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Dedicated to Professor Luminița Silaghi-Dumitrescu on the occasion of her 65th anniversary

THIAZOLE DERIVATIVES WITH ANTIFUNGAL ACTIVITY AGAINST *CANDIDA* SPECIES

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ABSTRACT. The antifungal activity of a series of thiazole, benzothiazole and benzothiazolyl-phenothiazine derivatives against *Candida albicans* and non-*Candida albicans* species causing invasive candidiasis was investigated using the diffusion method and the broth dilution procedure. Minimal inhibitory concentrations revealed antifungal activity against *C. albicans*, *C. guillermondii, C. krusei* and *C. parapsilosis* strains isolated from clinical materials (blood, urine and peritoneal fluids cultures). The tested hydrazine-thiazole derivatives showed promising antifungal activity against both fluconazole susceptible and resistive *Candida* species, while the benzothiazolyl-phenothiazine derivatives were not effective.

Keywords: *Thiazole, Benzothiazole, Phenothiazine, Candida species.*

INTRODUCTION

Health care associated infections have become a cause of major public concern due to complications inducing morbidity, mortality and increased treatment costs for both immunocompromised as well as non-immunocompromised

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patients. *Staphylococcus aureus, Enterococcus* species, *Escherichia coli*, *Pseudomonas aeruginosa and Candida* species are the most common groups of organisms that cause nosocomial infections in both adults and children. [1]. Candidiasis affected a significant number of individuals and the ratio of sepsis episodes of fungal etiology has risen [2]. Health care-associated candidiasis appears globally hierarchized as the third reported cause of invasive nosocomial infection [3,4]. Among *Candida* species, *C. albicans* was historically identified as predominant in causing invasive candidiasis, but more recently several non-*C. albicans* species such as *C. tropicalis, C. parapsilosis* complex*, C. glabrata* and *C krusei* were increasingly incriminated as responsible for invasive infections [5,6]. According to medical statistics, the top four *Candida* species involved in invasive infections were *C. albicans* (58.4%) followed by *C. parapsilosis* complex (19.5%), *C. tropicalis* (9.3%), and *C. glabrata* (8.3%) [7,8]. Minimal immune suppression seemed required to predispose an individual to infections and the high mortality associated to candidemia varied in different geographic areas (*e.g.* 15%-35% in Europe, up to 46% in USA and up to 54% in Brazil). [9]

In our previously reported studies we tested the antifungal activity of benzothiazol and benzothiazolyl-phenothiazine derivatives against *C. albicans* ATCC 14053 standard strain [10,11]. In this work we extended the screening of antifungal activity of selected hydrazino-thiazole, phenyl-benzothiazole and phenothiazinyl-benzothiazole derivatives against several *Candida species* isolated from clinical materials which were collected from patients with invasive fungal infections health cared in the *Hospital of Infectious Diseases* Cluj-Napoca, Romania.

RESULTS AND DISCUSSION

The chemical structures of the heterocyclic compounds selected for evaluation of antifungal activity against *Candida spp*. are presented in figure 1. The chemical synthesis of hydrazones **1-3** containing different substituents attached to the phenyl and thiazole unit, benzothiazolyl-phenothiazine derivatives **4,5** differentiated by the substitution pattern of the phenothiazine core and 2-phenyl-benzothiazole **6,** were described previously in some of our works [10,11].

The *Candida* strains subjected to this study were responsible for invasive infections in health-care patients with different comorbidities or immunosuppression in critical stages and were isolated from physiological fluids (blood, urine, peritoneal liquid, or bronco-alveolar lavage liquid) and the results are presented in Table 1. The minimal inhibitory concentrations (MIC) were determined according to CLSI standard [12]

