In vitro antifungal activity of 2,5 disubstituted amino-oksometyloso-arylo-thiadiazole derivatives

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Abstract

Purpose: The aim of the study was the determination of antifungal activity of new of 2,5 disubstituted amino-oksometyloso-arylo-thiadiazole (AOAT) derivatives against *Candida albicans,* non-*Candida albicans.*

Material and methods: The determination of antifungal activity AOATs against 20 *Candida albicans*, 18 non-*Candida albicans* was performed. Isolates were from different ontocenoses of patients were used for tests. AOATs were synthesized at Department of Chemistry University of Agriculture in Lublin.

Results: The mean MIC of AOATs against *Candida albicans* strains was 141.625 (37.5-200) mg/L on Sabouraud's medium (SB). The mean MIC of AOATs against non-*Candida albicans* strains was 153.3 (50-200) mg/L.

Conclusion: It seems that AOATs exert potent antifungal activity against the yeast-like fungi strains *in vitro*.

Key words: 2,5 disubstituted amino-oksometyloso-arylo-tiadiazoles, *Candida albicans*, non-*Candida albicans*.

Introduction

The incidence of nosocomial infections by the yeast-like fungi strains has surged over the past decade, from the eighth to the fourth most common cause of nosocomial bloodstream infection in the general hospital population [1]. In surgical patients, the incidence of *Candida* infections has increased from 2.5 to 5.6 per 1 000 discharges, with mortality rates of 30% to 75%. Most reports are drawn from general hospital populations, or general surgery, burns and oncology services [2-4]. However, one result of widespread use of powerful antifungal drugs administered for increasingly broader indications has been a dramatic increase in the isolation of resistant forms of *Candida* [5-7].

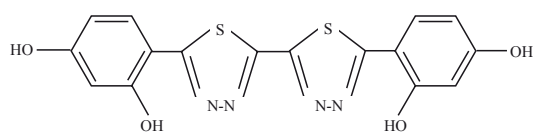
Advances made during the 1990's led to the introduction of a new allylamine, terbinafine, for the treatment of dermatophytoses and new lipid formulations of amphotericin B with improved safety profiles [8,9]. In addition, new classes of antifungal agents such as the candins (pneumocandins and echinocanidins), the nikkomycins, and the pradamicins-benanomicins are being studied. However, the resistance of the yeasts to fungal agents is increasing. This still need to develop new antimycotics.

The aim of the study was the determination of antifungal activity of new of 2,5 disubstituted amino-oksometyloso-arylo-thiadiazole (AOAT) derivatives against *Candida albicans*, and non-*Candida albicans* strains.

Material and methods

AOATs were synthesized at Department of Chemistry University of Agriculture in Lublin. An example of chemical structure of compound No 509 (bis(5-(2,4-dihydroxyphenyl)-2-1,3,4-thiadiazole) was presented in *Fig. 1*.

Figure 1. **Chemical structure of bis(5-(2,4-dihydroxyphenyl)-2-1,3,4-thiadiazole (No 509)**



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In order to define the antifungal activity of 2,5 disubstituted amino-oksometyloso-arylo-thiadiazole (AOATs) derivatives, we tested against 20 fresh clinical isolates of *Candida albicans*, and 18 isolates of non-*Candida albicans*. We used 30 different compounds of AOAT for tests. The yeasts were identified to the species level by the Candi*Select* (Bio-Rad), Fungiscreen 4H (Bio-Rad), Auxacolor (Bio-Rad) tests. Prior to antifungal susceptibility testing, each isolate was passaged on SB medium to ensure optimal growth characteristics.

AOATs were used in the tests. These compounds were dissolved in 1% DMSO. Susceptibility testing was performed by the agar dilution method. Minimum inhibitory concentrations (MICs) were determined by the agar dilution procedure according to National Committee for Clinical Laboratory Standards (NCCLS) reference document M27 [10]. Sabouraud's medium-SB (Bio-Rad) was used. Starting inocula were adjusted by the spectrophotometric method densitometr (BioMerieux) to 1x 10^5 CFU/ml. Concentrations of AOATs were ranging from 25 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 maximum final concentration of 1% had no effect on fungal growth. Control plates with SB medium without AOATs or with 1% DMSO were also prepared.

