

In vitro antifungal activity of new thiatriazole derivative agents

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ABSTRACT

Purpose: The assessment of the antifungal activity of 59 new synthesized compounds. We compared the antifungal activity of N,N-phenyl-1,2,3,4-thiatriazole-5-yl-2,4-b-resorcyl-carbothioamide (PTR), of n-3-(1,2,4-dithiazole-5-thione)- β -resorcylcarbothioamide (DTRTA), of N,N-phenyl-1,2,3,4-thiatriazol-5-yl-2,4-b-resorcyl-carbothioamide (PHARA) against *Candida albicans* strains *in vitro*.

Material and methods: We evaluated 59 samples of the compounds synthesized at the Department of Chemistry University of Agriculture in Lublin. In the first phase, we tested the selected three samples with the lowest value of MIC - PTR, DTRTA, and PHARA. A reference strain of *C. albicans* ATCC 10231 and 200 strains of *C. albicans* isolated from the patients was used.

Results: The MIC values of the tested samples fluctuated between 19.6 - 200 mg/L. The tested compounds showed moderate antifungal activity against *C. albicans* with MIC values of 19.6 mg/L for PTR, 22.0 mg/L for DTRTA, and 19.6 mg/L

for PHARA. We found significant ($p < 0.001$) differences between mean MIC values for PTR, DTRTA, PHARA on RPMI medium compared with MIC values on Sabouraud's and YNB medium. Similar results we found for the reference strains *C. albicans* ATCC 10231. The reference strain *C. albicans* ATCC 10231 had enzymatic activity of 14 from 19 hydrolases in the (APIZYM), after exposure PTR number of the active enzymes was 6, after exposure DTRTA - 9, after exposure PHARA - 6, respectively. Isolates of *C. albicans* from the patients had enzymatic activity of 16 from 19 hydrolases, after exposure PTR number of active enzymes was 11, after exposure DTRTA - 15, and after exposure PHARA - 11.

Conclusion: The synthesized compounds PTR, DTRTA and PHARA exert a moderate antifungal activity against the *C. albicans* strains *in vitro*.

Key words: new compounds, antifungal agents, *Candida albicans*, MIC

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INTRODUCTION

Yeasts are the most frequent organism involved in invasive fungal infections. *Candida albicans* - is the fourth most common pathogen among micro-organisms isolated in intensive care units *Staphylococcus aureus*, *Enterococcus species* and Gram-negative rods. An increase in infections due to non- *albicans* species of *Candida* and other yeast species has been observed [1]. Fungal infections are a leading cause of mortality in patients with chemotherapy, neutropenia and HIV. Candidiasis and aspergillosis account for most invasive fungal infections [2]. The discovery of the azole antifungal compounds, CAS 65277-42-1, 87625, and 86386-73-4, allowed for a broader spectrum of antifungal treatment and a shorter treatment duration [3]. These drugs act by inhibiting cytochrome P450-dependent ergosterol synthesis and cytochrome c oxidase and peroxidase enzymes. This disruption of enzymic processes ultimately leads to fungal cell death. Itraconazole has improved activity against moulds (*Aspergillus spec.*, *Sporothrix schenckii*) and dimorphic yeasts (e.g. *Histoplasma*, *Blastomyces*, and *Coccidioides spec.*) when compared with ketoconazole. It is used in the treatment of fungal infections localized to the toenails and fingernails [4].

During the last decade, a marked increase in resistance of *C. albicans* and non- *albicans Candida* species to azole and other antifungal treatment has been observed [5,6]. The search and development of new antifungal agents is expected to offer new opportunities for both prophylaxis and treatment of fungal infections in the immunocompromised host.

Series compounds with alfa-resorcylthiocarbonyl moiety from group of thiobenzanilides substituted in the N-aryl ring [7-9] and N-heterocyclic amides [10] were achieved in our laboratory. They show a wide spectrum of antifungal activity in relation to moulds [7], yeasts [8], dermatophytes [3-4] and strong inhibition action comparable with commercial antimycotic drugs [4]. Taking into account the wide application of antifungal medicines with azole moiety, N,N-phenyl-1,2,3,4-thiazotriazole-5-yl- β -resorcylcarbothioamide was produced as a compound with expected antifungal activity.

