In vitro anti-Candida albicans activity of new thiatriazole derivative agents

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A- Conception and study design; **B -** Collection of data; **C -** Data analysis; **D -** Writing the paper;
E- Review article; **F** - Approval of the final version of the article; **G** - Other (please specify)

ABSTRACT

Purpose: We tested 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*.
Materials and methods: We synthesized PTR, DTRTA and PHARA at the Department of Chemistry University of Agriculture in Lublin. 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 250 strains of *C. albicans* isolated from the patients was used. The enzymatic activity of the yeast-like fungi was performed by API ZYM test (bioMériux).
Results: The mean MIC *C. albicans* ATCC 10231 on Sabouraud's Medium was 12.5 mg/L and YNB Medium and RPMI medium - 6.25 mg/L. The mean MIC *C. albicans* on Sabouraud's Medium - exposure to PTR - 19.77 mg/L; exposure to DTRTA -21.06 mg/L, exposure to PHARA - 21.54 mg/L; on YNB Medium - exposure to PTR - 17.79 mg/L; exposure to DTRTA - 16.23 mg/l, exposure to PHARA - 18.92 mg/L and RPMI Medium - exposure to PTR - 12.73 mg/L; exposure to DTRTA -10.93 mg/l, exposure to PHARA - 10.65 mg/L. The reference *C. albicans* strain ATCC 10231 had 5 enzymes inhibited - after exposure to PTR inhibited the enzymatic activity of 13 enzymes, exposure to DTRTA inhibited the enzymatic activity of 10 enzymes and exposure to PHARA inhibited the enzymatic activity of 13 enzymes. The *C. albicans* isolates had 3 enzymes inhibited - after exposure to PTR **-** 5 enzymes was inhibited, exposure to DTRTA - 9 enzymes was inhibited and exposure to PHARA - 4 enzymes was inhibited.
Conclusion: The synthesized compounds PTR, DTRA and PHARA exert a moderate antifungal activity against the *C. albicans* strains *in vitro.*
Key words: Thiatriazole, antifungal activity, *Candida albicans*, *in vitro*

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INTRODUCTION

Yeasts are part of human normal flora and invasive infections arise when barrier leakage or impaired immune function occurs [1]. Candida albicans is common gastrointestinal flora that cause a wide range of severe manifestations when disseminated into the bloodstream.

Candida albicans and Candida species are common pathogen among micro-organisms isolated in intensive care units. Candidemia and candidiasis are major causes of nosocomial infections linked to a number of risk factors such as venous catheters, antimicrobial therapy, parenteral nutrition or immunosuppressive therapies [2]. In recent years, also an increase in infections due to non- *albicans* species of *Candida* has been reported [1]. *Candida* species are the fourth leading cause of circulatory infections [3].

Candidemia is associated with high rates of illness and death and has an attributable mortality rate >30%–40% in the United States [4]

In a Norwegian national study, comparison of the two periods found that the average incidence of candidaemia cases per 100 000 inhabitants increased from 2.4 (1991-2003) to 3.9 (2004-2012). Furthermore, the increase in incidence in the latter period was significantly higher in patients aged over 40 years [5] .

C. albicans is more frequent in patients aged up to 18 years, the frequency of C. parapsilosis decreases with age, and *C. glabrata* is more common in the elderly [6].

The discovery of the azole antifungal compounds 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 oxidative and peroxidative enzymes. This disruption of enzymatic processes ultimately leads to fungal cell death. Itraconazole has improved activity against moulds and dimorphic yeasts 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 immunocomprised host.

Series compounds with alfa-resorcylothiocarbamoyl 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 anti-fungal 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 anti-fungal medicines with azole moiety, N,N-phenyl-1,2,3,4-thiatriazole-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 anti-*Candida* activity of these new thiatriazole derivatives.

