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Institutul de Chimie Macromoleculara
"PETRU PONI"

Centrul de Cercetări Avansate pentru
Bionanoconjugate și Biopolimeri
Intelcentru

Synthesis and *in vitro/in vivo* assessment of conjugates with antifungal activity

PhD Thesis Summary

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Iași

2015

ROMANIAN ACADEMY

"PETRU PONI" INSTITUTE OF MACROMOLECULAR CHEMISTRY

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Nr. 5600 / 3 XII 2015

To _____

This is to inform you that on December 18th, 2015, at 11:00, in the library of the "Petru Poni" Institute of Macromolecular Chemistry of Iasi **Mr. Bogdan Minea** will publicly defend the thesis entitled „**Synthesis and *in vitro/in vivo* assessment of conjugates with antifungal activity**" in order to be awarded the scientific degree of PhD.

The Doctoral Committee will consist of:

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SCIENTIFIC SUPERVISOR	**Dr. Mariana Pinteală, senior researcher**, "Petru Poni" Institute of Macromolecular Chemistry of Iasi
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According to the Regulations of the Romanian Academy regarding the organisation of doctoral studies and thesis defence, please find enclosed a summary of the PhD thesis. Your comments and appreciations will be highly appreciated.

You are kindly invited to attend the public presentation of the PhD thesis.

DIRECTOR,

Dr. Anton Airinei

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Acknowledgements

While conducting my scientific research and writing this doctoral thesis, I received the help and support of many people and institutions. Therefore, I would like to take the opportunity to express due thanks and gratitude.

My deepest appreciation and sincere gratitude go to my doctoral supervisor, Dr. Mariana Pinteală for her great and sustained effort to provide the best conditions for scientific research and professional development. I would also like to thank Dr. Pinteală for all the late evenings spent in her office proofing my manuscripts and this thesis.

I would like to thank to the group from the Laboratory of Antimicrobial Chemotherapy ("Ion Ionescu de la Brad" University, Iasi, Romania) for their invaluable help. I would especially like to express my high regard and deep appreciation towards Dr. Mihai Mareș, Dr. Valentin Năstasă, Dr. Andra Bostănaru and Mrs. Nina Davidescu. I also thank Dr. Ramona Moraru.

I am especially grateful to my colleagues from the Institute of Macromolecular Chemistry „Petru Poni" of Iași and the Center of Advanced Research in Bionanoconjugates and Biopolymers, for their support and friendship. Special thanks to Dr. Narcisa Marangoci, Dr. Irina Roșca, Drd. Alina Nicolescu, Dr. Dragoș Peptanariu, Dr. Adrian Fifere, Dr. Cristian Varganici, Dr. Rodinel Ardeleanu, Dr. Mihaela Silion and Dr. Mirela Zaltariov for all their help and collaboration.

I would also like to acknowledge the help of Dr. Andrei Neamțu and Dr. Andreea Corciovă from "Gr. T. Popa" University of Medicine and Pharmacy of Iasi in performing computational and dissolution studies.

I thank the Romanian Academy for the financial support granted throughout my doctoral studies.

I thank the Ministry of National Education of Romania - CNCSIS-UEFISCDI for financial support through the PN-II-ID-PCCE-2011-2-0028 project - Biologically inspired systems for engineered structural and functional entities.

I also thank "Gr. T. Popa" University of Medicine and Pharmacy of Iasi for their financial support through the project "Programme of excellency in the multidisciplinary doctoral and post-doctoral research of chronic diseases", contract no. POSDRU/159/1.5/S/133377, beneficiary "Gr. T. Popa" University of Medicine and Pharmacy of Iasi, project co-financed from the European Social Fund through the Sectoral Operational Programme Human Resources Development (SOP HRD) 2007-2013.

Last but not least, I would like to thank my family for their unconditional love and support.

CONTENTS

List of abbreviations

^{1}H-NMR	nuclear magnetic resonance spectroscopy
BP	breakpoint
BSI	bloodstream infections
CD	cyclodextrin
CFU	colony forming unit (microbial cell)
CI	confidence interval
DEEP	deep-seated infections
DMEM	Dulbecco's modified Eagle's medium (cell culture medium)
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DSC	differential scanning calorimetry
EUCAST	the European Committee on Antimicrobial Susceptibility Testing
FLC	fluconazole
GYPA	glucose + yeast extract + mycological peptone + agar (microbiological culture medium)
HCCA	alpha-cyano-4-hydroxycinnamic acid
HIV	Human Immunodeficiency Virus
i.p.	intraperitoneal administration route
ICU	intensive care unit
ITS	internal transcribed spacer
LD_{90}	lethal dose 90%
LSU	large subunit of the ribosomal DNA
MALDI-TOF MS	matrix-assisted laser desorption/ionization time of flight mass spectrometry
MCT-β-CD	monochlorotriazinyl-β-cyclodextrin
MIC	minimal inhibitory concentration
MSP	main mass spectra
p.o.	*per os* (oral administration route)
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PCZH-NO3	protonated propiconazole nitrate
ROESY	2D rotating frame Overhauser effect NMR spectroscopy
RPMI	Roswell Park Memorial Institute medium (microbiological and cell culture medium)
SBE7-β-CD	Sulfobutylether-β-cyclodextrin
spp.	*species pluralis*
SUP	superficial infections
VOR	voriconazole
YPD	Yeast Peptone Dextrose (microbiological culture medium)
β-CD	β-cyclodextrin
β-CD-SNa	β-cyclodextrin sulfated sodium salt

Introduction

Fungal infections are an important health issue fuelled, paradoxically, by the advancements in medical care. The great majority of clinically relevant fungi are opportunistic pathogens, that is, they are part of the normal human microbiota, but they can cause infections if the defence mechanisms of the host become in some way impaired. That is the case with patients that receive immunosuppressive therapy, broad spectrum antibiotics, chemotherapy, patients with diabetes or HIV infection, etc. [1].

The fourth source of bloodstream infections as prevalence, *Candida* yeasts are the most frequent fungal pathogen in humans [2], with *C. albicans* as the dominant species. The prophylactic administration of antifungals to an increasing population of patients generates antifungal resistance by creating a selective pressure, which favours those non-*albicans Candida* species that are naturally less susceptible to the drugs, such as *C. glabrata*, or strains with acquired resistance-inducing mutations [3,4]. The increasing rates of resistance underline the need for new antifungal agents.