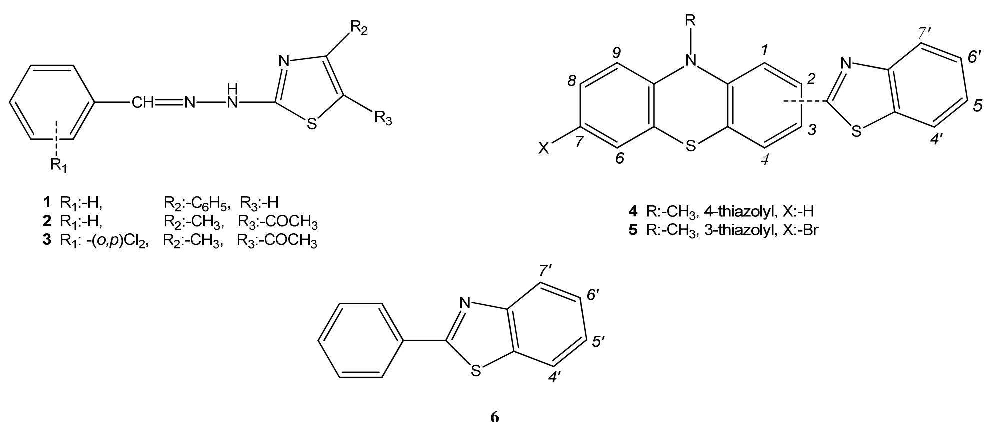

Figure 1. The chemical structures of the tested antifungal agents.

Table 1. Minimal inhibitory concentration (MIC) of different antifungal drugs against isolated *Candida* strains

***Candida* strain** source	MIC (µg/ml)					
	Fluconazole	Voriconazole	Caspofungin	Micafungin	Amph-B	5-FC
C. *albicans* Blood culture	1 (S)	0.12 (S)	0.25 (S)	0.06 (S)	2 (I)	1 (S)
C. *glabrata* Blood culture	16 (I)	0.5 (S)	0.83 (S)	0.012 (S)	1 (S)	1 (S)
C. glabrata Br-alv lavage	4 (DDS)	0.12 (S)	0.25 (S)	0.06 (S)	0.5 (S)	1 (S)
C. glabrata Blood culture	4 (DDS)	0.12 (S)	0.25 (S)	0.06 (S)	1 (S)	1 (S)
C. guillermondii Urine culture	32 (R)	0.12 (S)	0.25 (S)	0.06 (S)	2 (I)	1 (S)
C. krusei Peritoneal fluid	8 (R)	0.12 (S)	0.25 (S)	0.12 (S)	0.5 (S)	8 (I)
C. lusitaniae Blood culture	32 (R)	0.008 (S)	0.12 (S)	0.06 (S)	0.5 (S)	256 (R)
C. norvegiensis Urine culture	16 (DDS)	0.25 (S)	0.25 (S)	0.12 (S)	1 (S)	4 (S)
C. parapsilosis Blood culture	1 (S)	0.12 (S)	1 (S)	0.5 (S)	1 (S)	1 (S)
C. parapsilosis Blood culture	1 (S)	0.12 (S)	0.5 (S)	0.5 (S)	0.5 (S)	1 (S)
C. albicans ATCC 10231	1 (S)	0.12 (S)	0.25 (S)	0.06 (S)	0.12 (S)	1 (S)

S=sensible; I=intermediate; R=resistant; DDS=dose-dependent sensibility; Amph-B= Amphotericin B; 5-FC= 5-Fluorocytosine

Three of the non-*C.albicans* strains appeared to be resistant to fluconazole, the most largely employed antifungal drug.

The *in vitro* susceptibility of *Candida* species to the synthetic compounds **1-6** was screened using the diffusion method and the results are presented in Table 2.

Table 2. Inhibition zone diameter (mm) resulted by diffusion method using 10 µl solution 2.5 mg/ml of compound **1-6**.

***Candida* strain** source	**Inhibition zone diameter (mm)**						
	Fluconazole	**1**	**2**	**3**	**4**	**5**	**6**
C. albicans Blood culture	25	6	4	12	8	6	6
C. glabrata Blood culture	4	0	0	0	0	0	7
C. glabrata Br-alv lavage	4	0	4	0	0	0	6
C. glabrata Blood culture	4	0	4	0	0	0	6
C. guillermondii Urine culture	2	6	4	12	0	0	0
C. krusei Peritoneal fluid	0	6	8	6	0	0	7
C. lusitaniae Blood culture	0	0	0	0	0	0	0
C. norvegiensis Urine culture	6	6	4	4	0	0	0
C. parapsilosis Blood culture	31	6	7	12	0	0	0
C. parapsilosis Blood culture	24	7	7	12	0	0	0
C. albicans ATCC 10231	30	2	2	12	0	0	8

Neither of the tested compounds **1-6**, nor fluconazole were active against *C. lusitaniae,* while in the case of *C. albicans* and *C. parapsilosis* strains all the tested compounds **1-6** showed inhibition zones with considerably smaller diameter in comparison to the antimycotic drug.

