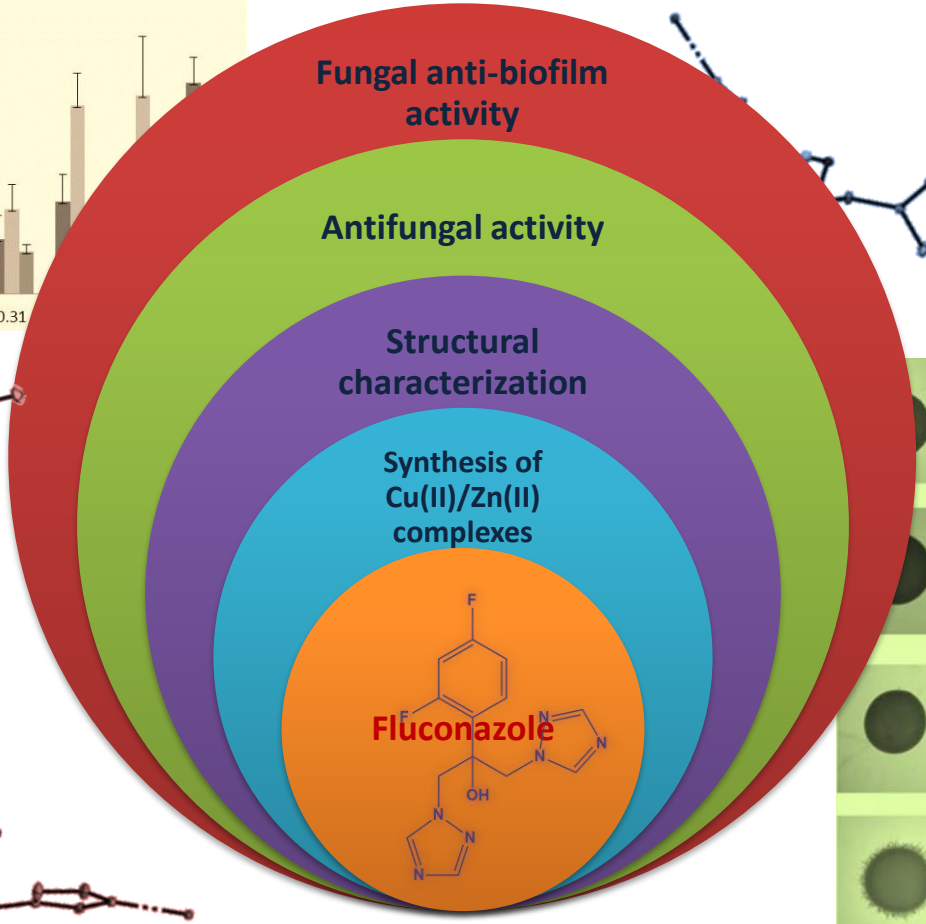
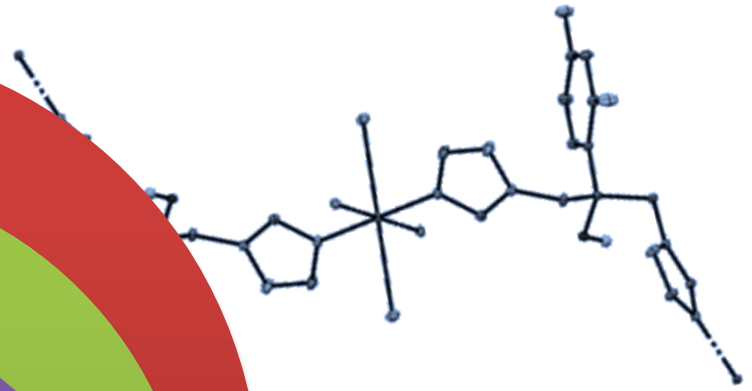
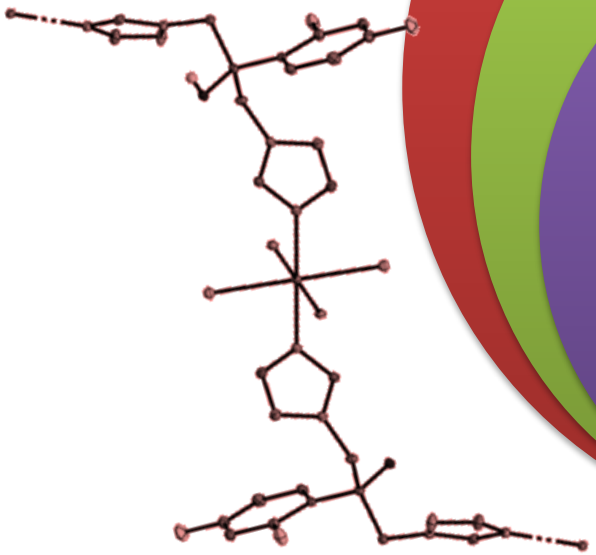
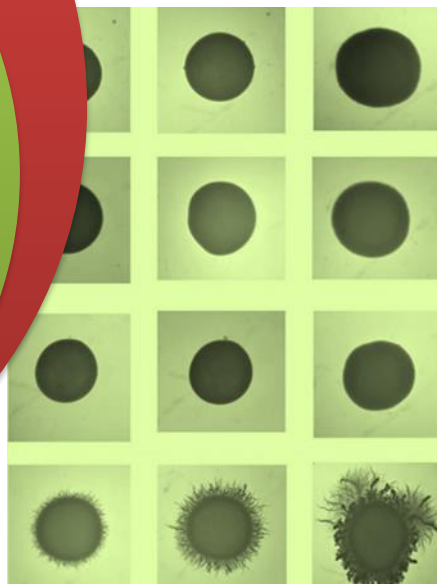