Azoles, diazoles (imidazoles) and triazoles, are the largest and most widely used class of antifungal agents. Azoles inhibit ergosterol biosynthesis by blocking a key enzyme, lanosterol-14α-demethylase. The lack of ergosterol primarily damages the plasma membrane and also, indirectly, the cell wall of fungal cells [5–8].

Most clinically used azoles (with fluconazole as the sole exception) have the major inconvenient of poor water solubility, which severely reduces their bioavailability. To overcome this problem, various methods were proposed, including the formation of host-guest inclusion complexes with cyclodextrins [9–11].

In this context, this doctoral thesis investigated protonated propiconazole nitrate (PCZH-NO_3) as a new triazolic antifungal agent against pathogenic yeasts and how its biological properties are influenced by the complexation with cyclodextrins. The present study is a continuation and a development of previous research that was part of another doctoral thesis published in our group by Dr. Narcisa Marangoci [12]. The work of Dr. Marangoci presented the obtaining of PCZH-NO_3 by nitration of propiconazole, the preparation and characterisation of an inclusion complex of PCZH-NO_3 with β-cyclodextrin (β-CD) and a brief preliminary evaluation of its antifungal effect. The current thesis investigates in detail the biological properties of the β-CD inclusion complex with *in vitro* and

in vivo studies. The preparation and characterisation of three new inclusion complexes and their *in vitro* biological properties are also presented.

Thesis outline

The thesis is composed of two main parts. The first part resumes scientific literature data regarding the chemical structure of azoles, the mechanism of their antifungal effect and their spectrum of activity. Information about the chemical structure of cyclodextrins, the complexation phenomenon and its influence on the biological properties of drugs is also summarised.

The second part presents the original contributions and consists of four chapters (2-5). Chapter 2 contains an extensive analysis of the *in vitro* antifungal activity of the β-CD/PCZH-NO_3 inclusion complex and a comparison with commercial azole formulations. Chapter 3 investigates the *in vivo* therapeutic efficiency of the β-CD/PCZH-NO_3 complex on a murine model of invasive candidosis. Chapter 4 describes the preparation and characterisation of three new inclusion complexes of PCZH-NO_3 with β-CD derivatives. It also includes a brief *in silico* assessment. Chapter 5 presents the *in vitro* biological properties of the inclusion complexes described in chapter 4. Chapters 2-5, each, contain a **Materials and methods** and a **References** section relevant to their content. The thesis ends with a general conclusions chapter.

Chapter 2

As part of the first study covering Romania with regard to species distribution and the azole susceptibility pattern of fungal clinical isolates [13], the β-CD/PCZH-NO_3 conjugate was tested against a collection of 551 clinical yeast isolates received from hospitals throughout the country. The conjugate was tested alongside fluconazole (FLC) and voriconazole (VOR), two commercial azoles with extensive clinical use. The commercial formulation of VOR, where it is complexed with sulfobutylether-β-CD (SBE7-β-CD/VOR), was used during the tests. Antifungal testing was performed by following the European EUCAST EDef 7.1 guidelines [14]. The interpretation of the MIC values was done according to the EUCAST breakpoints whenever they were available [15]. The most frequently isolated *Candida* species were *C. albicans*, *C. parapsilosis, C. glabrata*, *C. tropicalis*, *C. krusei*, *C. kefyr* and *C. lusitaniae*. The most frequently occurring non-*Candida* spp. isolates were of *Saccharomyces cerevisiae*.

MIC distribution

The standardised European testing method recommended by EUCAST revealed some common features of the commercial drugs and our experimental complex, namely the high susceptibility of *Candida albicans* and the reduced susceptibility of many non-*Candida* and non-*albicans Candida* species (Figures 1 and 2).

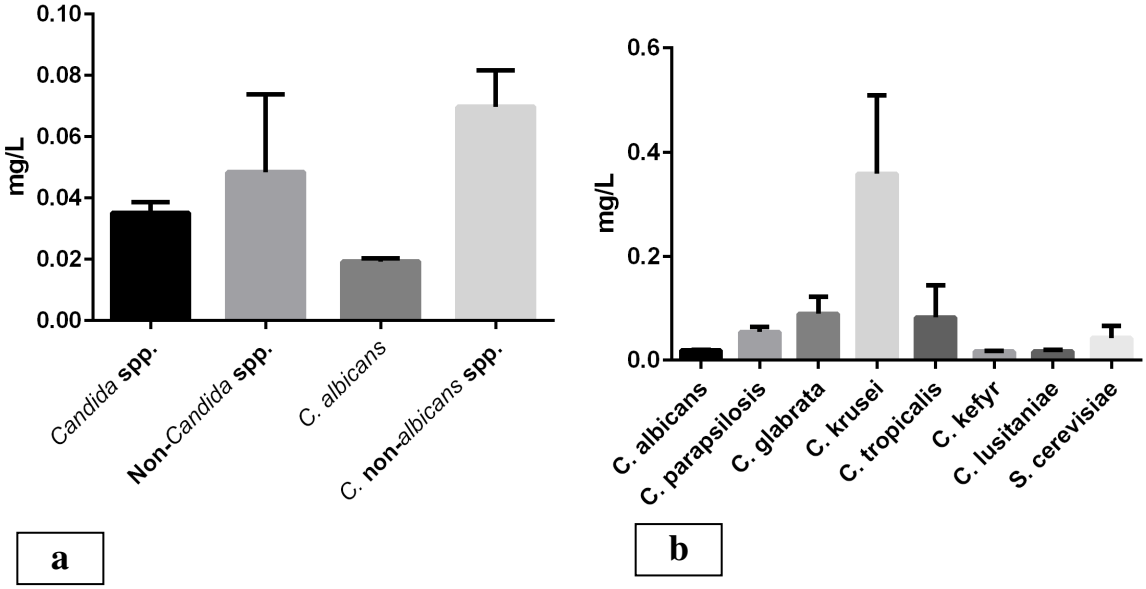

Figure 1 The antifungal activity of β-CD/PCZH-NO_3. Geometric means of the MICs with 95% CIs against **[a]** clinically relevant groups and **[b]** individual species.