The aim of this study was the synthesis and comparison of the antifungal activity of these new thiazotriazole derivatives.

MATERIAL AND METHODS

In the first stage, we tested the activity of antifungal agents against 10 *Candida* strains. We evaluated 59 samples of the agents. MIC values

ranged between 19.6 ± 10.7 mg/l to 200 ± 0 mg/l. Of the samples tested in the first phase, we selected three samples with the lowest value of MIC - N,N-phenyl-1,2,3,4-thiazotriazole-5-yl- β -resorcylcarbothioamide (PTR), N-3-(1,2,4-dithiazole-5-thione)- β -resorcylcarbothioamide (DTRTA), N,N-phenyl-1,2,3,4-thiazotriazole-5-yl- β -resorcylcarbothioamide (PHARA).

A reference strain of *C. albicans* ATCC 10231 and 200 strains of *C. albicans* isolated from the patients.

N,N-phenyl-1,2,3,4-thiazotriazole-5-yl- β -resorcylcarbothioamide (PTR) 0.01 mol of sulphanyl-bis-2,4-dihydroxybenzenethiyl (1) and 0.025 mol of N-1,2,3,4-thiazotriazole-5-ylaniline (2) (Sigma-Oldrich, Steinhein) were heated until boiling (3 h) in methanol (50 cm³). Post-reaction mixture was filtered when hot and the filtrate was concentrated until dry. Compound precipitated was washed using water and re-crystallized from dilute (2:1) methanol (75 ml). Sulphanyl-bis-2,4-dihydroxybenzenethiyl (1) as the starting material was prepared according to patent [11].

N-3-(1,2,4-dithiazole-5-thione)- β -resorcylcarbothioamide (DTRTA) 0.025 mol of 3-amino-1,2,4-dithiazole-5-thione (2) and 0.01 mol of bis-(β -resorcylcarbothiyl)thionyl (1) was added into 50 ml of methanol and heated to boiling (3 hrs). After reaction completed, the mixture was hot filtered and added with 100 ml of water. Separated compound was filtered, washed with water and re-crystallized from dilute (2:1) methanol (60 ml). Bis-(β -resorcylcarbothiyl)thionyl as the starting material was prepared according to patent [11].

N,N-phenyl-1,2,3,4-thiazotriazole-5-yl- β -resorcylcarbothioamide (PHARA) 0.01 mol of sulphanyl-bis-2,4-dihydroxybenzenethiyl (1) and 0.025 mol of N-1,2,3,4-thiazotriazole-5-ylaniline (2) (Sigma-Oldrich, Steinhein) were heated until boiling (3 h) in methanol (50 cm³). Post-reaction mixture was filtered when hot and the filtrate was concentrated until dry. Compound precipitated was washed using water and re-crystallized from dilute (2:1) methanol (75 ml). Sulphanyl-bis-2,4-dihydroxybenzenethiyl (1) as the starting material was prepared according to patent [11].

Anal. (C₁₄H₁₀N₄O₂S₂, M=330.32) % N 28.50; m. p. 84-85°C; ¹H-NMR, DMSO-d₆ (δ , ppm): 11.86 (s, OH), 10.75 (s, OH), 7.91-7.80 (m, 3H), 6.46-6.33 (m, 5H); IR (cm⁻¹): 1666, 1469, 1439 ν C=N, 1048 ν C=S; MS (EI, m/z): 320, 268, 244, 184, 153, 137, 124, 109, 69, 51. ¹H-NMR spectrum of the compound was recorded with a Varian spectrometer (400 MHz). Chemical shift (ppm) was determined in relation to TMS. Solutions were prepared in DMSO-d₆ and D₂O. Infra-red spectrum (KBr pellet) was made in range of 4000-600 cm⁻¹

using Perkin-Elmer 683 spectrophotometer. EI-MS spectrum was recorded with an AMD-604 mass spectrometer (electron ionisation at 70 eV, 33-800, temp. 28°C).