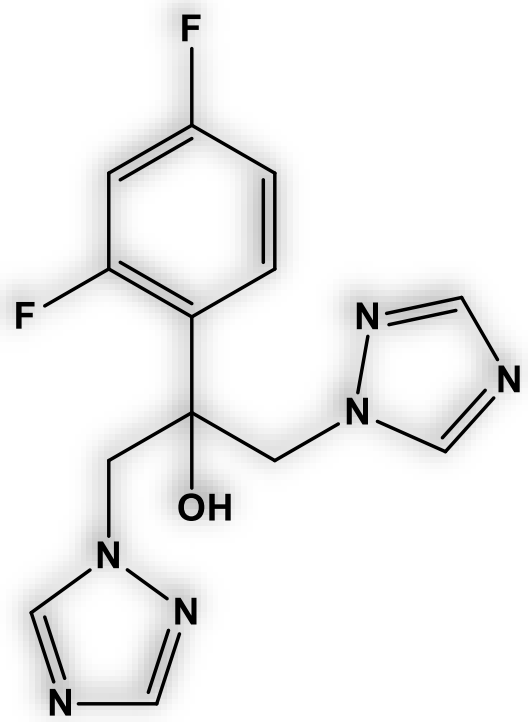


Abstract: In order to overcome resistance of the clinically used antifungal triazole agents, we synthesized copper(II) and zinc(II) complexes with fluconazole (flz), $\{[CuCl_2(flz)_2]\cdot 5H_2O\}_n$ (**1**) and $\{[ZnCl_2(flz)_2]\cdot 2C_2H_5OH\}_n$ (**2**). These complexes were obtained from the reactions between $CuCl_2\cdot 2H_2O$ or $ZnCl_2$ with this antifungal agent in 1 : 2 molar ratio in ethanol at room temperature. The compounds were characterized by elemental analysis, NMR, IR and UV-Vis spectroscopy and mass spectrometry. The crystal structure of complexes was determined by a single-crystal X-ray diffraction analysis. The antimicrobial effect of both complexes and fluconazole was evaluated against different *Candida* species, as well as Gram-positive and Gram-negative bacteria by means of minimal inhibitory concentrations (MICs). The obtained results have shown that, in most cases, the coordination of fluconazole to Zn(II) and Cu(II) ions leads to the enhancement of its antifungal activity. Both complexes showed strong inhibitory activity against *C. albicans* biofilm formation at concentrations lower than MIC values, as well as strong inhibition of *C. albicans* filamentation.

Keywords: Zinc(II) complex; Copper(II) complex; Fluconazole; Antifungal agents; Biofilms

Introduction

- Invasive fungal infections represent a serious problem for modern-day healthcare
- Therapeutic options for the treatment of fungal infections are presently limited to only four classes of compounds
- Each of these drug classes has significant therapeutic limitations, including serious toxic-side effects, resistance development and limited routes of administration
- Fluconazole (flz) belongs to the first-generation azoles and is developed for the treatment of *Candida* infections