Two-tailed test was used to compare mean MIC values. 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 6.0)

Results

The analytical data of compounds 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). Details of number compounds used for fungal tests are shown in *Tab. 1*. AOATs had a mean MIC of 141.62 mg/L for 20 of *Candida albicans* strains on SB *(Tab. 1)*. AOATs had MIC over the test range of 37.5-200 mg/L for *Candida albicans* isolates on SB. AOATs had a men MIC of 153.3 mg/L for 18 non-*Candida albicans* strains. We found that AOATs had MIC over the test range of 50-200 mg/l for non-*Candida albicans* clinical isolates on SB (*Tab. 2*).

Discussion

In our study, we demonstrated the antifungal activity of new thiadiazole derivatives against *Candida albicans*, and non-*Candida albicans in vitro*. The MICs values of this sample were comparable with currently used antifungal drugs (e.g. itraconazole and fluconazole). These compounds had different MICs against the yeast-like fungi strains. We should mention *Candida albicans* strains used in the present study were resistant to several antimycotics.

In mycological reports there are a lot data on resistance problem of the yeast-like fungi to antifungal agents [11-13]. 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 [1]. A correlation has been demonstrated between the amount of phospholipase produced and virulence in *Candida albicans* strains and other yeast species. Certain fungi such as: *Mucor, Rhizopus, Aspergillus, Penicllium* and *Candida*, have the ability of releasing hydrolytic enzymes into environment, which break down multimolecular compounds – polysaccharides, proteins, lipids, hydrocarbons [1]. Our findings are in accordance with earlier studies on antimicrobial activity of thiadiazole derivatives [13-15].

In previous study [13] various new 1,4-disubstituted thiosemicarbazide and 2,5-disubstituted-1,3,4-thiadiazole derivatives were synthesized and evaluated for their *in vitro* antimicrobial activity. The structure of compounds was confirmed by elemental analyses and spectroscopic techniques. Some of the synthesized compounds were found to be active against *Candida albicans*. Similar findings were reported by Mamolo et al. [14]. In recent report [15] the two series of 4,6--disubstituted 1,2,4-triazolo-1,3,4-thiadiazole derivatives were synthesized and checked for their efficacy as antimicrobials in vitro. These compounds significant inhibition against all the strains tested, when compared to standard drugs. Furthermore, recently Rzeski et al. [16] reported anticancer activity of thiadiazole derivatives. Anticancer activity studies of 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT), as one of the most promising derivatives from the N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole set, have been continued. The tested compound inhibited proliferation of tumor cells derived from cancers of nervous system (medulloblastoma/rhabdosarcoma, neuroblastoma, and glioma) and peripheral cancers including colon adenocarcinoma and lung carcinoma. The anticancer effect of FABT was attributed to decreased cell division and inhibited cell migration. In anticancer concentrations it exerted a trophic effect in neuronal cell culture and had no influence on viability of normal cells including astrocytes, hepatocytes, and skin fibroblasts. Moreover, a prominent neuroprotective activity of FABT was observed in the neuronal cultures exposed to neurotoxic agents like serum deprivation and glutamate.

In conclusion, it seems that AOATs exert potent antifungal activity against the yeast-like fungi strains *in vitro*. Further studies are needed to select the most potent compounds from these derivatives.