The yeasts were identified to the species level by the CandiSelect (Sanofi Diagnostics, Pasteur). The tested compounds was dissolved in 1% DMSO. Susceptibility testing was performed by the agar dilution method. For yeasts, dermatophytes and moulds MICs were determined by the agar dilution procedure according to National Committee for Clinical Laboratory Standards (NCCLS) reference document M 27 [12].

Sabouraud's medium (SB), YNB - Yeast Nitrogen Base Medium and RPMI was used. Starting inocula were adjusted by the spectrophotometric method densitometr to 1×10^5 CFU/ml.

Concentrations of PTR were ranging from 0.025 to 200 mg/l. Plates were incubated at 37°C and read after 24 h incubation. A solvent control was included in each set of assays; the DMSO solution at the maximum final concentrations of 1% had no effect on fungal growth.

The enzymatic activity of the yeast-like fungi was performed by API ZYM test (bio Mériux). API ZYM is a semi-quantitative micro-method designed for the assessment of enzymatic activities. This method is applicable to all specimens (tissues, cells, biological fluids, micro-organisms, washings, soil, oil, etc.). It allows the systematic and rapid study of 19 enzymatic reactions using only very small sample quantities. (Tab. 1)

Table 1. Hydrolytic enzymes and their substrates assayed using API ZYM test.

No	Enzyme assayed	Substrate
I	Phosphatase alkaline	2-naphtylophosphate
II	Esterase (C4)	2-naphtylbutyrate
III	Esterase lipase (C8)	2-naphtylcapylate
IV	Lipase (C14)	2-naphtylmyristate
V	Leucine arylamidase	L-leucyl-2-naphtylamide
VI	Valine arylamidase	L-leucyl-2-naphtylamide
VII	Cystine arylamidase	L-cystyl-2-naphtylamide
VIII	Tyripsin	N-benzoyl-DL-arrginine-2-naphtylamide
IX	Chymotripsin	N-glutaryl-phenylalanine-2-naphtylamide
X	Phosphatase acid	2-naphtylphosphate
XI	Naphtol-AS-BI-phosphodrolase	Naphtyl-AS-BI-phosphate
XII	α -galactosidase	6-Br2-naphtyl- α D-galactopyranoside
XIII	β -galactosidase	2-naphtyl- β D-galactopyranoside
XIV	β -glucuronidase	Naphtol-AS-BI- β D-glucuronide
XV	α -glucosidase	2-naphtyl- α D-glucopyranoside
XVI	β -glucosidase	6-Br-2-naphtyl- β D-glucopyranoside
XVII	N-acetyl- β -glucosaminidase	1-naphtyl-N-acetylo- β D-glucosaminide
XVIII	α -mannosidase	6-Br-2-naphtyl- α D-mannopyranoside
XIX	α -fucosidase	2-naphtyl- α -L-fucopiranoza

The API ZYM strip is composed of 20 microtubes where the bottom forms a sort of support especially designed to contain the enzymatic substrate and a buffer. This support allows for contact between the enzyme and the general insoluble substrate. All procedures were done according to the manufacturer's instructions. The results were determined by using the API ZYM color scale ranging from 0 (negative) to 5 (maximum), depending on the amount of substrate metabolized where: 1 corresponds to 5 nmol, 2 to 10 nmol, 3 to 20 nmol, 4 to 30 nmol and 5 to > 40 nmol.

We evaluated the enzymatic activity of the yeast-like fungi strains, before and after addition of PTR, DTRTA, PHARA.

Student-t test (two-tailed) was used to compare mean MIC values, Wilcoxon's paired test was used to compare enzymatic activity before and after exposure of sample in sore scale.