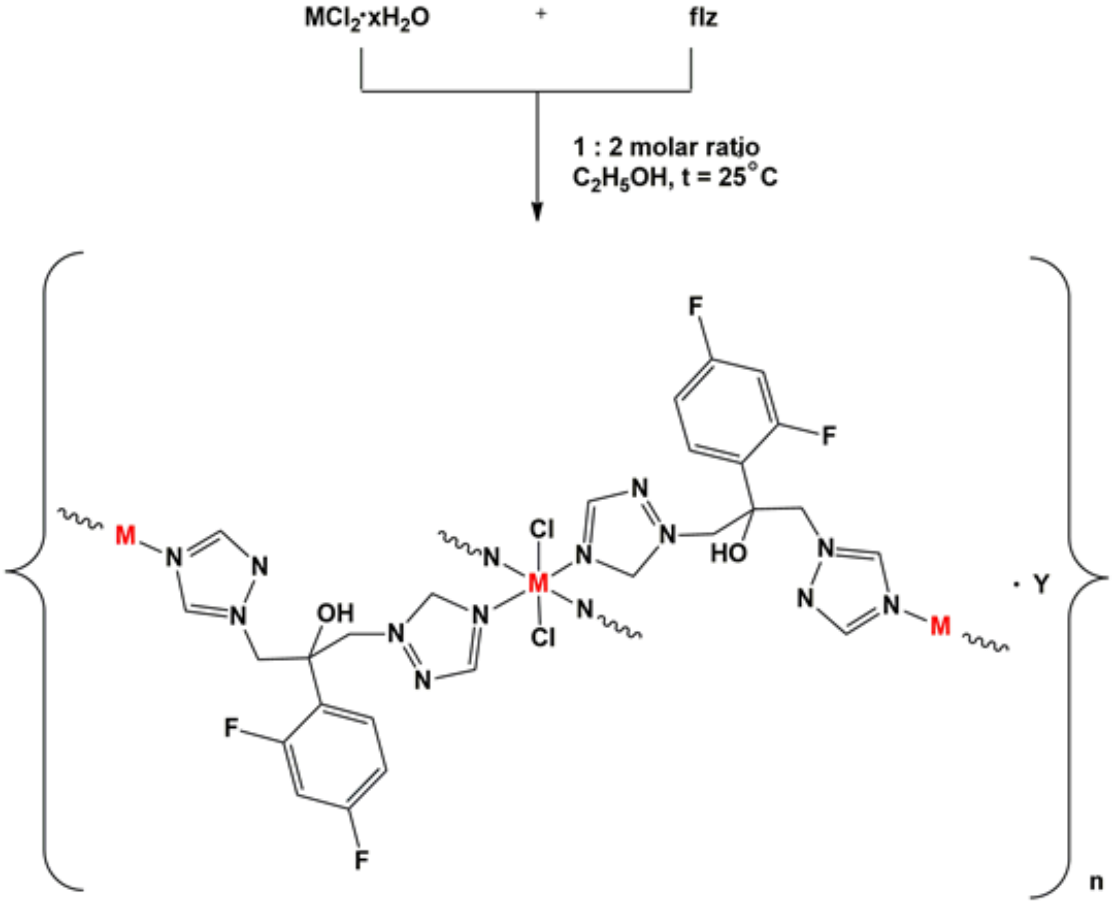
fluconazole (flz)

M.K. Kathiravan et al., Bioorg. Med. Chem. 20 (2012) 5678.

Results and discussion

Synthesis of metal complexes

Reaction between $CuCl_2 \cdot 2H_2O$ or $ZnCl_2$ with flz was perfomed in 1 : 2 molar ratio, respectively, in ethanol at room temperature