MATERIALS AND METHODS

We used three new synthetized chemical compounds:

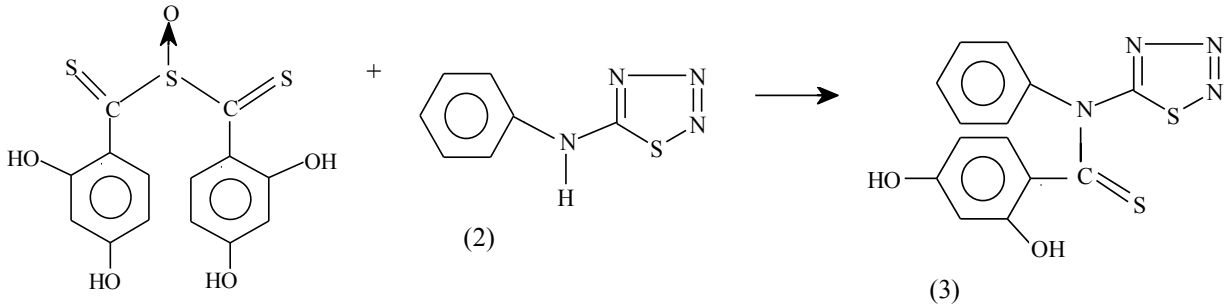
- N,N-phenyl-1,2,3,4-thiatriazole-5-yl-β-resorcyl-carbothioamide (PTR)
- N-3-(1,2,4-dithiazole-5-thione)-β-resorcylcar-bothioamide (DTRTA)
- N,N-phenyl-1,2,3,4-thiatriazol-5-yl-β-resorcyl-carbothioamide (PHARA).

Reference strain of *C. albicans* ATCC 10231 and 250 strains of *C. albicans* isolated from the patients were used for tests.

Chemistry

N,N-phenyl-1,2,3,4-thiatriazole-5-yl-β-resorcylcarbothioamide (PTR) 0.01 mol of sulphinyl-bis-2,4-dihydroxybenzenethioyl (1) and 0.025 mol of N-1,2,3,4-thiatriazol-5-ylaniline (2) (Sigma-Oldrich, Steinhein) were heated until boiling (3 hrs) in methanol (50 cm^3). 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). Sulphinyl-bis-2,4-dihydro-xybenzenethioyl (1) as the starting material was prepared according to patent [11]. PTR - N,N-phenyl-1,2,3,4-thiatriazole-5-yl-2,4-β-resorcylcar-bothioamide 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).

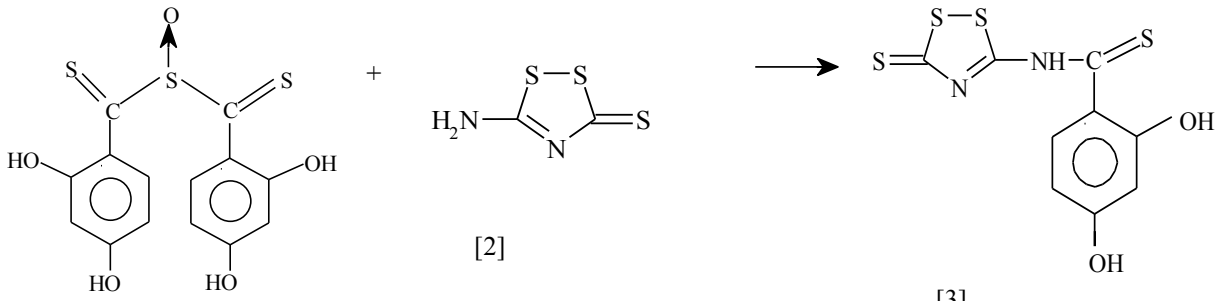
N-3-(1,2,4-dithiazole-5-thione)-β-resorcylcar-bothioamide (DTRTA) 0.025 mol of 3-amino-1,2, 4-dithiazole-5-thione (2) and 0.01 mol of bis-(β-rresorcylcarbothioyl)thionyl (1) was added into 50 ml of methanol and heated to boiling (3 hrs). After reaction completed, the mixture was hot filtered and added with 100 ml of water. Separated compound was filtered, washed with water and re-crystallized from dilute (2:1) methanol (60 ml).