Significance was defined as a p value of 0.05. These analyses were performed on a personal computer with a commercially available statistics program (Statistica 7.1 PL)

RESULTS

N,N-phenyl-1,2,3,4-thiazotriazole-5-yl-2,4-resorcylcarbothioamide (PTR) was obtained in the reaction according to Figure 1. The analytical

data of compound were in agreement with the proposed structure. The purity was confirmed by HPLC and HPTLC chromatography in the

reversed-phase system (RP-8, RP-18, methanol-water).

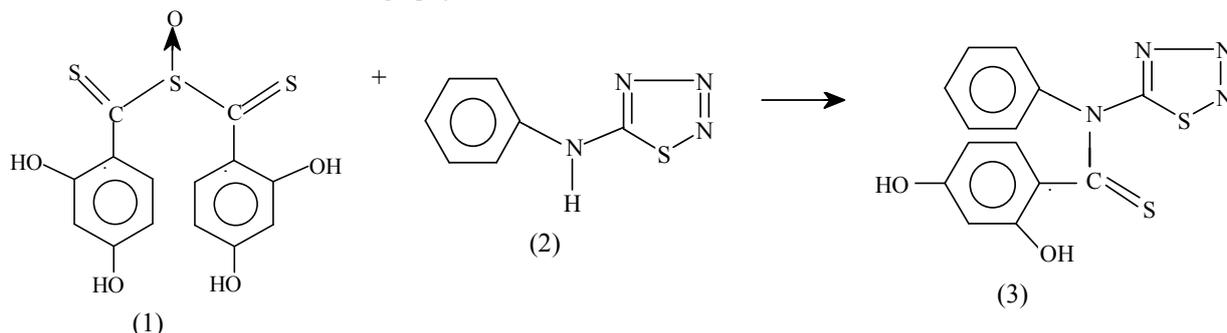


Figure 1a. Synthetic route and the structure of N,N-phenyl-1,2,3,4-thiazotriazole-5-yl-β-resorcylicarbothioamide.

N-3-(1,2,4-dithiazole-5-thione)-β-resorcylicarbothioamide (DTRTA) was obtained in the reaction according to Figure 1. The analytical data of compound were in agreement with the proposed

structure. The purity was confirmed by HPLC and HPTLC chromatography in the reversed-phase system (RP-8, RP-18, methanol-water).

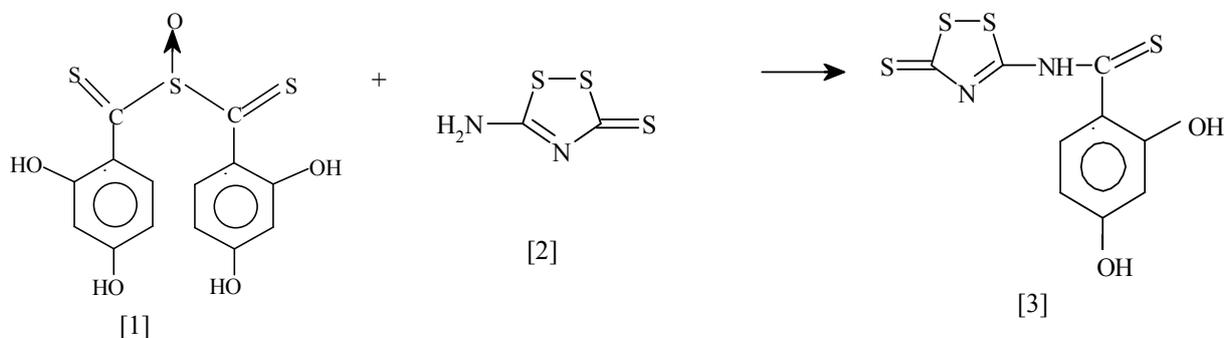


Figure 1b. Synthetic route and the structure of N-3-(1,2,4-dithiazole-5-thion)-β-resorcylicarbothioamide (DTRTA).