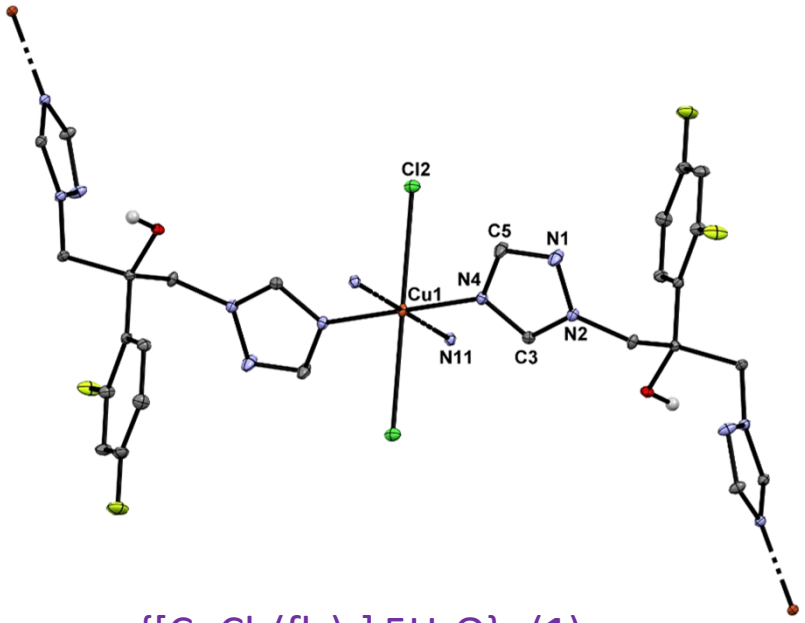
1 and 2

complex 1: M = Cu(II), x = 2, y = $5H_2O$

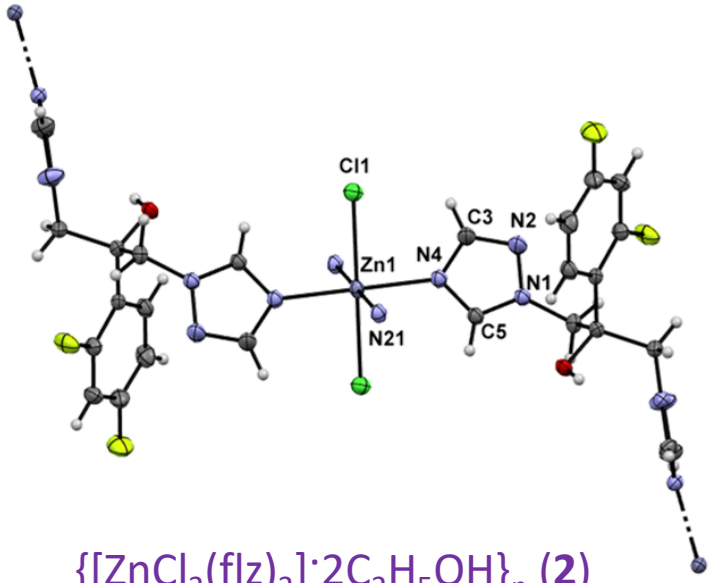
complex 2: M = Zn(II), x = 0, y = $2C_2H_5OH$

- ✓ The crystals of the complex **1** were obtained after the blue precipitate from the reaction was recrystallized in the mixture of acetonitrile/water, while those of **2** were obtained after evaporation of the mother solution

- ✓ The structure of the complexes was confirmed by mass spectrometry, IR and UV-Vis spectroscopy and single-crystal X-ray diffraction analysis, while the complex **2** was additionally characterized by ^{1}H and ^{19}F NMR spectroscopy