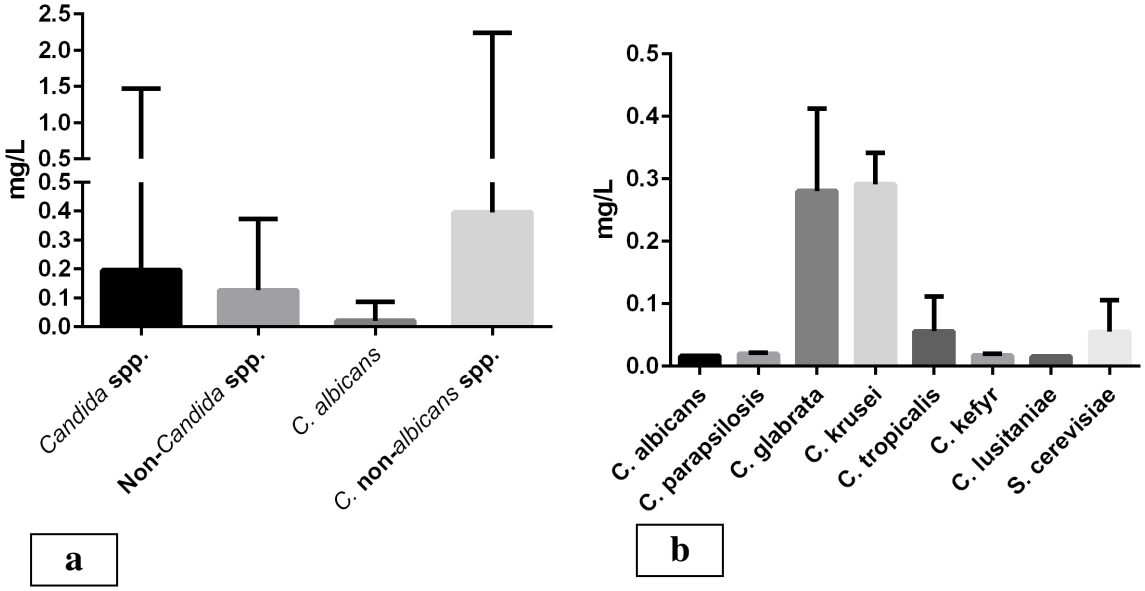

Figure 2 The antifungal activity of SBE7-β-CD/VOR. Geometric means of the MICs with 95% CIs against **[a]** clinically relevant groups and **[b]** individual species.

β-CD/PCZH-NO₃ vs. SBE7-β-CD/VOR

The antifungal activity of our complex was generally similar to that of the VOR complex, a commercial formulation with extensive clinical use (Figure 8). The absolute values of the β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences were small (Figure 8a), which suggests that PCZH-NO_3 could be therapeutically effective at low doses, similar to voriconazole. Approximately 60% of the β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences of log2 dilutions (Figure 8b) were zero, which means that the antifungal activity of β-CD/PCZH-NO_3 was identical to that of SBE7-β-CD/VOR for approximately 60% of the tested isolates.

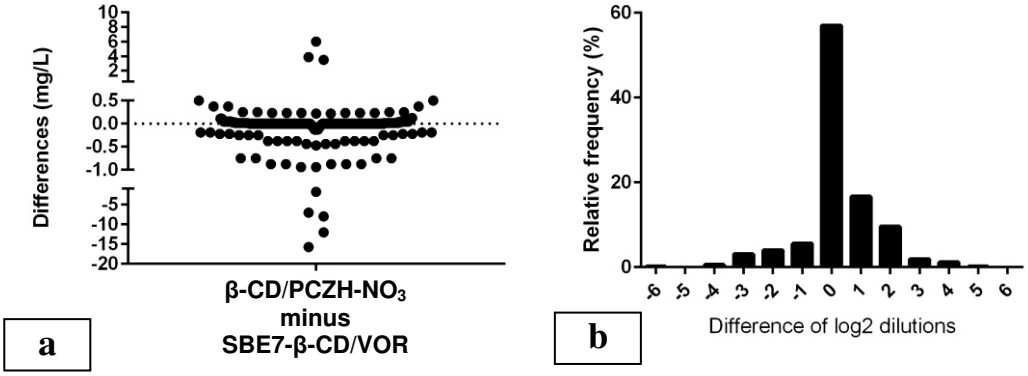

Figure 8. β-CD/PCZH-NO_3 vs. SBE7-β-CD/VOR – antifungal activity against the *Candida* spp. group: **[a]** absolute values of β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences (mg/L); **[b]** β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences of log2 dilutions.

The experimental compound was particularly active and superior to the VOR complex against *C. glabrata* isolates ($P < 0.0001$, Fig. 14), which are known to often have a reduced susceptibility to azoles [16].

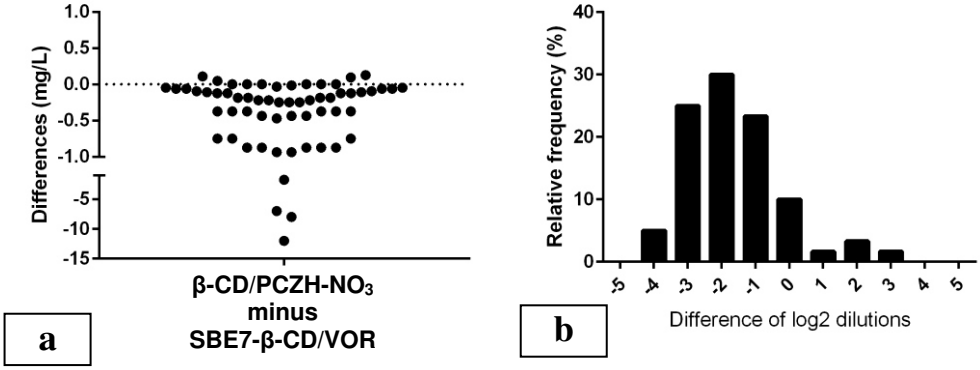

Figure 14. β-CD/PCZH-NO_3 vs. SBE7-β-CD/VOR – antifungal activity against *C. glabrata*: **[a]** absolute values of β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences (mg/L); **[b]** β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences of log2 dilutions

The experimental complex was significantly more effective than SBE7-β-CD/VOR against many isolates with resistance to fluconazole (P = 0.032, Figure 22).