(1) (2) (3)

Figure 1: Synthetic route and the structure of N,N-phenyl-1,2,3,4-thiatriazole-5-yl-β-resorcylcarbothioamide

Bis-(β-resorcylcarbothioyl)thionyl as the starting material was prepared according to patent [11]. N-3-(1,2,4-dithiazole-5-thione)-β-resorcylcarbothioamide (DTRTA) was obtained in the reaction according to Figure 2. 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).

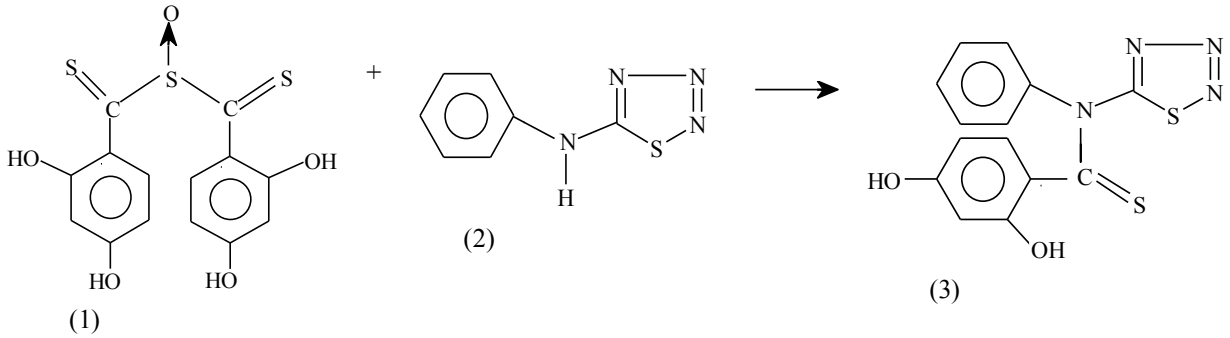


[1] [2] [3]

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

N,N-phenyl-1,2,3,4-thiatriazol-5-yl-β-resorcylcarbothioamide (PHARA) 0.01 mol of sulphinyl-bis-2,4-dihydroxybenzenethioyl (1) and 0.025 mol of N-1,2,3,4-thiatriazol-5-ylaniline (2) (Sigma-Oldrich, Steinhein) were heated until boiling (3 hrs) in methanol (50 cm^3). 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).

Sulphinyl-bis-2,4-dihydroxybenzenethioyl (1) as the starting material was prepared according to patent [11]. N,N-phenyl-1,2,3,4-thiatriazol-5-yl-2,4-β-resorcylcarbothioamide (PHARA) was obtained in the reaction according to Figure 3. 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).



(1) (2) (3)

Figure 3. Synthetic route and the structure of N,N-phenyl-1,2,3,4-thiatriazol-5-yl-β-resorcylcarbothioamide

Anal. ($C_{14}H_{10}N_4O_2S_2$, M=330.32) % N 28.50; m. p. 84-85°C; ^{1}H-NMR, DMSO-d_6 *(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. ^{1}H-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-d_6 and D_2O. 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).

Antifungal activity

The yeasts were identified to the species level by the Candi*Select* (Bio-Rad, Warsaw, Poland).

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 M27 [12].

Sabouraud's medium (SB), YNB - Yeast Nitrogen Base Medium and RPMI was used. Starting inocula were adjusted by the spectrophotometric method densitometr to 1x 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 (bioMériux). API ZYM is a semi-quantitative micromethod designed for the assessment of enzymatic activities. This method is applicable to all specimens (tissues, cells, biological fluids, microorganisms, washings, soil, oil, etc.). It allows the systematic and rapid study of 19 enzymatic reactions using only very small sample quantities (Table 1). 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.