N,N-phenyl-1,2,3,4-thiazotriazol-5-yl-2,4-β-resorcylicarbothioamide (PHARA) was obtained in the reaction according to Figure 1. The analytical data of compound were in agreement with the

proposed structure. The purity was confirmed by HPLC and HPTLC chromatography in reversed-phase system (RP-8, RP-18, methanol-water).

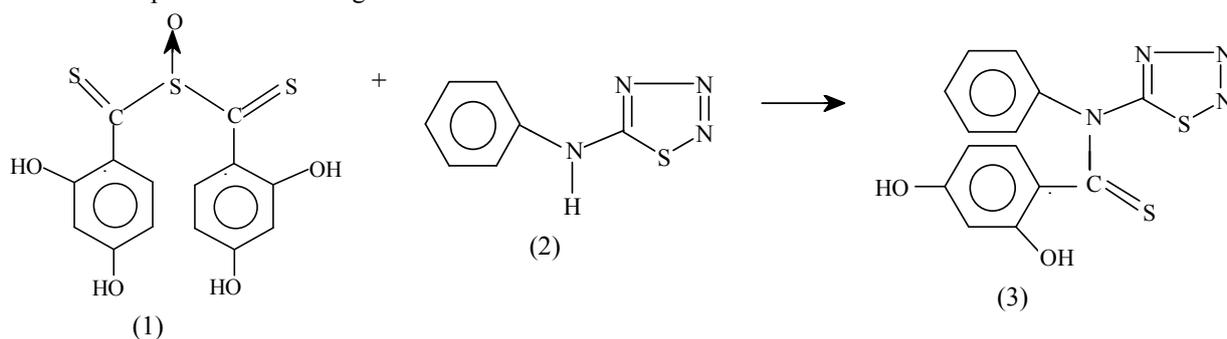


Figure 1c. Synthetic route and the structure of N,N-phenyl-1,2,3,4-thiazotriazol-5-yl-β-resorcylicarbothioamide.

PTR had a mean MIC of 12.5 mg/L for reference *C. albicans* 10231 ATCC strain on SB, 6.25 mg/L on YNB, respectively. PTR had MIC over the test range of 6.25- 50 mg/L for *C. albicans* isolates on SB. A mean MIC for *C. albicans* isolates was 19.6 mg/L on SB, and 16.9 mg/L on YNB. The MIC of PTR against the *C. albicans*

reference strain was 6.25 mg/L, and *C. albicans* strains – 14.9 mg/L in RPMI (Tab. 2).

DTRTA had a mean MIC of 12.5mg/L for reference *C. albicans* 10231 ATCC on SB, 6.25 mg/L on YNB, respectively. DTRTA had MIC over the test range of 3-50 mg/L for *C. albicans* isolates on SB. A mean MIC for *C.*

albicans isolates was 22.01 mg/L on SB, and 17.8 mg/L on YNB. Mean MIC for reference *C. albicans* 10231 strains on RPMI medium for DTRTA were 6.25 mg/L, for *C. albicans* strains 16.0mg/L (Tab. 2). PHARA had a mean MIC of 12.5 mg/L for reference *C. albicans* 10231 ATCC strains on SB, 6.25 mg/L on YNB, respectively. PHARA had MIC over the test range of 6.25 - 50 mg/L for

C. albicans isolates on SB. A mean MIC for *C. albicans* isolates was 19.6 mg/L on SB, and 16.9 mg/L on YNB. The MIC of PHARA against *C. albicans* reference strains were 6.25 mg/L, and *C.albicans* strains - 14.9 mg/L on RPMI. (Tab.2) Significant (p<0.001) differences between PTR, DTRTA, PHARA MIC values on RPMI medium and on Sabouraud's and YNB medium was found.

Table 2. MICs against *Candida albicans* and reference strain *Candida albicans* ATCC 10 231.