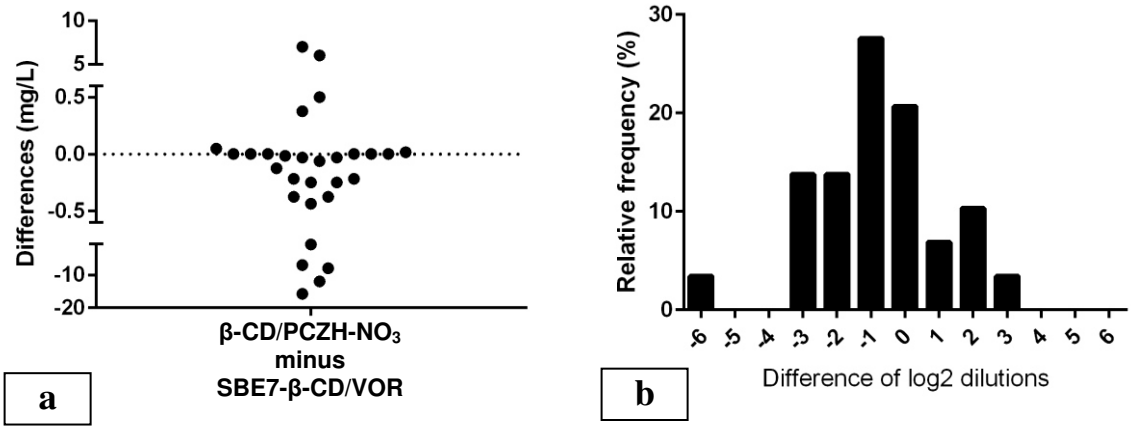

Figure 22. β-CD/PCZH-NO_3 vs. SBE7-β-CD/VOR – antifungal activity against non-*krusei* *Candida* FLC-resistant isolates: **[a]** absolute values of β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences (mg/L); **[b]** β-CD/PCZH-NO_3 minus SBE7-β-CD/VOR differences of log2 dilutions.

Chapter 3

The *in vivo* therapeutic efficiency of the β-CD/PCZH-NO_3 inclusion complex was assessed on a murine model of disseminated (invasive) candidosis and compared to that of the SBE7-β-CD/VOR complex.

Therapeutic efficiency: β-CD/PCZH-NO_3 vs. SBE7-β-CD/VOR

To compare the therapeutic efficiency of β-CD/PCZH-NO_3 with that of SBE7-β-CD/VOR, the two compounds were intraperitoneally (i.p.) administered to two groups of mice preinoculated with an LD_{90} of *C. albicans* SC5314. The mortality rates of each group were tracked for 15 days.

Due to the nephrotoxicity of the parental β-CD, the *in vivo* therapeutic efficiency of the inclusion complex of VOR was superior to that of the β-CD/PCZH-NO_3 complex (Figure 1).

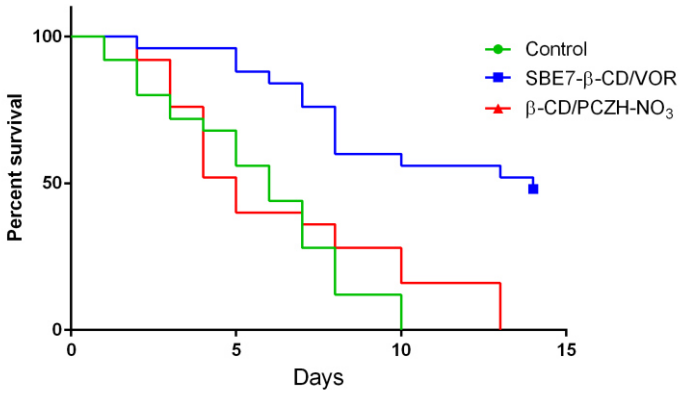

Figure 1. Therapeutic efficiency: β-CD/PCZH-NO_3 vs. SBE7-β-CD/VOR. Survival curves.

Therapeutic efficiency of β-CD/PCZH-NO_3: intraperitoneal administration vs. oral administration

To compare the therapeutic efficiency of the two administration routes, a similar procedure to that described above was used. Two groups of mice were inoculated with a LD_{90} of *C. albicans* SC5314 and then received treatment. The mortality rates and the fungal charge per gram of renal tissue were then monitored. For the group that received the inclusion complex by oral administration, the dose of PCZH-NO_3 was double compared to the group that received the complex via a parenteral route (intraperitoneal administration).

Despite the nephrotoxicity of β-CD and the dosage differences, the survival rates were similar for both groups. Thus, the parenteral administration of the experimental complex proved significantly more efficient than oral administration (Figure 2).

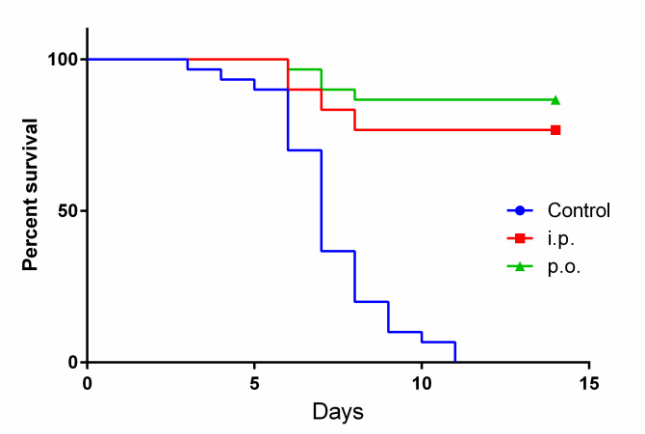

Figure 2 Therapeutic efficiency of β-CD/PCZH-NO_3: intraperitoneal (i.p.) vs. oral (p.o.) administration. Survival curves.

Chapter 4

To improve the efficiency of PCZH-NO_3, three new inclusion complexes were prepared, in which the parental β-CD was replaced with three of its derivatives, namely sulfobutylether-β-CD (SBE7-β-CD), β-CD sulfated sodium salt (β-CD-SNa) and monochlorotriazinyl-β-CD (MCT-β-CD). The inclusion complexes were prepared by freeze-drying. The complexation was confirmed by nuclear magnetic resonance spectroscopy (^{1}H-NMR), 2D rotating frame Overhauser effect spectroscopy (ROESY) NMR and differential scanning calorimetry (DSC).

DSC Studies

The DSC studies revealed inclusion efficiency values close to 100% (Figure 11).