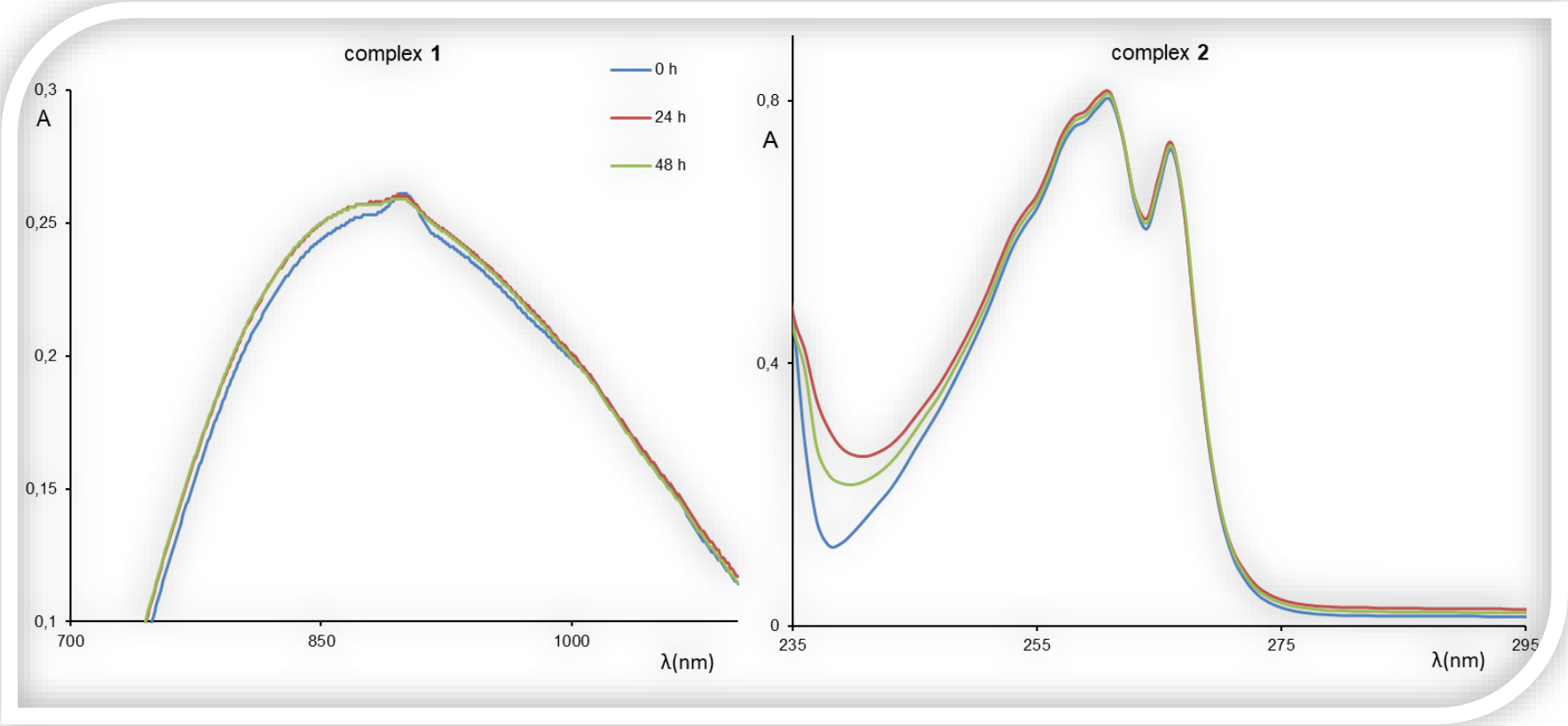


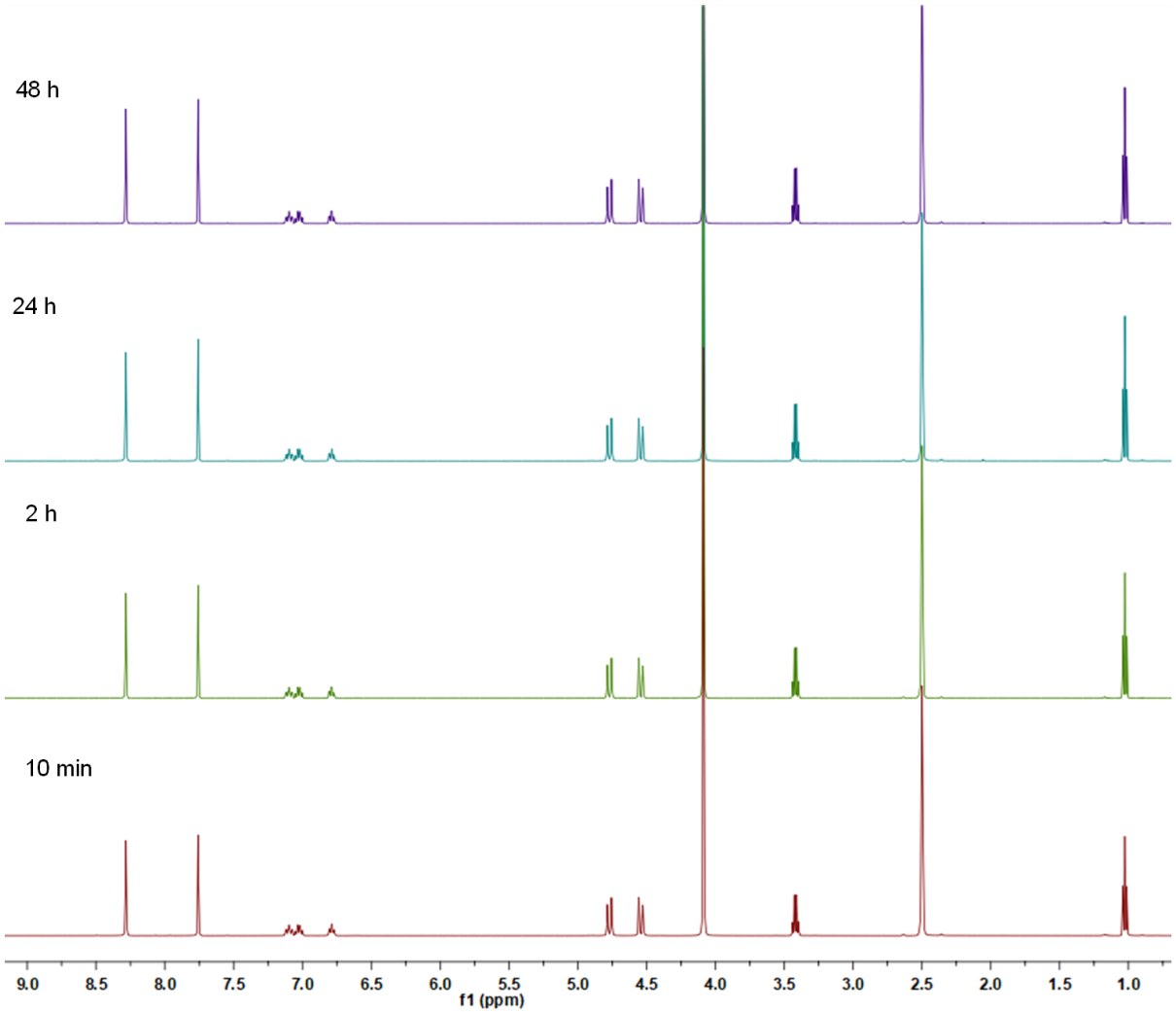
$\{[CuCl_2(flz)_2]\cdot 5H_2O\}_n$ (**1**)