Strains	MEAN MIC [mg/l]								
	Sabouraud's medium			YNB medium			RPMI medium		
	PTR	DTRTA	PHARA	PTR	DTRTA	PHARA	PTR	DTRTA	PHARA
<i>Candida albicans</i> ATCC 10231	12.5 ± 0			6.25 ± 0			6.25 ± 0		
<i>Candida albicans</i> strains isolated from patients n=200	19.6 ± 10.7	22.0 ± 75	19.6 ± 10.7	19.6 ± 0.7	17.8 ± 7.4	16.9 ± 7.9	14.9 ± 6.2*	16.0 ± 5.3*	14.9 ± 6.2*

*P<0.001 vs PTR, DTRTA, PHARA MIC values on Sabouraud's and YNB medium

The reference *C. albicans* strain ATCC 10231 had enzymatic activity of 14 enzymes. The highest enzymatic activity had esterase, lipase, leucine and valine arylamidase and N-acetyl-β-glucosaminidase. Exposure to PTR inhibited the enzymatic activity of 6 enzymes, exposure to DTRTA inhibited the enzymatic activity of 9 enzymes. Exposure to PHARA inhibited the enzymatic activity of 6 enzymes. (Tab.3, Fig.4)

Before PTR exposure, *C. albicans* isolates had enzymatic activity of 16 enzymes, after exposure to PTR 8 enzymes was inhibited. The highest enzymatic activity had leucine arylamidase, esterase lipase, esterase, valine arylamidase and cystine arylamidase. (Tab. 4, Fig.4)

Before exposure to DTRTA, *C. albicans* isolates had enzymatic activity of 16 enzymes, after exposure to DTRTA 4 enzymes was inhibited. The highest enzymatic activity had leucine arylamidase, esterase, esterase lipase, α-glucosidase and N-acetyl-β-glucosaminidase. (Tab. 4, Fig.4)

Before PHARA exposure, *C. albicans* isolates had enzymatic activity of 16 enzymes, after exposure to PHARA 8 enzymes was inhibited. The highest enzymatic activity had leucine arylamidase, esterase lipase, esterase, valine arylamidase and cystine arylamidase. (Tab.4, Fig.4)

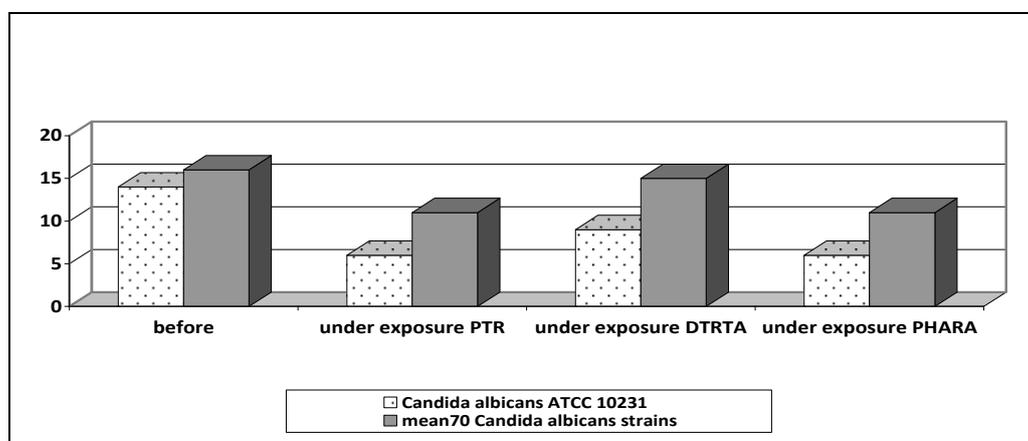


Figure 4. Number of active hydrolases in the (APIZYM) before and after exposure the tested compounds- PTR, DTRTA, PHARA.

Table 3. Enzymatic activity of *C. albicans* ATCC 10231 before and under exposure to PTR, DTRTA, PHARA.