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-naphthylamide
VI	Valine arylamidase	L-leucyl-2-naphtylamide
VII	Cystine arylamidase	L-cystyl-2-naphthylamide
VIII	Tripsin	N-benzoyl-DL-arrginine-2-naphthylamide
IX	Chymotripsin	N-glutaryl-phenylalanine-2-naphthylamide
X	Phosphatase acid	2-naphthylphosphate
XI	Naphtol-AS-BI-phosphodydrolase	Naphthyl-AS-BI-phosphate
XII	α-galactosidase	6-Br2-naphthyl-αD-galactopyranoside
XIII	β-galactosidase	2-naphthyl-βD-galactopyranoside
XIV	β-glucuronidase	Naphthol-AS-BI-βD-glucuronide
XV	α-glucosidase	2-naphthylyl-αD-glucopyranoside
XVI	β-glucosidase	6-Br-2-naphthyl-βD-glukopyranoside
XVII	N-acetyl-β-glucosaminidase	1-naphthyl-N-acetylo-βD-glucosaminide
XVIII	α-mannosidase	6-Br-2-naphthyl-αD-mannopyranoside
XIX	α-fucosidase	2-naphthyl-α-L-fucopiranoza

Strains were biotyped according to Williamsonis classification [13] distinguishing 8 biotypes (A to H) based on the analysis of five enzymes: esterase (II), valine arylamidase (VI), naphthol phosphohydrolase (XI), a-glucosidase (XV), and N-acetyl- β-D-glucosaminidase (XVII). Additional biotypes (I to N) described by Kurnatowska and Kurnatowski [14] as well as biotypes described by Krajewska-Kulak et al. [15], by Batura-Gabryel [16], and Bajer *et al.* [17] were also included in the assessment (Table 2).

Statistical analysis

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 < 0.05. These analyses were performed on a personal computer with a commercially available statistics program (Statistica 7.1 PL).

Table. 2. List of biotypes based on the available literature

BIOTYPES ENZYMATIC	ENZYMES				
	E 2 Esterase	**E 6 Valine arylamidase**	**E 11 Naphtol-AS-BI-phosphohydrolase**	**E 15 α-glucosidase**	**E 17 N-acetyl—ß-glucosaminidase**
according to Williamson et al. [13]					
A	+	+	+	+	+
B	+	-	+	+	+
C	+	+	+	-	+
D	+	+	-	+	+
E	+	+	+	-	-
F	+	+	+	+	-
G	+	-	+	+	-
H	+	+	-	-	-
according to Kurnatowska and Kurnatowski [14]					
I	-	-	-	-	+
J	-	-	-	+	+
K	+	+	-	+	-
L	+	-	+	-	+
M	+	-	+	-	-
N	+	-	-	-	+
according to Krajewska-Kułak et al. [15]					
O	+	-	-	-	-
P	+	-	-	+	-
R	-	+	+	+	+
according to Krajewska-Kułak et al. [15], and Batura-Gabryel et al. [16]					
S	+	+	-	-	+
T	+	-	-	+	+
acccording to Brajer et al. [17]					
T	-	+	+	-	-
U	-	+	+	-	+
W	-	+	-	+	-

RESULTS

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 and RPMI, respectively. PTR had MIC over the test range of 6.25-50 mg/L for C. albicans isolates on SB (Tab. 3).

A mean MIC for *C. albicans* isolates was 19.77±11.38mg/L on SB (5-50 mg/L), and 17.79±7.38 mg/L (3-50 mg/L) on YNB and 12.73±5.51 mg/L (6.25-25 mg/L) on RPMI (Tab. 3).

Table 3. MICs against *Candida albicans* and reference strain *Candida albicans* ATCC 10 231

	MEAN MIC [mg/l]								
	Sabouraud's medium			**YNB medium**			**RPMI medium**		
Strains	**PTR**	**DTRTA**	**PHARA**	**PTR**	**DTRTA**	**PHARA**	**PTR**	**DTRTA**	**PHARA**
reference *Candida albicans* ATCC 10231	12.5 ± 0			6.25 ± 0			6.25 ± 0		
Candida albicans strains isolated from patients N=250	19.77 ± 11.38	21.06 ± 12.20	21.54 ± 14.61	17.79 ± 7.38	16.23 ± 8.00	18,92 ± 10.66	12.73 ± 5.51*	10.93 ± 6.19*	10.65 ± 7.73 *

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

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

A mean MIC for *C. albicans* isolates was 21.06±12.20 mg/L on SB (3-50 mg/L), and 16.23±8.00 mg/L (6.25-25 mg/L) on YNB and 10.93±6.19 mg/L (6.25-25 mg/L) on RPMI (Tab. 3).