$\{[ZnCl_2(flz)_2]\cdot 2C_2H_5OH\}_n$ (**2**)

✓ The intensity and the position of the absorption maxima of **1** and **2** and the shape of spectra remained unmodified during the investigated time, being in accordance with the stability of these complexes in solution





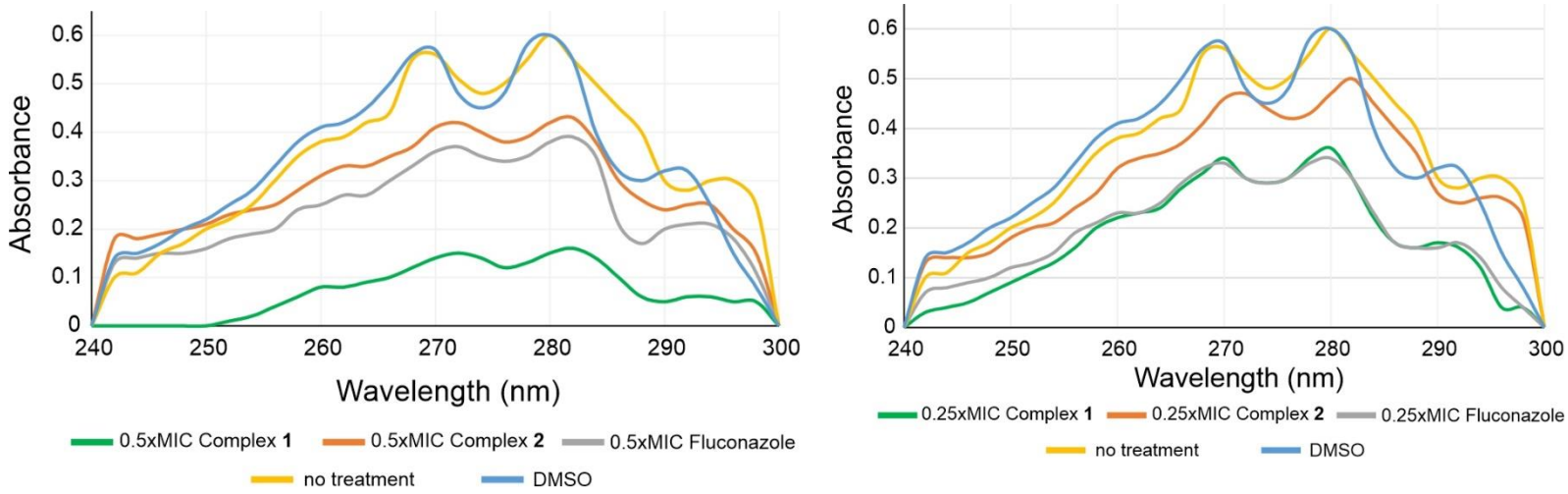
Stability of complex **2** in DMSO-d_6/D_2O (*v/v* 3:1) over a period of 48 h followed by ^{1}H NMR spectroscopy

Antifungal (MIC, µg/mL) *vs* cytotoxicity (LC_{50} values, µg/mL)