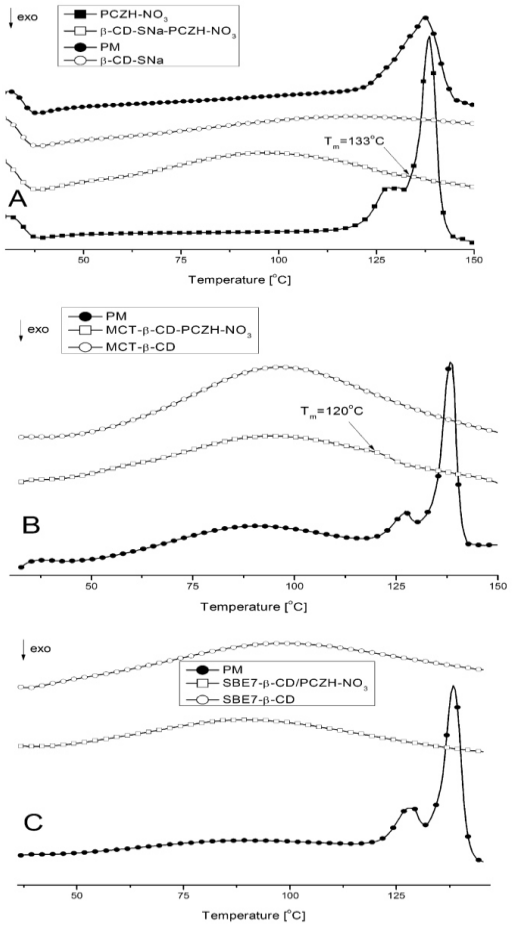

Figure 11. DSC heating curves of: **[A]** PCZH-NO_3, β-CD-SNa/PCZH-NO_3, physical mixture (PM) of β-CD-SNa and PCZH-NO_3, β-CD-SNa; **[B]** MCT-β-CD/PCZH-NO_3, MCT-β-CD, physical mixture of MCT-β-CD and PCZH-NO_3; **[C]** SBE7-β-CD/PCZH-NO_3, SBE7-β-CD and physical mixture of SBE7-β-CD and PCZH-NO_3.

In silico computational studies

Docking and molecular dynamics simulations (with water as an explicit solvent) of the inclusion complexes of PCZH-NO_3 with β-CD and SBE-β-CD were performed. The computational studies suggested the coexistence of different types of inclusion complexes, depending on the PCZH-NO_3 moiety that enters in the cyclodextrin cavity. The most energetically favourable conformation is with the aromatic ring of the dichlorophenyl moiety embedded in the cavity at the sugar ring level (Figure 7).

NMR experiments

Information offered by the computational studies was supported by NMR experiments. Both theoretically calculated and experimentally NMR titration measured K_a values display a consensus view of the PCZH-NO_3 complexation with higher affinity of the guest molecule for SBE7-β-CD compared to β-CD. The SBE7-β-CD derivative showed a better complexation capability due to additional stability induced by the interactions of the dioxolanyl and triazolic cycles with the glycosidic oxygens or the $–SO_3^-$ groups of the cyclodextrin, depending on the complexation mode.

The ^{1}H-NMR spectra of different mixtures of PCZH-NO_3 and cyclodextrins were recorded by keeping the concentration of PCZH-NO_3 constant and varying the concentrations of the cyclodextrins. The double-reciprocal plots of the NMR version of the Benesi-Hildebrand equation [17] gave straight lines (Figure 5), confirming the 1:1 stoichiometry of the inclusion complexes [18].

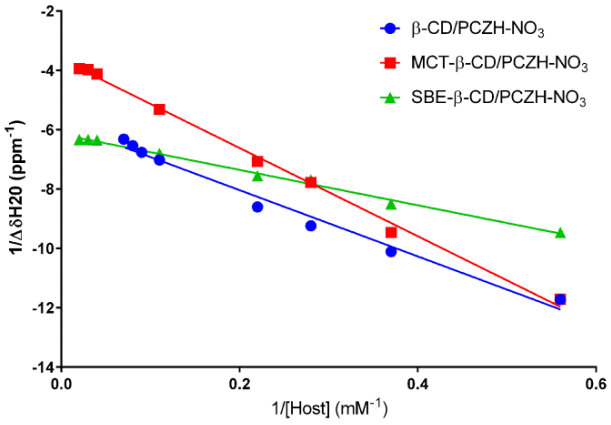

Figure 5. llustration of the Benesi-Hildebrand data treatment.