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Table 1. **MIC value of thiadiazole compounds against 20 of *Candida albicans* strains on Sabouraud's medium**

No sample	No strain																				Mean MIC Value mg/L
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
469	200	200	200	100	200	100	100	200	100	100	50	100	100	100	50	50	100	200	100	50	**120**
470	200	200	200	200	200	200	200	200	100	200	200	200	200	200	200	200	200	200	200	100	**190**
471	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	**100**
472	200	200	200	100	200	200	100	200	100	100	100	100	100	200	50	100	100	200	100	50	**135**
474	200	200	200	50	100	100	50	100	50	50	50	100	50	100	50	50	100	100	100	50	**92.5**
479	100	100	100	50	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	**97.5**
490	100	200	200	100	100	100	100	200	100	100	100	50	50	100	100	100	100	100	100	100	**110**
492	25	25	50	25	25	25	50	50	50	50	25	25	25	25	25	25	50	25	25	25	**32.5**
499	100	100	100	100	100	100	100	100	100	100	50	50	50	100	100	100	100	100	100	50	**90**
501	100	200	200	25	100	25	25	200	50	50	50	100	25	200	100	25	25	200	50	25	**88.75**
506	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
507	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
509	50	50	50	25	50	50	25	25	25	25	50	50	50	50	25	25	25	50	25	25	**37.5**
528	100	100	100	100	100	100	100	100	50	100	100	100	100	100	100	100	100	100	100	100	**97.5**
529	200	200	200	200	200	200	200	200	100	200	200	200	200	200	100	200	200	200	200	200	**190**
530	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
533	200	200	200	100	200	200	100	200	100	100	100	100	100	200	100	200	200	200	100	100	**155**
534	200	200	200	200	200	200	200	200	100	200	200	200	200	200	200	200	200	200	200	200	**195**
535	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
536	100	100	100	50	50	50	50	100	50	100	100	100	100	100	50	50	50	100	50	50	**75**
537	200	200	200	200	200	200	200	200	100	200	200	200	200	200	100	100	100	200	200	200	**180**
538	200	200	200	100	200	200	100	200	100	100	100	100	100	200	100	100	100	200	100	200	**145**
539	200	200	200	100	100	100	100	200	100	200	100	100	200	200	200	200	200	200	100	100	**155**
540	100	100	100	50	50	100	50	100	50	50	50	50	100	50	50	50	50	100	50	50	**67.5**
541	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
542	200	200	200	200	200	200	100	200	100	100	100	100	100	200	100	200	100	200	200	100	**155**
544	200	200	200	100	200	200	200	200	100	100	100	200	200	200	100	100	100	200	200	100	**160**
545	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
546	200	200	200	200	200	200	200	200	200	200	100	200	100	200	200	200	200	200	200	200	**190**
550	200	200	200	200	200	200	200	200	100	100	200	200	200	200	200	200	200	200	200	200	**190**
																					141.625

Table 2. **MIC value of thiadiazole compounds against 18 of non-*Candida albicans* strains on Sabouraud's medium**

No sample	No strain																		Mean MIC value mg/L
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
427	200	200	200	100	200	100	100	100	200	50	50	100	200	200	200	200	200	200	**155.6**
429	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	**200**
446	200	200	200	100	200	100	100	100	200	100	200	200	50	50	200	200	200	200	**155.6**
438	100	100	100	100	100	100	50	100	100	100	100	100	100	100	100	100	50	100	**94.4**
468	200	200	200	100	200	100	100	200	100	200	200	200	100	50	200	50	200	200	**155.6**
438	200	200	200	200	200	200	200	100	50	100	200	200	200	200	200	200	200	100	**175**
528	200	200	200	200	200	100	200	100	100	50	200	200	200	200	200	200	100	100	**163.9**
499	200	200	200	200	200	200	100	50	50	50	200	200	200	200	200	200	100	100	**158.3**
492	50	100	50	50	50	50	50	50	50	50	100	50	50	50	100	50	50	50	**58.3**
536	200	200	200	200	200	100	100	50	50	50	100	100	100	200	200	200	100	100	**136.1**
474	200	200	200	200	200	100	200	100	100	50	200	200	200	200	200	200	200	200	**175**
479	200	200	200	200	200	100	200	200	100	100	200	200	200	200	200	200	200	100	**172.2**
501	200	200	200	200	200	200	200	100	50	50	200	200	200	200	200	200	100	100	**166.7**
509	200	200	200	200	200	200	200	50	100	50	200	200	200	200	200	200	200	200	**177.8**
540	200	200	200	200	200	100	100	50	50	100	200	200	200	200	200	200	100	100	**155.6**
																			153.3

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