Test organism: / Complex ligand	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019	*C. krusei* ATCC 6258	*C. albicans* RFP	*C. albicans* GFP	*C. albicans* 1C	*C. albicans* 1F	*C. albicans* 11	*C. albicans 13*	*MRC-5*
Fluconazole (flz)	2.87	5.72	40.1	0.25	0.25	6.53	6.53	6.53	6.53	980
Cu(II) complex (1)	3.71	1.05	3.72	0.19	0.19	2.38	2.38	2.38	2.38	77.3
Zn(II) complex (2)	66.9	2.34	66.9	0.21	0.21	2.68	2.68	2.68	2.68	96.4

- ✓ The coordination of fluconazole to Zn(II) and Cu(II) ions leads to the enhancement of its antifungal activity
- ✓ Selectivity indices > 400 in the case of *C. albicans* RFP and GFP

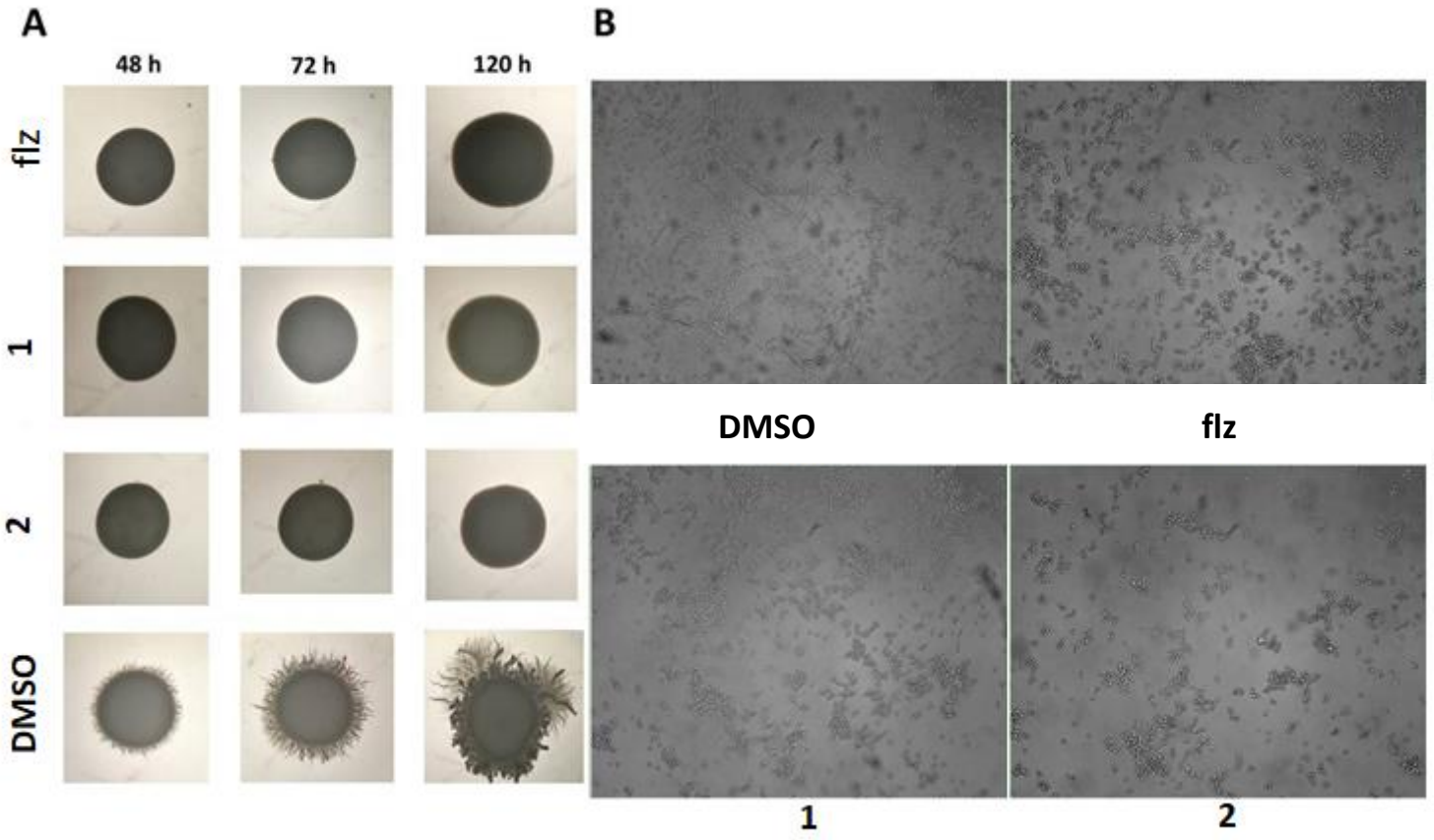
UV spectrophotometric ergosterol profiles of *C. albicans* treated with subinhibitory concentrations of fluconazole and complexes 1 and 2