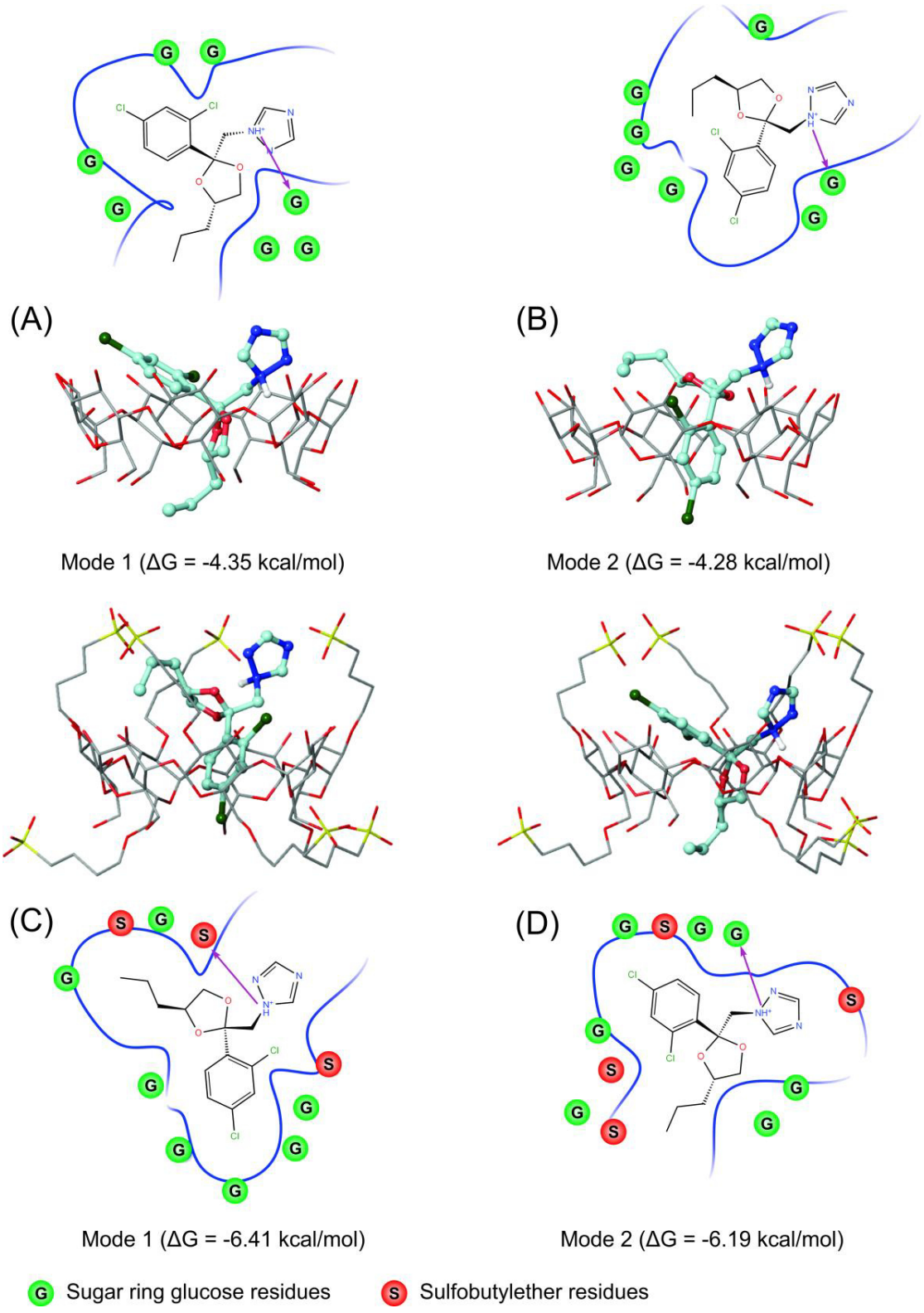

Figure 7. Conformations of inclusion complexes of **[A, B]** β-CD/PCZH-NO_3 and **[C, D]** SBE7-β-CD/PCZH-NO_3 obtained by docking simulations. The 2D diagrams of each complex emphasize the interactions of different groups of PCZH-NO_3 with pyranose and/or sulfobutylether residues. Arrows indicate hydrogen bonds between PCZH-NO_3 and CD.

Chapter 5

The antifungal activity of the three new inclusion complexes against pathogenic yeasts, growing in planktonic phase and in biofilm phase, as well as the cytotoxicity against cultured human cells were investigated and compared to those of the complex with the parental β-CD. The cells used for cytotoxicity evaluations were normal human dermal fibroblasts - NHDF.

Comparative antifungal activity against pathogenic yeasts in planktonic phase

Against planktonic yeasts, all four complexes exhibited antifungal activity at low and similar concentrations (Figure 1). The experiments showed that, in the majority of cases, the MIC values were in agreement with differences confined within the accepted ± 1 log2 dilution interval [19]. This suggested that the nature of the cyclodextrin does not significantly influence the *in vitro* behaviour of PCZH-NO_3 towards fungal cells.

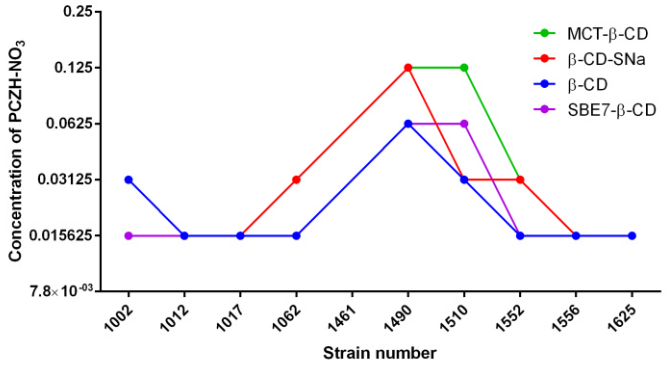

Figure 1. MIC agreement between the four tested inclusion complexes based on PCZH-NO_3 and β-CD or its derivatives against *C. albicans* isolates.

Comparative antifungal activity against biofilms

The four inclusion complexes of protonated propiconazole nitrate did not have antifungal activity against *C. albicans* biofilms at the tested concentrations. This confirms the reported low therapeutic efficiency of azoles against fungal biofilms [20,21]. In biofilms, the biosynthesis of ergosterol molecule is downregulated, which leaves azoles without their therapeutic target [22].

An additional mechanism of action, based on inducing oxidative stress in fungal cells, was reported for one imidazole derivative [23], which gives it antifungal activity against biofilms. The lack of antifungal activity against biofilms leads to the conclusion that protonated propiconazole nitrate acts exclusively by blocking 14α-demethylase and inhibiting ergosterol biosynthesis, and does not have a secondary mechanism of action, at least not one that is relevant for biofilm eradication.

Cytotoxicity

Compared to the parental propiconazole, the modified PCZH-NO_3 had a significantly lower toxicity towards human cells, which demonstrated that nitration can reduce the cytotoxicity (Figure 5).

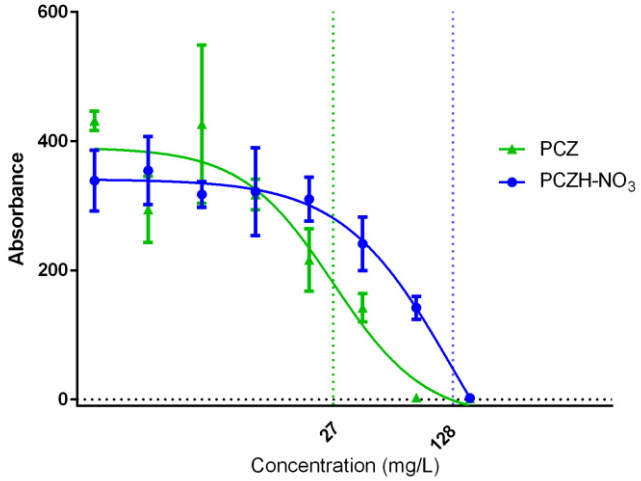

Figure 5. The cytotoxicity of propiconazole (PCZ) and protonated propiconazole nitrate (PCZH-NO_3) - nonlinear fit of the dose response curves. The dotted lines indicate the IC_{50} values.

The cytotoxicities of the four inclusion complexes of PCZH-NO_3 are presented in Figure 6. The nonlinear regression curves showed the PCZH-NO_3 complexed with the parental β-CD to be more toxic than the same compound complexed with the three β-CD derivatives. The lack of significant differences in the antifungal susceptibility tests and the differences in cytotoxicity between the complex with the parental β-CD and the complexes with β-CD derivatives suggest that the type of cyclodextrin may be more important for the interaction of the compounds with the infected host than it is for the actual antifungal activity.