Hydrazones **1-3** and phenyl-benzothiazole **6** exhibited a moderate antifungal activity against *C. guillermondii* and *C. krusei* strains which seemed resistant to fluconazole, while *C. glabrata* strain appeared slightly sensitive to phenyl-benzothiazole **6.**

Benzothiazolyl-phenothiazine derivatives **4, 5** appeared inactive against any of the isolated non-*C. albicans* strains.

Hydrazones **2, 3** and phenyl-benzothiazole **6** pointed by the diffusion method screening as potential antifungal agents against *C. albicans* and non-*C. albicans* strains: *C. guillermondii, C. krusei* and *C. parapsilosis* were further employed in MIC determination experiments using the broth dilution method. The results thus obtained are summarized in table 3.

Table 3. Minimal inhibitory concentration (MIC) for hydrazones **2**, **3** and phenyl-benzothiazole **6**.

Candida strain	MIC (µg/ml)			
	Fluconazole	**2**	**3**	**6**
C. albicans Blood culture	1 (S)	0.16	0.8	64
C. guillermondii Urine culture	32 (R)	32	0.16	64
C. krusei Peritoneal fluid	8 (R)	0.8	32	1.2
C. parapsilosis Blood culture	1 (S)	0.5 (S)	1 (S)	64
C. parapsilosis Blood culture	1 (S)	0.5 (S)	0.5 (S)	64
C. albicans ATCC 10231	1 (S)	32	1.2	64

S=sensible; R=resistant;

Hydrazones **2, 3** gave the impression of effective antifungal agents against the two non-*C.albicans* strains resistant to fluconazole: *C. guillermondii* and C. *krusei* and proved to be even more effective than the commercial antifungal drug against *C. albicans* and *C. parapsilosis* strains isolated from the blood of health-care patients.

Phenyl-benzothiazole **6** appeared effective only against *C. krusei* isolated from peritoneal fluid.

CONCLUSIONS

The antifungal activity of heterocyclic compounds containing the thiazole unit screened *in vitro* pointed out the sensibility of *C. albicans* and non-*C. albicans* strains isolated from clinical materials towards hydrazino-thiazole derivatives. Substituted benzothiazole derivatives appeared to be less effective and the presence of the phenothiazine unit did not favour the antifungal activity.

EXPERIMENTAL SECTION

Compounds **1-6** were synthesized according to our previously reported procedures [10,11].

The *Candida* species strains were isolated from clinical materials which were collected from patients with invasive fungal infections health cared in the *Hospital of Infectious Diseases* Cluj-Napoca.

The *Candida* species strains were isolated from blood, urine, peritoneal liquid and bronco-alveolar lavage liquid cultures.

The identification and the susceptibility profile of the isolated *Candida* strains to six different commercial antifungal drugs was determined using the automated systems Vitek 2 YST and AST-SY01.

Antifungal activity tests:

The fungus strains were cultivated on Sabouraud dextrose agar with incubation at 37±2^{0}C for 48h in aerobic conditions.

a) The Diffusion method was carried out according to previously reported procedure [11] using a fungus culture which has been adjusted to 0.5 McFarland standard inoculated in Muller Hinton agar plates; 10 μl from 2.5 mg/ml solution of synthetic compounds in DMSO were disposed on sterile paper disks. Standard Fluconazol 25 μg/disc (Oxoid, England) was employed. After incubation at 37±2^{0}C for 72 hours the plates were examined for inhibition zone, which was measured and recorded in mm.

The tests were repeated three times to ensure reliability.

b) Broth dilution procedure was carried out according to the protocols previously described [11]. MIC was determined using microdilution trays using a stock solution 2.5 mg/ml in DMSO and distilled water. Each tray included a growth control well and a sterility (uninoculated) well.

The rights of the patients regarding the confidentiality of personal information were respected in agreement to Helsinki declaration of Ethical Principles for Medical Research Involving Human Subjects

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