Table 4. 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 *C. albicans* 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 *C. albicans* 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 *Candida* 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 *Candida* under exposure PHARA																			
N=1	0	1	1	0	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0

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 and RPMI, respectively. PHARA had MIC over the test range of 6.25-50 mg/L for *C. albicans* isolates on SB (Tab. 3).

A mean MIC for *C. albicans* isolates was 21.54±14.61mg/L on SB (3-100 mg/L), and 18.92±10.66 mg/L (6.25 - 50 mg/L) on YNB and 10.65±7.73 mg/L (6.25 - 25 mg/L) on RPMI (Tab. 3).

We found significant ($p<0.001$) differences between PTR, DTRTA, PHARA MIC values on RPMI medium and 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-glucosamindase. 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.4).

Before PTR exposure, *C. albicans* isolates had enzymatic activity of 16 enzymes and 3 enzymes was inhibited (N-acetyl-β-glucosaminidase, β-glucuronidase, α-fucosidase), after exposure (Tab. 5):

- to PTR - 5 enzymes was inhibited (Chymotripsin, N-acetyl-β-glucosaminidase, β-glucuronidase, α-mannosidase, α-fucosidase)
- to DTRTA - 9 enzymes was inhibited (Lipase C14, Trypsin, Chymotripsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase)
- to PHARA - 4 enzymes was inhibited (α-galactosidase, β-glucuronidase, β-glucosidase, α-fucosidase).

Table 5. Enzymatic activity of 250 *Candida albicans* strains before and under exposure to PTR, DTRTA, PHARA

Scale/no strains	**Enzyme activity/ Mean values of the enzymatic activity of Candida albicans strains**									
n=250	**before exposure**									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
	1.15 ±0.36	2.47 ±0.59	2.47 ±0.59	0.78 ±0.62	3.64 ±0.92	1.89 ±0.66	1.84 ±0.76	0.304 ±0.46	0.21 ±0.41	1.98 ±0.74
	XI	**XII**	**XIII**	**XIV**	**XV**	**XVI**	**XVII**	**XVIII**	**XIX**	
	1.71 ±0.56	0	0.09 ±0.53	0	2.23 ±0.86	0.67 ±0.65	3.396 ±1.49	0.42 ±0.49	0	
	under exposure to PTR									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
	0.4 ±0.49	1.84 ±0.60	1.61 ±0.57	0.196 ±0.397	3.09 ±0.97	1.22 ±0.42	0.77 ±0.42	0.052 ±0.22	0	0.71 ±0.63
	XI	**XII**	**XIII**	**XIV**	**XV**	**XVI**	**XVII**	**XVIII**	**XVIII**	
	0.84 ±0.69	0	0.18 ±0.74	0	0.86 ±0.77	0.14 ±0.35	0,71 ±0.78	0	0	
	under exposure to DTRTA									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
	1.0 ±0	1.54 ±0.62	1.71 ±0.63	0	1.82 ±1.23	0.76 ±0.59	0.52 ±0.78	0	0	0.21 ±0.41
	XI	**XII**	**XIII**	**XIV**	**XV**	**XVI**	**XVII**	**XVIII**	**XVIII**	
	0.40 ±0.49	0	0	0	0.40 ±0.49	0	2.17 ±1.1	0	0	
	under exposure to PHARA									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
	1.12 ±0.34	2.44 ±0.67	2.49 ±0.61	0.804 ±0.60	3.53 ±1.14	1.92 ±0.69	1.83 ±0.80	0.28 ±0.45	0.24 ±0.43	1.78 ±0,82
	XI	**XII**	**XIII**	**XIV**	**XV**	**XVI**	**XVII**	**XVIII**	**XVIII**	
	1.63 ±0.61	0	0.12 ±0.66	0	2.12 ±0.80	0	2.72 ±1.77	0.24 ±0.42	0	