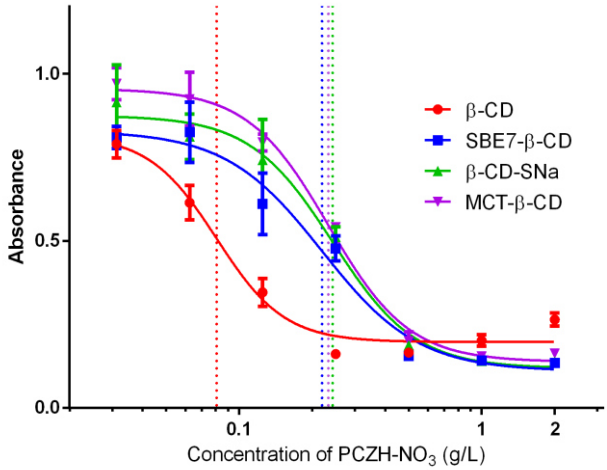

Figure 6. The cytotoxicity of the four inclusion complexes based on PCZH-NO_3 and β-CD or its derivatives - nonlinear fit of the dose response curves. The dotted lines indicate the IC_{50} values.

General conclusions

- The inclusion complex of a new antifungal triazole, protonated propiconazole nitrate (PCZH-NO_3), with β-cyclodextrin (β-CD) was prepared by freeze-drying;
- The biological properties of the inclusion complex – β-CD/PCZH-NO_3 (*in vitro* antifungal activity, cytotoxicity and *in vivo* therapeutic efficiency) were investigated;
- The investigation of the antifungal properties was part of the first study covering Romania with regard to species distribution and azole susceptibility pattern of fungal clinical isolates;
- The *in vitro* antifungal activity was tested alongside fluconazole (FLC) and voriconazole (VOR), two commercial azoles with extensive clinical use;
- The commercial formulation of VOR, where it is complexed with sulfobutylether-β-cyclodextrin (SBE7-β-CD), was used;
- The standardised European testing method recommended by EUCAST revealed some common features of the commercial drugs and our experimental complex, namely the high susceptibility of *Candida albicans* and the reduced susceptibility of many non-*albicans Candida* species;

- The antifungal activity of our complex was generally similar to that of the VOR complex, a commercial formulation with extensive clinical use;
- The β-CD/PCZH-NO_3 complex was more effective than SBE7-β-CD/VOR against many isolates with resistance to fluconazole;
- The experimental compound was particularly active and superior to the VOR complex against *C. glabrata* isolates, which are known to often have a reduced susceptibility to azoles;
- The comparative studies showed that PCZH-NO_3 could be, not only a cheaper replacement for VOR, but also a complementary treatment option;
- The good antifungal activity against *C. albicans* and *C. glabrata* suggested a potentially successful use of the experimental compound in the treatment of urinary tract yeast infections, where these two species account for the majority of cases;
- Due to the nephrotoxicity of the parental β-CD, the *in vivo* therapeutic efficiency of the β-CD/PCZH-NO_3 complex was inferior to that of the SBE7-β-CD/VOR complex;
- At the same time, however, despite the nephrotoxicity of β-CD, the parenteral administration of the experimental complex proved significantly more efficient than oral administration;
- To improve the therapeutic efficiency of PCZH-NO_3, three new inclusion complexes were prepared, in which the parental β-CD was replaced with three of its derivatives, namely sulfobutylether-β-CD (SBE7-β-CD), β-CD sulfated sodium salt (β-CD-SNa) and monochlorotriazinyl-β-CD (MCT-β-CD);
- The inclusion complexes were prepared by freeze-drying;
- The complexation was confirmed by nuclear magnetic resonance spectroscopy (^{1}H-NMR), 2D rotating frame Overhauser effect spectroscopy (ROESY) NMR and differential scanning calorimetry (DSC);
- The DSC studies revealed inclusion efficiency values close to 100%;
- Information offered by NMR was supported by *in silico* docking and molecular dynamics simulations (with water as an explicit solvent);

- The computational studies suggested the coexistence of different types of inclusion complexes, depending on the PCZH-NO_3 moiety that enters in the cyclodextrin cavity;
- The values of the association constants were 510, 1050 and 250 M^{-1} for β-CD, SBE-β-CD and MCT-β-CD complexes, respectively;
- The association constant of the β-CD-SNa/PCZH-NO_3 inclusion complex could not be determined since the variation of the chemical shifts of the dichlorophenyl protons did not follow a linear trend, possibly due to the high concentration of the anionic groups close to the CD rim, which can induce perturbations on the chemical shifts of the PCZH-NO_3 protons;
- The SBE7-β-CD derivative showed a better complexation capability due to additional stability induced by the interactions of the dioxolanyl and triazolic cycles with the glycosidic oxygens or the $-SO_3^-$ groups of the cyclodextrin, depending on the complexation mode;
- The most energetically favourable conformation is with the aromatic ring of the dichlorophenyl moiety embedded in the cavity at the sugar ring level;
- The antifungal activity of the three new inclusion complexes against pathogenic yeasts, growing in planktonic phase and in biofilm phase, as well as the cytotoxicity against cultured human cells were investigated and compared to those of the complex with the parental β-CD;
- Against planktonic yeasts, all four complexes exhibited antifungal activity at low and similar concentrations, which suggested that the nature of the cyclodextrin does not significantly influence the *in vitro* behaviour of PCZH-NO_3 towards fungal cells;
- The four inclusion complexes did not have antifungal activity against *C. albicans* biofilms which led to the conclusion that PCZH-NO_3 acts exclusively by blocking the 14α-demethylase enzyme and, consequently, inhibiting ergosterol biosynthesis;
- Compared to the parental propiconazole, the modified PCZH-NO_3 had a significantly lower toxicity towards human cells, which demonstrated that nitration can reduce the cytotoxicity;

- When complexed with the parental β-CD, PCZH-NO_3 had a significantly higher cytotoxicity, compared with the inclusion complexes with β-CD derivatives; this confirmed the detrimental effect of β-CD and the importance of the nature of the cyclodextrin for parenteral applications.