Scale/ no strains	ENZYME ACTIVITY																		
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
Enzymatic activity of <i>C. albicans</i> ATCC 10231 before																			
N=1	1	3	3	1	3	4	2	2	0	1	3	1	0	0	2	2	3	0	0
Enzymatic activity of <i>C. albicans</i> ATCC 10231 under exposure PTR																			
N=1	0	1	1	0	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0
Enzymatic activity of reference <i>Candida</i> under exposure DTRTA																			
N=1	1	1	1	0	1	2	1	1	0	0	0	1	0	0	0	0	1	0	0
Enzymatic activity of reference <i>Candida</i> under exposure PHARA																			
N=1	0	1	1	0	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0

Table 4. Enzymatic activity of 70 *Candida albicans* strains before and under exposure to PTR, DTRTA, PHARA.

Scale/ no strains	ENZYME ACTIVITY																		
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
Mean values of the enzymatic activity of 70 <i>Candida albicans</i> strains before																			
n=70	1.1 ±0.4	2.5 ±0.6	2.5 ±0.6	0.8 ±0.6	3.7 ±0.9	1.9 ±0.7	1.9 ±0.7	0.3 ±0.5	0.3 ±0.1	1.45 ±0.1	1.7 ±0.6	0	0.2 ±0.8	0	2.2 ±0.7	0.6 ±1.5	3.5 ±1.5	0.4 ±0.5	0
Mean values of the enzymatic activity of 70 <i>Candida albicans</i> strains under exposure to PTR																			
n=70	0.6 ±0.6	1.9 ±0.6	2.3 ±0.7	0.6 ±0.7	3.1 ±1.5	1.6 ±0.9	1.5 ±1.1	0	0	1.2 ±1.0	1.2 ±0.8	0	0	0	1.4 ±1.1	0	0.5 ±0.8	0	0
Mean values of the enzymatic activity of 70 <i>Candida albicans</i> strains under exposure to DTRTA																			
n=70	0.4 ±0.5	1.9 ±0.6	1.6 ±0.6	0.2 ±0.4	3.1 ±1.1	1.2 ±0.4	0.8 ±0.4	0.1 ±0.2	0	0.7 ±0.6	0.8 ±0.7	0	0.3 ±1	0.2 ±0.7	0.9 ±1	0.1 ±0.3	0.9 ±1.0	0	0
Mean values of the enzymatic activity of 70 <i>Candida albicans</i> strains under exposure to PHARA																			
n=70	0.6 ±0.6	1.9 ±0.6	2.3 ±0.7	0.6 ±0.7	3.1 ±1.5	1.6 ±0.9	1.5 ±1.1	0	0	1.2 ±1.0	1.2 ±0.8	0	0	0	1.4 ±1.1	0	0.5 ±0.8	0	0

DISCUSSION

In this study, we found that the new thiatriazole derivatives - PTR, DTRTA, PHARA exert a moderate antifungal activity against *C. albicans* strains *in vitro*.

We have also found that these agents inhibited the enzymatic activity of selected hydrolases.

Among factors known to contribute to the pathogenicity of yeast, enzymes play a significant role, possibly being harmful to host tissues when they are liberated by the fungi. A correlation has been demonstrated between the amount of phospholipase produced and virulence in *C. albicans* strains and other yeast species [13]. Certain fungi such as *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium* and *Candida species*, have the ability of releasing hydrolytic enzymes into the environment, which break down multimolecular compounds such as polysaccharides, proteins, lipids, and hydrocarbons [13]. Azole resistance was first seen in patients with AIDS, especially those with very advanced disease who had considerable exposure to fluconazole, but azole resistance has now also been

noted in other very immunocompromized patients, such as those undergoing bone marrow transplantation [4].

A number of resistance mechanisms have been well described [1]. These include over expression of the target enzyme of the azoles (14- α demethylase), point mutations in this or other fungal enzymes, or the appearance of efflux pumps that rapidly eliminate the drug from the cell. These pumps can be fluconazole-specific, which means that other azoles can still be active or can act to remove all azole drugs.