In the case of *C. albicans* 10 231 ATCC two hundred pre-exposure displayed activity for biotype A, after exposure to PTR - biotype K, after exposure to DTRTA -biotype S and after after exposure PHARA - biotype K (Tables 6,7).

Two hundred fifty pre-exposure *C. albicans* strains displayed 96.8% activity for biotype A and
3.2% for biotype F; after exposure (Table 6,7):

- to PTR - displayed 31.6% activity for biotype A, 25.2% for biotype F, 11.6% for biotype H, 10.4% for biotype D, 9.2% for biotype S, 6.8% for biotype E and 5.2% for biotype C
- to DTRTA- displayed 40.4% activity for biotype A, 26.4% activity for biotype S, 24.4% activity for biotype N, 6.4% activity for biotype I and 2.4% activity for biotype O
- to PHARA - displayed 80% activity for biotype A, 17.1% activity for biotype F, 0.8% activity forbiotype T and on 0.4% activity for biotype C,D,K,L,S.

Table 6. General biotype distribution of 250 *Candida albicans* strains before and under exposure to PTR, DTRTA, PHARA

BIOTYPES ENZYMATIC	STRAINS								
	reference *C. albicans* 10 231 ATCC N=1					*C. albicans* N=250			
	before exposure	after exposure				before exposure	after exposure		
		PTR	DTRTA	PHARA			PTR	DTRTA	PHARA
according to Williamson et al.									
A	**1**					**242**	**79**	**101**	**200**
B									
C							**13**		**1**
D							**26**		**1**
E							**17**		
F						**8**	**63**		**43**
H							**29**		
according to Kurnatowska and Kurnatowski									
I								**16**	
K		**1**		**1**					**1**
L									**1**
M									
N								**61**	
according to Krajewska-Kułak									
0								**6**	
according to Krajewska-Kułak et al. and Batura-Gabryel *et al*.									
S			**1**				**23**	**66**	**1**
acccording to Brajer *et al*.									
T									**2**

Table 7. Change biotypes of *Candida* strains after exposure to PTR, DTRTA, PHARA

reference *C. albicans* 10 231 ATCC N=1					
before exposure		post exposure			
biotyp	No	biotyp	PTR No	DTRTA No	PHARA No
A	1	K	1		1
		S		1	

C. albicans N=250					
before exposure		post exposure			
biotyp	No	biotyp	PTR No	DTRTA No	PHARA No
A	**242**	A	77	80	195
		C	12	12	1
		D	26	26	
		E	15	15	
		F	63	63	42
		H	27	27	
		K			1
		L			1
		S	23	23	1
		T			2
F	**8**	A	2	2	5
		C	1	1	
		D			2
		E	2	2	
		F	1	1	1
		H	2	2	

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 [17]. 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 [17]. 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 immune-compromized 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-∝ 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 [18]. They assessed *anti-Candida* activity of 6-amino-2-n-pentylthiobenzothiazole, benzylester of (6-amino-2- benzothiazolylthio) 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 [19] 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 minimum inhibitory concentrations (MICs) between 400-600 μg/ml.

Azolium salts and neutral 2-aryl derivatives of benzimidazole, benzothiazole and benzoxazole were synthesized by Cetinkaya *et al.* [20]. 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. tropicals*. The compounds 1f, 1g, 1l, 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. tropicals* 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 [21]. 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 echinocanidins), the nikkomycins, and the pradamicin-benanomicins are being studied [22].

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/xylidine 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 [23].

Many cationic peptides with antimicrobial properties have been isolated from bacteria, fungi, plants, and animals [24]. 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.

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.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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