Scientific activity

Contributions within the subject of the PhD thesis

Articles in ISI indexed publications (published/accepted)

1. **Minea B**, Marangoci N, Peptanariu D, Rosca I, Nastasa V, Corciova A, Varganici C, Nicolescu A, Fifere A, Neamtu A, Mares M, Barboiu M, **Pinteala M**. Inclusion complexes of propiconazole nitrate with substituted β-cyclodextrins. Synthesis, *in silico* and *in vitro* assessment of antifungal properties. NEW J CHEM - **accepted**, DOI: 10.1039/C5NJ01811K. **IF = 3.086**

2. **Minea B**, Nastasa V, Kolecka A, Mares M, Marangoci N, Rosca I, **Pinteala M**, Hancianu M, Mares M. Etiologic agents and antifungal susceptibility of oral candidosis from Romanian patients with HIV-infection or type 1 *diabetes mellitus*. POL J MICROBIOL - **accepted**. **IF = 0.697**

3. **Minea B**, Nastasa V, Moraru RF, Kolecka A, Flonta MM, Marincu I, Man A, Toma F, Lupse M, Doroftei B, Marangoci N, **Pinteala M**, Boekhout T, Mares M. Species distribution and susceptibility profile to fluconazole, voriconazole and MXP-4509 of 551 clinical yeast isolates from a Romanian multi-centre study. Eur J Clin Microbiol Infect Dis. 2015 Feb;34(2):367–83. **IF = 2.668**

Abstracts published in the proceedings of scientific events

1. **Minea B**, Moraru R, Năstasă V, Doroftei B, Marincu I, Mareş M. Comparative evaluation of the antifungal activity of voriconazole and a new propiconazole derivative (MXP405) against fluconazole-resistant yeast isolates. Rev Romana Med Lab. 2013;21(2/4)suppl.:S140. **IF = 0.239**

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1. Moraru R, **Minea B**, Năstasă V, Doroftei B, Mares M. Comparative evaluation of the antifungal activity of voriconazole and a new propiconazole derivative (MXP-4509) against 278 *Candida albicans* clinical isolates. Lucrări Științifice Universitatea de Științe Agricole și Medicină Veterinară „Ion Ionescu de la Brad" Iași, Seria Medicină Veterinară, vol. 56 (15), 2013.

2. Moraru R, **Minea B**, Năstasă V, Doroftei B, Mares M. Comparative evaluation of the antifungal activity of voriconazole and a new propiconazole derivative (MXP-4509) against fluconazole-resistant yeast isolates. Lucrări Științifice Universitatea de Științe Agricole și Medicină Veterinară „Ion Ionescu de la Brad" Iași, Seria Medicină Veterinară, vol. 56 (15), 2013.

Presentations

1. **Minea B**. Oral candidosis in diabetic or HIV-infected patients. Etiologic agents and antifungal susceptibility. The final conference of the POSDRU/159/1.5/S/133377 project " Programme of excellency in the multidisciplinary doctoral and post-doctoral research of chronic diseases", Iași (Romania), 3-5 December 2015.

2. **Minea B**, Moraru R, Năstasă V, Doroftei B, Marincu I, Mareş M. Comparative evaluation of the antifungal activity of voriconazole and a new propiconazole derivative (MXP405) against fluconazole-resistant yeast isolates. The 3rd National Conference of Medical Mycology, Sibiu (Romania), 20-22 June 2013.

3. **Pinteala M**, Marangoci N, Fifere A, Durdureanu-Angheluta A, **Minea B**, Simionescu BC. Advanced Research in Bionanoconjugates and Biopolymers. The 5th International Conference of Education, Research and Innovation (ICERI), Madrid (Spain), 19-21 November 2012.

4. Mares M, Nastasa V, Moraru R, **Minea B**, Miron L. Comparative In Vitro Activities of Fluconazole, Voriconazole, and MXP-4509 against 256 Candida Isolates from Romanian Tertiary Hospitals. The 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, CA (USA), 09-12 September 2012.

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2. Marangoci N. L., Corciova A., **Minea B.**, Fifere A., **Pinteala M.**, Inclusion complexes of hesperidin with cyclodextrin derivatives. Structural characterisation, antioxidant and antibacterial properties. The 33rd National Chemistry Conference, Călimănești Căciulata (Romania), 1-3 October 2014

Other contributions, related to the field of the PhD thesis

Articles in ISI indexed publications

1. David G, Fundueanu G, **Pinteala M**, **Minea B**, Dascalu A, Simionescu BC. Polymer engineering for drug/gene delivery: from simple towards complex architectures and hybrid materials. Pure Appl Chem. 2014;86(11):1621–35. **IF = 2.492**

2. Durdureanu-Angheluta A, Uritu CM, Coroaba A, **Minea B**, Doroftei F, Calin M, Maier SS, **Pinteala M**, Simionescu M, Simionescu BC. Heparin-anthranoid conjugates associated with nanomagnetite particles and their cytotoxic effect on cancer cells. J Biomed Nanotechnol. 2014 Jan;10(1):131–42. **IF = 5.338**

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1. **Minea B**, **Pinteala M**, Chiriac A. Filaggrins in atopic dermatitis. Primăvara Dermatologică Ieșeană, 2nd Edition, Iași (Romania), 18-22 April 2013.

Posters

1. **Minea B**, Ursu L, Doroftei F, Chiriac A, Solovan C, Chiriac AE, **Pinteala M**. Allergic onycholysis caused by cyanoacrylate nail glue (in artificial nails) –the use of scanning electron microscopy. 22nd Congress of the European Academy of Dermatology and Venereology „Dermatovenereology in a changing world", Istanbul (Turkey), 2-6 October 2013.

Member in research projects

1. Project PN-II-ID-PCCE-2011-2-0028, Biologically Inspired Systems for Engineered Structural and Functional Entities, Coordinator: "Petru Poni" Institute of Macromolecular Chemistry of Iași, project manager: Dr. Mariana Pinteală.

Professional training stages

1. Techniques & Applications of Molecular Biology, The University of Warwick, Coventry (UK), 14 - 17 July 2014.

2. Antifungal Resistance and its Challenges in the Management of Invasive Fungal Infections, European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Sibiu (Romania), 20 - 22 June 2013.

3. Microscopy Training Course, VIB-Ghent University (COST Action BM0903), Gent (Belgium), 29 - 31 May 2013.

4. The initiation of etiotropic therapy in fungal infections, University of Medicine and Pharmacy of Tîrgu Mureș, Tîrgu Mureș (Romania), 18 - 24 martie 2013.

5. Immunology & Genetics of Atopic Dermatitis, Mediterranean Institute for Life Sciences (COST Action BM0903), Split (Croatia), 10 - 12 September 2012.

6. Human Cell Cultures, "Nicolae Simionescu" Institute of Cellular Biology and Pathology, Bucharest (Romania), 21 November - 9 December 2011.

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