- ✓ Fluconazole and the corresponding complexes **1** and **2** reduced the total amount of ergosterol at subinhibitory concentrations, with copper(II) complex **1** being the most potent
- ✓ The general mode of the activity of fluconazole has been retained within the complexes, while the presence of Cu(II) ion might add some additional inhibitory activity

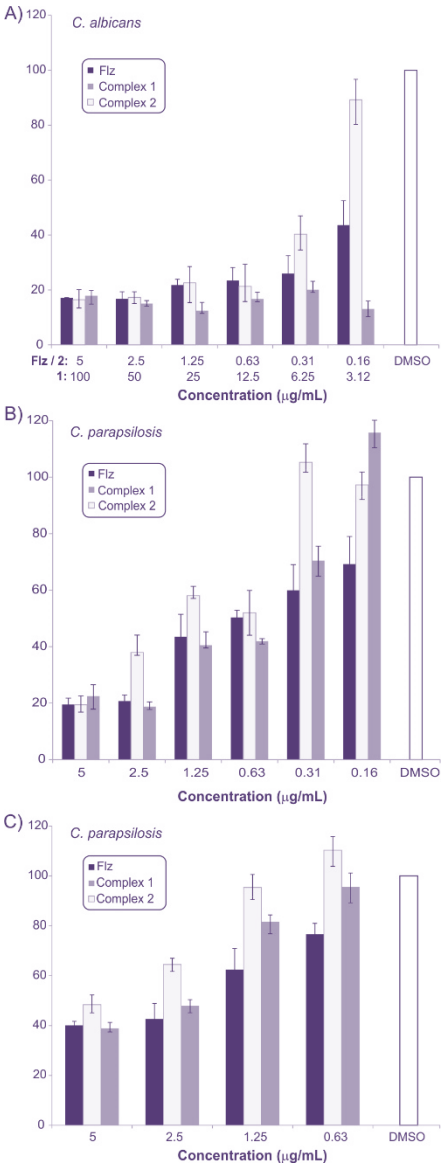
B.A. Arthington-Skaggs et al., J. Clin. Microbiol. 37 (1999) 3332.

Inhibition of *C. albicans* filamentation (Spider solid and RPMI medium)



- ✓ Strong inhibition of filamentation of *C. albicans* ATCC 10231 was observed in the presence of subinhibitory (0.5 x MIC value) concentrations of fluconazole and complexes **1** and **2**
  A) on the Spider medium and
  B) in RPMI broth

Effect of tested compounds on destruction of pre-formed biofilms



Effect of fluconazole and complexes **1** and **2** on Candida biofilms.
A) *C. albicans* biofilm formation;
B) *C. parapsilosis* biofilm formation and
C) *C. parapsilosis* biofilm destruction

✓ Activity is detected against pre-formed *C. parapsilosis* biofilms

Conclusions

- Copper(II) and zinc(II) complexes with fluconazole (flz), $\{[CuCl_2(flz)_2]\cdot 5H_2O\}_n$ **(1)** and $\{[ZnCl_2(flz)_2]\cdot 2C_2H_5OH\}_n$ (**2**) were synthesized and structurally characterized

- Both complexes have polymeric structure in the solid state, with four flz molecules monodentately coordinated to the metal center *via* the triazole nitrogen atom and two chlorido ligands

- In most cases, complexes **1** and **2** possessed higher antifungal activity than fluconazole itself, being 3-fold more active against the clinical isolates of *Candida albicans*

- The general mode of the activity of fluconazole has been retained within the complexes, while the presence of Cu(II) ion might add some additional inhibitory activity

- Both complexes showed strong inhibition of *C. albicans* biofilms formation and filamentation of this fungi at subinhibitory concentrations, what is highly desirable property of a novel antifungal agent

Acknowledgments

This research has been financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreements No. 451-03-68/2020-14/200042, 451-03-68/2020-14/200122 and 451-03-68/2020-14/200378) and by the Slovenian Research Agency (grant P1-0175). The EN→FIST Centre of Excellence, Trg OF 13, SI-1000 Ljubljana, Slovenia, is acknowledged for the use of the SuperNova diffractometer. This research has also received funding from the Serbian Academy of Sciences and Arts under strategic projects programme - grant agreement No. 01-2019-F65 and project of this institution No. F128.

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