Our results are in accordance with a previous study [14]. They assessed *anti-Candida* activity of 6-amino-2-n-pentylthiobenzothiazole, benzylester of (6-amino-2- benzothiazolythio) acetic acid and of 3-butylthio-(1,2,4-triazolo)-2,3-benzothiazole and compared to that of 2-mercaptobenzothiazole. They were active against other *Candida* strains. First compound exhibited inhibitory activity on germ-tube formation and mycelial growth in the *C. albicans* strains, while others were not active in these tests. All the compounds tested were highly active on a nystatin-resistant *C. albicans* mutant.

Kucukbay and Durmaz [15] assessed 40 organic or organometallic derivatives of benzimidazole and benzothiazole and 5 rhodium (I) and ruthenium (II) complexes for their in vitro antifungal activity against *C.albicans*. Four of the tested compounds, the rhodium containing compounds 30, 31, 32 and 33, were found effective at the MICs between 400-600 µg/ml.

Azolum salts and neutral 2-aryl derivatives of benzimidazole, benzothiazole and benzoxazole were synthesized by Cetinkaya et al. [16]. The salts 1 and the neutral compounds 2 were evaluated for their in vitro antimicrobial activity against the standard strains: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *C. albicans* and *C. tropicalis*. The compounds 1f, 1g, 1h, 1m, 1n, 2a, 2b, 2c, 2e, 2f showed antimicrobial activity against *E. faecalis* ATCC 29212, *S.aureus* ATCC 29213, *E. coli* ATCC 25922, *P.aeruginosa* ATCC 27853, *C. albicans* and *C.tropicalis* with MICs ranging between 50 to 200 mg/l.

New pyrimido [2,1-b] benzothiazole and benzothiazolo [2,3-b] quinazoline derivatives have been synthesized and tested for their antitumor and antiviral activities by el-Sherbeny [17]. The compounds 5c and 8d exhibited a broad spectrum antitumor activity with full panel (MG-MID) median growth inhibition (GI50) of 11.0 and 11.9 µmol/l, respectively. On the other hand, compounds 5c and 5d showed potential activity against Herpes simplex type-1(HSV-1) with 61 and 50% reduction in the viral plaques, respectively.

Advances made during the 1990s led to the introduction of a new allylamine, terbinafine, for the treatment of dermatophytoses and new lipid formulations of amphotericin B with improved safety profiles. In addition, new classes of antifungal agents such as the candins (e.g. pneumocandins and echinocandins), the nikkomycins, and the pradamicin-benranomicins are being studied [18].

Search for new antimicrobial agents led to the synthesis of series of N-1, C-3 and C-5 substituted bis-indoles. Their evaluation for antifungal and antibacterial activities resulted in the optimization of pyrrolidine/morpholine/N-benzyl moiety at the C-3 end and propane/butane/xylylidine groups as linkers between two indoles for significant inhibition of microbial growth. Preliminary investigations have identified three highly potent antimicrobial agents. Dockings of these molecules in the active sites of lanosterol demethylase, dihydrofolate reductase and topoisomerase II indicate their strong interactions with these enzyme [19].

Many cationic peptides with antimicrobial properties have been isolated from bacteria, fungi, plants, and animals [20]. This report surveyed the

literature to highlight the peptides that have antifungal activity and greatest potential for development as new therapeutic agents. Thus, to be included in the evaluation, each peptide had to fulfil the following criteria: (i) potent antifungal activity, (ii) no, or minimal, mammalian cell toxicity, (iii) of ≤ 25 amino acids in length, which minimises the costs of synthesis, reduces immunogenicity and enhances bioavailability and stability in vivo, (iv) minimal post-translational modifications (also reduces the production costs). The ~80 peptides that satisfied these criteria are discussed with respect to their structures, mechanisms of antimicrobial action and in vitro and in vivo toxicities. Certainly, some of these small peptides warrant further study and have potential for future exploitation as new antifungal agents.

However, the resistance of the yeasts to fungal agents is increasing. This still need to develop new antimycotics.

CONCLUSION

In our opinion, the new compounds PTR, DTRTA, PHARA exert a moderate antifungal activity against *C. albicans* strains *in vitro*. Further studies are needed to evaluate the antifungal activity in animal models.

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